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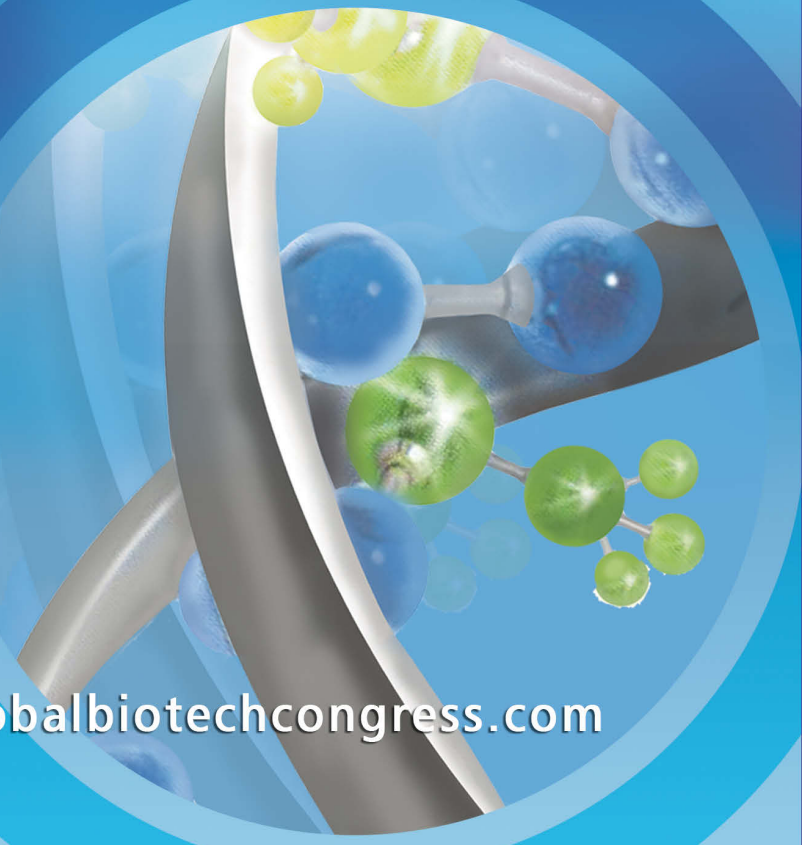

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PLENARY LECTURES

PL-55

OBESITY DUE TO ADENOVIRUS 36 INFECTION: CLINICAL PRESENTATION AND MECHANISMS

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The concept of obesity due to virus infection is now well accepted. Early studies in animals showed that canine distemper virus, Rous-7 virus, Borna virus, and scrapie agents caused obesity by damaging areas of the brain, predominantly the hypothalamus. Adenoviruses are particularly interesting as they appear to cause obesity *via* peripheral mechanisms and do not appear to cause brain damage. SMAM-1, an avian adenovirus, causes obesity in chickens and one study showed that humans with antibodies to SMAM-1 were heavier than uninfected individuals. The most exciting work has been done on human adenoviruses. Adenovirus 36 (Adv36) was the first human adenovirus reported to cause obesity in animals. Multiple studies have been done in humans associating Adv36 with human obesity as described below. Adv37 and Adv5 were shown to cause obesity in animals (chickens and mice respectively), but do not appear to be associated with human obesity. Adv36 has been administered to chickens, mice, rats, and monkeys, causing obesity in 60%-90% of lower animals and 100% of monkeys. Multiple studies in humans from across the world show that about 30% of obese humans and about 15%-20% of non-obese humans have been infected with Adv36. A large majority of studies show that prior Adv36 infection is associated in some way with obesity and this effect is strongest in children. Two meta-analyses show that Adv36 is associated with human obesity, with Odds Ratios of 1.9 and 1.6 in infected *vs* uninfected individuals. One meta-analysis showed that in children the Odds Ratio for obesity was 1.95 for infected *vs* uninfected. There is disagreement about the effects of Adv36 on metabolic variables. Animal studies show a consistent effect on serum lipids, with a paradoxical reduction of serum cholesterol and triglycerides in infected *vs* uninfected. In humans, some studies show lower serum cholesterol and/or triglycerides, whereas others show no effect or higher serum lipids in infected *vs* uninfected. Children's studies generally show higher levels of lipids with Adv36 infection. There also is some disagreement on the effects of Adv36 on glucose metabolism. Some studies have shown no effect of Adv36 on serum glucose and insulin, but most show that serum insulin levels and insulin resistance by HOMA are lower in animals or humans infected with Adv36. Again this is a paradoxical effect since both lipids and glucose metabolism would be expected to be worse with obesity. Mice that were made mildly diabetic with streptozocin, then infected with Adv36 actually had a decrease in serum glucose compared to uninfected mice. The mechanisms of Adv36 induced obesity and the effects on glucose metabolism appear to be due to direct action of the virus on peripheral cells. With the initial virus infection, there is an intense viremia that results in infection of most organs of the body. Seven months after experimental Adv36 infection in monkeys, viral DNA, but not live virus, could be recovered from brain, lung, liver, muscle, and adipose tissue. With initial infection of cells by Adv36, the viral DNA travels to the host nucleus and initiates a series of actions. In upper respiratory epithelium, the virus turns off apoptotic protective mechanisms, allowing the virus to rapidly replicate and make large numbers of copies of itself. Eventually the infected cell dies and releases the viral particles into the bloodstream where they travel throughout the body. In differentiated cells of most organs, the virus does not appear to replicate, or does so slowly. In multiple cell types the virus increases the number of glucose transporters in the cell membrane *via* stimulation of the Ras pathway. Glucose transport into cells is facilitated, thus lowering the need for insulin for glucose transport and giving the appearance of increased insulin sensitivity. Once glucose enters the cell, it is transformed into fatty acids by Adv36 induced stimulation of fatty acid synthase (FAS). FAS is the final step in production of fatty acids from glucose. Multiple cell types, including adipocytes, show increased stores of lipids. PPAR-Āis stimulated by Adv36, which results in differentiation of human adult stem cells into adipocytes. All of the actions are due to the viral gene, early gene 4, open reading frame 1 (E4orf1). If E4orf1 is blocked with siRNA, the effects of the virus are blocked. Transfecting the E4orf1 gene into cells, usually by lentivirus, results in the spectrum of Adv36 effects. Currently there are no antiviral agents that can be used to treat Adv36 infection or block its effects. Research is ongoing to identify active agents against Adv36. The long term solution for this disease will be final development of a vaccine which has been shown to produce antibodies in animals and block the obesity effect in mice.

Scope of the Topic: Adv36 causes obesity in multiple animal species. About 30% of obese people and about 15% of non-obese people are infected. Children show a stronger correlation of Adv36 with obesity. Adv36 improves glucose metabolism and reduces the effects of diabetes. Anti-virals and a vaccine are under development.

CONFLICT OF INTEREST

Richard Atkinson is the owner of Obetech, LLC. This company provides assays for adenoviruses that produce obesity and has several patents and patent applications regarding virus-induced obesity including diagnostic assays, vaccine, and antiviral agents.

PL-155

NATURAL PRODUCT BASED PHARMACOPHORES - *WHAT ARE THE MOST PRODUCTIVE APPROACHES?*

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From centuries natural products are serving as excellent sources of human medicines, and only about 200 years ago scientific progress allowed drug research to adopt its present shape. Today almost 50% of modern therapeutic agents are either natural products or their derivatives. The initial systematic drugs studied were derived from plants, some are still being used today, such as morphine, salicylic acid, quinine, digitoxin and pilocarpine. In spite of the successful outcomes from natural products as a rich source for drug leads, the pharmaceutical industries have abandoned or deemphasized natural product based drug discovery in favor of combinatorial libraries of synthetic compounds. However, this approach was eventually found to be less effective to improve the success rate in new drug discovery. Therefore, pharmaceutical R&D is facing an unprecedented decline in identification of new molecular entities (NME) against prevailing and emerging diseases. The change in the strategy of target identification may provide more useful start-points. The more productive approach is the phenotypic i.e. target–naive screening approach which can identify initial hits that can be useful start points for further development. Since the last two decades, we have been working in natural product-based drug discovery by using the phenotypic approach. This has led to the discoveries of several NMEs.

Epilepsy is among the leading neurological disorders in the world. About 50 million people worldwide have epilepsy with almost 90% of these people being in developing countries. Epilepsy is non-curable disease, but can be controlled and managed with medications. Unfortunately most of anticonvulsant drugs available in the market are synthetic in nature, and associated with severe side effects and have to be used whole life to control the seizures. Through extensive studies on medicinal plants of family Ranunculaceae, anticonvulsant natural products, isoxylitones, were discovered from medicinal plants *Delphinium denudatum*, and as well as in non alkaloidal aqueous extracts of *Aconitum cochleare*, *Aconitum laeve*, and *Delphinium nordhagenii*. *Delphinium denudatum* was found to exhibit a good anticonvulsant activity in *in vivo* animal models of epilepsy. Bioassay-guided isolation studies on the roots of this plant, afforded a non-toxic and non-alkaloidal aqueous extract, which exhibited strong anticonvulsant activities in *in vivo* animal models of epilepsy, such as MEST test, scPTZ, scBIC, scPTX, and scSTN tests. Further purification of aqueous extract led to isolation of a strongly anticonvulsant isomeric mixture of *E/Z* isoxylitones which were then synthesized to investigate their anticonvulsant activities in *in vitro* and *in vivo* models. Studies have shown a potent anti-epileptogenic activity of isoxylitones in scPTZ-induced kindling model in mice and they also found to affect some of the underlining molecular changes that are induced following the seizures. These compounds were also subjected to various toxicological studies and compound did not exhibit LD_{50} up to the dose of 1,000 mg/kg and were found to be harmless to the model animals with higher potency as antiepileptic compounds than the currently available drugs.

Multidrug resistance (MDR) is a challenging problem for the healthcare sector. It is very common in most important pathogens, such as vancomycin-resistant *Enterococci* and *Staphylococcus aureus*. Exposure and inappropriate use of the antibiotics is the major cause of MDR, both in developed and developing regions. We have been focusing our efforts on the discovery of natural and synthetic compounds, active against MDR bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* (resistant to over 20 antibiotics). About 1400 fully characterized natural and synthetic compounds were evaluated by high throughput screening against MDR *S. aureus* and *P. aeruginosa*. We have discovered some potent, reproducible and highly active MDR inhibitors of flavonoids, monoterpenes, sesquiterpenes, quinolones, thiourea and organometallic derivatives. Mechanism-based studies on selected compounds of both synthetic

and natural origin were also carried out to assess the compound-induced effects on membrane potential, efflux pump inhibition, etc. We also studied the reversal of multidrug resistance by using the MDR inhibitors, which boost the activity of existing antibiotics.

During this plenary presentation, underlying philosophy and approach of our research on cost-effective discovery of lead molecules at the interface of chemistry and biology will be discussed.

PL-1

ADVANCES IN CLINICAL APPLICATIONS OF SYNTHETIC THYMOSINS IN TREATING LIFE-THREATENING DISEASES

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Significant biochemical and clinical advances in the preparation and use of two of the synthetic thymosins in clinical medicine have occurred. Thymosin Ā1 (TĀ1) and Thymosin Ä4 (TÄ4) have been synthesized by solid-phase methodology and have reached the clinic. TĀ1 (Trade name Zadaxin) is approved in 35 countries for the treatment of hepatitis B and C, and as an immune stimulant and adjuvant. The most recent reports of clinical trials with TĀ1 are pointing to important, hitherto unrecognized, applications in a number of diseases and disorders, including severe sepsis, acute respiratory distress syndrome, peritonitis, acute cytomegalovirus infection, TB, and lung infections in critically ill patients. It is also emerging as a promising chemo-protective agent in patients undergoing chemotherapy and in the treatment of late stage melanoma in combination with chemotherapy. TÄ4 is the first of the synthesized Ä-thymosins to reach the clinic. Many of its activities directly affect the repair and regeneration cascade following injury. For example, TÄ4 guides progenitor stem cells from the outer layer of the heart to repair tissue sites within the heart after a heart attack and stimulates oligodendrogenesis in the brain. TÄ4 also has been found to protect cells and tissues from further damage and to reduce apoptosis, inflammation, and microbial growth. In experimental studies in mice, rats, rabbits and pigs, TÄ4's activities centering around wound healing have provided the scientific foundation for ongoing and projected human trials (phase I and II) in the treatment of eye injuries, dermal wounds, repair of heart following and acute myocardial infarction and in the brain following stroke, trauma or neurological diseases such as multiple sclerosis, and peripheral neuropathies. In the results of two early phase II trials in patients with dry eye, TÄ4 was found to significantly improve several signs and symptoms of dry eye, as well as to show positive trends in other outcome measures. The availability of a number of synthetic thymosin peptides like TĀ1 and TÄ4 has significantly accelerated animal experimentation in the field and is helping researchers to consider a number of new and novel clinical applications. In recently publishes studies, TÄ4 has been shown to be an effective promoter of corneal healing in patients with chronic, medically unresponsive, non-healing corneal defects related to loss of corneal innervation primarily associated with diabetes and neurotrophic keratitis due to herpes zoster, and in patients with moderate to severe dry eye secondary to GVH.

PL-2

LIPID-MODIFYING THERAPIES AND CARDIOVASCULAR PREVENTION: 2014 UPDATE

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The field of lipid management and cardiovascular prevention continues to move rapidly ahead. In the past year, a major development has been the release of controversial new guidelines from the American College of Cardiology and the American Heart Association on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults. These guidelines include important modifications in risk

assessment designed to better estimate risk in women and African Americans. A radically new feature is the elimination of low-density lipoprotein cholesterol targets, which have been replaced by recommendations regarding the intensity of therapy in four groups of individuals that can benefit from statin treatment.

Data regarding the relative efficacy and safety of statins and other lipid-lowering drugs, including niacin, resins, fibrates, and ezetimibe, continue to accumulate. Recently introduced agents, including the microsomal triglyceride transfer protein inhibitor lomitapide and the antisense therapeutic mipomersen, have been approved for the treatment of homozygous familial hypercholesterolemia for more than a year in the United States, with recently published studies providing additional information on their use in this population of very high-risk individuals. Cholesteryl ester transfer protein inhibitors, anti-inflammatory agents, and drugs targeting PCSK9 are advancing rapidly in clinical development and have the potential to address the residual cardiovascular risk that persists even with optimal statin therapy.

PL-105

MECHANISMS OF BARIATRIC SURGERY: IT'S IN THE CHEMISTRY, NOT THE PHYSICS

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Bariatric surgical procedures, including Roux-en Y gastric bypass (RYGB), vertical sleeve gastrectomy (VSG) and others, are the most effective treatments for obesity, with the vast majority of weight lost as fat. In patients with type 2 diabetes, these operations lead to full remission at one year in a substantial portion of patients, an effect that appears to be out of proportion to the associated weight loss alone. Although long considered to act through mechanical restriction of food intake and malabsorption of ingested nutrients, recent clinical observations suggest that both RYGB and VSG exert their therapeutic effects by altering the physiological regulation of energy balance and glucose homeostasis. To explore the mechanisms of action of these procedures, we have developed rat and mouse models of these operations and examined their effects on food intake, food preference, energy expenditure and glucose homeostasis. In both rats and mice, RYGB induces weight loss by decreasing food intake and increasing resting energy expenditure. The alterations in food intake are associated with decreased appetitive drive and altered food preferences. Late after surgery, these animals exhibit a change in preference from a high-fat, high-sugar diet to a normal chow diet. The RYGB-associated increase in energy expenditure results from stimulation of diet-induced thermogenesis, suggesting that this operation works in part by enhancing the normal thermogenic response to ingested nutrients. Like human patients, animals with type 2 diabetes exhibit a dramatic improvement in fasting blood glucose and glucose tolerance after both RYGB and VSG. These changes are associated with increased hepatic insulin sensitivity as well as improved pancreatic Ā-cell function and glucose-induced insulin secretion. The molecular mechanisms of these profound effects are just beginning to be understood. Although these operations are associated with elevated postprandial concentrations of GLP-1 and decreased circulating ghrelin, genetic deletion studies demonstrate that signaling by these hormones is not required for their effectiveness. Rather, the underlying mechanisms appear to include alteration of the intestinal microbiota, enhanced glucose transport into small intestinal enterocytes, bile acid signaling through the nuclear receptor FXR, and neural signaling through the melanocortin type 4 receptor. Dissection of the myriad physiological effects of these operations and identification of the cellular and molecular mechanisms underlying their effects will facilitate the development of novel, less invasive therapies for obesity, diabetes and related metabolic disorders that reproduce the profound and durable benefits of these operations, without the need for the surgery itself.

PL-3

IMPORTANT ROLES OF MICRORNA IN NEURODEGENERATIVE DISEASES

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Non-coding RNAs play essential roles in neurodegenerative disorders (Salta and Strooper, 2012). MicroRNAs (miRNAs) are an abundant class of small RNAs that mediate potent inhibitory effects on global gene expression. Recent advances in molecular methods allow us to study the contribution of these miRNAs to gene expression in neurodegenerative diseases, such as Alzheimer's disease (AD), which is the most prevalent form of dementia. One of the major hallmarks of AD is the presence of amyloid-Ā (AĀ) peptide plaques. Neurochemically, AD is believed to result from the misregulation of the production or clearance of AĀ. The rate-limiting step in AĀ production of is the cleavage of the amyloid- Ā precursor protein (APP) by a Ā-secretase or Ā-site APP-cleaving enzyme (BACE1). Expression studies suggest that dysregulation of proteins involved in AĀ production, such as APP and BACE1, may contribute to excess AĀ deposition. Elucidating how expression of these proteins is regulated will ultimately reveal new drug targets. Our aim is to utilize the novel approach of studying the regulation of these gene products by miRNAs. Here we review miRNA-mediated regulation of APP and BACE1. Using multiple bioinformatic tools and a series of functional studies in neuronal and glial cultures, we reported specific microRNA species regulate APP levels, such as miR-101 and miR-153 (Long, Ray and Lahiri, *J. Biol. Chem.,* 2012). We have recently reported the discovery of novel BACE1-specific miRNAs (Long, Ray and Lahiri, *J. Biol. Chem.,* 2014). Briefly, we prepared a chimeric BACE1 3'-UTR reporter construct by inserting the 3.9 kb BACE1 3'-UTR downstream of a reporter *Renilla* luciferase gene and then delivered the reporter construct along with several miRNAs predicted to target the BACE1 3'-UTR into human cell lines. Several "hits" (e.g. miR-298, miR-339-5p) resulted in reduced reporter expression. We further validated the reporter expression data for miR-339-5p by Western analysis of native BACE1 levels, which were significantly reduced following miR-339-5p delivery, with a significant reduction in potentially toxic AĀ levels. Delivery of miR-339-5p mimic also significantly inhibited expression of BACE1 protein in human primary brain cultures. Finally, miR-339-5p levels were found to be significantly reduced in brain specimens isolated from AD patients as compared to age-matched controls. Therefore, miR-339-5p regulates BACE1 expression in human brain cells and is most likely dysregulated in at least a subset of AD patients. These results reveal a novel regulatory interaction between two important AD-related genes (APP and BACE1) and specific endogenously expressed miRNA species. These novel miRNAs are likely to serve as novel therapeutic targets for AD.

This work is supported by grants from Alzheimer's Association and NIH to Dr. D.K. Lahiri.

PL-56

THE BIGGER IS BETTER IN THE CANCER TARGETING DRUGS: THE EPR EFFECT FOR THE PRIMARY AND METASTATIC TUMORS FOR TREATMENT AND IMAGING, AND BEYOND

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The enhanced vascular permeability and retention (EPR) effect is more universal tumor targeting mechanism for most solid tumors using macromolecular biocompatible drug conjugates or nanoparticles than so called molecular target drugs or antibody conjugated drugs. It utilizes vascular permeability of tumor vasculatures, which is uniquely different from the blood vasculature of normal tissues. This difference is not only seen in the anatomical architecture, but also seen in the production of vascular mediators excreted by the tumor tissue. Normal tissue surrounding the tumor tissue may be affected by these permeability factors, such as nitric oxide, bradykinin, vascular endothelial growth factors (VEGF), prostaglandins, and others [1].

Enhanced vascular permeability means extravasation of macromolecules including plasma proteins and other macromolecules (nanoparticles) or lipidic particles into interstitium of the solid tumors. Then what would happen to those nanoparticles. In the normal tissues, little extravasation of nanoparticle would occur usually. However, if it does occur as in the inflamed tissue, their clearance will be *via* the lymphatic system or reticuloendothelial system. One should recall that the lymphatic system is the most preferred site for cancer metastasis. Cancer cells traverse lymphotropically into the lymphatic system then to the regional lymph node, where they would propagate and become lymphatic metastatic nodules. This means once the nano-drugs are leaked out of blood vessels near the tumor tissue they will be targeted to the metastatic lymph nodes. Therefore, such nano-drugs may be advantageous candidates as an anti-lymphatic metastasis [2]. It should be noted, however, that larger lymph metastatic tumor nodules are fed *via* the neovasculature (blood vessels) [3], not by the lymphatic system that is also the subject of EPR effect [1]. Whichever the tumor feeding system, i.e. *via* the lymphatic or blood vessels, nanoparticles are therefore more advantageous than low MW drugs.

The EPR effect can be augmented to 2-3 fold by infusing permeability modulator such as nitric oxide generators (e.g. nitroglycerin), or by elevating the blood pressure, e.g. from 110 to 150 mmHg using angiotensin II. These tactics were found useful also in human clinic [1,5]. Further, angiotensin I-converting enzyme (ACE)-inhibitor, such as enalapril, would inhibit degradation of bradykinin (BK). As a consequence, the level of BK in tumor would increase, which will result in 2-3 fold enhancement of EPR effect (nano-drug delivery to tumor). None of these methods cause adverse effect [1].

Further, some hypovascular tumors, such as pancreatic and prostate cancer, metastatic cancer in the liver, and also tumors with heterogeneous EPR effect, that show least EPR effect and less cancer drug delivery, may become more tumor accessible by utilizing these tactics.

Metastatic tumors in experimental tumor model also exhibit the EPR effect, of which data will be presented. Consequently, polymer (styrene-co-maleic acid, SMA) conjugated pirarubicin (a derivative of doxorubicin (DOX), 4'-O-tetrahydropyranyl doxorubicin) at 20mg/kg of free THP equiv. iv dose only once at day 20 eradicated all lung metastatic tumor by day 50.

To best utilize the EPR effect we have recently synthesized another polymer conjugated pirarubicin using HPMA (hydroxypropylmethacrylamide) polymer [P-THP], which is designed to release THP at tumor site, namely by its unique environmental low pH and hydrolytic enzymes of tumor. P-THP, which accumulate more in tumor selectively by EPR effect, released free THP from the conjugates near in tumor tissue will be taken up into the tumor cells about ～ 100 times faster than free DOX. Thus cytotoxicity is seen only at tumor site. This EPR driven tumor selective accumulation is seen even 48 hr to 72 hr after iv infusion, where no normal organs showed drug accumulation after 24 hr.

Preliminary results of compassionate use of this P-THP conjugate for stage IV patients of end stage prostate cancer and lung cancer in a hospice indicate promising results, and no indication of toxicity at effective dose, 30～50 mg/1.8m^2 of free THP equivalent.

Photodynamic therapy (PDT) has been known for more than a century; which has longer history than cancer chemotherapy, and in PDT, it requires photosensitizers (PSs). The examples of commonly used PSs are Photofrin® and Laserphyrin®, not nano-drugs, and they emit fluorescence and generate oxygen radicals when illuminated at excitable light. The problem of these PSs is that they do not accumulate in the tumor selectively; rather they are distributed throughout the body evenly, but more preferably in the liver. And they are quickly excreted into the bile and then into the feces. Another problem is that current PDT uses He/Ne or YAG laser. However, they do not fully utilize excitation wavelength of PSs due to insufficient spectral fitting.

We have developed PS conjugated with HPMA polymer, SMA polymer, or PEG, and PS of our choice is zinc protoporphyrin (ZnPP), which have multiple cancer suppressing mechanisms other than generating oxygen radical upon light excitation (by blue fluorescent light, xenon light of normal endoscopic light). Spontaneously formed breast cancer in rats *in vivo* (autochthonous) and other cancers were completely eradicated by once or twice light irradiation after only one iv infusion of this polymer ZnPP conjugates at 20mg/kg. No toxicity was seen.

In conclusion, there are definitely advantageous aspects of nanodrugs as described above, however, sophistication and wide knowledge of tumor physiology, biology and biochemistry should be fully incorporated [4]. Augmentation of tumor delivery, in low EPR or heterogeneous tumor, will be possible to make nanodrugs more accessible to tumor 2-3 fold. We have demonstrated this in clinic using SMANCS in Lipiodol which was infused arterially and by elevating the blood pressure, for highly advanced stage IV patients, or with NO generating agents, and they found incredibly effective [5].

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PL-103

POWER OF MOLECULAR PATHOLOGICAL EPIDEMIOLOGY (MPE) APPROACH TO DISCOVER DISEASE BIOMARKERS FOR PRECISION MEDICINE

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This lecture introduces "Molecular Pathological Epidemiology (MPE)" (= Molecular Pathology + Epidemiology)" (Ogino *et al*. J Natl Cancer Inst 2010; Ogino *et al*. Nat Rev Clin Oncol 2011; Field *et al*. JAMA 2013; etc.) as simply as possible. Any given human disease represents fundamentally heterogeneous process, as implicated by the "Unique Disease Principle" (Ogino *et al*. Expert Rev Mol Diagn 2012; Ogino *et al*. Mod Pathol 2013). MPE dissects complex interplay between environmental, dietary and lifestyle factors, molecular pathogenic alterations, and disease occurrence and progression. MPE is a logical next step of genome-wide association studies ["GWAS-MPE Approach" (Ogino *et al*. Gut 2011)]. MPE has proved itself to be a promising approach to identify biomarkers for precision medicine (Chan *et al*. NEJM 2007; Liao *et al*. NEJM 2012; Nishihara *et al*. NEJM 2013, etc.). It is increasingly possible to design MPE database worldwide using routine molecular testing data, as molecular pathology testing is becoming routine clinical practice. It is essential to build large-scale population-based databases including medication use, lifestyle factors, molecular pathology, and clinical outcome. Such databases can generate novel information on potential chemopreventive or therapeutic benefits of drugs, which can be further tested by experimental models and clinical trials. Because disease heterogeneity is a ubiquitous phenomenon, the MPE paradigm should become routine to advance epidemiology in the 21st century, and move us towards personalized prevention and treatment.

PL-154

NEW CONCEPTS IN THE PATHOPHYSIOLOGY OF TYPE 2 DIABETES: INSIGHTS FROM HUMAN STUDIES

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Type 2 diabetes (T2D) is a major public health problem worldwide. However, the underlying molecular defects that confer T2D risk remain unknown. While diabetes risk factors are well-recognized, including family history, abnormal intrauterine environment, obesity, inactivity, and aging, the underlying defects that predispose to diabetes remain unknown. Insulin resistance, particularly in muscle, is an important contributor to T2D pathophysiology, occurring years before reduced insulin secretion or glucose intolerance, and also predicts T2D. Therefore, understanding

molecular mechanisms underlying insulin resistance is essential to develop new approaches for prevention and treatment of T2D.

The association of insulin resistance and risk for T2D with obesity and inactivity indicates an important, and potentially pathogenic, link between fuel and energy homeostasis and the emergence of metabolic disease. Given the central role for mitochondria in fuel metabolism, alterations in cellular mitochondrial oxidative function may contribute to the pathogenesis of T2D. Consistent with this hypothesis, analysis of genomic, metabolomics, and *in vivo* data has demonstrated evidence for alterations in oxidative metabolism in individuals at risk for T2D.

This presentation will focus on pathways altered in tissues of humans with established T2D, as well as those with insulin resistance and genetic risk, which we have identified in unbiased analyses of human tissue samples obtained from patients across the spectrum of insulin sensitivity and risk for type 2 diabetes. One such pathway is marked by overexpression of genes regulated by serum response factor (SRF), its coactivator MKL1, and its upstream regulator ABRA (actin-binding Rho-activated protein, also known as STARS). Importantly, ABRA is the top-ranking differentially expressed gene in muscle of humans with T2D, and its expression correlates with insulin resistance. Our recent studies in both animal and cellular models suggest that the ABRA-MKL1-SRF pathway is a very potent regulator of metabolism.

PL-153

MULTIFUNCTIONAL PHARMACEUTICAL NANOPREPARATIONS

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The use of pharmaceutical nanocarriers, including liposomes and polymeric micelles, for the delivery of a broad variety of both soluble and poorly soluble pharmaceuticals is our days a well-established paradigm for enhancing the *in vivo* efficiency of many drugs. The next challenge now is to develop a new generation of nanopharmaceuticals making those multifunctional and stimuli-responsive. In other words, such nanocarriers, depending on the specific requirements, can circulate long; target the site of the disease via both non-specific and/or specific mechanisms, such as enhanced permeability and retention effect (EPR) and ligand-mediated recognition; respond local stimuli characteristic of the pathological site by, for example, releasing an entrapped drug or deleting a protective coating to facilitate the contact between drug-loaded nanocarriers and target cells; provide an enhanced intracellular delivery of an entrapped drug; and even target individual organelles inside cells. Additionally, these carriers can include contrast moieties to follow their real-time biodistribution and target accumulation. Among new developments to be considered are: drug- or DNA-loaded delivery systems additionally decorated with cell-penetrating peptides for the enhanced intracellular delivery; "smart" multifunctional drug delivery systems, which can reveal/expose temporarily hidden functions under the action of certain local stimuli characteristic for the pathological zone; new means for controlled delivery and release of siRNA; organelle-targeted nanocarriers; nanocarriers co-loaded with siRNA and chemotherapeutics to treat multidrug resistant cancers; and theranostic nanopreparations.

PL-57

EGFR-TYROSINE KINASE INHIBITOR AGENTS AND NEUROGENIC INFLAMMATION ASSOCIATED WITH HYPOMAGNESEMIA

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Background: Erlotinib (ERL, Tarceva™), approved as a first line treatment, maintenance treatment, and 2nd- or 3rd-line treatment for advanced-stage non-small cell lung cancer, is a reversible tyrosine kinase inhibitor targeting the EGFR receptor (EGFR) which is up-regulated in the majority of lung, colorectal and head and neck cancers. However, EGFR activation is also required for active epithelial Mg-absorption/re-absorption mediated by the transient receptor potential melastatin 6 (TRPM6) channel in the kidney and colon. We determined whether prolonged treatment with ERL causes hypomagnesemia, oxidative stress and cardiac dysfunction in rats, and if blockade of the neurokinin-1 (substance P [SP]) receptor is protective.

Methods and Results: ERL was administered in the diet (~10 mg/kg/day) to normomagnesemic rats for 9 wks. Plasma magnesium in ERL-treated rats decreased gradually after 10 days and became significant between 3-9 weeks: -9% at wk 3, -13% at wk 5, -16% at wk 7 and -26% at wk 9. Moderate but significant increases in plasma substance P (SP) were detected at week 3 (+27%) and week 9 (+25%). At the end of 9 weeks, neutrophils isolated from whole blood exhibited a 3-fold higher basal, and a 2-fold higher stimulated (by PMA) superoxide generating activity. Concomitantly, total plasma 8-isoprostance content, a marker of systemic lipid peroxidation, rose significantly to 210%. The effect of SP receptor blockade was assessed by dietary administration of Emend™ (as aprepitant, a SP receptor antagonist, ~ 2mg/kg/day).

Emend mildly (NS) attenuated (up to 35%) ERL-induced hypomagnesemia, but significantly attenuated SP increases, neutrophil activation and 8-isoprostane elevation. Echocardiography revealed significant decreases in left ventricular ejection fraction (LVEF: -11%) and % fractional shortening (%FS: -17%) after 7 weeks, indicative of systolic dysfunction, and significant reduction (-17.5%) in mitral valve E/A ratio at week 9, indicative of diastolic dysfunction.

Concomitantly, left ventricular posterior wall thinning accurred consistent with early sign of dilated cardiomyopathy. Treatment with Emend completely prevented both systolic and diastolic dysfunction and partially attenuated anatomical changes caused by ERL treatment.

Conclusion: Since hypomagnesemia alone can cause neurogenic inflammation, our study suggested that chronic ERL treatment induced moderate but progressive hypomagnesemia, which in turn triggered SP-mediated oxidative inflammation and significant levels of cardiac dysfunction. Our study also demonstrated, for the first time, that administration of a clinically used SP receptor blocker, Emend, effectively prevented the ERL-induced systemic oxidative stress and cardiac dysfunction.

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PL-104

IMPORTANCE OF HUMORAL IMMUNITY IN *M. TUBERCULOSIS* INFECTION

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Mycobacterium tuberculosis (Mtb) infection is a major world public health problem. One third of the world's population is thought to have latent tuberculosis, a condition where individuals are infected by the intracellular bacteria without active disease but are at risk for reactivation, if their immune system fails. Here, we discuss the role of non-specific inflammatory responses mediated by cytokines and chemokines in response to interaction of innate receptors expressed in macrophages and dendritic cells (DCs). We also review current information regarding the importance of cytokines in the development of protective T cell mediated responses to Mtb and their influence in the formation and stability maintenance of granuloma. Will discuss novel insights of the mechanisms of failure of Mtb control, including the immune dys-regulation induced by the treatment with biologic drugs in different autoimmune diseases. Future functional studies focused in the mechanisms involved in the early control of Mtb infection and the interplay with host innate and acquired immunity may be helpful to understand the pathogenesis of TB particularly the role of humoral immunity and IL-17 in latent tuberculosis. A possible new vaccine could be based on booster immunizations with tuberculin together with Ag-85B-ESAT-6.

Most important is the fact that although cellular immunity is very important in the pathogenesis of active and latent tuberculosis, will discuss evidence that humoral immunity is also important. In this regard, recent evidence (Comas *et al*., Nature Genetics 2010) demonstrated that conserved genes of *M. tuberculosis* coding the epitopes that induce T cell dependent are naturally selected; mycobacteria benefits from the immune response. Also, cellular immunity deos not control drug resistant strains of MTB. Therefore, it is possible that more efforts should be made to study humoral immune responses in MTB infections particularly comparing individuals that had been BCG vaccinated with those without and comparing the immune responses in relation to the socioeconomic class, the pro-inflammatory state related to the microbiome.

KEYNOTE LECTURES

KNL-60

Track: Cancer Targeted Drug Delivery

TARGETING RESISTANT CANCER WITH SPECIFIC DRUG-EFFLUX INHIBITORS AND APOPTOSIS INDUCERS

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Overexpressed ABC ("ATP-binding cassette") transporters are involved in multidrug-resistant tumors by pumping anticancer drugs out of the cells. For early discovered ABCB1/"P-glycoprotein", third-generation drug-efflux inhibitors are under clinical development.

For more recently identified ABCG2/"breast cancer resistance protein", we have screened different series of flavonoids and derivatives, such as flavones, rotenoids and acridones, and more recently chalcones [1, 2] and chromones [3, 4], as inhibitors of mitoxantrone efflux from transfected HEK293 human cells and as chemosensitizers of cell proliferation, to establish 3D-Quantitative Structure-Activity Relationships. Two types of selective, non-competitive, inhibitors have been characterized, either inhibiting or stimulating the basal ATPase activity. The most potent inhibitor is indeed efficient *in vivo* on *SCID* mice, xenografted with human ABCG2-transfected cells, by chemosensitizing tumor growth to the drug-substrate irinotecan [5]. These selective inhibitors constitute good drug candidates, with low intrinsic toxicity, as sensitizers of cell proliferation to conventional chemotherapeutics.

The "Multidrug Resistance Protein 1" ABCC1 is able to catalyze the efflux of either glutathione conjugates or free glutathione together with hydrophobic substrate drugs. We have identified modulators such as verapamil [6, 7] mimicking substrates and inducing a fast and massive efflux of intracellular glutathione from ABCC1-overexpressing cells, leading to a selective cell death through apoptosis called "collateral sensitivity". Since verapamil is known for its cadiotoxic effects, we investigated other types of modulators such as xanthones [8], flavones and flavonoid dimers. Glutathione efflux appeared to be necessary, but not sufficient alone, to trigger apoptosis, indicating the contribution of other partner(s) or signaling pathway(s). Such apoptosis inducers may constitute a new type of anticancer drugs operating through an original strategy aimed at selectively targeting and eliminating multidrug-resistant tumors overexpressing the ABCC1 transporter [9].

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KNL-58

Track: Drug Discovery in Preclinical Research

ANTI-VZV VACCINE AS ANTI-HERPES THERAPY

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The phylogenetic tree of the members of the family *Herpesviridae* shows a close relationship between *Human herpesvirus*- (HHV-) 3 also known as *Varicella zostervirus* (VZV), and HHV-1 and HHV-2. The possibility of using the anti-VZV (anti-HHV-3) vaccine against orobuccal HHV-1 and genital HHV-2 was suggested.

A prospective study was conducted from January 2005 through January 2011. Twenty-four patients afflicted with HHV-1 and HHV-2 recurrences over a period of several years averaging 5–8 recurrences per year, agreed to receive the anti-VZV vaccine. They were compared with 26 non vaccinated patients presenting HHV recurrences 2–5 times a year. All 50 patients were checked by serological testing for anti-HHV-1, anti-HHV-2, and anti-VZV antibodies.

From 2005 through 2011, for all 24 anti-VZV vaccinated patients, the number of herpes relapses fell to 0 and was correlated with an increased anti-VZV antibody level and clinical recovery of all patients, whereas no improvement was observed for the 26 non vaccinated herpes patients.

A defective anti-VZV genetic immune power in these patients was suggested in correlation with a significant increase of anti-VZV serological antibody levels among the vaccinated patients ($P < 0.001$) and with the clinical recovery of all these herpes patients. As suggested recently [1], an increase in anti-HHV-3 antibodies with the anti-HHV-3 (anti-VZV) vaccine boosts HHV-1/HHV-2 defence against recurrent herpes diseases.

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KNL-75

Track: Chemistry

HEPARIN-BASED THERAPEUTICS WITH IMPROVED PROPERTIES

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Heparin, a highly sulfated polysaccharide anticoagulant, commands worldwide market of ~$7B. Currently, heparin and related low molecular weight heparins (LMWHs, such as enoxaparin, tinzheparin, dalteparin), require an extraction from pig intestine. The ultra-low molecular weight heparin (ULMWH) fondaparinux, a chemically synthesized pentasaccharide, is expensive and has limited clinical applications. We have developed an efficient chemoenzymatic synthesis of heparin, LMWH, and ULMWH. Here, we show our efforts to prepare low molecular weight heparin with improved pharmacological properties (*i.e.,* defined pharmacokinetics, liver clearance and protamine reversibility) using chemoenzymatic synthesis. These improved properties might expand the use of LMWH in patients with compromised renal function. Our approach relies on chemical synthesis and transformations relying on biosynthetic enzymes,

including heparosan synthases, sulfotransferases and epimerase. Chemoenzymatic synthesis is reliable over a wide range of scales and should be useful in both research and pharmaceutical applications. Furthermore, this biotechnological process should allow the preparation of this critical drug under cGMP. This controlled technological process should afford improved products and help prevent the introduction of impurities, contaminants and adulterants, possible through less highly regulated processes as illustrated by the 2008 heparin contamination crisis.

KNL-71

Track: Chemistry

MODERN METHOD FOR COMPONENT ANALYSIS AND IDENTIFICATION OF SUBSTANCE USING THE THZ SIGNAL WITH BROAD SPECTRUM AND MODERN ASSESSMENT CRITERIA

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In the past decade, the THz Time-Domain Spectroscopy has been widely used in problems of the detection and identification of explosives, drugs and other dangerous chemical and biological substances. THz-TDS technology is based on the analysis of frequency spectrum of THz radiation passed through or reflected from the substance. However, there is an essential disadvantage, which makes difficulties for reliable identification in many cases using this technology. For example, some dangerous materials have THz Fourier spectra, which is similar to spectra of ordinary harmless materials or have not pronounced absorption frequencies. We proposed method, named as SDA-method, which is free from this disadvantage. It allows to analyze the dynamics of many spectral lines simultaneously and to obtain the spectrogram - the unique 2D THz signature of the substance. Early, the SDA-method was successfully applied for identification of explosives, including those hidden under opaque covers; substances in compound media; the mixture of substances with similar Fourier spectra in GHz and THz range of frequencies. We also showed the possibility to use the spectrogram for the detection and identification by the reflected THz signal.

In this report, the SDA-method is applied for the detection and identification of illicit drugs - methamphetamine (MA), methylenedioxyamphetamine (MDA), 3, 4-methylenedioxymetham-phetamine (MDMA) and Ketamine by the transmitted THz signal. The drugs MA, MDA and MDMA have similar Fourier spectra in the range 0.1-3.0 THz, and their spectra do not have obvious absorption characteristics. However, we show that there is possibility to identify these drugs if the special criteria are used for the probability assessment for substances analyzing.

KNL-172

Track: Anti-Infectives

TECHNOLOGIES FOR NEW AND IMPROVED VACCINES

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Vaccines are without a doubt the most successful of mankind's medical interventions. However, despite more than two centuries of effective use of vaccines, many substantial challenges remain. These include: 1) improvement of existing but suboptimal vaccines (e.g., tuberculosis, influenza), 2) discovery and development of new vaccines against targets to address large unmet medical needs (e.g., HIV, malaria, cancer), and 3) rapidly responding to new pathogens (e.g., newly emerging

microbes, bioweapons). Recent advancements have demonstrated proof of concept for active immunization in the treatment of cancers. Taking full advantage will require the application of new technologies and paradigms in the areas of tumor antigen identification and optimization, novel potent and safe adjuvants, and enhanced vaccine delivery systems.

KNL-84

Track: Structural Biology

ALLOSTERIC INHIBITION OF PROTEIN-PROTEIN INTERACTIONS IN TRANSLATION INITIATION FOR DESIGN OF ANTI-TUMOR AGENTS

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Eukaryotic translation is regulated by features of the mRNA 5'UTR and by the concentrations and state of initiation factors. The eIF4E protein recruits the small ribosomal subunit to the 5' end of the mRNA *via* interactions with eIF4G and eIF3. Elevated concentrations of eIF4E have been found in several forms of cancer. The activity of eIF4E is regulated by the 4E-binding proteins (4EBPs), which are targets of kinase in signaling pathways and are validated tumor suppressors. We hypothesized that weakening the interaction of eIF4E with eIF4G would selectively reduce the translation of oncogenes. Using high-throughput screening we discovered small molecules that inhibit the eIF4E/eIF4G interaction. The inhibitors termed 4EGIs displace eIF4G from eIF4E but do not affect the interaction with 4EBP-1. Binding of the initial hit compounds and analogs to eIF4E were studied with NMR, X-ray crystallography and other biophysical techniques. The compounds and analogs were tested in *in vitro* and *in vivo* assays. Indeed, the molecules discovered exhibit activity against melanoma, breast, lung, prostate cancer and acute myelogenous leukemia (AML). The lead compounds inhibit eIF4E/eIF4G interactions in xenograft tumors in mice and reduce tumor growth. 4EGI-1 inhibits tumor expression of oncogenic proteins such as cyclin E, cyclin D1, c-myc and Bcl-2. Intra-peritoneal treatment with 4EGI-1 did not exhibit toxic effects in mice. Recently we determined high-resolution structures of eIF4E/inhibitor complexes and discovered that the inhibitors act by an allosteric mechanism. Furthermore, we find enhanced activity of 4EGI-1 against breast cancer stem cells at hypoxic conditions, such as found in solid tumors.

KNL-88

Track: Inflammation and Immunology

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) DNA BLOCKS IN NONAGERIANS AND CENTENARIANS OF MEXICO: ROLE OF SOCIOECONOMIC CLASS

Edmond J. Yunis[1], Edmond J. Feris[2], Nora Alvarez[3], Sandra Romero[4], Joaquín Zúñiga[5], Esteban Jesús Ortega Hernández[6], Juan García Lara[6], Mónica Escamilla Tilch[6], Julio Granados[6], Sharon Alosco[7], Marina Ohashi[7], Tatiana Lebedeva[7] and Neng Yu[7]

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The knowledge of genetic fixity, that is the use of frequency of DNA Blocks within the MHC (short arm of human chromosome 6) to measure population genetic diversity, should be taken into account in studies of longevity. Genetic admixture and control of infections explains the increasing incidence of autoimmune diseases in Europe (mixture of MHC autoimmune genes, MHCA with non-MHC autoimmune genes, NMHCA). And, in Mexicans not only MHCA are important in autoimmune disease but together with other genes (NMHCA genes) and the socioeconomic class of cohorts are markers of longevity. In this report, using the known DNA blocks of HLA in genetically admixed Mexicans, we found that genes within the class II region, present in autoimmune diseases in developed countries contribute to the increased frequency of class II DNA blocks (DRB!*15:01, DQB!*06:02, DRB*01:01 and *102, DQB1*05:01 in high socioeconomic class and DRB1*04:04, DQB1*03:02 and DRB1*06:03, DQB1*03:01 in low socioeconomic class in nonagenarians and centenarians in Mexicans. Also. the decrease of the added frequencies of Class I DNA blocks contributed by absence of the class I block B*07:02, C*07:02 is important in the low socioeconomic class. This finding explains the higher frequency of the ligands of group 2 of HLA-C of NK receptors (KIRs) which are important in the longevity of the low socioeconomic class. Therefore, our results are consistent with the concept that 'cohort morbidity phenotype' represents inflammatory processes that persist from early age into adult life producing different genetic effects related to the socioeconomic class of young controls compared with groups of genetically admixed nonagenarians and centenarians of Mexico.

INVITED LECTURES

IL-70

Track: Drug Discovery in Preclinical Research

IDENTIFICATION OF AN Atg8-Atg3 PROTEIN-PROTEIN INTERACTION INHIBITOR FROM THE MEDICINES FOR MALARIA VENTURE MALARIA BOX ACTIVE IN BLOOD AND LIVER STAGE *PLASMODIUM FALCIPARUM* PARASITES

Adelaide U.P. Hain, David Bartee, Natalie G. Sanders, Alexia S. Miller, David J. Sullivan, Jelena Levitskaya, Caren Freel Meyers and Jürgen Bosch

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Atg8 is a ubiquitin-like autophagy protein in eukaryotes that is covalently attached (lipidated) to the elongating autophagosomal membrane. Autophagy is increasingly appreciated as a target in diverse diseases from cancer to eukaryotic parasitic infections. Some of the autophagy machinery is conserved in the malaria parasite, Plasmodium; the best-characterized autophagy protein in *P. falciparum* (*Pf*) is Atg8. Although Atg8's function in the parasite is not well understood, it is essential for Plasmodium growth and survival and partially localizes to the apicoplast, an indispensable organelle in Apicomplexans. Here we describe the identification of inhibitors from the Malaria Medicine Venture (MMV) Malaria Box against the protein-protein interaction of *PfAtg8* with its E2-conjugating enzyme, *Pf*Atg3 by surface plasmon resonance (SPR). Inhibition of the protein-protein interaction of *PfAtg8* with *PfAtg3* prevents *PfAtg8* lipidation with phosphatidylethanolamine (PE). These small molecule inhibitors share a common scaffold and have activity against both blood and liver stages of infection by *P. falciparum*. We have derivatized this scaffold into a functional platform, while retaining *in vitro* parasite growth inhibitory activity for further optimization.

IL-127

Track: Inflammation and Immunology

PRECISION MEDICINE: THE FUTURE OF MEDICINE

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A new strategy is needed for the development of validated biomarkers (BMs) which will assist in optimal decision making process during preclinical phase of in drug development as well as in effective execution of personalized medicine in clinical phase across complex disease areas.

A systems biology approach that views biology as an information science to examine biological systems as a whole and their interactions with the environment is one way to approach for the discovery and development of BMs that can address these needs. This approach has particular power in the search for network-based informative BMs of diseases and treatment as well as BMs for selection of right patients who will likely respond to a specific treatment.

Recent advances in multiple omics technologies including epigenetics, genomics, transcriptomics, proteomics, cytometry, metabolomics, and imaging with the help of bioinformatics and biostatistics have improved the discovery and development of robust BMs for complex chronic diseases.

However, a consistent framework for the validation, acceptance and qualification of BMs for regulatory use is still required to promote innovative research and application of BMs in preclinical and clinical phase of drug development.

IL-111

Track: Diabetes and Obesity Drug Discovery & Therapy

PREVENTION OF OBESITY AND TYPE 2 DIABETES MELLITUS: IS IT FEASIBLE?

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Obesity is spreading throughout the world, and type 2 diabetes is suspected to affect at least half a billion people worldwide by 2030. A majority of U.S. women of childbearing age are overweight or obese, and these women are likely to gain excessive weight when they're pregnant, making it harder for them to return to their prepregnancy weight after delivery. Postpartum weight retention portends an increased BMI at the inception of future pregnancies and, during pregnancy, excessive weight gain, along with other risk factors such as gestational diabetes, can alter fetal growth and metabolism, leading to higher adiposity in the offspring. Some studies have identified prenatal risk factors for obesity ranging from lifestyle factors such as the mother's smoking status to psychosocial factors including antepartum depression, medical conditions such as gestational diabetes, physiological stress (as reflected by fetal exposure to glucocorticoids), and epigenetic markers such as gene-specific DNA methylation levels in umbilical-cord tissue. After birth, rapid weight gain in the first 3 to 6 months of life is a potent predictor of later obesity. The perinatal hormonal milieu may very well be a contributing factor, and higher leptin levels in umbilical-cord blood, have been shown to be associated with slower gain in infant weight-for-length and lower adiposity at the ages of 3 years and 7 years. In contrast, higher leptin levels at 3 years of age were associated with faster gains in BMI from 3 to 7 years, suggesting that leptin resistance develops between birth and 3 years of age. Moreover, the introduction of solids before 4 months was associated with a sixfold increase in the odds of obesity 3 years later. Emerging risk factors for obesity include exposure to endocrine disruptors, and the gut microbiota. Prevention of type 2 diabetes is possible in obese patients, and the risk may be decreased by 33% through diet *per se* and by 50% through diet and higher level of physical activity. Healthy food habits consist in 1) decreasing the daily caloric intake, the caloric intake after 9:00 pm, the consumption of food with high glycemic index, saturated and trans fatty acids, sodium, sugar-sweetened beverages and too much alcohol (> 36 g/day), 2) increasing the frequency of breakfast, the consumption of food with low glycemic index and low energy density, dietary fiber, fruit and vegetables, monounsaturated and polynsaturated fatty acids, and coffe, and 3) respecting the Mediterranean dietary patterns. The cited healthy food may decrease body weight and the risk of obesity.

IL-125

Track: CNS Drug Discovery and Therapy

$GABA_A$ RECEPTORS AS TARGETS FOR PROCOGNITIVE DRUGS

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In addition to being the most obvious and main symptom of developmental disorders such as Down Syndrome or later stage dementias, such as Alzheimer's, cognitive impairment is a core symptom of a varying array of disorders, such as Autism Spectrum Disorders and schizophrenia. Moreover, with the life expectancy in developed countries increasing, slowing down age-related cognitive decline has become a point of major interest, in order to increase the quality of life and independency, and extend the productive years of an ever-aging population. As such, development of procognitive drugs has been a major area of interest for drug development, with identification of a diverse set of targets, including various neurotransmitter systems and signalling molecules.

$GABA_A$ receptors are the main mediators of fast neuronal inhibition in the central nervous system and are classified into 6 subtypes based on the Ā-subunit they carry (Ā1-6). As targets of barbiturates and benzodiazepines, these receptors have been a main player in the treatment of different psychiatric disorders for decades. However, these drugs are not selective for specific $GABA_A$ receptor subtypes and affect a large number of these receptors in different brain areas simultaneously. This leads to an unfavourable side-effect profile and the possible masking of potential effects, as different $GABA_A$ receptor subtypes are distributed differentially in the brain and have been shown to have diverging functions through studies from our laboratory and others in the last 2 decades. One particular subtype, $Ā_5$-$GABA_A$-Rs, shows specific and almost exclusive expression in the hippocampus, the brain area mediating the formation of long-term memories in humans and other mammals. Here, we will attempt to give a short overview of studies investigating the role of $Ā_5$-$GABA_A$-Rs in learning and memory, with studies from our laboratory using genetic and pharmacogenetic methods, pharmacological studies in rodents and primates from other laboratories, and the effects observed in clinical trials testing drugs targeting this receptor. Preclinical and early-stage clinical studies suggest that inverse agonists of $Ā_5$-$GABA_A$-R can improve working memory and executive function, and could be effective in treating cognitive dysfunction in disorders such as Down Syndrome, without the adverse side-effect profile of classical benzodiazepines. However, according to some newer findings from our laboratory, the improvement in learning and memory can come at other cognitive costs. Thus, we will discuss earlier findings in the light of this caveat and focus on the potential of this drug target as cognitive enhancers for specific conditions.

IL-106

Track: CNS Drug Discovery & Therapy

MULTI-LEVELED RNA METABOLISM CHANGES IN ALZHEIMER'S DISEASE

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Mutations in genes involved in RNA metabolism have been shown to be involved in numerous neuronal disorders, but it remained unknown if acquired, rather than inherited impairments in RNA metabolism processes are causally involved in non-familial neurodegenerative processes. We have identified a significant reduction of the splicing regulators hnRNP A1/B2 in the entorhinal cortex of Alzheimer's disease (AD) patients that associates with synaptic impairments, cognitive decline and memory loss [1]. HnRNP A1/B2 depletion was mediated by cholinergic deficiencies and involved a decline of the acetylcholinesterase-targeted neuroimmune modulator microRNA-132 [2,3]. In mouse models, miR-132-mediated cognitive decline occurred through acetylcholinesterase targeting [4], and profiling Alzheimer brain transcripts demonstrated consistent miR-132 decrease [5,6]. Recently we have utilized novel high throughput sequencing strategies to identify extensive pathology and cognition-related alternative polyadenylation usage in AD. Taken together, our findings demonstrate multiple neurodegeneration-related alterations in RNA processing which may play a role in AD pathogenesis and disease progression.

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IL-184

Track: CNS Drug Discovery & Therapy

TNF-ĀAS A GATEKEEPER TO MITIGATING NEUROINFLAMMATION

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Inflammatory signals generated within the brain and peripheral nervous system direct diverse biological processes and are critical to the maintenance of homeostasis in healthy development and aging. Key amongst the inflammatory molecules is tumor necrosis factor-! (TNF-!), a potent pro-inflammatory cytokine that, *via* binding to its cognate receptors, is judged to be a principal regulator of cellular pathways that control an array of disparate activities associated with cell viability, gene expression, synaptic integrity and ion homeostasis. Although a self-limiting neuroinflammatory response commonly results in the resolution of an extrinsically or intrinsically initiated insult by the removal of toxic material or injured tissue to reinstate brain homeostasis and optimal function, on the occurrence of an unregulated reaction, where the immune response persists, a state of improper chronic neuroinflammation can arise. Such un-orchestrated neuroinflammatory action may provoke neuronal dysfunction and death, and initiate a self-propagating cascade of detrimental pathogenic events. Chronic neuroinflammation is a distinctive characteristic common across a broad range of debilitating neurological disorders (e.g., stroke and head trauma) as well as neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis), in which TNF-! expression has been determined to be dramatically elevated. Although it remains unclear whether neuroinflammation is the driving force behind these disorders, compelling evidence implicates both neuroinflammation and TNF-! in exacerbating disease progression. The development of small drug-like compounds that lower TNF-! levels systemically and in brain is providing the opportunity to elucidate the role of TNF-! in protective homeostasis signaling cascades as well as its targeting for intervention and treatment of a wide number of disorders.

IL-157

Track: Diabetes and Obesity

DIET-RELATED DISEASES AND THEIR PREVENTION/ATTENUATION BY NUTRACEUTICALS

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Metabolic syndrome (MS), even including obesity and diabetes, has become a serious health emergency in western and westernized countries. Inappropriate dietary habits represent one of the major causes of MS, and, therefore, use of nutraceuticals and functional foods has been increasing as one of the main therapeutic measure. Obesity is a generalized inflammatory condition also complicated by the modification of the gut microbiota which tends to aggravate the phlogistic status. On these bases, in obese patients reduction of inflammation and correction of the gut microbiota seem to be the major target of a series of nutraceuticals. In this framework, polyphenols (flavonoids and resveratrol), that are largely disseminated in the vegetal kingdom, are endowed with antioxidant and anti-inflammatory activities. In our own studies, red wine polyphenols or fermented grape marc (FGM) polyphenols have intensively been investigated in *in vitro* and in *in vivo* models for assessing their effects on the immune system. Quite interestingly, polyphenols are able to

induce differentiation and activation of peripheral human T regulatory (TREG) cells with release of interleukin (IL)-10, an anti-inflammatory cytokine. Moreover, release of free radicals from neutrophils and monocytes is decreased by pretreatment of human leukocytes by FGM. On the other hand, in murine experimental colitis oral administration of FGM leads to a dramatic decrease of inflammation by abrogation of intestine length reduction. Furthermore, in inflamed colon homogenates, a decrease of two inflammatory cytokines, tumor necrosis factor-alpha and IL-1 beta has been documented.

In addition, it is well known that polyphenols are able to induce changes of gut microbiota composition, thus, increasing the number of anti-inflammatory bacteria.

In conclusion, polyphenols for their anti-inflammatory activities and ability to stabilize the gut microbiota may represent appropriate nutraceuticals for the attenuation of the phlogistic conditions in obese people.

ACKNOWLEDGEMENTS

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IL-107

Track: Diabetes and Obesity Drug Discovery & Therapy

CONSEQUENCES OF OBESITY ON CENTRAL INSULIN AND LEPTIN SIGNALING

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In the wake of an obesity epidemic, we encounter a steady increase in the prevalence of metabolic syndrome and type 2 diabetes. A central feature of type 2 diabetes is insulin resistance, a state in which tissues in the body exhibit abnormal responses to normal levels of insulin. Obesity-associated activation of inflammatory pathways represents a key step in the development of insulin resistance in peripheral organs, partially via direct activation of Toll like receptor (TLR) signaling by fatty acids. In the Central Nervous System (CNS) we demonstrated that central application of palmitate, an abundant fatty acid in obesity, inhibits leptin-induced anorexia and Stat-3 activation in the hypothalamus, which was dependent on the principal TLR adaptor molecule MyD88. Mice deficient for the MyD88 specifically in the CNS (MyD88Ā^{CNS} mice) were protected from high fat diet (HFD) induced weight gain and HFD-induced leptin and insulin resistance, thus defining neuronal MyD88-dependent signaling as a key regulator of diet induced leptin and insulin resistance. Nevertheless, we questioned how central leptin resistance mechanistically induces insulin resistance. Our subsequent work identified the mitochondrial chaperone Hsp60 as a key integrator in central leptin and insulin signaling. We demonstrated that type 2 diabetic mice suffer from hypothalamic insulin resistance and mitochondrial dysfunction due to downregulation of Hsp60, which was caused by impaired leptin signaling. Acute downregulation of Hsp60 *in vitro* and *in vivo* in the hypothalamus induced insulin resistance, indicating that mitochondrial dysfunction can cause insulin resistance in the hypothalamus. Strikingly, type 2 diabetic patients also exhibited decreased expression of Hsp60 in the brain, indicating that this mechanism is also important in humans with diabetes. Thus, obesity can induce leptin resistance via MyD88 signaling in the hypothalamus and in turn causes mitochondrial dysfunction and insulin resistance via dysregulated Hsp60. These data provide a novel pathway of leptin/insulin crosstalk in the CNS which may impact on hypothalamic control of energy homeostasis in obesity and insulin resistant states.

IL-64

Track: Cancer Targeted Drug Delivery

THREE STEPS CANCER SELECTIVE MECHANISMS OF POLYMER CONJUGATED PIRARUBICIN AND SUPERIOR *IN VIVO* ANTITUMOR EFFECT

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Genetic diversity and accelerated mutagenicity in real cancer patients make the strategy of single molecular target drug formidable for cure of cancer. Further, conventional low molecular weight cytotoxic agents, which distribute normal tissues and organs throughout the body, and no tumor tropic accumulation, result in more severe adverse effect in patients. Therefore, a need for truly cancer selective anticancer agent is more urgent than ever. In this context, we have been working on biocompatible macromolecular anticancer drug-conjugates, which can utilize the leakiness of tumor blood vessels as a prime target due to their enhanced accumulation in cancer tissue, when the macromolecular drugs of > 50KDa, are injected iv. Second uniqueness of solid tumors is slightly acidic nature of the tumor tissue (～pH 6.5). We utilize this character for spontaneous hydrolysis of a bond (hydrazon) between the drug (pirarubicin, THP) and the polymers (e.g. HPMA polymer). Third character is the more rapid rate of endocytotic uptake of nanoparticle by tumor cells than non-dividing normal cells. Fourth character is to utilize more upregulated nucleotide transporter in the membrane of tumor cells, than that of normal cells, that would carry THP into tumor cells. These unique characters of tumor selectivity can be fully utilized in the present THP polymer conjugate (P-THP), which yielded excellent *in vivo* data as well as unpreceded clinical result.

Comparison of tissue distribution of free THP and P-THP after administered iv to tumor (S-180) -bearing mice showed, little tumor uptake of free THP, whereas P-THP showed exceedingly good tumor uptake, even 72 hr after drug administration; liberated THP was found only in the tumor. It also shows complete tumor eradication at 15mg/kg iv only once.

Preliminary clinical trial of only three patients at this moment showed remarkable results. All patients are stage 4 advanced cancer in our hospice. All cases resumed treatment in October as compassionate use and received infusion of P-THP 3 times at 50-75 mg free THP equiv. per patient, with interval of 2 weeks. All cases responded well. Case 1, prostate cancer, had numerous metastatic nodules in the pleural cavity and PSA value of 1460. Remarkable results were seen in PSA value decreased to 0.67, and most of the metastatic nodules disappeared in the lung after iv administration of three times. Case 2 was a lung cancer patient with meningeal carcinomatosis and multiple tumor nodules in the lung: symptom of meningeal carcinomatosis disappeared and size and numbers of nodules of lung tumor decreased clearly as seen by CT. Case 3, ovarian cancer with the tumor marker CA125, showed its decrease from the maximum 950 to 270. No one showed sign of serious adverse effect. Considering their clinical stage (end stage) and failure of all previous conventional treatments, present P-THP treatments appear very promising, and warrant further evaluation.

Keywords: Decreased toxicity, EPR effect and enhancer, stage IV lung cancer, stage IV prostate cancer, therapeutic effect, phase zero, pirarubicin-polymer conjugate, preliminary clinical evaluation, THP-HPMA-polymer conjugate, tumor selective delivery.

IL-123

Track: Inflammation & Immunology

PRO-INFLAMMATORY CYTOKINES ACTIVATE MULTIPLE SIGNALING PATHWAYS IN CULTURED HUMAN CHONDROCYTES

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The destruction of articular cartilage is one of the hallmarks of inflammation in rheumatoid arthritis (RA) and late-stage osteoarthritis (OA). In the milieu of inflammation, articular chondrocytes respond

to the elevated synovial fluid levels of the pro-inflammatory cytokines such as tumor necrosis factor-Ā (TNF-Ā) and interleukin-6 (IL-6) by up-regulating matrix metalloproteinase gene expression and induction of apoptosis. Using a quantitative Western blot approach, the present study determined the extent to which recombinant human TNF-Ā (rhTNF-Ā), rhIL-6, and two IL-6-type proteins, rh-adiponectin (rh-APN) and rh-oncostatin M (rh-OSM) activated the MAPK and JAK/STAT signaling pathways in the human juvenile immortalized chondrocyte cell lines, T/C28a2 and C28/I2, and in chondrocytes derived from human late-stage OA cartilage. P38 kinase-Ā, JNK1/2 and STAT3, but not STAT1 or STAT5, were activated by rhTNF-Ā in human OA chondrocytes. Although STAT1, STAT3 and STAT5 were constitutively phosphorylated in T/C28a2 chondrocytes, rhIL-6 and rhAPN modestly increased the phosphorylation of STAT3. However, STAT3 phosphorylation was markedly increased by rhIL-6 in C28/I2 chondrocytes. RhIL-6 also increased the content of unphosphorylated-STAT1A (U-STAT1A) and U-STAT1B in C28/I2 chondrocytes maintained in suspension culture. The IL-6 receptor blocking monoclonal antibody, tocilizumab, the combination of rhIL-6 and tocilizumab, or soluble IL-6 receptor (sIL-6R) plus rhIL-6, largely reduced the content of U-STAT1A. Three forms of U-JNK were identified in C28/I2 chondrocytes, M_r, ~51kDa, ~46kDa, and ~36kDa. Neither rhIL-6, nor rhAPN or rhOSM increased the phosphorylation of these JNK forms or p38 kinase-Ā However, rhOSM, rhAPN, and, to a lesser extent, rhIL-6, increased the phosphorylation of ERK1/2 in C28/I2 chondrocytes which was blocked by U0126, a small molecule inhibitor of MEK1/2. (Supported by a grant from Genentech/Roche Group, Takeda Pharmaceuticals of North America, and P30 EY-11373 from the National Eye Institute).

IL-115

Track: Diabetes and Obesity Drug Discovery & Therapy

POTENTIAL ROLE OF NATURAL BIOACTIVE COMPOUNDS ON PREVENTION OF OBESITY

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Overweight and obesity and their associated metabolic disorders are increasing at an alarming rate. Given their worldwide epidemic proportions, they appear to be a major threat to public health. According to the Center for Disease Control (CDC), more than 35% of U.S. adults and about 17% of children are obese. While several dietary and exercise regimens for weight reduction are available on the market, the progress and maintenance of weight loss often meets with less success. Natural bioactive phytochemicals present in foods have recently been discovered for their potential health benefit effects on the prevention of chronic disorders such as cancer, cardiovascular disease, inflammatory and metabolic disorders including obesity. Several natural polyphenols from plant sources such as catechins, resveratrol, curcumin, and anthocynines, which are bioactive phytochemicals, have been shown to modulate physiological and molecular pathways and are involved in energy metabolism, adiposity, and obesity. The potential *in vivo* beneficial effects of these polyphenols on adiposity and obesity as complementary agents in the up-regulation of energy expenditure have emerged by investigating these compounds in cell cultures, animal models of obesity, and in some human clinical and epidemiological studies. It should be noted that the adverse effects of high doses of purified and concentrated forms of these polyphenols have been reported. Nonetheless, adopting early behaviors to include foods containing these types of polyphenols in combination with a healthy lifestyle may help prevent weight gain and maintain ideal body weight.

IL-62

Track: Drug Discovery in Preclinical Research

OUTLINE OF THE ERYTHROPOIETIC PROCESS UNDER STEADY-STATE AND STRESS CONDITIONS

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Red cells are the specialized cellular elements of the blood responsible for both the delivery of oxygen to and the removal of CO_2 from all tissues of the body. Over time, their number in the blood is maintained constant by the balance of two ongoing processes: the destruction of old red cells, mainly in the spleen, and the generation of new of new red cells within the bone marrow by a process defined erythropoiesis.

Erythropoiesis is part of a more general process during which hematopoietic stem cells generate all the cellular elements of the blood. Generation of erythrocytes, like that of the other blood elements, is accomplished through a complex interplay between hematopoietic stem cells and regulatory elements. These regulatory elements include lineage-non restricted growth factors produced by stromal cells, the circulating hormone erythropoietin, a growth factor specific for erythroid cells produced by the kidney, and the extracellular matrix that provides functional support within the bone marrow microenvironment.

In addition to mechanisms assuring that under steady state conditions new red cells are produced in numbers equivalent to those that are being destroyed by senescence, there are regulatory mechanisms that increase red cell production in response to acute and chronic blood losses. These mechanisms involve activation of erythropoietin production by the kidney and of an alternative pathway of differentiation that favours proliferation over maturation defined stress erythropoiesis.

Recent advances in our knowledge on erythropoiesis and how this process is altered under conditions of stress have been instrumental to the development of culture conditions that allow production *in vitro* from currently discarded stem cell sources of red cells in numbers theoretically sufficient for transfusion. This presentation will summarize the scientific milestones in our understanding of human erythropoiesis that have made possible to conceive such cell therapy goal.

IL-158

Track: Hot Topics in Drug Targets

TARGETING GALECTIN-3 ATTENUATES BOTH CORNEAL FIBROSIS AND RETINAL GLIOSIS

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Purpose: Corneal scarring and retinal gliosis are fibrotic proliferations which commonly lead to significant vision loss. As recent studies have revealed that a carbohydrate-binding protein, galactin-3, plays a critical role in lung and kidney fibrosis, the current study was designed to determine whether a small molecular inhibitor of galectin-3 can prevent fibrosis in the cornea and retina in an experimental mouse model.

Methods: Alkaline burn injury (0.15 N NaOH, 1.5 min) was used to induce corneal fibrosis and retinal gliosis in murine eyes using a procedure described by Mohan *et al*. A galectin-3 inhibitor, TD139 (325 ng in 10Ãl), or vehicle (10Ãl PBS containing 0.5 % DMSO) were administered by sub-conjunctival injections every other day. Corneal opacity was scored by slit lamp examination. Corneas and retinal tissues were harvested on day 14 post treatment and subjected to Western blot analysis to detect the expression of ÄSMA, a marker of corneal fibrosis, and glial fibrillary acidic protein (GFAP), a marker of retinal gliosis.

Results: Eyes receiving TD139 demonstrated significantly reduced corneal opacity scores on day 14 (opacity scores on day 14: Vehicle treatment 2.101±0.21; TD139 treatment 1.14±0.19; N=28; p = 0.002). Control corneal and retinal tissue expressed negligible levels ÄSMA and GFAP, respectively. As expected, following alkali burn injury, corneal and retinal tissues expressed substantial amounts of ÄSMA and GFAP, respectively. Western blot analysis revealed that treatment with TD139, sifnificantly reduced ÄSMA expression in the cornea (TD139 treatment: 0.61±0.25 *vs* vehicle 1.0 normalized units) and GFAP expression in the retina (TD139 treatment: 0.45 ±0.05 *vs* vehicle 1.0 normalized units).

Conclusions: Our data provide proof-of-concept that targeting galectin-3 by the novel, small molecule inhibitor, TD139, ameliorates pathological corneal fibrosis as well as retinal gliosis. These findings suggest a potential new therapeutic strategy for prevention of these and perhaps other ocular cicatricial processes.

IL-156

Track: CNS Drug Discovery & Therapy

REVERSAL OF STRESS-INDUCED BEHAVIOR BY TARGETING NEUROSTEROIDOGENESIS: IMPLICATIONS FOR NOVEL PTSD THERAPY

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Levels of allopregnanolone (Allo) are reduced in the cerebrospinal fluid (CSF) of patients with posttraumatic stress disorder (PTSD), a severe, undertreated condition that affects millions, yet is without a consistently effective therapy. Selective serotonin reuptake inhibitors (SSRIs) are the only medications currently approved by the FDA for treatment of PTSD, though they are ineffective in a substantial proportion of PTSD patients, which underscores the need for new effective therapies. Allo is produced in emotionally relevant corticolimbic neurons of the frontal cortex, hippocampus and basolateral amygdala where it positively and allosterically modulates gamma-amino-butyric acid (GABA) action at $GABA_A$ receptors. This suggests that restoring downregulated corticolimbic Allo levels in PTSD patients may be beneficial. Allo biosynthesis is also decreased in association with the emergence of PTSD-like behaviors in response to protracted social isolation stress in mice. Interestingly, social isolation also causes changes in the frontocortical and hippocampal expression of $GABA_A$ receptor subunits, resulting in reduced benzodiazepine-mediated sedation and anxiolysis. Allo acts at a larger spectrum of $GABA_A$ receptor subunits than benzodiazepines, and increasing corticolimbic Allo levels in socially isolated (SI) mice by injecting Allo or stimulating Allo biosynthesis with a selective brain steroidogenic stimulant (SBSS), such as S-norfluoxetine at low non-serotonergic doses, improves PTSD-like behavior. This suggests that synthetic analogs of Allo, such as ganaxolone, may also improve behavioral dysfunction in the SI mouse model. We found that ganaxolone induced a dose-dependent improvement in SI-induced anxiety-like behavior and inhibited SI-induced aggression. An EC_{50} dose of ganaxolone also normalized the exaggerated contextual fear conditioning in SI mice. At these doses, ganaxolone failed to change locomotion in an open field test. Therefore, unlike benzodiazepines, ganaxolone may improve dysfunctional emotional behavior at non-sedating concentrations and provide an alternative treatment for PTSD patients who cannot adequately synthesize Allo. Hence, an Allo analog such as ganaxolone may offer a safe therapeutic GABAergic alternative to SSRIs or benzodiazepines for the treatment of PTSD or other disorders in which Allo synthesis may be impaired.

IL-66

Track: Drug Discovery in Preclinical Research

IDENTIFICATION, MECHANISM OF ACTION AND ANTI-TUMOR ACTIVITY OF A SMALL MOLECULE INHIBITOR OF HIPPO, TGF BETA, AND WNT SIGNALING PATHWAYS

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Embryonic signaling pathways, in particular those mediated by Wnt and TGF beta, are known to play key roles in tumor progression through the induction of epithelial-mesenchymal transition (EMT). Their simultaneous targeting could therefore represent a desirable anti-cancer strategy. Based on recent findings that both Wnt and TGF beta associated pathways are regulated by Hippo signaling in mammalian cells, we reasoned that targeting the latter would be more effective in inhibiting EMT. In a search for such inhibitors, we identified a small molecule (C19) with remarkable inhibitory activity not only against Hippo, but also against Wnt and TGF beta pathways. C19 inhibited cancer cell migration, proliferation, and resistance to doxorubicin *in vitro,* and exerted strong anti-tumor activity in a mouse tumor model. Mechanistically, C19 induced GSK 3 beta mediated degradation of the Hippo transducer TAZ, through activation of the Hippo kinases Mst/Lats and the tumor suppressor kinase AMPK upstream of the degradation complex. Overall, this study identified C19 as a multi-EMT pathway inhibitor with a unique mechanism of action. The observation that both AMPK and Mst/Lats mediate the effects of C19 sheds light on a potential cross-talk between metabolic and organ size control pathways in relation to cancer progression and suggests that C19 may have applications not only for the treatment of EMT associated diseases such as cancer and fibrosis but also for metabolic disorders such as obesity and diabetes.

IL-44

Track: CNS Drug Discovery and Therapy

A NOVEL 5' UNTRANSLATED REGION TRANSLATION CO-BLOCKER OF THE AMYLOID PRECURSOR PROTEIN AND PRION (PRP); INTERFACE THERAPY OF SPONGIO-FORMOPATHIES AND ALZHEIMER'S DISEASE

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We show evidence that iron influx directly drives the translational expression of both the neuronal Alzheimer's amyloid precursor protein (APP) and the prion protein PrP that can cause transmissible spongiform encephalopathies. Each of these neurodegenerative proteins were recently reported to have a key role in neuronal iron transport. We identified, via a classic 'release-of-repressor' mechanism, interaction between APP and PrP mRNAs with iron-regulatory protein-1 (IRP1) whereas IRP2 controlled the mRNAs encoding the L- and H-subunits of the iron storage protein, ferritin. Here, we report a high throughput screen and characterization of a highly potent benzimidazole-based tricyclic compound that co-jointly inhibited PrP and APP translation by selectively targeting the uniquely configured iron-responsive element RNA (IRE) stem loops in both the 5' untranslated regions (UTR)s of APP and PrP mRNAs. This compound was designated as BL-1, which was assessed by Western blotting to co-inhibit neural APP and PrP translation in SH-SY5Y

cells at picomolar concentrations without affecting viability or the expression of alpha-synuclein and ferritin. We are currently establishing the IC-50 and efficacy of BL-1 to reduce the production of toxic A∀ in SH-SY5Y neuronal cells and primary neurons compared with other well-tolerated tricyclic APP 5'UTR-directed translation blockers, including the benzoxazole paroxetine, that were shown to limit amyloid burden in mouse models of Alzheimer's disease (AD). These current studies provide key data to develop small molecules that selectively and coordinately reduce neural APP (and A∀Äin addition to prion production at 10-fold lower concentrations than previously characterized translation blockers. Our data evidenced a novel therapeutic strategy of potential impact for amyloidosis, an event seeded by prion after cleavage of A∀ from APP. This is predicted to be particularly relevant in people with trisomy of the APP gene on chromosome 21, which is a phenotype long-associated with Down syndrome (DS) that can also cause familial Alzheimer's disease.

IL-28

Track: CNS Drug Discovery & Therapy

BEYOND CLASSICAL BENZODIAZEPINES: NOVEL THERAPEUTIC POTENTIAL OF GABAA RECEPTOR SUBTYPES

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#-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. A variety of physiological processes and pharmacological responses have been shown to be mediated by $GABA_A$ receptors. The identification of specific physiological and pharmacological functions of $GABA_A$ receptor subtypes provides a rationale for the development of novel therapeutic strategies using $GABA_A$ receptor subtype-selective ligands. In the absence of truly subtype-specific ligands, we developed a combined genetic/pharmacological approach to identify functions of GABAA receptor subtypes. By introducing point mutations into $GABA_A$ receptor ! subunits which render defined $GABA_A$ receptor subtypes insensitive to modulators such as diazepam while maintaining sensitivity to the physiological neurotransmitter GABA, functions of $GABA_A$ receptor subtypes can be dissected genetically *in vivo*. For example, it was found that the sedative action of diazepam is mediated by ! 1-containing $GABA_A$ receptors, whereas its anxiolytic-like action is mediated by ! 2-containing $GABA_A$ receptors, demonstrating that anxiolysis and sedation are pharmacologically separable. Furthermore, pronounced analgesia can be achieved in models of inflammatory and neuropathic pain by specifically targeting spinal $GABA_A$ receptors containing ! 2 and/or ! 3 subunits. Global ! 3 knockout mice displayed a hyperdopaminergic phenotype and a prepulse inhibition deficit, which could be rescued with haloperidol. Sensorimotor and latent inhibition deficits have been found in ! 5 subunit partial knockdown mice, suggesting that ! 3- and ! 5-containig $GABA_A$ receptors might be useful targets in schizophrenia.

! 1-containing $GABA_A$ receptors on GABAergic neurons in the VTA inhibit the activity of these GABAergic neurons and thus indirectly disinhibit dopaminergic VTA neurons and drug-evoked synaptic plasticity in excitatory afferents onto dopaminergic neurons underlying drug reinforcement, indicating that ! 1-sparing compounds might have less abuse liability. Moreover, in addition to ! 1-containing $GABA_A$ receptors, ! 2-containing $GABA_A$ receptors in the nucleus accumbens are required for the reward-related properties of benzodiazepines.

In summary, identification of separable key functions of $GABA_A$ receptor subtypes suggest that receptor subtype-selective compounds could overcome limitations of classical benzodiazepines. Furthermore, they might be valuable for novel indications such as chronic pain.

IL-135

Track: Protein and Peptide Sciences

MIMICKING HIV STRATEGY TO SUPPRESS THE IMMUNE RESPONSE: NOVEL PEPTIDES FOR THE TREATMENT OF AUTOIMMUNE DISEASES

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Viruses have evolved several strategies to modify cellular processes and evade the immune response in order to successfully infect, replicate, and persist in the host. In the case of the human immunodeficiency virus (HIV), modulation of T-cell responses occurs via distinct mechanisms, one of which we found to involve inactivation of T-cells already at the stage of virus-cell fusion. Using biological and computational studies we have found that Hydrophobic portions of the gp41 protein of the viral envelope that contributes to membrane fusion also modulate T-cell responsiveness. These include the fusion peptide, the transmembrane domain and the loop region. We found that synthetic peptides derived from these domains have the ability to bind the T-cell membrane and to interact with the T-cell receptor (TCR) complex and inhibit T-cell proliferation and IFNÄsecretion. Importantly, the inhibitory mechanism differs between the different peptides. The immunosuppressive activity of these gp41 derived peptides can be exploited for the design of novel tools for down-regulation of undesired inflammation. This includes T-cell mediated autoimmune diseases, such as Multiple Sclerosis and Adjuvant Arthritis.

IL-32

Track: CNS Drug Discovery & Therapy

DEVELOPMENT OF THE SELECTIVE Ā1-ADRENERGIC PARTIAL AGONISTS AS A NOVEL THERAPEUTIC APPROACH FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common age-related neurodegenerative disease, affecting more than 24 million people worldwide. Unfortunately, existing drugs for AD only offer relatively small symptomatic relief and frequently have undesirable side effects. These limitations demonstrate a pressing need to develop new therapeutics for AD. Dysfunctions of the noradrenaline (NA) system have been strongly implicated in AD not only for cognitive symptoms but also for underlying pathological processes. Pathological changes in the NA system such as loss of NA neurons in the locus coeruleus, decreased cortical levels of NA, and changed expressions of ADRs has been well-characterized in both human patients and AD mouse models. In particular, beta1-adrenergic receptor (beta1-ADR) has been shown to play a key role in the disease. Recent studies from our laboratory suggest that restoration of the NA signaling with a beta1-ADR agonist in AD can provide promising therapeutic benefits which are not yet explored. We have shown that xamoterol, a selective beta1-ADR partial agonist, can rescue memory deficits associated with the disease in two independent preclinical models of AD. At the molecular level, xamoterol selectively activates a c-AMP signaling cascade while maintaining minimal activity on the beta-arrestin signaling pathway. By activating the cAMP pathway, xamoterol induces cellular and molecular events crucial for neurotransmission such as production of cAMP and phosphorylation of cAMP response element binding protein (CREB). On the other hand, the lack of effects on the Ā-arrestin signaling pathway will allow xamoterol to bypass receptor desensitization and provide sustained therapeutic efficacy. In addition to the neuronal effect, we have also shown that xamoterol is enhancing the beneficial neuroinflammation in mouse AD brains. Based on these unique pharmacological properties, we hypothesized that xamoterol can produce functional and disease modifying efficacy in AD. We have now put together a developmental plan to support the IND-enabling and proof of concept clinical studies contingent on obtaining regulatory approval for

xamoterol. This proof of concept studies is shown to be effective could open new doors for development of a therapeutic approach for AD which has not been yet explored.

Keywords: Adrenergic receptor, IND, Alzheimer's disease, herapeutics, brain.

IL-149

Track: Hot Topics in Medicinal Chemistry

EXPLOITING PROPERTIES OF THE DIRUTHENIUM-IBUPROFEN METALLODRUG TARGETED TO GLIOMA

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Ruthenium compounds have open new perspectives in the field of metal-based cancer chemotherapy as alternative drugs to the clinic used platinum chemotherapeutic agents. Two main classes of ruthenium compounds have been reported at the literature: a) Ru(III)-chloride-*N*-heterocycle based drugs, that have reached clinical trials due to anti-metastatic activity or cytotoxic properties overcoming drawbacks associated to platinum treatment; b) Ru(II)-organometallics which have also been found to show antitumor properties. None of these compounds, however, was reported to be active against glioma. Gliomas are primary brain tumors difficult to treat by conventional procedures. The most aggressive malignant intracranial tumor glioblastoma multiforme (GBM, WHO grade IV), for example, leads to a patient average survival of only one year after surgical resection. The urgent need of developing new chemotherapeutic drugs with novel modes of action prompted us to study the effects of a new class of metallodrugs on glioma models. We have used the strategy of combining carboxylic organic pharmaceuticals with ruthenium to prepare new biologically active compounds. The ability of pharmaceuticals derived carboxylate ligands to bridge two metal ions and stabilize metal-metal bonded centers has been exploited to prepare an unique class of metallodrugs that contain mixed-valent $Ru_2(II,III)$ cores capable of carrying four drug active ligands per dimetal unit [1]. Investigation of the effects on glioma models has shown that the coordination of carboxylate anions derived from drugs such as non-steroidal anti-inflammatories [2] or the Ā-linolenic acid [3] to the dimetal core leads to an enhancement of the activity through synergistic effects with the metal center. Features of synthesis, physico-chemical properties and insights into the mechanism of action, exploiting interactions with small biomolecules and serum proteins, as well as the most recent findings of our research on antitumor properties related to glioma, including human glioma cells and *in vivo* studies, will be discussed for the lead compound, Ruibp, of the formula $[Ru_2Cl(ibp)_4]$, ibp = anion derived from the organic drug ibuprofen (HIbp). (This work has been supported by Brazilian financial agencies FAPESP, CNPq and CAPES).

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IL-40

Track: CNS Drug Discovery and Therapy

REGULATION OF BACE1 AND A PRODUCTION BY GSK3À AND ITS PHARMACEUTICAL IMPLICATION FOR ALZHEIMER'S DISEASE

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Alzheimer's Disease (AD) is the most common neurodegenerative disorder leading to dementia. Deposition of amyloid Ā protein (AĀ) to form neuritic plaques in the brains is the pathological hallmark of Alzheimer's disease (AD). AĀ is generated from sequential cleavages of the Ā-amyloid precursor protein (APP) by the Ā- and À-secretases. Beta-site APP cleaving enzyme 1 (BACE1) is the Ā-secretase essential for AĀ generation. Increased AĀ levels could facilitate AD pathogenesis and inhibition of AĀ generation may have therapeutic implications for AD treatment. Our studies showed that regulation of BACE1 expression plays an important role in AD pathogenesis and could be a valid target for AD drug development. We found that BACE1 tightly controlled APP processing and AĀ production in normal condition, and selection of Ā-secretase cleavage site by BACE1 had a dramatic effect on AĀ production in the pathological condition. Upregulating BACE1 expression by hypoxia facilitated neuritic plaque formation and potentiated behavioral deficits in AD pathogenesis. Furthermore, we found that GSK3Ā signaling regulated BACE1 gene expression and AD pathogenesis and that inhibition of GSK3 signaling reduced AĀ neuropathology and alleviate memory deficits in AD model mice.

IL-152

Track: Innovative Drug Discovery and Nanotechnology

NANOPLATFORMS FOR PRECISION MEDICINE

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Biocompatible nanomaterials are key components of novel approaches to addressing the major problems of disease diagnosis and therapy for personalized medicine. We have developed several nanoplatforms that offer potential for significant improvements in multi-modal imaging and targeted delivery of therapeutics. Theranostic nanoplatforms combine multiple functionalities including multi-modal imaging using MRI, SPECT and FMT, magnetic targeting to the disease site, delivery of the drug payload through sustained as well as triggered drug release.

We have developed a new approach to chemoradiation therapy (CRT), termed Biological *In-Situ* Image Guided Radiation Therapy, that involves the coating of spacers routinely used during radiation therapy with nanoparticles that release radiosensitizing drugs (e.g. docetaxel DTX for Prostate Cancer), that is synchronized with the radiation therapy schedule, with almost no systemic toxicity. This new nanoparticle approach is an exciting new combinatorial therapy for cancer as well as other diseases where image-guided radiation therapy is currently a preferred choice of treatment.

We have recently prepared injectable nanoformulations of poorly soluble PARP inhibitors for blocking DNA repair in a variety of cancers. Combination platforms with DNA damaging agents (cisplatin) have also been developed. The nano-PARPi formulation acts as a chemo and radio-sensitizer enabling several therapeutic approaches for personalized medicine: MonoTherapy (PARPi only), Combination ChemoTherapy (PARPi + Pt), and Chemo-Radiation Therapy (PARPi + Pt + radiation).

Multi-modal nanoplatforms incoroporating MR, SPECT, optical and PET imaging moieties, as well as chemotherapeutics, have been developed. The iron oxide nanoparticles incorporated in the nanoplatforms have been used to demonstrate novel positive contrast MR imaging, and on-demand triggered release of chemotherapeutics. These nanoplatforms have been shown to enable quantitative image-guided drug delivery.

A new doctoral program has also been established incorporating new courses and interdisciplinary research in Nanomedicine.

Supported by the National Science Foundation, National Cancer Institute, DoD Prostate Cancer Research Program, Mazzone Foundation and CIMIT.

IL-67

Track: Traditional Chinese Medicine

THE CHEMICAL INVESTIGATIONS ON TRADITIONAL MEDICINES FROM WORLDWIDE DIFFERENT ORIGINS

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Traditional medicine plays very important role in the prevention and treatment of diseases in the world. The complex pharmacodynamic entities still attract the interests of chemists in our research team. “Cha de Bugre” (the leaves of *Casearia sylvestris*) is a traditional appetite suppressant in Brazil. 23 new compounds were obtained from the dried powder of “Cha de Bugre”, including fifteen new diterpenoids, 2 new C13 *nor*-isoprenoids, and 6 new neolignans, together with 28 known compounds. “Arjuna”, the bark of *Terminalia arjuna*, is a traditional Indian cardiotonic in heart failure. In our continuing chemical investigations, 8 new compounds including 5 novel 18,19-secooleanane type triterpenoids, 2 new oleanane triterpenoids, and one new ursane triterpenoids, together with one known lupane triterpenoid, 9 known oleanane triterpenoids, and 4 other known compounds were isolated from “Arjuna”. “Xue Tong”, the stem of *Kadsura heteroclita*, is a Chinese Tujia ethnomedicine used for rheumatoid arthritis. 25 new triterpenoids, lignans and sesterpenoids, as well as 26 know compounds were isolated and identified from it.

ACKNOWLEDGEMENTS

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SESSION LECTURES

SL-134

Track: Drug Discovery in Preclinical Research

CYCLIC IMIDES AS NOVEL CLASS OF COX-2 INHIBITORS

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A group of cyclic imides was designed for evaluation as selective COX-2 inhibitors and investigated *in vivo* for their anti-inflammatory activity. Several Compounds related to structures C were proved to be potent COX-2 inhibitors with IC50 range of 0.1-4.0 mM. *In vitro* COX-1/COX-2 inhibition structure-activity studies identified compounds containing homosulfonamide fragment as highly potent (IC50 = 0.1mM), and an extremely selective [COX-2 (SI) > 1000] comparable to celecoxib [COX-2 (SI) >384], COX-2 inhibitor that showed superior anti-inflammatory activity (ED50 = 72.4mg/kg) relative to diclofenac (ED50 = 114mg/kg).

SL-68

Track*: Cancer Targeted Drug Delivery*

TARGETING AMPK IN HCC: A POTENTIAL ROLE FOR COMBINED ASPIRIN AND METFORMIN THERAPY

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Hepatocellular carcinoma (HCC) ranks the 2nd most common cancer among Egyptian population with a rising incidence mostly due to high prevalence of viral hepatitis and its complications. Chemotherapy is one of the palliative approaches for un-resectable tumors, but the efficacy of current HCC chemotherapy is only modest and HCC remains an unmet medical need.

AMP-activated protein kinase (AMPK), an energy sensor that plays a key role in metabolism, emerged as an attractive target molecule for cancer prevention and treatment.

Aspirin and Metformin, having an excellent and a safer therapeutic index with few side effects, were reported to have a protective effect against development of cancer. Therefore, due to their potential effect on AMPK, we investigated the possible *in vitro* anticancer activity of combined Aspirin and Metformin therapy on HepG2 cell line by MTT assay. Furthermore, we assessed AMPK, p-AMPK and mTOR protein expression levels, apoptosis induction (Caspase 3 Activity Assay), and autophagy (TEM) as molecular targets for Aspirin/Metformin in both HepG2 cell line and Egyptian patients' HCC tumor samples.

Combined treatment by Aspirin/Metformin inhibited cell growth and induced both apoptosis signaling and autophagy. These findings suggest that Aspirin/Metformin combined treatment might be a promising anticancer strategy for Egyptian HCC's patients.

SL-133

Track: CNS Drug Discovery & Therapy

REELIN AND NEUROSTEROIDS IMPROVE BEHAVIORAL DYSFUNCTIONS INDUCED BY ANABOLIC ANDROGENIC STEROIDS

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Steroid abuse represents a growing public health concern because of the widespread worldwide recreational use. Long-term steroid use has been associated with negative effects such as increased irritability, impulsive aggression, anxiety disorders, irrational fear, and major depression. Furthermore, abusers who stop using steroids face withdrawal symptoms and can experience an increased risk of suicide.

Corticolimbic neurons express neurosteroid biosynthesis, which is altered in psychiatric conditions, including post-traumatic stress disorder (PTSD) and depression. Administration of anabolic androgenic steroid (AAS), including testosterone propionate (testosterone), nandrolone, and stanozolol affect the expression of 5Ā-reductase-type-I in glutamatergic corticolimbic neurons of the prefrontal cortex, hippocampus, and basolateral amygdala (BLA), which results in a downregulation of the GABAergic neurosteroid allopregnanolone. Increased aggression and fear responses are abolished by normalizing corticolimbic allopregnanolone levels with allopregnanolone treatment or selective brain steroidogenic stimulants (SBSSs), including S-norfluoxetine. Furthermore, a single bilateral BLA microinfusion of reelin, a glycoprotein expressed in GABAergic neurons with trophic functions at postsynaptic dendritic spines, abolishes aggression in AAS-treated mice in the absence of locomotion impairment. Ongoing studies in our laboratories are investigating the role of steroid-regulated antimicrobial peptides (AMPs), such as ! -defensins in peripheral tissue. Like NPY, these peptides are also expressed in the forebrain where they may play a role in the regulation of behavior. Altogether, our results suggest that increasing neurosteroids or neuropeptides, such as reelin and possibly ! -defensin may regulate corticolimbic circuitry and improve behavioral dysfunctions that relate to AAS abuse.

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Keywords: Aggressive behavior, anabolic androgenic steroid (AAS), biomarkers, emotional behavior, neuropeptides, neurosteroidogenesis, neurosteroids, psychiatric conditions, reelin, selective brain steroidogenic stimulants (SBSSs).

SL-137

Track: CNS Drug Discovery & Therapy

THERAPEUTICAL EFFECTS OF MINOCYCLINE AND FK506 ON REHABILITATION FROM SPINAL INJURY IN RATS

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Injury to the spinal cord results in immediate physical damage (primary injury), followed by a prolonged posttraumatic inflammatory disorder (secondary injury).

The present study was designed to investigate the neuroprotective effects of minocycline and FK506 (tacrolimus) individually and in combination on rehabilitation from experimental spinal cord injury (SCI). Young adult male Sprague-Dawley rats, weighing 250-280g were subjected to experimental SCI by exposing extradural area of the spinal cord for compression. The drugs minocycline (50 mg/kg) and FK506 (1 mg/kg) were administered orally in combination as well as individually to the SCI group daily for three weeks. The

rehabilitation parameters included behavioral as well as biochemical assessments. Behavioral motor functions (BBB and Tarlov scorings) were assessed in a blind manner every alternate day for 29 days after SCI. For biochemical studies, the drugs were administered 5 minutes after SCI and after an interval of 4 hours, the animals of all groups were sacrificed and 1.5 cm segment of the spinal cord centered at the injury site was removed for biochemical analysis of monoamines like 5-hydroxytryptamine (5-HT) and 5-hydroxy-indolacetic acid (5-HIAA), and oxidative stress indices like thiobarbituric acid - reactive substances (TBARS), total glutathione (GSH) and myeloperoxidase (MPO). All behavioral results indicated that both drugs induced a significant rehabilitation effect from the SCI with respect to time. The biochemical results also supported the behavioral findings by showing significant recovery in the levels of monoamines as well as in the oxidative stress indices. Overall, the comparative rehabilitation effects of the present drugs from SCI were in the order of FK506+Minocycline > Minocycline > FK506.

It is concluded that minocycline along with FK506 should be considered as an ideal cocktail therapy to treat acute SCI and may provide significant rehabilitation from SCI in humans.

Keywords: Drug therapy, spinal cord injury, behavioral rehabilitation, oxidative stress, monoamines.

SL-50

Track: Hot Topics in Natural Products

ANTI-HEPATOTOXIC PLANTS AND DERIVED DRUGS FOR LIVER DISEASES

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There are certain diseases of the liver that are not cured by the modern system of medicine. Keeping in view this problem, a large number of medicinal plants have been explored by several scientists. Consequently, some potent active chemical components have been obtained possessing a promising antihepatotoxic activity. In the present lecture the literature of such chemical and biological investigation on medicinal plants and related techniques will be covered wherein all major aspects of medicinal plants that are useful for the treatment liver ailments will be under taken.

The lecture will be delivered on "*Naturally Occurring Antihepatotoxic Drugs and Related Techniques*". Different aspects on medicinal plants possessing antihepatotoxic activity will be covered namely description of containing the antihepatotoxic activity, their uses, their action on liver, important active chemical constituents tested for their action on liver, techniques used for evaluating the antihepatotoxic activity, isolation techniques, structural elucidation methods and cultivation of some important plants. Lecture will be valuable for both researcher and pharmaceutical industries, who are interested in the development of drugs for different diseases of liver based on natural remedies. Many potent chemical constituents will be described in detail for their therapeutic uses, which could lead to the development of pharmaceutical preparations for the treatment of various liver ailments. In brief the lecture will contain the following major aspects:

1: ***Introduction, Liver diseases and causes of liver diseases:*** It will describe the different kinds of liver diseases in detail, their causes, origin and possible mechanisms and related consequences.

2: ***Crude formulations used in the treatment of Liver diseases:*** This will include the description of medicinal plants used in preparing the crude pharmaceutical formulations manufactured by many pharmaceutical companies, which are available in the market by their trade names. About 35 such pharmaceutical crude preparations will be described.

3: ***Plants possessing antihepatotoxic activity:*** About 300 plants possessing antihepatotoxic activity will be included. The model implemented for determining the activity, parts of the plant investigated and pharmacological screening and pure active components will be described.

4: ***Important chemical constituents possessing antihepatotoxic activity:*** This will describe the detailed study of some well established plants with their active chemical components. Their phytochemical, pharmacological investigations and clinical studies have been described in detail.

5: ***General isolation and characterization techniques of chemical components from plants:*** This will describes the general isolation techniques, structure elucidation by spectral and chemical methods of the chemical components from plants, so that the researcher could be able to isolate and identify the phytocomponents for further biological studies.

6: ***Synthetic compounds containing antihepatotoxic activity:*** In recent years some chemical compounds have been synthesized based on the template of naturally occurring compounds, which have exhibited promising antihepatotoxic activity. This will describe their synthesis and testing of their biological activities with respect to their action on liver.

7: ***Techniques used for the determination of antihepatotoxic activity:*** The techniques which are generally used for determining concentration of different liver enzymes like Serum Glutamate Oxaloacetate Transferase (SGOT), Serum Glutamate Pyruvate Transferase (SGPT), Alkaline Phosphatase (SAP), lipid profile (cholesterol, triglycerides and total lipids), total proteins, total albumin, and bilirubin etc, have been described in detail. In addition, the method for histopathological examination of the liver will also be narrated for comparing the biochemical parameters with histopathological studies.

8: ***Cultivation of Some important plants used for the treatment of liver diseases:*** This will describe know how and relevant techniques for cultivation of medicinal plants. It also includes the detailed cultivation of medicinal plants used for the treatment of liver ailments.

9: ***Other natural sources for the drugs, which could be used for the treatment of liver disease:*** Some work on marine natural drugs and other animal products for liver ailments have carried out. This chapter describes in detail the work carried out up to date with pertinent reference work.

10: ***Recent Trends in Tissue Culture Techniques:*** Some chemical components possessing well established and potent antihepatotoxic activity have been produced by tissue culture techniques to enhance the yield of a particular component. This will describe such chemical components in detail and their relevant techniques for their production by tissue culture techniques.

SL-160

Track: CNS Drug Discovery & Therapy

NEUROSTEROIDS DURING DEVELOPMENT: IMPLICATIONS FOR BEHAVIOR AND STRESS SENSITIVITY

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Exposure of developing female rats to estradiol during the perinatal period induces long-lasting dysregulation of gonadal axis and alters brain and peripheral concentrations of steroid hormones. A single administration of ! estradiol 3-benzoate (EB) on the day of birth to female rats induces a marked a persistent decrease in the concentrations of allopregnanolone and progesterone in the cerebral cortex, hypothalamus and hippocampus of juvenile and adult rats. These alterations are associated with increased agonistic behaviors in the resident-intruder test and a drastic reduction in spontaneous and induced female sexual behaviors, while locomotor activity, anxiety- and mood-related behaviors, as well as seizures sensitivity are not affected. Neonatal administration of EB also changes the expression of specific subunits of the GABAA receptor and enhances the sensitivity of adult rats to the anxiolytic, sedative-hypnotic, amnesic, but not anticonvulsant, effects of diazepam. Finally, neonatal administration of EB increases sensitivity of adult rats to acute stress, as demonstrated by the greater enhancement in brain allopregnanolone levels and in extracellular concentrations of dopamine and norepinephrine in the prefrontal cortex of EB-treated rats compared to vehicle-treated rats following exposure to foot shock stress. These effects of estradiol suggest that it plays a major role in the regulation of brain allopregnanolone concentrations during development and in the expression of behavior and stress sensitivity in adult female rats.

SL-178

Track: Anti-Infectives

COMPUTATIONAL CALCULATIONS OF MOLECULAR PROPERTIES, BIOACTIVITY SCORES AND DOCKING STUDY OF NEW AND REFERENCE CEPHALOSPORINS ON PENICILLIN BINDING PROTEINS AND VARIOUS Ā-LACTAMASES

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An approach of using computer-aided drug design (CADD) to measure molecular docking scores and molecular calculations is employed to establish a new method of predicting activity on PBPs and the degree of resistance against certain Ā-lactamases of the future cephalosporins. The molecular properties predictions, drug-likeness score on the bases of Lipinski's rule and bioactivity prediction through molinspiration website are to be calculated for various cephalosporins. These cephalosporins are selected to represent the five generations (cephalexin, cefuroxime, ceftriaxone, ceftazidime, and ceftobiprole) and a set of previously synthesized and evaluated (Alwan, S.M., Molecules; 2012, 17, 1025-1038) were further subjected to extensive evaluation by calculating the molecular properties and docking study on PBPs and certain Ā-lactamases. The synthesized cephalosporins fulfill Lipinski's rule on certain molecular properties and showed fairly good drug-likeness scores. Few of these cephalosporins have acceptable topological polar surface area (TPSA), OH-NH interaction and n-violation values according to this rule. Moreover, the molecular properties, particularly TPSA and molecular masses of the reference cephalosporins did not comply with Lipinski's rule, as their values are much higher than required, particularly, ceftazidime and ceftobiprole. Docking study of the cephalosporins on PBPs was conducted using 1-click-docking website to evaluate their potency and the docking results indicated that these cephalosporins have docking scores of -6.85 to -7.37, while the docking scores of the reference cephalosporins were -5.75 to -7.47. However, few cephalosporins showed low docking scores of -7.075 to -7.35, indicating more affinity binding than the others in the series, which means that they have confirmed the reasonable antibacterial activity. In comparison with the above mentioned results, ceftobiprole has the lowest score of -7.47, indicating its strong affinity binding. Docking study of the new cephalosporins on Ā-lactamses produced by *E. coli, K. pneumonia* and *P. auroginosa* was conducted and the results suggest that these cephalosporins recorded the lowest docking scores (average of -8.35) on Ā-lactamases produced by *P. auroginosa*. Moreover, few compounds recorded the lowest docking scores of -8.65 and -8.85, which are comparable with the reference cephalosporins. Moreover, ceftriaxone and ceftobiprole have the lowest docking scores of -9.32 and -9.40, respectively, while, cephalexin recorded docking scores of -7.60 on *P. auroginosa*. The cephalosporins recorded higher docking scores on Ā-lactamases produced by *E. coli* (-7.15 to -7.85) and *K. pneumonia* (-5.72 to -7.4) and few of these may be resistant to Ā-lactamases, particularly, those produced by *P. auroginosa.*

In conclusion, the application of this approach of molecular calculation and bioactivity score together with molecular docking on PBPs and Ā-lactamases may provide excellent method that is practical, rapid and informative. Moreover, this method may compensate for calculating the MIC values of affinity binding with either PBPs or Ā-lactamases by providing the docking scores with comparative data for the reference cephalosporins. This CADD method may be very useful to select the most potent and Ā-lactamase-resistant cephalosporins prior their chemical synthesis.

SL-151

Track: Protein and Peptide Sciences

MULTIVALENT DISPLAY ON SYNTHETIC PNA BACKBONES TO PROBE MEMBRANE RECEPTOR BINDING

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Multiple, simultaneous interactions between ligands and receptors at the surfaces of cells are necessary for numerous biological functions, such as cell attachment, growth, and intracellular communication. To provide insight into these complex associations, we are developing a highly programmable scaffold for the display of receptor ligands. By controlling ligand location, valency, and density within our scaffold, we are able to generate chemical libraries that provide information about the multivalent interactions between ligands and membrane receptors. Our approach uses ligand-modified peptide nucleic acids (L-PNA). Peptide nucleic acids (PNAs) are synthetic oligomers in which nucleo bases are attached to a peptidic backbone consisting of alternating glycine and ethylene diamine units. PNAs bind to complementary nucleic acid sequences following Watson-Crick hydrogen bond pairing rules. The L-PNA modification we have developed is the introduction of a gamma-lysine side chain into a position of the PNA backbone that will not interfere with DNA binding and which also serves as an attachment point for the multivalent display of ligands. A lysine side chain is one of the most versatile attachment points for covalent modification in peptides and proteins. By attaching protein binding ligands onto the gamma-lysine side chains of a PNA, we can use an array of oligonucleotide sequences to probe for multivalent effects in protein receptor binding. Using L-PNA, the multivalent effects associated with ligand binding to an integrin receptor and an adenosine GPCR receptor will be presented.

SL-143

Track: Protein and Peptide Sciences

SWITCHING THE ANTIMICROBIAL ACTIVITY OF GRAMICIDIN S BY LIGHT

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Many drugs have considerable side effects by exerting a certain activity also off target, as is the case with various antibiotics and chemotherapeutic agents. It would therefore be desirable to be able to switch the activity of such a compound - including its side effects - ON and OFF using light. In analogy to photodynamic therapy, it would be possible this way to control the biological activity in an organism carefully in time and space, by administering the inactive form of the drug and switching it ON only when and where it is required. Here, we present the development of such photo-switchable antimicrobial peptide, based on a novel photo-active amino acid that can be incorporated into the polypeptide backbone. Antimicrobial peptides are an essential part of innate immune system and defend the organism against bacteria, viruses, and fungi. They are promising therapeutic sources for new antibiotics, as they permeabilize bacterial membranes mechanically and do not evoke rapid resistance. However, they tend to suffer from cytotoxic side effects by damaging also eukaryotic cells to some extent. We demonstrate here that these problems, which have prevented clinical applications, can be eliminated by remotely switching the membranolytic activity ON and OFF. We designed and synthesized a reversibly photo-isomerizable amino acid analogue based on a diarylethylene scaffold. The amino acid analogue was incorporated into the cyclic backbone of the antimicrobial peptide Gramicidin S. Several analogues of this peptidomimetic were synthesized, their photochromic features were recorded in the ON and OFF states, and the corresponding molecular conformations were analyzed by CD spectroscopy and MD simulations. Antimicrobial assays and hemolysis tests showed that the biological activity can be directly and reversible controlled by

irradiation with visible and UV light. These results on Gramicidin S pave the way towards the development of new strategies for treating bacterial infections or other localized pathologies.

SL-138

Track: Drug Discovery in Preclinical Research

PHARMACOLOGICAL EVALUATION OF DRUGS & COMBINATIONS IN PATIENT SAMPLES OF HEMATOLOGICAL MALIGNANCIES BY AUTOMATED FLOW CYTOMETRY

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The novel flow cytometry ExviTech® platform has incorporated key innovations to predict or evaluate the response of primary patient tumor cells to compounds and combinations analyzing both efficacy and hematotoxicity by dose-response curves and kinetics. We use whole sample without isolating leukocytes, which was vitiating previous efforts for >30 years. This approach has achieved 84% correlation with clinical patient outcome in AML 1st line treatment Cytarabine plus Idarubicin, a level of clinical correlation not achieved before with *ex vivo* testing. We have profiled the pharmacological activity of more than 50 drugs in more than 1,000 patient samples of AML, MM, CLL, ALL, NHL, MF and PV. We now offer this body of knowledge to characterize the behavior of your compounds in these patient samples. Assays include depletion, apoptosis, proliferation, differentiation, autophagy, hypomethylating agents, etc. Optimal combinations of new compounds with other drugs or drug candidates are identified by measuring synergism among combinations, and also complementarity with other individual drugs (drugs active in those samples where the lead compound is resistant).

In summary, we have developed an improved methodology to measure the pharmacological activity of drugs and drug combinations in hematological patient samples as well as modeling their pharmacological behavior.

Keywords: Drug discovery, hematological malignancies, hematotoxicity.

SL-184(a)

Track: CNS Drug Discovery ad Therapy

SP1 INHIBITORS AS MODULATORS OF APP AND BACE1 LEVELS IN HUMAN CELLS: A NOVEL DRUG TARGET IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is believed to result from the misregulation of the production of amyloid-! (A!), which forms the plaques seen in AD brains. The rate-limiting step in the production of A! is the processing of amyloid-! precursor protein (APP) by ! -site APP-cleaving enzyme (BACE1). Understanding how expression of these proteins is regulated will eventually expose new drug targets. The transcription factor specificity protein 1 (SP1) coactivates the expression of the APP and BACE1 gene. We tested SP1-mediated regulation of APP with Mithramycin A, a selective inhibitor of SP1, and Tolfenamic acid, an inducer of SP1 degradation in human glioblastoma cells U373 and in human neurosphere (NSP) cultures. We are focused on not simply globally blocking BACE1 activity, but on controlling how the expression of BACE1 is regulated. We seek to demonstrate the activity of SP1-mediated activation at specific regions of the

BACE1 promoter with various doses of Mithramycin A, Tolfenamic acid, and other selective pharmacological inhibitors of SP1. NSPs were cultured in Neurocult basal media plus differentiation supplement (Stem Cell Technologies). U373 (ATCC) cells were cultured and transfected, and Western blot analysis was performed as previously described (Long *et al.,* JBC-2014). Mithramycin A (Santa Cruz) and Tolfenamic acid (Sigma Aldrich) were prepared in 1 ĀM and 5 ĀM doses. After 72-hour treatment or transfection, cell viability was assessed using CTG assay (Promega), and protein lysates made. Western blot analysis reveals a significant decrease in the expression of APP in U373 and NSP treated with Mithramycin A. NSP treated with Mithramycin A also exhibit a decrease in BACE1 expression. Treatment with Tolfenamic acid, however, does not significantly decrease APP or BACE1 expression in either cell model. APP siRNA effectively knocks down APP expression in U373 and NSP cultures. BACE1 siRNA and SP1 siRNA did not significantly affect APP levels. CTG showed no significant changes in cell viability among treatment groups in U373 and NSP. We show that expression of APP is decreased after treatment with the SP1 inhibitor Mithramycin A in both U373 and human neurospheres cells. However, APP expression is not affected by treatment with Tolfenamic acid, perhaps due to the differences in the mechanisms between these SP1-inhibiting drugs. We also show that transfection with siRNAs can successfully change the expression of APP and BACE1 in human cells. It is essential to discover whether small RNAs or drugs targeting SP1 could be used to decrease amyloid load and possibly alleviate symptoms associated with Alzheimer's disease.

SL-165

Track: Diabetes and Obesity Drug Discovery & Therapy

OXYTOCIN AND BDNF: NEW PLAYERS IN THE TREATMENT OF OBESITY AND OSTEOPOROSIS

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This study aimed to analyze the effect of ethanolic extract of leaves honje (Etlingera hemisphaerica) to detoxify mercury chloride (HgCl2) in the blood of mice (Mus musculus).

In this study we examined whether Oxytocin (Oxy) and Brain-derived Neurotrophic Factor (BDNF) play a physiological role in the regulation of bone mass and obesity. It is now accepted that several neurotransmitters and hormones are involved in bone homeostasis. One hormone that regulate bone metabolism is leptin which control food intake and energy expenditure in humans and animals. The sympathetic signaling has a negative impact on bone mass and the central leptin action on bone is mediated by the sympathetic signaling, while peripheral leptin is anabolic to bone. Our rational was that central deletion of BDNF in Bdnf2lox/2lox/93 mice produces a metabolic phenotype characterized by obesity, hyperphagia and elevated leptin similar to that of the high bone mass ob/ob mice, and we examined the skeletal phenotype and sympathetic tone in the Bdnf2lox/2lox/93 mice. In sum, we show that deletion of central BDNF expression in mice results in increased bone mass and white adipose tissue, with no significant changes in sympathetic signaling. The therapeutic implication of these finding relates to the possible actions of peripherally administered BDNF, that is hypothesized to exert dual beneficial effect through reversal of low bone mass and obesity. Similarly, we show that a mouse model of oxytocin KO develop late onset obesity, hyperleptinaemia, insulin resistance and low sympathetic tone. In conclusion, whereas central Oxy can contribute to the central regulation of bone metabolism mediating the antiproliferative action of sympathetic tone on bone formation, peripheral Oxy is a promising candidate for the treatment of osteoporosis. Deficiency in the Oxy and BDNF/estrogen pathways is observed in the aged female rats characterized by osteoporosis, obesity and neurodegeneration. Whether peripherally Oxy and BDNF/ estrogen administration may show beneficial effects in aged female rats is not known and is under investigation in our laboratories.

Keywords: BDNF, oxytocin, obesity.

SL-120

Track: Cancer Targeted Drug Delievery

IN-CELL OPTICAL IMAGING OF FLUORESCENT ANTICANCER AGENTS FOR DRUG DEVELOPMENT AND THERAPY

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A novel fluorescent molecule, 3,6-bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC), selectively lights up cancer cells upon irradiation, which can be used for point-of-care screening of cancer cells [1,2]. Since delocalized lipophilic cations could induce mitochondria mediated apoptosis of cancer cells, we have synthesized a BMVC derivative with a dodecyl carbon chain terminated with a methyl-piperidinium cation in N-9 position of carbazole, BMVC-12C, which selectively targets to mitochondria of cancer cells with strong fluorescence, but is also trapped in lysosomes of normal cells with weak fluorescence [3]. BMVC-12C can induce mitochondrial dysfunctions and cause cancer cell death without harming normal cells. However, the mechanism is unlikely due to mitochondria mediated apoptosis.

Optical imaging provides a fantastic tool to visualize cellular uptake, localization, and distribution of ligand for better understanding of ligand-target interactions in various cells. For example, quantitative measurements of cellular response from images and biological activity from bioassays of various cells may allow us to elucidate the relationship between mitochondrial accumulation and cytotoxicity for drug development. Using a mitochondrial membrane permeability transport pore inhibitor, cyclosporine A, confocal images suggest that BMVC-12C can enter the matrix of mitochondria and possibly interact with mitochondria DNA. A possible mechanism is proposed for BMVC-12C induced mitochondria dysfunctions in cancer cells. In addition, BMVC-12C shows no fluorescence in the nucleus of living cells, while it shows fluorescence in the nucleus of dead cells. Thus, fluorescence images inspire us to monitor ligand induced cell death. Such outcomes may have implications for cancer diagnosis and treatment.

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SL-76

Track: Cancer Targeted Drug Delivery

A POTENTIAL THERANOSTIC AGENT FOR CANCER RESEARCH: BMVC

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Development of theranostic agents for imaging, diagnostics, and therapy has become an emerging field in biomedical application. We have synthesized a potential theranostic agent, 3,6-bis(1-methyl-4-vinylpyridium) carbazole diiodide (BMVC), for the purpose of stabilizing the G-quadruplex (G4) structure of human telomeres to inhibit telomerase activity as an antitumor agent and verifying the presence of G4 structure in human telomeres as a fluorescence probe. BMVC is a poorly fluorescent molecule in tris-buffer, but shows a bright fluorescence by increasing two orders of magnitude upon binding to DNA. In addition, fluorescence imaging showed that BMVC specifically targets nucleic acids in the nucleus of cancer cells based on the treatment of DNAse and RNAse. Using optical methods, distinct fluorescence decay times of BMVC allowed us

to verify the presence of G4 structure in human telomeres. Of interest is that BMVC can not only stabilize the G4 structure of human telomeres to inhibit proliferation of cancer cells but also stabilize the G4 structure in the promoter region of the *WNT1* gene to inhibit the WNT1-mediating migration and invasion [1]. Moreover, BMVC can selectively enter the nucleus of cancer cells, interact with oligonucleotides, and light up cancer cells upon irradiation for cancer diagnosis. Using BMVC test, a good discrimination between malignant and benign specimens with sensitivity of 89.4% (42/47) and specificity of 93.3% (56/60) was obtained for diagnosis of malignant pleural effusion [2]. This BMVC test is currently applied to the clinical samples from thyroid of outpatients. Considering the large contrast of BMVC fluorescence between cancer and normal cells, BMVC was further applied to detect cell carcinogenic transformation [3]. It is most likely that BMVC is a fluorescent theranostic agent.

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SL-170

Track: Hot Topics in Drug Targets

LYMPHANGIOGENESIS IS REGULATED BY GALECTIN-8-DEPENDENT CROSSTALK BETWEEN VEGFR-3 AND PODOPLANIN

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Lymphangiogenesis plays a vital role in diverse pathological conditions and there is currently intense interest in characterizing its molecular mechanism. Here, we demonstrate that galectin-8 is a potent lymphangiogenic factor. Galectin-8 was markedly upregulated in inflamed human and mouse corneas, and inhibitors of galectin-8 reduced suture-induced lymphangiogenesis in mouse corneas. In corneal micropocket assays and in 3D sprouting assays, galectin-8 promoted lymphangiogenesis in a carbohydrate-dependent manner. In contrast, Galectins-1, -3, and -7 were not lymphangiogenic. Galectin-8 was identified as a key mediator of VEGF-C/VEGFR-3 signaling. Galectin-8 inhibitors reduced VEGF-C-induced lymphangiogenesis. Conversely, exogenous galectin-8 markedly enhanced VEGF-C-induced lymphangiogenesis in a carbohydrate-dependent manner. We further demonstrate that galectin-8 binds to glycans of VEGFR-3 as well as of another lymphangiogenic molecule, podoplanin, and that VEGF-C and galectin-8-mediated lymphangiogenesis is reduced in podoplanin knockout mice and in podoplanin knockdown lymphatic endothelial cells (LECs). Importantly, when added to LECs, galectin-8 caused segregation of VEGFR-3 and podoplanin on plasma membranes and activated AKT and ERK pathways. Collectively, these data suggest that galectinal-8 binds to specific glycan ligands on cell-surface VEGFR-3 and podoplanin and segregates them into discrete signaling complexes to activate lymphangiogenesis. In summary, lymphangiogenesis is regulated by galectin-8-dependent crosstalk between VEGFR-3 and podoplanin.

SL-175

Track: Anti-Infectives

OZONATED TRI-DISTILLED WATER COMBINING WITH THE STANDARD TREATMENT INCREASING *H. PYLORI* ERADICATE RATE

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Background & Aims: Ozone was known as a physical bactericide. The paper was to find whether ozonated tri-distilled water combining with the standard esomeprazolebased triple therapy could increase eradicating rate of *Helicobacter pylori* (Hp) in gastric mucosa.

Method: 132 patients were confirmed Hp infection positive by ^{14}C urea breath test and pathological examination, then divided into 4 groups with 33 patients in each group. Group 1 and group 2 included patients with chronic gastritis, while group 3 and group 4 include those with duodenal ulcer. The patients from group 1 and group 3 were given standard esomeprazole-based triple therapy taking amoxycillin 1.0 two times a day, clarithromycin 0.5 two times a day, esomeprazole 20mg one time a day, and hydrotalcite chewable tablets 1.0 three times a day. The patients from group 2 and group 4 were given ozonated tri-distilled water combining with standard esomeprazolebased triple therapy: 300ml ozonated tri-distrilled water twice daily, amoxycillin 1.0 twice daily, clarithromycin 0.5 twice daily, esomeprazole 20mg once daily, hydrotalcite chewable tablets 1.0 thrice daily. The period of anti-Hp treatment was 2 weeks and then the patients from all four groups would continue taking esomeprazole and hydrotalcite for 4 weeks. ^{14}C urea breath test was performed for each patient after 4 weeks of drug withdrawal application.

Results: ^{14}C urea breath test were negative for 26 patients in group 1, the rate of elimination of Hp was 79%; in group 3, 24 patients, rates of elimination of Hp 73%; 32 patients in group 2, rates of elimination of Hp 97%; and 31 patients in group 4, rates of elimination of Hp 94%. The chi-square test exhibited a significant difference both between group 1 and group 2 ($p<0.05$), and between group 3 and group 4 ($p<0.05$). The Hp eradication of drinking ozonated tri-distilled water combining standard esomeprazole-based triple therapy were higher than that of standard esomeprazolebased triple therapy alone.

Conclusion: Drinking ozonated tri-distilled water combining with the standard treatment can make Hp eradicate rate increased markedly.

Keywords: Gastric mucosa, *Helicobacter pylori,* ozonated tri-distilled water.

SL-92

Track: Academic CRO/Industrial Collaborations in Drug Discovery

CARDIOVASCULAR SURVEILLANCE DURING USE OF NON-CARDIAC THERAPEUTICS: A GROWING CHALLENGE IN THE ERA OF POPULATION AGING

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Numerous novel therapeutics for preventing or treating common age-related diseases have emerged over the last several decades. Along with the increasing demand for and use of such therapeutics is the ever growing challenge to maintain balance between drug efficacy and safety, particularly with respect to cardiovascular safety in older adults. This challenge has been highlighted by the widely publicized incidence of adverse cardiovascular events during the use of certain non-steroidal anti-inflammatory agents for pain, thiazolidinediones for diabetes, and chemotheraptic agents for

cancer. We will review the lessons learned from past experiences and also discuss proposed new strategies for optimizing cardiovascular safety during drug development, evaluation, and post-marketing use. The presentation will focus on strategies for integrating novel clinical, imaging, and biomarker approaches to surveillance.

SL-145

Track: Medical Imaging

NOVEL IMAGE ANALYSIS TECHNIQUES FOR DETECTING AGE- AND DISEASE-RELATED CHANGES IN CARDIAC STRUCTURE AND FUNCTION: IMPLICATIONS FOR STUDIES OF DRUG EFFICACY AND SAFETY

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High-sensitivity phenotyping of cardiac function is essential for evaluating the efficacy and safety of novel therapies in both experimental and clinical studies of cardiovascular disease. Conventional cardiac ultrasound methods and techniques have played a critically important role for not only understanding the pathophysiology of common cardiac diseases but also identifying potential targets for therapy. More recent advances in image analysis techniques now offer the ability to detect early changes in cardiac structure and function with greater sensitivity than that offered by conventional methods. These techniques include quantitation of myocardial microstructure and strain-based analyses of myocardial deformation. In mouse models of cardiac disease, advanced imaging can detect disease-related cardiac changes and response to rescue drug therapy at earlier time points when compared to conventional imaging. In clinical studies, advanced imaging has been used to detect early benefits of anti-hypertensive therapy as well as early adverse cardiac responses to novel non-cardiac drug interventions. Novel cardiac imaging techniques may serve an especially important role in distinguishing disease- or therapy-related cardiac changes from intrinsic age-related alterations in cardiac structure and function.

SL-185

Track: Combinatorial Chemistry

THE ROLE OF HYDROFLUIDS IN THE ORIGIN OF ALZHEIMER'S DISEASE

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Water, which is the body main component, uses its arteries and veins as a piping system to transport minerals, vitamins, hemoglobine, viruses, bacteria and other substances and particles throughout the body. It also picks up wastes which have to be eliminated. Water's role in the body is similar to the one it has in city water networks: Drinking and waste water. I've covered this topic of water conduits in 10 books and 100 published articles.

By applying the knowledge on the use of fluids and many chemical products, it is possible to be able to obtain useful information regarding our health. How can the large amount of water that is in our bodies be used to our advantage? By using it to transport products which could be useful toward our purpose.

I'm concerned about sicknesses such as Alzheimer's disease. Its true origin is unknown; people close to me have suffered from it. Being an engineer, I've tried to analyse it from that perspective using my experience and knowledge of fluids, chemistry and biophysical-chemistry and its applications on clay minerals by molecular or atom changes for its stabilization. On soil chemical stabilization I've published 40 articles in English, gathered in 8 books.

Successful research by using molecules and atoms in soil treatment can also be applied similarly to the atoms and molecules of our cells. Alzheimer's disease is not caused by viruses or bacteria. Thus, it could be eliminated by non-organic chemical products.

Alzheimer's is caused because the body fluids (except blood) become alkaline. This generally occurs due to our diet. This can be remedied by making it less alkaline. I have managed to do this by using chemical products I'm familiarized with because of my previous use in clay micelles research. During my work in soil research, I evaluated 50 chemical products. Antidotes to Alzheimer's can be found by selecting, some of the 50 chemical products used by the author, acids, acidic salts, alkalis and alkaline salts.

While studying Alzheimer's disease, I noticed that it was a sickness which was in the same anti-group as cancer. It can be said that cancer and Alzheimer are like enemies in the same cell tribe. Both cannot be found together. They thrive in opposite environment of certain body fluids.

A list of the chemicals I used in my theses will be attached. They have different grades of acidity or alkalinity. Those that are most effective in counteracting fluid acidity or alkalinity in the body are shown.

SL-63

Track: Cardiovascular Drug Discovery & Therapy

MYOCARDIAL REPERFUSION INJURY: CARDIAOPROTECTIVE ACTIVITY OF BANABA (LAGERSTROEMIA SPECIOSA L) AND ROLE OF ITS GLUCOSE REGULATING POTENTIAL IN REPERFUSION INJURY

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The worldwide prevalence of metabolic syndrome is enhancing the vulnerability of vast population to cardiovascular diseases. Most of the diabetic population is susceptible to micro and macrovascular insults culminating into endothelial and coronary artery disease. Similarly deranged energy homeostasis of diabetic hearts utilizes fatty acids escalating oxygen demands rendering the diabetic to be extremely susceptible to myocardial ischemia and reperfusion (I/R) injury. Exploration of potent antidiabetic herbs such as Banaba (Lagerstroemia speciosa L) could be valuable therapeutic tool to answer high unmet needs of I/R injury.

Ethanolic extract of Banaba evaluated on left descending coronary artery model of I/R in Wistar rats. Banaba (100 mg/kg) was intraperitoneally administered seven days prior and 30 minutes after induction of I/R injury. Banaba significantly improved oxidative biomarkers, restricted infarct area and inflammatory damage indicated by reduced activities of myeloperoxidase and creatine kinase. Significant reduction in DNA fragmentation suggests anti-apoptotic activity. The marked alleviation of myocardial I/R injury by Banaba could be attributed to potent antioxidant, antiinflammatory actions. Improvement in hemodynamic markers suggests involvement of beneficial endothelial effects of Banaba treatment in I/R injury. This protective activity could be improved by exploring new drug delivery system for Banaba and investigating it on diabetic individuals.

Keywords: Apoptosis, banaba, inflammation, myocardial ischemia, reperfusion injury.

SL-30

Track: Cancer Targeted Drug Delievery

SYNTHESIS, ANTITUMOR ACTIVITY AND MOLECULAR MODELING STUDY OF SOME NOVEL QUINAZOLINE DERIVATIVES BEARING TRIMETHOXYANILIDE FRAGMENT

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A novel series of 2-(3-benzyl-4(3H)-quinazolin-2-ylthio)-N-(3,4,5-trimethoxphenyl)anilide were designed, synthesized and evaluated for their *in vitro* antitumor activity. 2-(3-Benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N-(3,4,5-trimethoxyphenyl)acetamide (7), 2-(3-Benzyl-6,7-dimethoxy-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N-(3,4,5-tri-methoxyphenyl)acetamide (8) and 3-(3-Benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N-(3,4,5-trimethoxy-phenyl)- propanamide (11) possessed amazing broad-spectrum antitumor activity more active than the known drug 5-FU with GI50, (10.47, 7.24 and14.12 mM), TGI (58.8, 36.30 and 60.25 mM) and LC50 (>100, 87.09 and 95.49 mM) values, respectively. On the other hand, Compounds 6 and 10 yielded selective activities toward CNS, renal and breast cancer cell lines, whereas compound 9 showed selective activities toward to leukemia cell lines. Molecular docking methodology was performed for compounds 7, 8 and 11 into ATP binding site of EGFR-TK which showed similar binding mode to erlotinib.

Keywords: *In vitro* antitumor evaluation, molecular docking, NCI, quinazoline.

SL-130

Track: Drug Discovery in Preclinical Research

A DEXAMETHASONE-DEPENDENT MACROPHAGIC NICHE PROMOTES ERYTHROID EXPANSION BY STIMULATING ERYTHROBLAST CYTOKINESIS

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The regulation of erythroid cell proliferation is modulated by growth factors and by the presence of accessory cells. Cultures of human $CD34^{pos}$ cells stimulated with erythroid growth factors plus dexamethasone, a model for stress erythropoiesis, generate numerous erythroid cells plus a modest increase in macrophages (~3%, 1:1 positive and negative for CD169). Interactions occurring between erythroblasts and macrophages in these cultures and the biological effects associated with these interactions were documented by live phase-contrast videomicroscopy. Macrophages expressed high motility interacting with hundreds/thousands of erythroblasts per hour. $CD169^{pos}$ macrophages established multiple rapid "loose" interactions with proerythroblasts leading to formation of transient erythroblastic island-like structures. By contrast, $CD169^{neg}$ macrophages established "tight" interactions with mature erythroblasts becoming engulfed with these cells. Loose interactions of $CD169^{pos}$ macrophages were associated with proerythroblast cytokinesis (the M phase of cell cycle) suggesting that these interactions may promote proerythroblast duplication. This hypothesis was tested by twenty-four hour co-cultures in which the addition of as few as 10^3 macrophages significantly increased levels of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide incorporation and frequency in S/G2/M and in cytokinesis expressed by the proerythroblast population. Macrophages exerted these effects in a dexamethasone-dependent fashion. These results indicate that, in addition to promoting proerythroblast proliferation directly, dexamethasone stimulates expansion of these cells indirectly by favoring the generation of macrophages supporting their cytokinesis. Strategies to exploit these data to devise culture conditions that would improve the generation of red cells for transfusion *ex-vivo* will be discussed.

SL-22

Track: Cancer Targeted Drug Delievery

THE TRIEPOXIDE DERIVATIVE, TEROXIRONE, AS A POTENTIAL TUMOR THERAPEUTIC AGENT

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The triepoxide derivative, 1,3,5-triglycyl-s-triazine-trione (teroxirone), has been reportedly used to cure patients recovering from leukemia and lymphomas. To investigate how the drug acts in solid tumors, we found that the growth of human non-small-cell-lung-cancer cells can be inhibited by teroxirone in cells models with IC50's ranging from 0.1 to 2 μM in various cell lines. The cytotoxicity was proved mediated by apoptotic cell death as a result of DNA damage. Teroxirone first elevated reactive oxygen species and caused mitochondrial membrane potential drop. The subsequent transient elevation of p53 activates downstream p21 and procaspase-3 cleavage with increased Bax/Bcl-2 ratio. Cells pre-incubated with caspase-3 inhibitor can overcome the apoptotic phenotype by restraining cells at G2/M phase. Furthermore, we showed the cytotoxicity of teroxirone occurred in H1299 cells with stable ectopic expression of p53, but not those of mutant p53. The *in vivo* experiments in nude mice model showed that teroxirone suppressed the growth of xenograft tumors. What is more, teroxirone treatment suppressed the proliferation of liver cancer cells with IC50's ranging between 1 and 3 μM in various cell lines. More experiments indicated that low concentrations of teroxirone inhibited self-renewal of cancer stem cells in dose-and time-dependent manners.

Being proved a potential therapeutic agent by suppressing cell growth through apoptotic death at low concentrations, teroxirone implies a novel alternative in reversing tumorigenic progression of human cancers.

Keywords: Anti-cancer chemotherapy, liver cancer, lung cancer, Teroxirone.

SL-85

Track: Drug Discovery in Preclinical Research

NOVEL STRATEGIES TO EXPAND ERYTHROID CELLS *EX-VIVO* FOR TRANSFUSION

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Induced pluripotent stem cells (iPSC) provide an attractive source for generating red blood cells (RBCs) through *ex-vivo* expansion. A limitation however is that culture condition-based methods for induce erythroid fate determination are inefficient. We hypothesize a more direct and efficient approach will be to induce the erythroid program using a combination of transcription factors that specifies red cell fate. While an evolutionarily conserved transcription factor code governs erythroid specification in all vertebrates it is not clear which factors are driving this process and which are merely permissive. With the aim to identify a combination of factors that can be used to induce red cell production from human iPSCs we used a stringent system to identify the minimal combination of transcription factors that mediate cellular reprogramming of mouse fibroblasts directly to erythroid progenitor cells. By transducing fibroblasts from Erythropoietin receptor reporter YFP mice with combinations of 63 different factors we identified a combination of 4 transcription factors (not revealed here due to patent issues) that directly reprogram fibroblasts to erythroid progenitor cells. Epo receptor positive cells emerge 5 days after transduction and hemoglobinized cells with erythroid morphology appear 3-5 days later. There is no sign the cells emerge from a multipotent precursor, instead the 4 factors induce direct erythroid transdifferentiation. Our discovery reveals the core of

the genetic signal that induces and maintains red blood cell specification and our next step is to validate these findings in human cells and to utilize the code to enhance RBC production from iPSCs. In conclusion we present a new concept for generating and studying RBCs and a tool that could be utilized to enhance *ex-vivo* RBC production.

SL-147

Track: Protein and Peptide Sciences

CONTROLLING RESISTANT BACTERIA WITH NOVEL DESIGNED MULTIFUNCTIONAL COMPOUNDS: FROM BETA-LACTAMASE INHIBITORS TO IMMUNOMODULATORY AND ANTIMICROBIAL PEPTIDES

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Peptide rational design was here used to guide the creation of novel compounds that could help on resistant bacteria control. Firstly, two novel short Ā-lactamase inhibitors with five amino acid residues length were generated. Molecular modeling associated to peptide synthesis improved bactericidal efficacy in addition to amoxicillin, ampicillin and cefotaxime. Docked structures were consistent with calorimetric analyses against bacterial ! -lactamases. These two compounds were further tested in mice. Whereas commercial antibiotics alone failed to cure mice infected with *Staphylococcus aureus* and *Escherichia coli* expressing Ā-lactamases, infection was cleared when treated with antibiotics in combination with peptides, clearly suggesting that peptides were able to neutralize bacterial resistance. Moreover, host-defense peptides from mastoparan and clavanin families were redesigned in order to improve antimicrobial activities and decrease mammalian cell toxicity. Both peptides were evaluated in sepsis and wound model infections showing the ability to control the infection caused by Gram-positive and -negative pathogenic bacteria. Moreover in all cases, immune response was also evaluated. In summary, the unusual peptides here described provide leads to overcome Ā-lactamase-based resistance, a remarkable clinical challenge.

SL-34

Track: Cancer Targeted Drug Delivery

NOVEL TUBULIN-TARGETING SCAFFOLD DERIVED FROM THE RIGIDIN FAMILY OF MARINE ALKALOIDS

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A general method of synthesis of marine alkaloid rigidins was developed. Natural alkaloids Rigidin A,B,C,D and over 40 synthetic analogues based on the 7-deazaxanthine, 7-deazaadenine, 7-deazapurine, and 7-deazahypoxanthine skeletons were synthesized and tested against different cancer cell lines. Analogues based on the 7- deazahypoxanthine skeleton exhibited nanomolar potencies against cell lines representing cancers with dismal prognoses, tumor metastases, and multidrug resistant cells. Studies aimed at elucidating the mode(s) of action of the 7-deazahypoxanthines in cancer cells revealed that they inhibited *in vitro* tubulin polymerization and disorganized microtubules in live HeLa cells. Experiments evaluating the effects of the 7-deazahypoxanthines on the binding of [^{3}H]colchicine to tubulin identified the colchicine site on tubulin as the most likely target for these compounds in cancer cells. The results of the docking studies utilizing the colchicine site on ! -tubulin were consistent with the observed structure-activity relationship data, including an important finding that derivatization at C2 with linear alkyl groups leads to the retention of activity, thus permitting the attachment of a biotin-containing linker for the subsequent proteomics assays. Because many microtubule-targeting

compounds are successfully used to fight cancer in the clinic, the new chemical class of antitubulin agents represented by the 7-deazahypoxanthine rigidin analogues has significant potential as new anticancer agents.

Keywords: New anticancer agents, marine alkaloids, anti-tubulin compounds.

SL-33

Track: High-Throughput Screening & Laboratory Automation

DEVELOPMENT OF A RAPID *IN VIVO* CHEMICAL SCREENING METHOD FOR THE IDENTIFICATION OF ANTIMETASTATIC COMPOUNDS

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To date, most high-throughput screening models for cell migration, an integral component of cancer metastasis, are based on *in vitro* studies of cells. These studies generated many 'hits', however, they lack relevant whole organism physiology to further validate the findings and many of the assay positives do not replicate when tested *in vivo*. Thus, an *in vivo*, phenotype driven screen will present better novel targets for therapeutic intervention. We developed a robust *in vivo* assay to identify new cell migration inhibitors and conducted a high-throughput screen using transgenic zebrafish and the migrating posterior lateral line primordium as a readout for migratory inhibition. We screened FDA approved drugs and other bioactive compounds, as well as a collection of natural products and a set of kinase inhibitors to identify compounds that blocked migration. Demonstrating the utility of this approach, we confirmed that inhibition of the Src pathway prevented normal lateral line migration and decreases tumor metastasis *in vivo*. We also identified that inhibition by novel flavonoid-derivatives molecules and a cluster of structurally related kinase inhibitors disrupted primordium migration. Thus, this approach demonstrates that zebrafish can be used for large-scale, high-throughput screening for drugs that impact cancer metastasis.

Keywords: Zebrafish, high-throughput drug screening, cell migration, cancer metastasis.

SL-25

Track: Drug Delivery & Targeting

QUANTUM MECHANICAL AND MOLECULAR DYNAMICS APPROACH TO THE CHIRAL RECOGNITION BY INCLUSION COMPLEXATION

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Enantiomeric recognition of by complex formation with Ā-Cyclodextrin (Ā-CD) and C-molecule were studied by semi-empirical PM3 method and molecular dynamics simulation. The results show that R-enantiomer complex is more stable than S-enantiomer complex by 8.54 kJ/mol (based on Hartree-Fock energy) for inclusion of Propranolol. The thermodynamic calculations carried out at 1 atm and 298 K in vacuo by PM3 method indicates that both complexes are hardly occurring at room temperature due to their positive formation free energies. Ā-CD complex formation process is more favorable (being driven by enthalpy but not entropy) with R-enantiomer than with S-enantiomer, though both are free-energy-wise unfavorable. The relative stability of R-enantiomer complex was also established by the results of molecular dynamics simulation; the relative stability due to the van der Waals energy is manifested by 5.04 kJ/mol, astonishingly being rather close to the Hartree-Fock energy difference (8.54 kJ/mol) as the

counterpart energy. Similar calculations were also carried out using the C-molecule which has been presented as a means for drug delivery and enantiomeric recognition.

SL-142

Track: Women's Health Drug Discovery & Therapy

BIOSUPERIORS: FSH-GEX– RESULTS FROM CLINICAL PHASE II STUDIES

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Background: Glycosylation is one of the major post-translational modifications of biotherapeutics important for bioactivity, bioavailability, immunogenicity and patient coverage. By establishment of the GlycoExpress toolbox (GEX) we have generated a set of glycoengineered human cell lines for the high yield production of fully human glycoproteins to optimize the glycosylation of antibodies and non-antibody biotherapeutics for improvement of the clinical efficacy and side effects.The fully human follicle stimulating hormone FSH-GEX™ produced in these cell lineshas finished the dose finding clinical phase II trial

The data show that FSH-GEX™, even at half the biologic dose (75 IU FSH-GEX™ daily), is at least as active as the comparator Gonal-f (150 IU daily) in all FSH mediated parameters and endpoints.

FSH-GEX™ dose of 112.5 IU daily, showed superiority over all FSH mediated aspects and endpoints compared to a 33% higher Gonal-f® standard dose including follicular response, as well as high biochemical (57.5%) and ongoing (50.0%) pregnancy rates. Particularly important for future clinical use are the statistically significantly improved numbers of retrieved oocyte complexes (+29.5%) and high quality metaphase II oocytes (+24.5%), as well as a strong trend for more pronuclear (PN) 2 oocytes (+21%) which could be observed in this dose regimen.

SL-177(a)

Track: Traditional Chinese Medicine

INFLUENCE OF HERBAL-CAKE-SEPARATED-MOXIBUSTION ON THE CONTENT OF SP AND THE INTESTINAL SENSITIVITY IN RATS WITH FUNCTIONAL GASTROINTESTINAL DISORDERS DUE TO SYNDROME OF LIVER STAGNATION AND SPLEEN DEFICIENCY

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To study the effect of volatile oil and decoction of *Saussurea costus* on intestinal motion of rabbits, and to study the components absorbed into blood. Experimental system of BL-420F biological function has been used to observe the effect of volatile oil and decoction. HPLC method had been used to compare the differences in chromatograms profile. The essential oil could elevate the average amplitude of the normal intestinal motion; the decoction could reduce the average amplitude and frequency of normal intestinal motion. Essential oil could excite normal intestinal motion; decoction could inhibit the normal intestinal motion. The differently performed components into serum and metabolites should be the principal components of *S. costus*.

SL-176

Track: Pharmaceutical Research & Development

A STUDY ON THE PRESCRIPTION PATTERN OF DRUGS IN JIZAN GENERAL HOSPITAL OF JIZAN, KSA

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Journals and books are overcrowded with information concerning the bio-medical use of nanotechnologies. Some authors see these technologies as a fundamental way of advancement of medicine and pharmacology; others consider them as the basic treat for the ecological safety. However both viewpoints are one-sided, since they are formed in isolation from peculiarities of nanoparticle manufacturing. Nanotechnology provides vast possibilities and the mankind has no warrants for renunciation of them, therefore the problem of nanoparticles comes down to the guaranteeing of the manufacturing safety. Advantages and risks associated with nanoparticles have the same reasons: they are connected with their catalytic activity that is determined by their structural-morphological characteristics. Size, structure, shape (habit) are thermodynamically interrelated in the nanoworld, but technologies of obtaining particles with one single size are impossible. Besides, thermodynamics determines only the probability of an equilibrium state, but the origin of the equilibrium state is determined by the kinetics, i.e., by technologies, therefore particles of the same size can have different structure and habit. Defect structures influence also on a catalytic activity, but the specific regularities of a defect formation in the nanoworld are practically unknown.

An outgoing inspection is the integral part of any technology. It must secure conformity of the inspected production with the existing technical regulations and thereby ensure its compatibility with the biosphere. Safe technologies are only possible if their development is accompanied by the synchronous development of the outgoing and interoperation inspection. Microscopic methods are single methods, where the information goes from single objects to their massif (bottom-up technologies). SEM and TEM allow obtaining electron transmission diffraction patterns. Diffraction patterns depend also on the particle orientation, and only the habit determination provides unequivocal information. TEM image depends on diffraction conditions, but regularities of images in SEM are more single-valued. SEMs are used for the inspection in microelectronics where a number and sizes of objects is similar to them in nanotechnologies. Goals and specificity of inspections also are very like. Therefore SEMs are the single applicant to be an instrument of the outgoing and interoperation inspection in nanotechnologies. However the existing SEMs give information about an object contour only whereas safety assurance in nanoparticle manufacturing demands the information about a particle habit. Lack of techniques and instruments of the outgoing inspection is a principle obstacle for the developments of safe nanoparticle bio-medical technologies.

Theoretical solution of the problem of the outgoing inspection mass-production-oriented and based on SEM suggested in 2009 and was developed in posterior papers. It is built upon the confrontation of intensity distributions one of which corresponds to a parallel electron beam and others conform to a converging beam. The obtained results are processed by means of computer simulations and the particle massif is divided in the structural-morphological fractions. Structure and surface activity of each fraction is studied further additionally. It is possible to reveal portions of different particles, at least, equal to $\sim 10^{-7}$ of the general particle number in principle.

The method demands SEM modification therefore. There is a great distance between the physical idea and its hardware realization, and a transition from the idea to the hardware demands great intellectual and financial costs. Therefore the basic results of our works are the evidence of the principal possibility of the outgoing inspection in nanotechnologies and the indication of the way to its realization. Other methods of the outgoing inspections have not been suggested and evidently they are hardly probable. Discoveries of the new reciprocal actions between nanoparticles and living matter are carried out by heightened rates, and many results could be used in medicine with the provision of an adequate outgoing inspection. Besides, there are apprehensions that new technologies can be permitted in the medicine in some countries on the basis of the “maximum permissible concentration” (MPC) safety standard which is not effective in nanoworld. Bio-medical use of nanoparticles must be regulated by the **“**maximum permissible concentration of dangerous particles (MPCDP)**”** safety standard, but MPCDP is meaningless without an effective instrument to realization. Safe development of bio-medical technologies without a solution the problem of the outgoing production inspection is impossible.

This paper is augmented: by the information concerning the new achievements of the biomedical technologies demanding inspections, the description of constructive peculiarities of SEM intended for the method realization and the

more detailed description of the inspection methods. We hope this paper will promote solution to the major problem of bio-medical nanotechnologies.

Introduction: Inappropriate drug prescribing is a global problem affecting the healthcare system. This study was performed to assess the drug prescribing pattern in geriatric, pediatric and obstetrics and gynaecology department patients as chances of exposure to polypharmacy are more, therefore, this study was carried out to find out the rational use of prescribed drugs in Jazan General Hospital, KSA.

Methodology: A prospective cross sectional (descriptive) study was carried out and a total of 3070 prescriptions were collected for the study during November 2012 to October 2013.

1034, 1024, and 1012 prescriptions from geriatric, pediatric and obstetrics and gynaecology department patients respectively were collected.

1. Average number of drugs per prescription and Beer's criteria for geriatric patients.
2. Percentage of category of drugs prescribed as per WHO core indicator and USFDA.
3. Percentage of patient prescribed injectables.
4. Percentage of patient prescribed antibiotics.

Result and Discussion: The average numbers of drugs used per patient were 3.1, 7.4, and 3.3 for geriatric, pediatric and obstetrics and gynaecology department patients respectively.

Prescription pattern of the drugs for pediatric patients consists of Antibiotics, Analgesics and Antipyretics mainly. For geriatric patients among systemic route, commonly prescribed therapeutic class of medications were antibacterials (70.5%), and among oral route, pantoprazole was the most commonly prescribed medication (61.2%).

For obstetrics and gynaecology department patients the most frequently prescribed drugs were oral iron, folic acid preparations, antibiotics and analgesics.

Conclusion: There is a high level of exposure to medication in paediatric and geriatric population but in obstetrics and gynaecology department the average numbers of drugs per prescription were slightly higher compared to the standard set by WHO but majority of the drugs were prescribed as per USFDA category A (the safest category during pregnancy).

Keywords: Drug prescription pattern, Jazan general hospital, Nakul Gupta.

SL-52

Track: Hot Topics in Natural Products

HEPATOPROTECTIVE ACTIVITY OF *CLERODENDRON INERME* AGAINST PARACETAMOL INDUCED HEPATIC INJURY IN GUNIEA PIG FOR PHARMACEUTICAL PRODUCT

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Hepatitis is one of the major health problems in human which sometimes may lead to even death. Natural products may be the best source of remedies for the treatment of liver diseases. Thus identification of a potential therapeutic agent for the protection of liver from the hepatotoxins will provide a useful way for the prevention of these liver related illnesses. Our studies identified a plant with potential hepatoprotective activity. The etanolic extract of *Clerodendron inerme* leaves were screened for its hepatoprotective activity in paracetamol induced liver damage in Guniea pig at a dose of 200 mg/kg bw.

Preveiously we have taken trial in Swiss albino rats. The etanolic extract exhibited a significant protective effect by lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase and

total bilirubin. Liv.52 was used as positive control. The effects of the drug was judged by changes in serum marker ALT, AST, ALP, Protein and bilirubin levels. The extract did not show any mortality up to a dose of 2000g/kg bw.

Keywords:, *Clerodendron inerme*, hepatoprotective activity, paracetamol.

SL-164

Track: Recent Advances in Patient Treatment and Care

MICROPROPAGATION OF AN IMPORTANT MEDICINAL PLANT *PFAFFIA GLOMERULATA* AND ANTILEISHMANIAL ACTIVITY OF AGENTS ISOLATED FROM PFAFFIA GLOMERULATA

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Successful protocol is standardized for organogenesis from mature leaf explants and nodal segments explants of valuable Antileishmanial agents contain medicinal plant *Pfaffia glomerulata*. Nodal segment and leaf explants from *Pfaffia glomerulata* initially treated with Bavistin 0.1% and 0.1% Endosulphan and 0.05% Fluconazole were cultured in solidified modified murashige and skoog (MS) media, each with different hormonal combination for the establishment of cultures, green compact callus multiplication and rooting of plants. We got huge clusters of *Pfaffia glomerulata* with modified MS media supplemented with NAA, BAP, Kn, IAA at pH 5.7. These clusters of plantlets were obtained after 4 weeks of growth in the media. High multiplication efficiency, phenotypic stability and yield ensure the efficacy of the protocol developed for the production of medicinal herb *Pfaffia glomerulata*. 2-4 D (2mg/l) is responsible for callus induction and, callus were transferred to MS medium with lower concentrations of 2mg BAP and 1mg indole acetic acid (IAA) for embroid formation. Explants were cultured on MS medium supplemented with various concentrations and combinations of auxins and cytokinins. Among 0.25mg/l NAA and 2.5mg/l Kn produced highest percentage of green compact callus. However, the highest concentration of BAP (2mg/l), 1mg/l Kn and 1mg/l IAA increased the formation of shoots from callus. Shoot elongation and rooting was observed on modified MS medium supplemented with 1mg/l-2mg/l IAA Leishmanicidal drugs which are available in the maket have severe side effects and costly. To over come these problem leishmanicidal agents are extracted from the roots of *Pfaffia glomerulata*. The generated data will be discussed. The extracted drugs are 100% effective for Leishmanicidal activities.

SL-45

Track: Chemistry

MODERN ANALYTICS AND SYNTHESIS TOOLS IN DRUG DISCOVERY

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In today's drug discovery, the use of enabling laboratory technologies can offer an edge in a very competitive and scrutinized environment. The challenge is to ensure the discovery and validation of safe, effective, accessible, and scalable drug molecules at a very early stage. Technologies that automate mundane manual tasks, for instance: Performing laboratory experiments, analyze reaction components, capture and store experiment results electronically offer invaluable value to enhance

the productivity of the discovery process and the quality of products meeting our current therapeutic needs. Several case studies from drug discovery and development teams in industry and academia will be presented, for instance, ATR-FTIR spectroscopy to characterize multi-component (Petasis) and hazardous (hydrogenation in flow) reactions, which lead to fewer side-reactions and permitted facile isolation and purification of the final product. Other case studies will show the combined use of automated synthetic platforms and real time monitoring for high throughput reaction screening and optimization. We'll finally illustrate that such technologies provide large amount of data and require rapid data analysis, interpretation, as well as safe, automated and reliable collection on a server.

SL-8

Track: CNS Drug Discovery & Therapy

MANGOSTEEN PERICARP POWDER IMPROVED THE SPATIAL MEMORY RETRIEVAL OF 3ĀTG-AD MICE

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Mangosteen (Garcinia mangostana) is a tropical fruit native in Southeast Asia and reported to contain multiple health promoting properties. In this study we investigated the effects and molecular mechanisms of Mangosteen pericarp powder (MP) in AD mice. First, the pre-treatment of MP in the mouse hippocampal slice culture induced neuroprotective effect against the neurotoxicity of oligomeric AẠ42. We further applied MP to the 3ĀTg-AD mice for 8 months started from 5 month-old of age. We found dietary MP supplement improved mouse spatial memory retrieval associated with protected hippocampal pyramidal neurons. Further pathological and molecular characterization revealed that MP increased the molecules associate with anti-oxidative stress, cognitive-related pathway, and calcium binding protein. MP also reduced inflammatory response (IL-6, pp38, and COX2) and astrogliosis and the levels of AẠ42, APP, BACE, active form of GSK3! , and p-tau (262/202) in mouse hippocampus. These results show that the MP administration attenuated cognitive impairment might through multiple mechanisms including anti-amyloidgenic process and tau protein hyperphosphorylation. We suggest that MP supplement could be potential for delaying the progression of neurodegeneration.

SL-96

Track: Diabetes and Obesity Drug Discovery & Therapy

LONG-CIRCULATING BIODEGRADABLE NANOPARTICLES OF REPAGLINIDE (RPG): RATIONAL APPROACH FOR THE MANAGEMENT OF TYPE 2 DIABETES MELLITUS

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RPG is an oral hypoglycemic agent with excellent bioavailability (90-98%) and short plasma half life (2-6h). Present study was aimed to design a novel system to maintain peak plasma levels of RPG for the long-term management of diabetes mellitus. Two nanoparticle formulations were prepared by combining RPG with poly(lactic-co-glycolic)acid alone or as a copolymer with methoxy-polyethylene-glycol (NP1 and NP2, respectively); both formulations were subjected to *in-vitro* and *in-vivo* characterization. *In vivo* characterization was performed in streptozotocin (STZ) induced diabetic rats model. The mean particle size of the NP1 and NP2 were 387.8±11.9

and 310.2±12.4 nm respectively, with a zeta potential of -27.4±0.7 and -15.7±0.5 mV respectively. The entrapment efficiency and drug content of NP1 (58.7±1.3% and 27.4±2.3%, respectively) was better than that of NP2 (45.8±1.2% and 24.3±1.1%, respectively). Blood glucose levels of NP1 and NP2 treated STZ induced diabetic rats were reduced signif cantly compared with untreated STZ induced diabetic rats ($P<0.05$), but there was no difference between the two treatment groups ($P>0.05$). However, whereas NP1 was effective for a period of only 24 h, NP2 was effective for up to 1 week. The results of the present study indicate that NP2 effectively manages diabetes mellitus for up to 1 week.

SL-10

Track: Cancer Targeted Drug Delievery

SPONTANEOUS REMISSION OF TUMOR AS A MODEL FOR A NEW ANTI-CANCER TREATMENT

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The mechanism of spontaneous remission of tumor is proposed involving the cytotoxic lymphocytes (natural killer T-cells) activation. The model seems to suggest a new general treatment for cancer diseases.

Keywords: Cancer remission, natural killer cells, hypoglycemia, hyperglycemia.

SL-13

Track: Drug Delivery & Targeting

TREATMENT FOR TUMOR-BEARING LYMPH NODE BY LYMPHATIC ADMINISTRATION WITH A COMBINATION OF NANO/MICRO BUBBLES AND ULTRASOUND

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Lymphatic metastasis is a main metastatic route of many malignancies. Tumor cells migrated from new lymphatic vessels (LVs) reach lymph nodes (LNs). The tumor cells may spread in downstream LNs or distant organs from metastatic LN, leading to poor prognosis of patients. Therefore, it is important to develop an effective treatment of LN metastasis.

However, conventional chemotherapy of LN metastasis has poor selectivity or poor drug potency of target tissue because of drug dispersion over whole body due to systemic administration of drugs.

Here we show that the lymphatic administration of doxorubicin with a combination of nano/micro bubbles (NMBs) and ultrasound (US) enhances antitumor effect. The solution of doxorubicin and NMBs were injected into the subiliac LN to deliver it to the tumor-bearing proper axillary LN (proper-ALN) through LVs. US was exposed to the proper-ALN might collapse NMBs and generate subsequent cavitation bubbles. We found that mechanical stress could increase cell membrane permeability transiently, resulting in delivery of doxorubicin into the tumor cells efficiently. Furthermore, lymphatic administration inhibited acute toxicity compared to systemic administration. Our results demonstrate that the lymphatic administration of US with NMBs has a potential of treatment for LN metastasis.

Keywords: Chemotherapy, drug delivery, lymphatic metastasis, medical ultrasound, sonoporation.

SL-100

Track: Drug Discovery in Preclinical Research

CURCUMIN/BSA: NEW APPROACH FOR HEPATOCELLULAR CARCINOMA TREATMENT

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Background: The systemic availability of curcumin is very low after oral administration; this limits their therapeutic potential. Curcumin's chemo-preventive efficacy in almost all stages of carcinogenesis has received even more attention because of curcumin's nontoxic nature.

Aim: This study aims to increase the bioavailability of curcumin; the highest reproducible solubility modality will be applied on an experimental carcinogenesis models in order to evaluate its chemo-preventive, chemotherapeutic effects and antitumor potential.

Results: Our results found that, administrating of curcumin (200 mg/kg I.P. bound to 5% BSA in PBS, pH 7.4) results in a significant inhibitory effect on tumor *in vivo*. An anti-oxidant effect and anti-tumor effect of curcumin *in vivo* was observed. A significant reduction in anti-oxidants and tumor markers levels in tumor treated animals when compared with untreated ones. As well as Bcl2 expression was reduced while caspase-3 activity were increased. Also, chromosomal aberrations and liver tissues were recovered after curcumin treatment.

Conclusion: curcumin bound BSA has a strong inhibitory activity against tumors. The anti-tumor mechanism may be mediated by preventing oxidative damage and induction of apoptosis improved animals' chances of survival and they become healthier.

Keywords: Apoptosis, bovine serum albumin, curcumin, hepatocellular carcinoma.

SL-110

Track: CNS Drug Discovery & Therapy

REAPPEARANCE OF HIGH FEVER ON MIGRAINE PATIENTS, AFTER INDIVIDUALIZED HOMEOPATHIC TREATMENT, IS A VALUABLE PROGNOSTIC FACTOR.

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Methods: One hundred and twenty migraine patients assigned to receive individualized homeopathic treatment. Additional evaluation by a neurologist was performed at baseline, 6 and 12 months. Primary and secondary measures of migraine severity and impact on quality of life were recorded and analyzed.

Results: Eighty two patients opted only for homeopathic treatment until the completion of the study, with a baseline HIT-6 score of 67±4. Significant improvement was recorded at 6 months (HIT-6 45±8, $P<0.0009$ *vs* baseline, Wilcoxon signed ranks test), further established at 12 months (HIT-6 40.2±7, $P<0.0009$ *vs* 6 months). Migraine severity (VAS) decreased by 72% and frequency by 81% at 12 months ($P<0.0001$ *vs* baseline). Observed potential adverse effects were an initial 'aggravation' of migraine symptoms in 69%, recurrence of past medical diseases end especially infections or upper respiratory with high fever in 62%.

Original videos of many patients describing the unexpected reappearance of very high fever, after a long time, will be presented.

SL-122

Track: CNS Drug Discovery and Therapy

HOMEOPATHIC THERAPEUTIC AGGRAVATION AND REAPPERRANCE OF PATIENT'S SYMPTOMS OF PAST MEDICAL HISTORY, EXPLAINED BY PSYCHONEUROIMMUNOLOGY

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There are two peculiar incidences occurring only during individualized (one single remedy) and not complex homeopathic treatment. Initial aggravation of patients' symptoms and reappearrance of symptoms, fever included, of patients' past medical history. When appeared, these incidences are considered to be good prognostic factors.In a recent study of ours, taken place in a big public hospital and presented in an international migraine congress, 72 migraine patients treated only with individualized homeopathic treatment, 64% of them experienced therapeutic aggravation and about half of them experienced reappearance of symptoms of past medical history. Homeopathic individualized prescription is a choise of one, between 3500 remedies, and is based on an extended medical interview, with emphasis mostly on metabolic, neueroendocrinal, and immunological characteristics of each patient's particular symptoms. "Prescribing on the patient and not only on the disease" is a common but insufficient exrpression in homeopathic bibliography. "Prescribing on the psychoneuroendocrinoimmunological profile of patient's disease" is the more scientific expression. Original patient's videos will be presented.

SL-179

Track: Pharmaceutical Research & Development

INTELLIGENT SENSORY SYSTEM FOR PHARMACEUTICS AND DRUG THERAPY

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An innovative non-invasive multisensory micro-nanolaboratory E-TNY on a chip "electronic tongue" (e-tongue), "electronic nose" (e-nose) on surface acoustic waves (SAW), and LED "electronic eye" (e-eye) including an intelligent sensory system (ISS) with parallel information processing was developed. Sensory layers of e-nose and e-tongue respond to changes of SAW characteristics, e-eye logs reflection coefficients on frequency range of 240-2400 nm. Self-learning, self-diagnosis ISS functions parallel like human cerebral hemispheres using intuitive self-organizing neural networks of a whole mind. Experiments proved the information pattern recognition of any pharmaceuticals, their quality using human biomatters (blood, saliva, sweat) for a ubiquitous personalized medicine. Self-learning ISS takes less than 0.1 sec on information patterns generated in 1 sec from sensory data at most. The devised methodology of sensory information patterns, ISS realize the individual selection of pharmaceuticals, the diseases prediction, and the human health diagnosis. E-TNY with ISS are of worth for innovative drugs discovery, therapy, nanotechnology, ecology, biosafe personal taking medicine for the optimal recovery, maintaining nutrition and metabolism, producing effective and safe-health pharmaceuticals in nanomedicine researches etc. ISS provides a unique data protection of sensory information patterns, micro-nanostructures of any biomatters, and electronic medical maps of the human life activity including their QR-coding.

SL-182

Track: Pharmaceutical Research & Development

NOVEL SYNTHESIS OF PREACTIVATED THIOMERIC POLYMERS AS MEDICAL THERAPEUTIC AGENTS FOR DRY MOUTH SYNDROME

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Purpose: This study was aimed to investigate preactivated thiomers for their potential in the treatment of dry mouth syndrome.

Methods: Accordingly, chitosan-thioglycolic-mercaptonicotinamide conjugates (chitosan-TGA-MNA) were synthesized by the oxidative S-S coupling of chitosan-thioglycolic acid (chitosan-TGA) with 6-mercaptonicotin amide (MNA). Unmodified chitosan, chitosan-TGA (thiomers) and chitosan-TGA-MNA conjugates were compressed into test discs to investigate cohesive properties, cytotoxicity assays and mucoadhesion studies.

Results: Due to the immobilization of MNA, the chitosan-TGA-MNA conjugates exhibit comparatively higher swelling properties and cohesive properties corresponding unmodified chitosan. On the rotating cylinder, discs based on chitosan-TGA-MNA conjugates displayed 3.1-fold improved mucoadhesion time compared to thiolated polymers. Tensile study results were found in good agreement with rotating cylinder results. Moreover, preactivated thiomers showed higher stability. All polymers were found non-toxic over Caco-2 cells.

Conclusion: On the basis of achieved results the preactivated thiomeric therapeutic agent seems to represent a promising generation of mucoadhesive polymers which are safe to use for a prolonged residence time to target the mucosa requested for dry mouth syndrome.

Keywords: Thiomers, chitosan-thioglycolic acid, mercaptonicotinamide, preactivated thiomers, dry mouth syndrome.

SL-26

Track: Cancer Targeted Drug Delievery

POTENTIAL CHINESE HERBAL MEDICINES AGAINST GLIOBLASTOMA AND ITS CANCER STEM CELLS

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Glioblastoma (glioblastoma multiforme, GBM) is the most common and aggressive malignant primary brain tumor in adults. It is notorious for its genetic, cellular and phenotypic heterogeneity, which makes the disease difficult to target. Its invasiveness makes the traditional surgical procedure hardly succeeded, and its resistance to chemo-and radiotherapies also makes it a highly recurrent malignant disease with poor prognosis. The existence of glioblastoma stem cells (GSC or glioblastoma-initiating cells) may account for all these characteristics and become a suitable target for developing new treatment for GBM. Chinese herbal medicine is a traditional therapy and has been used widely in Chinese for few thousand years. Nowadays, it is not only important in disease treatment, but also becomes a promising biotech product remained to be revealed by evidence-based analysis. To investigate whether herbal extracts may serve as a new drug for the treatment of glioblastoma, especially targeting GSCs, we have set up a drug screening platform using human glioblastoma cell lines-derived tumor sphere cells to screen various Chinese herbal extracts. Three extracts belong to the Genus Elaeocarpus, Chamaecyparis and Cerbera have been identified, which effectively inhibited the growth of GSCs but with low or no toxicity to non-transforming cells. In addition, these extracts also inhibited the spheroid formation and cell migration ability of GSCs. The expression levels of stem cell markers such as CD133 and Sox2 were also reduced by the treatments of these extracts. The signaling

pathways affected by these extracts including Akt, ERK, cell cycle regulation and apoptosis. In conclusion, we have identified three effective Chinese herbal extracts which target GSCs effectively, and could be further developed as potential therapeutics of glioblastoma.

Keywords: Cancer stem cells, Chinese herb medicine, drug screen, glioblastoma.

SL-4

Track: CNS Drug Discovery & Therapy

NOVEL DRUG SCREENING TARGETING AMYLOID-Ā AGGREGATION FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most prevalent form of dementia associated with protein misfolding that interferes with diverse cellular processes. AD is pathologically characterized by extracellular amyloid-Ā (AĀ) plaques and intracellular neurofibrillary tangles formed from hyperphosphorylated tau protein. AĀ deposition causes neuronal death *via* a number of possible mechanisms including oxidative stress, excitotoxicity, energy depletion and apoptosis. Therefore therapeutic approaches to identify novel AĀ aggregate reducers may be effective for the disease treatment. In this study we developed AĀ biochemical and cell assays to screen potential aggregate reducers from plant extracts and synthetic compounds. For biochemical assay, thioredoxin and His tagged AĀ was used in conjunction with thioflavin T staining assay. For cell assay, Tet-On 293 cells with inducible AĀ-GFP expression was used combining automated microscopy and automated image analysis. In addition, changes of ROS/neuronal phenotype in Tet-On AĀ-GFP 293/SH-SY5Y cells were examined. Based on these experiments, we have identified several plant extracts/compounds with potential to inhibit AĀ aggregation, reduce ROS and improve neurite outgrowth.The effects of the identified extracts/compounds on mitochondrial function are currently being examined.

SL-180(a)

Track: Traditional Chinese Medicine

CYCLOARTANE TRITERPENOIDS AND THEIR GLYCOSIDES FROM THE RHIZOMES OF *CIMICIFUGA FOETIDA*

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The *Cimicifuga foetida* (called "Sheng-ma" in china) have been used as traditional medicine for treatment of wind-heat headache, sore throat, toothache and uterine prolapse. During the past two decades, although many phytochemical and pharmacological studies on the species were reported, it is unclear that whether its chemical constituents of the herb collected off Gansu province in northwestern China, one of the main produced areas, were different from the previous

reports. With this doubt, a phytochemical study on the rhizomes of the *C. foetida* produced in Gansu was carried out. Consequently, two new cycloartane triterpenoids, cimifoetidanols A and B (**1** and **2**), and eight new glycosides, cimifoetidanosides A-H (**3**-**10**), together with six known glycosides (**11**-**16**), were isolated from the EtOH extracts of the herb. Their structures were elucidated by NMR, MS, single-crystal X-ray diffraction analysis, and induced CD (ICD) spectroscopy of the $Mo_2(OAc)_4$ complex. Structurally, all the compounds possessed a cycloartane skeleton and the isolated glycosides belong to 3-*O*-*Ā*-D-xylopyranoside. Compounds **1**-**6** were obtained as three pairs of epimers featuring a 9,10-seco-7-membered B-ring part, especially compounds **3**-**6** isomerized at the C-3 and C-10 were never found in previous studies. Compounds **15** and **16** are firstly reported to have potential cytotoxicity against SMMC-7721 cell line with IC_{50} values of 5.5 and 6.3 μM, respectively.

SL-168

Track: Proteomics & Bioinformatics

UPLC-Q-TOF/MS BASED METABONOMIC PROFILING IN RAT SERUM TO REVEAL PHARMACOLOGICAL MECHANISMS OF *BERBERINE* FOR THE TREATMENT OF HIGH FAT DIET INDUCED NONALCOHOLIC *STEATOHEPATITIS*

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Introduction: Nonalcoholic fatty liver disease (NASH) is a kind of prevalence disease. Although much progress has been made in recent years, the specific therapeutic strategies were also not yet fully clear. NASH is closely allied to metabolic syndrome which linked obesity, insulin resistance, metabolic disorder, free radical reaction, and dyslipidemia, etc. Therefore, kinds of pharmacologic agents, such as insulin-sensitizer, antioxidant, *n-3* polyunsaturated fatty acids, ursodeoxycholic acid, lipase inhibitors, and lipid-lowering drug, are thought to have beneficial effects on the treatment for NASH. However, due to undesirable side effects and the limited effectiveness of current chemical drugs for NAFLD, researchers have focused on the development of natural drugs from herbs. *Berberine* ($C_{20}H_{18}NO_4$), an isoquinoline alkaloid, is one of the main bioactive constituents of *Uhizoma coptidis*, which has been used to treat NASH addressed in much more studies. However, the detailed metabolic mechanisms of *berberine* for treating NASH have not been reported, to date.

Methods: Here we report a metabolomics study on a high-fat diet (HFD) induced NASH rat model using UPLC-Q-TOF/MS techniques coupled with histopathology and biochemical analysis to reveal the mechanisms of *berberine* on the protective effects against NASH.

Results: The initial results showed that: 1) According to some conventional methods (histopathology, and biochemical serum parameters), *berberine* treatment plays a fighting role on HFD induced NASH due to its beneficial effects against insulin resistance, inflammation, and lipid metabolism. 2) Based on UPLC-Q-TOF/MS, the global metabolic profiling of NASH rat was detected and compared with the normal control rat, suggesting a metabolic disruption associated with amino acid, phospholipid, and unsaturated fatty acids in the HFD induced NASH rat. In contrast, *berberine* has the opposite effects on pathological process of NASH. Collectively, these effects lead to a net switch in the metabolic profiles involved creatine, 3-indoxyl-sulfuric acid, sphingomyelin (SM), phosphatidylcholine (PC), lysophosphatidylcholine (Lyso-PC), 13-hydroperoxy-9,11-octadecadienoic acid (13-HpODE), Eicosatrienoic acid, Docosatrienoic acid, and Eicosenoic acid.

Conclusion: Our strategy, proposed in this study, provided essential data for further molecular pharmacological study of *berberine*, and for R & D of NASH drugs.

SL-186(a)

Track: Traditional Chinese Medicine

THE METABOLIC AND PHARMACOKINETIC ANALYSIS OF CURCUMINOIDS BASED ON NANOPARTICLE FORMULATIONS BY LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY

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Curcuminoids, including curcumin, demethoxycurcumin and bisdemethoxycurcumin are the putative cancer chemopreventive agent with poor bioavailability, which makes the comprehensive metabolic and pharmacokinetic studies difficult and limited. In our research, three curcuminoids loaded nanoparticles formulations were prepared to improve the intestinal absorption and metabolic and pharmacokinetic analysis were conducted by LC/MS method.

The metabolites in rats and tumor-bearing mice after oral administration were investigated. A totally of 37 metabolites were identified in plasma, urine, feces, bile, liver, brain and tumor samples. Reduction, glucuronidation and sulfation were found to be the major metabolic reaction for curcuminoids through intestinal absorption. The glucuronide conjugates, sulfated conjugates and mixed conjugates, together with the related secondary metabolites including tetrahydro-, hexahydro- and octahydro- were unambiguously identified based on their characteristic fragmentations in MS^n analysis and UV absorbance. Based on these results, the metabolic pathway of curcuminoids *in vivo* were proposed. Furthermore, a validated LC-MS/MS method was established to determine three curcuminoids in tumor. The pharmacokinetics of curcumin, demethoxycurcumin, and bisdemethoxycurcumin were studied and the pharmacokinetic parameters were calculated. We found that the methoxy group ($-OCH_3$) on curcuminoids might possess strong tumor! affinity, and the curcumin possess the strongest tumor tissue affinity among three curcuminoids.

SL-12

Track: CNS Drug Discovery & Therapy

SIMILAR DRUG TARGETS FOR THE TREATMENTS OF SPINOCEREBELLAR ATAXIA TYPE 17, ALZHEIMER AND HUNTINGTON DISEASE

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Spinocerebellar ataxia type 17 (SCA17) is a neurodegenerative disorder caused by polyglutamine (poly Q) expansion in the TATA binding protein (TBP). Because the Chinese herbal medicines (CHMs) could be a new prospect for the neurodegenerative disorder (ND), we screened 40 kinds of CHMs which have been used for the treatment of ND and two kinds of CHMs, *Ginkgo biloba* and *Huperzia serrata*, were found to be effective for the treatment of SCA17 by *in vitro* cell model and *in vivo* transgenic mouse model, and furthermore, the active components of two CHMs were identified as EGb761 and Huperzine A, respectively. The active compounds suppressed the ER stress cell and apoptosis mediated by sodium glutamate in SH-SY5Y cells, and the aggregation of polyQ79-TBP induced by doxycycline of nTBP/Q79 SCA 17 cell model. The compounds also ameliorated SCA 17 transgenic mine on an accelerating rotarod behavior and footprint experiments. These two compounds are also effective in the treatment of Alzheimer disease and Huntington disease by previous studies, with considerable differences in the pathological and clinical symptoms but similarities in the mechanisms such as unfolded

protein response induced ER stress and neuronal cell apoptosis. Therefore, there could be similar targets for the treatment of several neurodegenerative diseases.

SL-18

Track: Cancer Targeted Drug Delivery

GARDENIA JASMINOIDES INHIBITS HEPATOCELLULAR CARCINOMA *VIA* THE AKT/MTOR PATHWAY

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Hepatocellular carcinoma (HCC) is the most common cancer worldwide, and there is no effective treatment for patients. In this study, we found that water extracts of the Chinese herbal medicine Gardenia jasminoides (GJ), which are frequently used to treat rheumatoid arthritis because of their anti-inflammatory activity, had an inhibitory effect on the migration and invasion of HCC Huh7 cells. To understand the underlying mechanism, we studied whether GJ-mediated inhibition of cell migration/invasion is related to inhibition of VEGF expression and F-actin reorganization. We demonstrated that GJ inhibited the phosphorylation of focal adhesion kinase (FAK) and Src kinase, resulting in disruption of F-actin rearrangement, and inhibited the AKT/mTOR pathway and decreased the translocation of Snail/Slug to the nucleus, leading to decreased VEGF-A expression. In addition, GJ decreased HIF-1! expression and translocation to the nucleus. *In vivo* validation using a mouse xenograft model showed that GJ had inhibitory effects on tumor growth and angiogenesis. Taken together, these results show that GJ has a potential use as a chemotherapeutic agent for HCC.

Keywords: AKT, Gardenia jasminoides, Hepatocellular carcinoma, mouse xenograft model, mTOR, VEGFA.

SL-20

Track: CNS Drug Discovery & Therapy

HERBAL MEDICINES FOR AMELIORATING AĀ-INDUCED NEUROTRANSMISSION OF GLUTAMATE

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Alzheimer disease (AD) is a deteriorate neurodegenerative disease in which the abnormal Ā-amyloid (AĀ) and tau protein plaque accumulation in brain tissues are currently prevailing hypothesis for causing this disease. Glutamate, the neurotransmitter of excitatory synapses in the central nervous system, binds to glutamate receptors such as AMPA, NMDA, Kainate, and mGluR1/3/5. Studies indicate that the neurotoxicity of AĀ is due to the over-stimulation of glutamates through NMDA receptors at postsynapses. Therefore, memantine, a NMDA receptor inhibitor, was developed and currently approved for treating the moderate-to-severe AD patients. Since Chinese herbal medicines (CHMs) have been widely using for thousands of years, we screened CHMs for reducing the AĀ-induced glutamate neurotransmission. Mice of postnatal day 1 to day 3 were sacrificed and performed primary culture of cortical neurons. Immunocytochemistry and Western blot were used to calculate and characterize the purity and property of glutamatergic neurons. DiBAC4(3), a slow-response voltage sensitive fluorescence dye, was adopted to establish an approach for screening CHMs which are effective on decreasing AĀ-induced neuron depolarization. Judging from vGLUT1/2 staining in immunocytochemistry, we obtained

a relative uniform population of glutamatergic neurons. Synaptotagmin staining also showed the formation of neuronal network within these neurons. The property of neurons/glutamatergic neurons was further confirmed by Western blot analysis using antibodies such as type III Ā-tubulin, PSD95, and AMPAR. Moreover, the fluorescence of DiBAC4(3) was increased when primary neurons were stimulated by either KCl or glutamate. We have established the primary culture in which around 90% of cellular population is glutamatergic neurons. We will further use this assay to screen CHMs for ameliorating AĀ-induced neurotransmission of glutamate.

SL-162

Track: Hot Topics in Drug Targets

EFFECT OF ADENOVIRAL VECTOR EXPRESSING SHORT HAIRPIN SIRNA TARGETING RRM1 GENE ON CELL VIABILITY AND CHEMOSENSITIVITY TO GEMCITABIN IN HUMAN NON-SMALL CELL LUNG CANCER CELLS

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Ribonucleotide reductase M1 (RRM1) is involved in the regulation of cell proliferation, cell migration and the synthesis of deoxyribonucleotides for DNA synthesis. It is also a cellular target for gemcitabine (GEM) and high levels of RRM1 expression are associated with resistance to GEM. In order to establish a new treatment method for GEM-resistant tumors, the efficacy of gene therapy was investigated using an adenoviral vector expressing short hairpin siRNA targeting RRM1. Tow RRM1-overexpressing human lung cancer tumor cells, adenocarcinoma cell MAC10 and squamous carcinoma cell RERF were used. A replication-deficient recombinant adenoviral vector expressing short hairpin siRNA targeting RRM1 (Ad-shRRM1) was constructed under the control of the human U6 promoter. Infection with Ad-shRRM1 effectively downregulated the RRM1 expression in both cell lines (about 90%). MTT assays demonstrated that treatment with Ad-shRRM1 significantly inhibited the growth of these two RRM1-overexpressing cell lines ($p<0.001$, respectively). Caspase 3/7 analysis revealed the infection with Ad-RRM1 into RRM1-overexpressing tumor cells to increase the percentage of apoptotic cells. Furthermore, combined treatment with Ad-shRRM1 and GEM demonstrated significantly greater inhibition of tumor cell growth in comparison to GEM treatment alone and Ad-shRRM1 treatment alone. In conclusion, the cancer gene therapy using Ad-shRRM1 has a strong antitumor effect against RRM1-overexpressing NSCLC cells through its antiproliferation and proapoptotic effects. Moreover, combined therapy with Ad-shRRM1and GEM may provide new treatment method for the GEM-resistant tumors.

Keyword: Apoptosis, chemorisistance proliferation, gemcitabine, lung cancer, RRM1.

SL-174(a)

Track: Traditional Chinese Medicine

THE CENTRAL MECHANISM OF PUNCTURING ACUPOINTS ALONG SHAOYANG MERIDIANS FOR MIGRAINEURS

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Objective: To explore the central mechanism of treating migraine with acupoints along related meridians.

Methods: Fourteen migraineurs without aura were received 20 times acupuncture by acupoints along shaoyang-meridian within 4 weeks. Clinical evaluation and resting-state functional Magnetic Resonance Imaging (fMRI) scans were performed after four-week observation at baseline, two-week treatment and four-week treatment in each group.

Headache related indexes, including headache days (HD), disabled days due to headache attack (DDHA), visual analogue scale (VAS), average attack lasting hours (AALH), headache scoring scale (HSS), migraine specific questionnaire (MSQ) and emotion evaluation were assessed. Region Homogeneity (ReHo) method was applied to analyze fMRI data. The clinical effects, cerebral regions of activation/deactivation and the correlation between effectiveness and brain areas were analyzed.

Results: (1) VAS and depression evaluation showed significance compared migraineurs after two-week treatment to those at baseline, the reduction of HD showed significant difference comparing four-week treatment to two-week treatment, and the reduction of HD, DDHA, VAS, AALH and HSS, as well as the improvement of MSQ showed statistical significance comparing four-week treatment to baseline; (2) middle brain, anterior cingulate cortex, caudate, putamen, anterior prefrontal cortex, and cerebellum were activation/deactivation compared two-week treatment to baseline; medulla, parahippocampus, anterior prefrontal cortex, and cerebellum were activation/deactivation compared two-week treatment to four-week treatment; right parahippocampus, prefrontal cortex and temporal cortex were activation/deactivation compared four-week treatment to baseline; (3) the reduction of HD positively correlated with ReHo value of parahippocampus ($r=0.65$, $P<0.01$), the improvement of MSQ scores positively correlated with ReHo value of OFC (BA11) ($r=0.53$, $P<0.01$).

Conclusion: The central mechanism of puncturing shaoyang acupoints for migraineurs may relate with the targeting modulation of brain stem-limbic-prefrontal cerebral cortices.

SL-139

Track: Protein and Peptide Sciences

PEPTIDES DERIVED FROM HIV-1 GP120 CO-RECEPTOR BINDING DOMAIN FORM AMYLOID FIBRILS AND ENHANCE HIV-1 INFECTION

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Amyloid fibrils play important roles in HIV-1 infection. We found peptides derived from the HIV-1 gp120 co-receptor binding region, which are defined as enhancing peptides (EPs), could form amyloid fibrils and remarkably enhance HIV-1 infection. EPs bound to the virus and promoted the interaction between HIV-1 and target cells. The antiviral efficacy of antiretroviral drugs (ARVs) was substantially impaired in the presence of EPs. Epigallocatechin gallate

(EGCG) could both inhibit the formation of fibrils composed of EPs and counteract the EP-mediated enhancement of HIV-1 infection. Our findings identify viral derived amyloid fibrils that hold potential for biochemical applications.

SL-114

CNS Drug Discovery & Therapy

CELL TYPE-SPECIFIC ACTIONS OF NEUROSTEROIDS: RELEVANCE FOR THERAPEUTICS

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Extrasynaptic GABAA receptors (GABAARs), particularly neurosteroid-sensitive Ā subunit-containing GABAARs, have been suggested to be promising therapeutic target for the treatment of numerous neurological and neuropsychiatric disorders, ranging from posttraumatic stress disorder (PTSD) to epilepsy. GABAAR Ā subunit-containing receptors mediate tonic GABAergic inhibition in many brain regions, including the dentate gyrus subregion of the hippocampus. Tonic GABAergic inhibition in dentate gyrus granule cells (DGGCs) is thought to maintain the "dentate gate" and breakdown in this control and been implicated in seizure generation. For example, alterations in the expression of GABAAR Ā subunit expression in DGGCs under conditions of fluctuating neurosteroid levels is associated with changes in network excitability and seizure susceptibility. However, extrasynaptic GABAARs are expressed widely throughout the brain in both principal neurons and interneurons. Previous studies underestimated the contribution of GABAAR Ā subunit-containing receptors on the regulation of interneurons in the hippocampus. Studies from our laboratory demonstrate a critical role for GABAAR Ā subunit-containing receptors in the regulation of interneurons in the hippocampus which dramatically impacts principal neuron activity. Mice with deficits in Ā subunit-containing extrasynaptic GABAARs specifically in interneurons (Gabrd/Gad mice) exhibit a disinhibition of interneurons in the hippocampus, which results in a profound increase in the inhibitory drive onto principal neurons, which reduces neuronal excitability and seizure susceptibility. These findings have significant translational impact given the enthusiasm for targeting extrasynaptic GABAARs for treatments. In fact, several clinical trials targeting these receptors have failed. Targeting GABAAR Ā subunit-containing receptors pharmacologically will have an effect on both principal neurons as well as interneurons, which is likely to produce a net opposite effect. These results question the usefulness of targeting extrasynaptic GABAARs for therapeutics.

SL-129

Track: CNS Drug Discovery & Therapy

NEUROSTEROIDS AS BIOMARKER CANDIDATES AND NEW THERAPEUTICS IN SCHIZOPHRENIA AND TBI

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Introduction: Preclinical and clinical data suggest that pregnenolone may be a promising therapeutic in schizophrenia and traumatic brain injury (TBI). Pregnenolone is neuroprotective, and enhances learning and memory, myelination, and microtubule polymerization. Treatment with pregnenolone elevates allopregnanolone (a neurosteroid that enhances $GABA_A$ receptor responses) and pregnenolone sulfate (a positive NMDA receptor modulator). Pregnenolone could thus potentially mitigate GABA dysregulation and/or NMDA receptor hypofunction in schizophrenia *via* metabolism to other neurosteroids, and also potentially ameliorate TBI sequelae through metabolism to allopregnanolone.

Objectives: 1. To conduct a randomized controlled trial (RCT) of adjunctive pregnenolone in schizophrenia; 2. To investigate neurosteroids as biomarker candidates in TBI secondary to blast injury.

Methods: 1. 120 participants were randomized to pregnenolone or placebo for 8 weeks (Institute for Mental Health, Singapore). Primary endpoints were changes in MCCB composite scores (cognitive symptoms), UPSA-B composite scores (functional capacity), and SANS total scores (negative symptoms). A modified intent-to-treat analysis approach was utilized. 2. Neurosteroids were quantified in serum samples from male OEF/OIF Veterans who had been exposed to a blast-related TBI during deployment (n=55) and in male OEF/OIF Veterans who had been deployed but who had not been exposed to a blast-related TBI in theater (GC/MS preceded by HPLC). Groups were matched for smoking status, time of blood draw, and age. Non-parametric Mann-Whitney analyses were conducted.

Results: 1. No significant changes compared to placebo were demonstrated in composite MCCB scores. In contrast, participants randomized to pregnenolone (n=56) demonstrated greater improvements in functional capacity (UPSA-B composite changes) compared to placebo (n=55), p=0.03. Pregnenolone was also superior to placebo in the communication subscale of the UPSA-B ($p<0.001$). Serum pregnenolone changes post-treatment predicted UPSA-B composite score changes in females ($rs=0.497$, $p<0.042$, n=17) but not in males. Mean total SANS scores were very low at baseline, and did not improve further post-treatment. Pregnenolone was well-tolerated. 2. Pregnanolone was significantly reduced in Veterans who had sustained a blast-related TBI in Iraq or Afghanistan compared to Veterans who had no history of blast-related TBI (p=0.001, n=55/group). Androsterone was also significantly reduced in Veterans who had sustained a blast-related TBI (p=0.001, n=55/group). Pregnenolone tended to be reduced in Veterans who had sustained a blast-related TBI (p=0.08).

Conclusions: 1. Pregnenolone improved functional capacity in participants with schizophrenia, but did not improve cognitive symptoms over an 8-week treatment period. Neurosteroid changes correlated with functional improvements in female participants. 2. Veterans who sustained a blast-related TBI demonstrated significantly reduced serum neurosteroid levels compared to Veterans who had been deployed to Iraq or Afghanistan but who had not been exposed to a blast-related TBI.

Summary: Neurosteroid interventions may exhibit promise as biomarker candidates and new therapeutic leads in schizophrenia and TBI.

SL-74

Track: Drug Discovery in Preclinical Research

DISCOVERING DRUGGABLE TARGETS FOR TRIGGERING ENUCLEATION OF IMMORTALIZED ERYTHROID CELLS

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Shortage of transfusable red blood cell (RBC) stock is serious problem in the world, hence developing new source/methods to supply RBCs infinitely is required. We have recently established immortalized erythroid cell lines from mouse embryonic stem (ES) cells (Hiroyama *et al*., *PLoS ONE*, 2008), human umbilical cord blood (CB) cells and induced pluripotent stem (iPS) cells (Kurita *et al*., *PLoS ONE*, 2013). These cell lines grow continuously and are already committed to the erythroid lineage. In addition, other groups have also reported successful establishment of immortalized erythroid cell lines (Hirose *et al*., *Stem Cell Reports*, 2013; Huang *et al*., *Mol. Ther.*, 2014), However, such immortalized cell lines usually possess a limited differentiation capacity, as efficiency of terminal differentiation (enucleation) is very low. Our group has succeeded to efficiently generate enucleated RBCs from human umbilical cord blood (UCB) hematopoietic stem/progenitor cells (Miharada *et al*., *Nat. Biotechnol*., 2006), however use of the protocol shows minimum effect on the cell lines, suggesting that the immortalized cell lines have different/irregular regulation in cell maturation from primary cells. We therefore need to identify what type of gene(s) are the direct trigger(s) of enucleation. We have recently been trying to identify critical regulator(s) triggering and undergoing enucleation of human erythroid cell lines by using a proteomics approach and a large screening assay using chemical compound library. Identifying potential chemical compound(s) will lead to discovering the mechanism of enucleation, which also contributes to develop a method for the *in vitro* RBC production.

SL-181

Track: Anti-Infectives

A NOVEL CHEMICAL SCAFFOLD WITH BROAD-SPECTRUM ANTIVIRAL ACTIVITY TARGETS RNA VIRUSES

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Hantaviruses are rodent born enveloped negative strand RNA viruses. They have evolved a unique translation strategy to boost the manufacture of viral proteins in the host cell during the course of viral infection. This translation strategy is operated by viral nucleocapsid protein (N), which lures the host translation machinery for the preferential translation of viral mRNA in the host cell cytoplasm where cellular transcripts are competing for the same translation apparatus. N protein specifically binds to both the viral mRNA 5' cap and a heptanucleotide sequence GUAGUAG at the viral mRNA 5' UTR. In addition, N protein also binds to the ribosomal protein S19 (RPS19), a structural component of the 40S ribosomal subunit. N associated ribosomes are efficiently loaded onto the viral mRNA 5' UTR by an unknown mechanism that promotes the translation of viral transcripts. We developed a tractable fluorescence based high throughput screening assay to monitor the interaction between N protein and the heptanucleotide sequence of the viral UTR. The assay was used to screen a chemical library to identify inhibitors that interrupt N-UTR interaction and prevent the manufacture of viral proteins by the N-mediated translation strategy. We identified a cluster of lead compounds that specifically bind to N protein and block its interaction with the viral UTR, thereby inhibit the N-mediated translation mechanism. The launch of structure activity relationship (SAR) campaign revealed the identification of a unique structural scaffold that has potential for further modification and development of high potency broad-spectrum antivirals. Interestingly, one of the lead inhibitors (K31) showed potent anti-hantaviral activity. This lead inhibitor specifically binds and induces structural perturbations to N protein that compromise its function. A single application of K31 inhibited viral

replication by 96% in 24 hours post-treatment in cell culture model. This lead inhibitor also inhibited hepatitis C virus (HCV) replication in cell culture model with similar potency. We are currently testing the efficacy of this lead inhibitor for other RNA viruses of potential biomedical importance. Our results demonstrate the identification of a novel chemical scaffold that has promise for the development of broad-spectrum antivirals targeting diverse families of RNA viruses.

Keywords: Hantavirus, Hepatitis C virus, RNA viruses.

SL-118

Track: CNS Drug Discovery & Therapy

ONO-2952, A NOVEL POTENT AND SELECTIVE TRANSLOCATOR PROTEIN 18KDA (TSPO) ANTAGONIST, PRODUCES ANTI-STRESS EFFECTS IN RATS

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Accumulating evidence has shown the pathophysiological significance of the balance in the levels of endogenous steroids in the central nervous system. TSPO is mainly located in the outer mitochondrial membrane of steroid producing cells, including glial cells, and regulates cholesterol transport from intracellular sources into mitochondria, a rate-limiting step in steroidogenesis. Neurosteroids act as allosteric modulators of excitatory and/or inhibitory neurotransmission and its levels are drastically changed by stress. These findings indicate that TSPO ligands could represent a novel class of pharmacological agents useful for the treatment of stress-related disorders. We evaluated the beneficial effects of ONO-2952, a novel selective TSPO antagonist in rat stress models.

ONO-2952 inhibited both pregnenolone accumulation and noradrenaline release in the brain of stressed rats. The inhibitory effect of ONO-2952 on stress-induced noradrenaline release was attenuated by co-treatment with TSPO agonist CB34. ONO-2952 dose-dependently suppressed stress-induced rectal hyperalgesia and defecation with brain TSPO occupancy of more than 50%. In addition, ONO-2952 suppressed hyperemotionality and anxiogenic-like behavior in olfactory bulbectomized rat. It should be noted that ONO-2952, unlike diazepam, did not affect passive avoidance behavior. The present findings indicate that ONO-2952 is a promising candidate for the treatment of stress-related disorders, such as irritable bowel syndrome, depression, and anxiety disorder.

SL-108

Track: Cancer Targeted Drug Delivery

LIGAND-LINKED POLYMERIC MICELLES FOR TARGETED DELIVERY OF PLATINUM ANTI-CANCER DRUG INTO MALIGNANT TUMOR

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The installation of ligand molecules such as antibodies, antibody fragments, aptamers, and peptides onto nanocarriers is now considered an important strategy to improve the selectivity of drug delivery. Among these ligands, Arg-Gly-Asp peptides are promising ligand molecules for targeting alpha v beta 3/alpha vbeta 5 integrins, which are overexpressed in angiogenic sites and tumors, such as intractable human glioblastoma (U87MG). In this study, the cyclic Arg-Gly-Asp (cRGD) was introduced to the maleimide-surface functionalized dichloro(1,2-diamino-cyclohexane)platinum(II)-loaded micelles (DACHPt/m) by Michael addition reaction. Intravital confocal laser scanning microscopy revealed that the cRGD-linked polymeric micelles (cRGD/m) accumulated rapidly and had high

permeability from vessels into the tumor parenchyma compared with the polymeric micelle having non-targeted ligand, "cyclic-Arg-Ala-Asp" (cRAD). As both cRGD/m and cRAD-linked polymeric micelles have similar characteristics, including their size, surface charge, and the amount of incorporated drugs, therefore, it is likely that the selective and accelerated accumulation of cRGD/m into tumors occurred via an active internalization pathway, possibly transcytosis, thereby producing significant anti-tumor effects in an orthotopic mouse model of U87MG human glioblastoma**.**

Keywords: Polymeric micelle, platinum drug, ligand.

SL-48

Track: Women's Health Drug Discovery & Therapy

IN VITRO AND *IN VIVO* EFFICACY OF A NOVEL SUPERBENZOPYRAN ANALOGUE TRX1 AGAINST PLATINUM-RESISTANT OVARIAN CANCER STEM CELLS

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Ovarian cancer is the most lethal of all gynecologic malignancies. Cellular heterogeneity is a primary cause for the chemoresponse profile and aggressiveness of ovarian cancer. We identified and cloned two subtypes of ovarian cancer cells that resemble ovarian tumor complexity. CD44-/MyD88- epithelial ovarian cancer (EOC) cells represent the chemoresponsive phenotype while CD44+/MyD88+ EOC stem cells exhibit tumor-initiating properties, are chemoresistant, and are the source of recurrence. Our objective was to identify novel therapeutics with efficacy against chemoresistant EOC stem cells, a pre-requisite to improve survival. From a panel of super-benzopyran analogues designed by iterative molecular-biological screening techniques we identified Trx1 as the most potent analogue.

Screening was performed using Incucyte^{TM} kinetic imaging platform and Celltox^{TM} dye labelling. *In vivo* efficacy was determined using a platinum resistant intra-peritoneal (i.p.) ovarian cancer xenograft model.

Out of 45 compounds screened, Trx-1 was the most effective at inducing cell death in all EOC stem cell clones (IC50 of 0.05 ug/ml *vs* 32 ug/ml Caboplatin). *In vivo*, Trx-1 significantly decreased tumor growth and tumor burden compared with control on completion of the study (0.54 g in Trx-1-treated *vs* 1.92 g in control p=0.001). Maximum Tolerable Dose (MTD) was determined and efficacy at concentration of 80 mg/kg.

We have identified a novel compound (Trx-1) that exhibited significant *in vitro* and *in vivo* efficacy against chemoresistant ovarian cancer stem cells. These findings open the possibility of using Trx-1 as a therapy against platinum/taxol resistant ovarian cancer. On-going studies are designed to identify better drug delivery options to further improve *in vivo* activity of Trx-1.

SL-21

Track: Drug Delivery & Targeting

INULIN AS MATRIX SYSTEM AND COMPRESSION COATING MATERIAL FOR COLON SPECIFIC DELIVERY OF 5-FLOUROURACIL

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Inulin (FrutafitTex®) has been evaluated as a carrier for the preparation of matrix tablets and as compression coating material for targeted drug delivery to the colon using 5-fluorouracil as the model drug. Drug release studies were carried out in 0.1M HCl for 2h, pH 7.4 Sorensen's buffer for 3h and then in PBS pH 6.8 or in simulated colonic fluid for the rest of the experiment to mimic the physiological conditions from the mouth to colon. The susceptibility of the polysaccharide to undergo biodegradation in the colon was assessed by conducting the drug release studies in the presence of rat ceacal contents in phosphate buffer saline (PBS) at pH 6.8. The results obtained indicated that inulin matrices could be useful for controlled drug delivery and various polymers such as carbopol, methylcellulose and pectin, could be used to modulate the drug release from the matrix tablet depending on the need. Furthermore, inulin and inulin:wax (4:1) mixture were capable of protecting the drug from being released in conditions mimicking the mouth to colon transit time and are susceptible to enzymatic attacks in the colon leading to complete release of the drug within 12 hours. The results clearly demonstrate that inulin has the potentials for drug targeting to the colon.

SL-9

Track: Drug Delivery & Targeting

THE EFFECT OF MONOMER COMPOSITION IN THE SYNTHESIS OF PACLITAXEL LOADED POLY (STYRENE-CO-METHYL METHACRYLATE) NOVEL NANOPARTICLES FOR CONTROLLED RELEASE

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Paclitaxel (Taxol®) is one of the most utilized anti cancer drugs with a broad spectrum of antitumor activity. Paclitaxel usually formulated in Cremopher EL because it suffers from low solubility in water and it has a lot of side effects. Low solubility leads the encapsulation and controlled release of Paclitaxel a rough process and not every kind of polymer can entrap it. In this study for the first time a novel route was used for nano-encapsulation of Paclitaxel into three different types of polymer particles. These are including poly (Methyl Methacrylate) (PMMA) and poly (Styrene) (PS) and poly (Methyl Methacrylate-co-Styrene) (PMMAS) with different monomer ratio and different drug contents in monomer have been investigated. Synthesis was performed via mini-emulsion polymerization method and spherical shape and the size of approximate 100 nm nanoparticles obtained. FT-IR tests have been performed to reveal the effect of monomer composition in different atomic bonds. DLS characterization showed a sharp statistical distribution in nanoparticles sizes. *In-vitro* drug release profile showed an increase in drug loading (to more than 10%) and encapsulation efficiency (to more than 80%) in co-polymers with higher molar mass of Styrene monomer after 96h.

Keywords: Encapsulation, methyl methacrylate copolymer, miniemulsion, nanoparticle, paclitaxel, styrene.

SL-166

Track: Hot Topics in Drug Targets

CHARACTERIZATION OF INFECTIOUS ENDOCARDITIS IN A NATIONAL REFERENCE CENTER

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Objective: To study the clinical, epidemiological and microbiological characteristics as well as the surgical medical treatment of patients admitted diagnosed with infectious endocarditis to deepen in its study and to contribute to a better treatment for these patients.

Methods: Twenty four patients from whole country, discharged with the diagnosis of infectious endocarditis. Data were collect from the medical records of the "William Soler" Children Hospital files and from the database of surgery service of heart center. The frequency of different manifestations of infectious endocarditis was determined according to: age groups, risk factors and the more frequent clinical, laboratory and microbiologic data. Also, the predominant valvular take, the etiology and the response to antibiotics were determined.

Results: The more involved age group was between 5 and 18 years; the previous heart disease was the more predominant factor; the more frequent symptoms and signs were: fever, anorexia and weight loss. The heart failure and the pulmonary embolism were frequent complications. Most of patients had an accelerated erythrosedimentation and the fourth of cases had negative blood cultures. The aortic and mitral valves were the more involved and the predominant clinical course was the subacute. In almost the half of patients the infection had a nosocomial origin. The more used antimicrobial agents were amikacin, vancomycin and ceftriaxone.

Conclusions: The infectious endocarditis is uncommon in our institution occurs more often in relation to congenital heart diseases. The more constant clinical facts were fever and a history of previous heart disease, the existence of both, especially if accompanies of an increase of the globular eritrosedimentacion, they should be considered strong indications for the diagnosis. To focus the surgical medical treatment from a beginning in these patients guarantees a better one predicts of the illness.

SL-42

Track: Hot Topics in Natural Products

TRANSDERMAL ABSORPTION ENHANCEMENT OF GEL CONTAINING NIOSOMES LOADED WITH *VOLVARIELLA VOLVACEA* EXTRACT

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Our previous study demonstrated the potent *in vitro* collagen biosynthesis stimulating and antioxidant activities of *Volvariella volvacea* extract. This study was performed the loading of *V. volvacea* extract, physicochemical characterization of the loaded niosomes and incorporated in gel. Rat skin transdermal absorption by Franz diffusion cells at 6 hours of *V. volvacea* extract loaded in niosomes (NV) and gel containing niosome of the extract (GNV) were compared with *V. volvacea* extract solution (SV). GNV and NV showed higher chemical stability of total phenolic contents than SV. NV was the mixture of unilamellar structures with negative zeta potential values and in the size range of 200-300 nm. Both GNV and NV retarded the cumulative amounts and fluxes of the total phenolic in the extract in the first hour of skin permeation, while enhanced the skin permeation at the 6th hour in the experiment. GNV gave the highest percentages of the total phenolic content through rat skin to the receiving solution followed by NV and SV, respectively. This study has demonstrated the potential of gel containing niosomes of the *V. volvacea* extract

appeared to be the suitable system for topical anti-aging application of *V. volvacea* extract because of the enhancement of chemical stability and rat skin transdermal absorption of *V. volvacea* extract.

Keywords: Cancer metastasis, cell migration, high-throughput drug screening, Zebrafish.

SL-95

Track: Hot Topics in Natural Products

USING LEAF ETHANOLIC EXTRACT HONJE (ETLINGERA HEMISPHAERICA) FOR MERCURY DETOXIFICATION ON MICE (MUS MUSCULUS) BLOOD CELLS

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This study aimed to analyze the effect of ethanolic extract of leaves honje (*Etlingera hemisphaerica*) to detoxify mercury chloride ($HgCl_2$) in the blood of mice (*Mus musculus*).

Methods: There were four stages of the study. *First stage* included Po, P1, P2, P3, and P4 group with 5 replications. In day 1, imunos 0.2 mg/g body weight (bw) was administered by gavage on P1, and the extract of 0.13, 0.26, and 0.39 mg/g bw were given by gavage on P2, P3, and P4 respectively. Leukocyte was counted on day 2. *Second stage* included Qo, Q1, Q2, Q3, and Q4 groups with 5 replications. In day 1, HgCl2 5 mg/g bw was given by gavage on Q1, Q2, Q3, Q4, then in day 2 the extract of 0.13, 0.26, and 0.39 mg/g bw were given by gavage on Q2, Q3, and Q4 respectively. Leukocyte was counted on day 3. Third stage included Ro, R1, and R2 group with 15 replications. In day 1, $HgCl_2$ 5 mg/g bw was injected on R1 and R2, and in day 2 the extract of 0.39 mg/g bw was given by gavage on R2. Leukocyte and erythrocyte were counted in days 3 and 5. *Fourth stage* included the blood samples of Ro, R1, and R2 in day 4 from the *third stage* were separated by one-dimensional electrophoresis to obtain a protein profile. Po, Qo, and Ro as the controls of the study were administered solvent only.

Results: Obtained data indicated that a number of leukocyte in P3 increased significantly compared to Po and P1, while the extract of 0.39 mg/g bw on P4 revealed a similar effect as well as imunos 0.2 mg/g bw on P1. $HgCl_2$ treatment increased significantly the number of leukocytes in Q1 compared to Qo, while the extract of 0.13, 0.26, and 0.39 mg/g bw in Q2, Q3 and Q4 respectively could restore the number of leukocytes similar with Qo. Further $HgCl_2$ administration could increase leukocyte and decrease erythrocyte on R1 and R2 compared to Ro, while the extract of 0.39 mg/g bw on R2 could recover the amount of blood cells as well as Ro. A proteomcs observation revealed that $HgCl_2$ treatment appeared a new protein 125 kDa and over expressed protein 48 kDa on R1, while the extract of 0.39 mg/g bw could modify R2 protein profile similar with Ro.

Conclusion: The leaf ethanolic extract *E. hemisphaerica* (0.39 mg/g bw) is capable to detoxify $HgCl_2$ (5 mg/g bw) on the blood cells of *M. musculus*.

Keywords: *E. hemisphaerica*, erythrocyte, ethanolic extract, mercury chloride, leukocyte, protein profile.

SL-46

Track: Hot Topics in Natural Products

EVALUATION OF ANTI-NOCICEPTIVE PROPERTY OF CURCUMA LONGA, *CENTELLA ASIATICA* AND THEIR COMBINATION IN RATS: A COMPARATIVE STUDY

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This study had been carried out to discern the anti-nociceptive effect of *Curcuma longa* (CL), *Centella asiatica* (CA) and their combination using acetic acid induced writhing test after acute and chronic treatment. In the chronic study, 60 wistar rats of either sex were divided in 10 groups and normal saline, CA (50, 100 & 200 mg/kg), CL (20, 40 & 80 mg/kg) and CA + CL (50+20, 100 + 40 & 200 + 80 mg/kg) were orally administered for eight days. On 8th day writhing test was performed 30 minutes after drug administration. In acute study, additional 30 rats were divided in 5 groups and normal Saline, CA 300 mg/kg, CL 200 mg/kg, CA + CL (300+200 mg/kg) and Tramadol 12.5 mg/kg were orally administered in single dose and writhing test was performed after 30 minutes. Both the herbal drugs *per se* and in combination, after single dose and after eight day treatment, expressed significant, dose dependent, peripherally mediated anti-nociceptive property (except CA in lowest dose). Combination of these drugs provided better reduction in pain as compared to their individual effects. Moreover, CL seems to be more effective than CA if these are to be used alone. As compared to tramadol, both these herbal drugs showed comparable effects.

Keywords: Anti-nociceptive, *Centella asiatica*, *Curcumin longa,* Writhing Test.

SL-97(a)

Track: Information and Immunology

GALECTIN-8 INHIBITS EXPERIMENTAL AUTOIMMUNE UVEITIS BY PROMOTING TREG

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Purpose: Galectins (Gal) such as Gal-1 and Gal-9 have been shown to play an immunomodulatory role by inhibiting effector T cells and promoting regulatory T cells (Treg), however the role of Gal-8 in T cell homeostasis is poorly understood. Experimental autoimmune uveitis (EAU) is a mouse model of uveitis that is driven by T_H17 cells. Pathology can be ameliorated by shifting the T cell response from T_H17 to Treg. Thus, the purpose of this study is to determine what role Gal-8 plays in T cell differentiation, and to determine whether Gal-8 treatment reduces EAU pathology.

Methods: Mouse $CD4^+$ T cells were isolated by negative selection and polarized to T_H1, T_H2, T_H17, and Treg in the presence and absence of recombinant Gal-8 to determine the effects of Gal-8 on T cell differentiation. In order to identify novel Gal-8 binding partners, the effects of Gal-8 on IL-2 receptor and TGFß receptor signaling, necessary for Treg polarization, were determined by western blotting. The ability of Treg cells to inhibit proliferation of activated T cells was assessed in *in vitro* suppression assays. EAU was actively induced by immunization with $IRBP_{1\text{-}20}$, and one group of mice were treated with vehicle, while the other group was treated with Gal-8.

Results: Recombinant Gal-8 promoted Th2 and Treg differentiation and induced apoptosis of T_H17 cells *in vitro*. Gal-8 sustained STAT5 phosphorylation downstream of IL-2 receptor, and enhanced Smad3 phosphorylation downstream of TGFß receptor to promote Treg polarization. Treg cells polarized in the presence of Gal-8 suppressed division of activated T cells. Treatment of uveitic mice with Gal-8 resulted in decreased pathology and expanded Treg cells in draining lymph nodes and retina, and decreased pathogenic T_H1 and T_H17 function at the retina.

Conclusion: Gal-8 induces apoptosis of T_H17 cells and promotes Treg differentiation, making it a good candidate for treating human uveitis without the side effects of long-term corticosteroids or immunosuppressive drugs.

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SL-14

Track: Cancer Targeted Drug Delivery

NOVEL, EFFECTIVE, AND ECONOMICAL ELECTRICALLY-ENHANCED GEMCITABINE UPTAKE IN PANCREATIC CELL LINES

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A recent study at the MD Anderson Cancer Center using a number of pancreatic cell lines indicated that resistance to chemotherapy is one of the major problems in the pancreatic cancer management. Gemcitabine is the standard chemotherapy for the treatment of pancreatic cancer and it has very meagre benefits initially, and eventually it is completely ineffective. A previous researcher mentioned that here are no good options for pancreatic patients using current standard of cure. This indicates that alternate techniques are urgently needed with the existing 5-10% survival rate for pancreatic cancer; towards this, we propose a novel, effective and economical treatment using electrical pulses. This technique, known as electrochemotherapy is the local application of high intensity, short duration electrical voltage pulses to the tumor tissue, to render the cell membranes permeable to otherwise impermeant or poorly permeant anticancer drugs, thereby facilitating a potent, localized, enhanced cytotoxic effect, compared to drug alone. Being physical, this therapy is applicable to all types of histologies and it has shown up to 1000 fold enhancement in the drug uptake, and causing cell death by an apoptotosis-like phenomena. Hence, there is no inflammatory reactions and since the treatment is confined to the electric field, there is little collateral tissue injury. Successful treatment of other cancers, such as melanomas, sarcomas and chest wall breast carcinoma attest to the effectiveness of this technique.

In this research, we studied the efficacy of Gemcitabine, the FDA approved chemodrug used for pancreatic cancer at 100ĀM concentration using electrical pulses on two human pancreatic cell lines, Panc 1 and Panc 28. These cells were chosen as they closely relate to the actual tumor environment and were also studied by MD Anderson Cancer Center. Using 1200V/cm, 100Ās (8 pulses at one second interval) and 500V/cm, 25m (2 pulses at one second interval) pulses we could obtain drastically reduced cell viabilities of 74% and 30% for Panc 1 cell line and 33% and 34% for Panc 28, compared to drug alone. With drug only, the viabilities were 79% and 66% respectively for Panc 1 and Panc 28 cell lines (control 100%).

These results indicate that using electrical pulses and low dose chemo drug, we could treat pancreatic cancers effectively and economically and this technique is easily transferable to clinics.

Keywords: Apoptosis, electrochemotherapy, gencitabine, panc 1 cell line, panc 28 cell line, pancreatic cancer.

SL-78

Track: Cancer Targeted Drug Delivery

IMMUNO-NANOPARTICLES FOR TARGETED INTRACELLULAR DELIVERY OF ANTICANCER DRUGS FOR IMPROVED BREAST TUMOR THERAPY

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Nanotechnology offers advantages, such as improved stability, favorable biodistribution, slower drug release kinetics, lower immunotoxicity, and targeting ability. Biodistribution and unwanted phagocytic uptake can be improved by pegylation of the nanoparticles, while targeting efficiency can be improved by antibody coating. Such engineered nanoparticles (NPs) that can target the anticancer drugs to tumor cells and reduce undesirable interactions with immune system are termed Immuno-nanoparticles. Our objective was to prepare and characterize exemestane loaded pegylated polycaprolactone (PCL) immuno-nanoparticles for targeted drug delivery for improved breast tumor therapy. The Nanoparticles were prepared by interfacial deposition of preformed polymer and were further surface modified with estrogen receptor (ER) antibody to form immuno-nanoparticles (ImmunoNPs) capable of targeting ER positive tumor cells. The NPs were evaluated by DSC, TEM and *in vitro* drug release studies. Qualitative and quantitative cellular uptake studies of NPs were conducted on MCF7 cells using fluorescent microscopy and FACS respectively. Nanoparticulate formulations were subjected to cytotoxicity studies (MTT assay) in both receptor positive (MCF7) and receptor negative (MDAMB231) cell lines. Cell cycle arrest and apoptosis studies were also performed. Results of Fluorescent imaging and quantitative cellular uptake studies confirmed higher cell uptake and higher cytotoxicity than drug solution or un-conjugated NPs. Combined results of *in vitro* cytotoxicity studies, cell cycle analysis and apoptosis studies confirmed the receptor mediated endocytosis of ER antibody conjugated ImmunoNPs. Thus, this nanoparticulate system can serve as an effective and promising delivery system capable of targeting estrogen receptor breast tumor and improving tumor localization of exemestane.

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Keywords: Breast cancer, exemestane, immuno-nanoparticles, pegylation, polycaprolactone, targeting.

SL-49

Track: Innovative Drug Discovery and Nanotechnology

ANTIOXIDANT DENDRIMERS

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Several reports attest to the benefits of antioxidants for prevention of a variety of human diseases including cancer and neurodegenerative diseases. Surprisingly, there are also many reports that express concerns over their use as preventative therapeutics. Popular naturally occurring antioxidants such as vitamins C and E and polyphenols such as quercetin are

able to neutralize harmful free radicals and prevent cellular damage. However, they are also capable of generating large amounts of harmful free radicals in the presence of transition metals such as iron and copper, resulting in cellular damage. This dual anti-oxidant and pro-oxidant behavior of these antioxidants is an important contribution to the antioxidant controversy. We have utilized a nanotechnology approach to design and synthesize over twenty antioxidants with a dendritic architecture known as antioxidant dendrimers. These macromolecules display a five-fold increase in free radical scavenging than the naturally occurring antioxidants. More importantly, they do not show pro-oxidant activities in the presence of physiological amounts of transition metals. This presentation will summarize their syntheses, characterization and protective effects on human low-density lipoproteins and DNA and provide structure-activity relationships of these novel compounds.

SL-99

Track: Hot Topics in Natural Products

ANTHRAQUINONE DERIVATIVES FROM THE *FUNGUS ALTERNARIA* SP. (XZSBG-1) ISOLATED FROM SALT LAKE SEDIMENTS AS A BIOLOGICAL EQUIVALENT OF THE MARINE ENVIRONMENT

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Seven secondary metabolites including three new anthraquinone and tetrahy-droanthraquinone derivatives (1-3) along with four known compounds (4, 5, 6, 7) were obtained from extracts of fungus strain Alternaria sp. XZSBG-1 from sediments of the Salt Lake, Tibet, as an equivalent of the marine environment. Their structures were established on the basis of one- and two-dimensional NMR spectroscopy, UV, CD, and mass spectrometry. Compound 2 is a new tetrahydroanthraquinone with a rare epoxy ether bond between C-4a and C-9a, exhibiting a considerable cytotoxicity against human MCF-7/ADR breast cancer cells with an IC50 value of 18.48 ĀM. Compound 3 is the first macrosporin dimer with a C-5, 5' linkage. The known Compound 4, showing the most potent inhibitory activity on Ä-glucosidase and also exhibiting moderate activity against human MCF-7/ADR breast cancer cells, may be a promising drug candidate for the drug discovery thanks to the association between Cancer and Dabetes.

Keywords: Anthraquinone derivatives, biological activity, *Fungus strain Alternaria* sp. XZSBG-1, isolation, structure elucidation, secondary metabolites.

SL-5

Track: Drug Delivery & Targeting

PACLITAXEL POLIGLUMEX (PPX): A PEPTIDE POLYMER-LINKED PACLITAXEL MACROMOLECULE: PRECLINICAL PROPERTIES AND CLINICAL DEVELOPMENT

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Introduction: PPX was designed to enhance the efficacy and safety of paclitaxel through conjugation to a biodegradable polymer, poly-l-glutamic acid. Poly-l-glutamic acid, a highly charged polyanionic peptide, was chosen as the polymer backbone because it is biodegradable, highly soluble, has one potential binding site for each glutamyl residue allowing optimization of both paclitaxel content and MW. Unlike non-biodegradable polymers, the breakdown product of PPX is l-glutamic acid that can enter normal cellular metabolism. Paclitaxel is conjugated by ester linkage to the !-carboxylic acid side chains, resulting in a stable conjugate. Because the conjugation site is through the 2' hydroxyl of paclitaxel, a site crucial for tubulin binding, the paclitaxel conjugate does

not interact with h-tubulin and is inactive. The median molecular weight of PPX chosen for development is approximately 48,000 Da, Conjugated paclitaxel represents approximately 37%, by weight, of the conjugate, equivalent to about one paclitaxel ester linkage per 11 glutamic acid residues. PPX is metabolized following endocytosis of the polymer-drug conjugate followed by release of active paclitaxel by lysosomal cathepsin B. The metabolism of PPX in non-tumor-bearing cathepsin B homozygous knockout mice is reduced but not eliminated, indicating that other metabolizing enzymes can also degrade PPX. Plasma levels of free paclitaxel are low and fall below the limits of quantification within several days. However, it can be assumed that the AUC for systemic exposure to paclitaxel includes all polymer bound drug and thus although neutropenia appears lower than an equivalent dose of free paclitaxel, the neuropathy observed is approximately equivalent although the onset is delayed and cumulative [reviewed in 11, 12, 14].

Results: PPX has been through definitive phase 3 development studies that have demonstrated that although it has certain advantages of paclitaxel in terms of ease of administration (10 minute without the need for routine pre-medications) and toxicity (minimal alopecia, neutropenia, nausea and vomiting), it has similar efficacy as both first and second line therapy in non-small cell lung cancer [6-8]. These results were insufficient to obtain regulatory approval in either the US or the EU and suggest that demonstrating an improvement in overall survival with polymer bound cytotoxic agents may continue to confound the field. A notable property of PPX is the ability to radiosensitive tumors far more effectively than any other reported agent, including native paclitaxel or docetaxel, without sensitizing normal tissues [15,16]. The ability of PPX to enhance the efficacy of radiation has been clinically evaluated in Phase II trials in esophageal cancer [4] and head and neck cancer, and is currently in a controlled trial *versus* temozolamide TMZ) in patients with glioblastoma multiforme (GBM) who do not have methylation of MGMT [1]. PPX has been shown to be active and well-tolerated in first line and relapsed ovarian cancer [5]. A phase 3 trial of PPX given for one year as maintenance therapy in patients with ovarian cancer following resection and standard chemotherapy with carboplatin and paclitaxel has completed accrual (1130 patients) and is in the follow-up period. Results are expected in late 2014 or early 2015 [2,3].

Conclusions: Overall PPX has been given to approximately 1200 patients and its prolonged development illustrates some of the difficulties associated with development of polymer based cytotoxic agents. Even with demonstration of major improvements in efficacy in preclinical models of polymer-conjugated drugs over standard formulations, translation of these effects to clinical benefit through clinical trials remains a difficult undertaking.

Keywords: Paclitaxel poliglumex, peptide polymer.

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SL-83

Track: Cancer Targeted Drug Delivery

PACRITINIB, A FLT3/JAK2 KINASE INHIBITOR AND TOSEDOSTAT, AN INHIBITOR OF INTRACELLULAR PROTEIN RECYCLING: NOVEL AGENTS IN ADVANCED DEVELOPMENT IN HEMATOLOGICAL NEOPLASIA. PRECLINICAL BIOLOGY, EARLY CLINICAL DATA AND DEVELOPMENTAL STRATEGIES

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As drug development in oncology has become increasingly complex and expensive and regulatory hurdles have become more onerous, to be successful, the development of novel targeted agents has become more reliant on identifying patient populations who are most likely to benefit from therapy, thereby enhancing effect size and decreasing the size and cost of pivotal studies. This presentation will discuss two novel agents under development for hematological neoplasia, and identification of specific patient populations with an unmet need for improved therapeutics, and who are most likely to benefit from these agents.

Pacritinib is a low nM inhibitor of the phosphorylation of native and mutated forms of JAK2/FLT3 as well as several other kinases associated with myeloid neoplasia and is in Phase 3 development in primary and secondary myelofibrosis. It is administered orally once daily, has a prolonged half-life, and is well tolerated with mild to moderate but easily manageable gastrointestinal side effect as the most common toxicity. It has impressive preclinical activity in murine models of both acute and chronic myeloid neoplasia. Of note, unlike other commercial and pre-commercial JAK inhibitors, pacritinib does not cause myelosuppression. In patients with myelofibrosis, pacritinib does not worsen anemia or cause thrombocytopenia. Therefore the focus of the phase 3 program is to demonstrate that although pacritinib is effective in all patients with myelofibrosis, it can be used where the other agents cannot be, in patients with moderate to severe thrombocytopenia.

Pacritinib, through suppression of signaling through both FLT3 and JAK2 is of great interest in AML, especially in the elderly where FLT3 mutations that occur in about 30% of patients are associated with a particularly poor prognosis. Prior FLT3 antagonists have been associated with a rapid response rate in FLT3 mutant AML but rapid emergence of resistance both by additional FLT mutations and overexpression of pJAK2. Pacritinib blocks both of these escape pathways and is currently under evaluation in AML patients with the FLT mutation who relapse after conventional therapy. If activity is demonstrated in relapsed patients, randomized trials will be initiated in untreated elderly patients with AML who manifest a FLT3 mutation. Exploratory studies in other myeloid malignancies associated with JAK/FLT3 upregulation including MDS and CMML are also planned.

Tosedostat (TST), the only aminopeptidase inhibitor in clinical development, inhibits enzymes that break down small polypeptidases to amino acids, the final step in protein recycling. In sensitive cells, it induces genes associated with amino acid starvation and kills through apoptotic mechanisms. Normal cells are not affected although many liquid and solid tumor cells are killed are low nM concentrations. Preclinical data demonstrate excellent activity in myeloid neoplasia and in multiple myeloma. Growth on stromal cells did not protect against tosedostat induction of apoptosis. TST is given orally, once daily, chronically, and generally is well tolerated. It has demonstrated activity in both relapsed acute myeloid leukemia and myelodysplasia and is synergistic with both cytotoxic and targeted agents. It appears to have higher response rates in patients with prior hypomethylating agent therapy. It is currently in Phase II trials in combination with cytarabine elderly patients with newly diagnosed AML and in patients with MDS who have relapsed following hypomethylating therapy. Clinical efficacy and safety data will be presented.

SL-173

Track: Pharmaceutical Research & Development

THE DESIGN AND EVALUATION OF NANO PARTICULATE DRUG DELIVERY SYSTEM FOR IMPROVING SYSTEMIC ABSORPTION

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On the basis of highest drug permeation, lowest droplet size, lowest polydispersity, lowest viscosity, and optimum surfactant and co-surfactant concentration, we selected formulation NE1 of Diclofenac Diethylamine which contained

isopropyl myristate (10%wt/wt) Tween 80 (7.5% wt/wt), Transcutol P (22.5%wt/wt), and distilled water (60% wt/wt), for use *in vivo* studies. The *in vivo* studies revealed a significant increase in the anti-inflammatory effects as compared to Diclofenac Diethylamine gel and nanoemulsion gel. From *in vitro* and *in vivo* data it can be concluded that the developed nanoemulsions have great potential for transdermal drug delivery. Ultrasonication is a relatively simple and effective technique for producing Diclofenac Diethylamine oil-in-water nanoemulsions. The particle size and size distribution of the nanoemulsions were influenced by the emulsifiers and their concentrations, as well as homogenization temperature, pressure and cycle. The Diclofenac Diethylamine nanoemulsions had moderate physical stabilities. The present work comprised the formulation and evaluation of Diclofenac Diethylamine patches. It was observed that basic physicochemical properties and diffusion profiles are extensively affected by different proportions of Excipient. Further *in-vitro* skin permeation studies suggested about permeation enhancing ability to get the effective rate of release of drug from the patches. In this new era of sustained release dosage form, prolonged delivery of Diclofenac Diethylamine may be possible by these transdermal drug delivery systems.

Keywords: Nano emulsion, nano particulate, nanogel.

SL-6

Track: Cancer Targeted Drug Delivery

PHYSICAL ACTIVITY INTERFERES WITH THE IMMUNOMODULATORY EFFECT OF ANTINEOPLASTIC DRUG NSC631570

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The activation of antitumor immune responses is a necessary condition for successful treatment of cancer patients. The main mechanism of action of cytotoxic anticancer drugs is an induction of cancer cell death. Additional effect of many of these agents is a modulation of immunological reactivity. Modulation of phagocytes functions is an important component of the therapeutic effect of antineoplastic drug NSC631570. Treatment with NSC631570 is provided both in the outpatient setting (associated with moderate physical activity of patients) and in the inpatient setting (associated with limited excursion). Clinical investigations revealed that the therapeutic effect of NSC631570 is higher when the treatment is provided in the inpatient setting. This study is aimed to investigate the impact of physical activity on the modulatory effect of NSC631570 on circulating phagocytes. Six healthy volunteers were recruited; each of them received a single dose of NSC631570 i/v. In the inpatient setting model our volunteers spent most of their time in bed. In the outpatient setting model they were performing one bout of standartized physical activity. ROS generation and phagocytosis were estimated by flow cytometry. In the inpatient setting model NSC631570 caused an increase of ROS generation and slight decrease of phagocytic activity in neutrophils and monocytes. After physical activity phagocytes were unresponsive or had an inverted reaction to the drug.

SL-116

Track: Cancer Targeted Drug Delivery

CANCER IMMUNOTHERAPY: TARGETING TUMORS EXPRESSING MUTANT P53

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Background: P53 is a tumor suppressor gene which is normally expressed at low levels in a latent inactive form, and plays a critical role in safeguarding the integrity of the genome. The tumor suppressor gene p53 is inactivated by missense mutations in more than 50% of all human tumors.

These mutations may not only disrupt normal anti-tumor p53 function but may confer to mutant p53 gain of functional tumorogenic activity.

Methodology: We isolated a human scFv named TAR1 that binds to the common mutant epitope FRHSVV and restores tumor suppressor functions to the mutant protein. Since p53 is a nuclear protein, TAR1 has to be internalized and targeted to the nucleus. The antibody contained a TAT peptide as a protein transduction domain at the N-terminus of TAR1 and a NLS (nuclear localization signal) at the C-terminus.

Results: *In vitro* studies showed that TAR1 binds specifically and differentially the mutant form of p53 with high affinity in the low nM range. The antibody facilitates transcriptional activity of mutant p53 and abrogates the tumorogenic gain of function activity of mutant p53. The restoration of p53 wild-type function to the highly accumulated mutant p53, even partly, could trigger therapeutic responses. *In vivo* studies lead to reduction of human tumor xenografts in nude mice by systemic application of TAR1 as proof of concept for immunotherapy of cancers related to mutant p53 etiology.

Conclusions: Our immunotherapy strategy is based on restoration of wild-type p53 functions to mutant variants towards cancer treatment.

SL-16

Track: CNS Drug Discovery and Therapy

IMMUNE SYSTEM AND ALZHEIMER'S DISEASE PERSPECTIVE OF IMMUNOTHERAPY

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The amyloid cascade hypothesis states that overproduction of amyloid-beta (AĀ) peptide or failure to clear this peptide, leads to Alzheimer's disease (AD) primarily through amyloid deposition, presumed to be involved in neurofibrillary tangles formation; these lesions are then associated with cell death which is reflected in memory impairment, the hallmarks of this dementia. The abundant evidence that A! aggregation/oligomerization is an essential early event in AD pathogenesis has prompted intensive search for therapeutics that target various conformations of AĀ Several labs have bred AD diseased models of transgenic mice that produce human AĀ and develop plaques and neuron damage in their brains as well as immunological aspects of the disease pathogenesis.

The immune system appears to participate in AD pathogenesis. There is evidence for partial tolerance against AĀ in mutant amyloid precursor protein (APP) transgenic mice as well as in AD patients. Animal models of the disease enabled the immunological concept for treatment of conformational diseases to gain more attention and immunization approaches are being pursued in order to stimulate clearance of brain amyloid plaques. In spite of the first clinical setback, this research field has clearly strengthened the hypothesis that A! plays a central role in AD and has stimulated a new area for development of Alzheimer's therapeutics. The renewed human phase clinical trials towards improved immunotherapeutic strategies which maintain the beneficial effects without adverse side effects are under further evaluation.

SL-119

Track: Diabetes and Obesity Drug Discovery & Therapy

IDENTIFICATION OF THE FIRST SMALL-MOLECULE MODULATORS OF THE OX40-OX40L COSTIMULATORY PROTEIN-PROTEIN INTERACTION

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Background: There is increasing evidence that small molecules can serve as effective modulators of certain protein-protein interactions (PPIs). Following our recent identification of the first small

molecule inhibitors of the CD40-CD40L costimulatory PPI within the chemical space of organic dyes (J. Mol. Med. 2009, 87, 1133), we are exploring the possibility of finding modulators for other receptor-ligand interactions within the TNF superfamily (TNFSF). Here, we describe the identification of a small-molecule OX40 modulator and the confirmation of its partial agonist character.

Methods: Cell-free screening assays were developed to identify OX40-OX40L inhibitors. Modified versions of this assay were used to elucidate the binding-partner and the binding-nature of active compounds. Several NF-ÄB reporter cell lines transfected with selected receptors within TNFSF were constructed and used to confirm and characterize activity and specificity. Immune-modulatory activity and partial agonist nature were further confirmed by *ex vivo* T cell polarization assays. Pilot animal study was conducted to test the potential effect of our most promising compound on prevention of T1D in NOD mice.

Results: Several compounds able to concentration-dependently modulate OX40-OX40L interaction were identified. Cell assays indicated that they are partial agonists with low micromolar activity and adequate selectivity. The partial agonism of our most promising compound has been confirmed in both *ex vivo* T cell polarization assays and exploratory NOD mouse study.

Conclusions: We report what to our knowledge are the first small-molecule compounds capable to interfere with OX40-OX40L binding and, more importantly, to also act as OX40 partial agonists. This is a particularly intriguing finding as small-molecule agonism or activation of PPIs is considered unusually challenging and there are only very few known examples. These results provide proof-of-principle evidence for the feasibility of small-molecule modulation of the OX40-OX40L costimulatory interaction and new tools to study OX40-mediated immune responses.

SL-24

Track: CNS Drug Discovery & Therapy

HDAC INHIBITION SUPPRESSES TAU MEDIATATED NEUROTOXICITY IN *DROSOPHILA*

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Down-regulation of histone deacetylase (HDAC) has been demonstrated to be beneficial for many neurodegenerations, including tauopahies. Nevertheless, the underlying mechanisms by which HDAC inhibition suppress tau toxicity have not been fully understood. To address this, we have systematically knocked down the expression of HDAC in *Drosophila* first, and found that the down-regulation of each HDAC, including HDAC1, HDAC2/6, HDAC3, HDAC4 and HDAC10/11, attenuated tau induced toxicity significantly. We further show that many novel HDAC inhibitors also suppress the tauopathy phenotype in *Drosophila*. HDAC inhibitors increased the expression of both Histone H3 and acetylated H3. The expression level of acetylated Ātubulin in the heads of *Drosophila* was also increased when treated with HDAC inhibitors. Interestingly, these HDAC inhibitors also increase the expression of phosphorylated tau species in *Drosophila*. Nevertheless, oral administration of HDAC inhibitors extended the lifespan of tauopathy fly model. These findings suggest that phosphorylated tau is not likely to be the major pathogenic factor of tauopathies. The beneficial effect of HDAC inhibition on tauopahies is possibly mediated through increasing the acetylations of H3 and Ātubulin. In sum, HDAC inhibitors are potential therapeutic agents for tauopathies.

SL-17

Track: Drug Delivery & Targeting

EFFECT OF HIGH-FLOW INTRA-ARTERIAL ACNU (NIMUSTINE HYDROCHLORIDE) THERAPY FOR SUPRATENTORIAL MALIGNANT ASTROCYTIC TUMORS

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The recent standard treatment for patients with malignant glioma consists of TMZ as a chemotherapeutic agent. However, for patients who have adverse effects, or are resistant to TMZ, there is a need for another chemotherapeutic treatment. Using experimental rat brain tumors, we showed that the high-flow intra-carotid infusion of a chemotherapeutic agent (SarCNU) reduces laminar flow and was useful to obtain a higher concentration, and a more sufficient distribution, of this chemotherapeutic agent in malignant brain tumors.

We have been using the high-flow intra-arterial (carotid) infusion chemotherapy (HFIA) for supratentorial malignant astrcytic tumors since 1997. Selected patients with supratentorial newly diagnosed GBM were treated through surgery to resection the tumor, followed by radiotherapy (60 Gy) with adjuvant and maintenance HFIA (ACNU 50mg - 100mg/20mlNS/1min).

Fourteen patients (mean age 51.2±11.0) with newly diagnosed supra-tentorial GBM were treated using HFIA. There was no permanent CNS side effect and no bone marrow side effect at a level of Grade 3 or higher. The median survival time was 37 months and the median progression-free survival time was 15 months.

Our findings suggest that HFIA may be another safe and useful option for the treatment supratentorial astrocytic tumors and also may provide more information regarding drug delivery in the brain and brain tumors.

Keywords: Glioblastoma, high-flow injection, intra-arterial chemotherapy, laminar flow.

SL-93

Track: Drug Discovery in Preclinical Research

THE ERYTHROID CELL MEMBRANE

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The biconcave erythrocyte has a unique flexible membrane necessary to withstand shear stress in capillaries. The unique property of the erythrocyte membrane is its cytoskeleton that is not based on a radial actin structure, but on spectrin lining up parallel to the membrane. Protein complexes in the cell membrane function as anchor points that connect this spectrin lattice to the lipid membrane and regulate this flexibility. In specific hereditary haemolytic diseases, this connection between the membrane and the spectrin lattice is disturbed.

Differentiation of erythroblasts to mature erythrocytes involves a major re-organisation of membrane proteins and of the actin-based cytoskeleton leading to the assembly of the erythrocyte specific alfa/beta-spectrin cytoskeleton (spectrin/actin lattice) connected to the intracellular junctional complex composed of protein 4.1/alfa-betaspectrins/adducins/tropomyosins and to the plasma-membrane via the band 3 macro complex (tetrameric band 3/ protein 4.2/ ankyrin/ GPA/ GPB/ LW/ CD47/ RhD/ RhCE/ RhAG/ Kell/ GAPDH/ de-oxygenated haemoglobin/ glycolytic proteins) and the GPC complex (GPC/p55/protein 4.1). In addition, this process is characterized by the complete loss of specific membrane proteins through specific sorting (e.g. CD71, specific integrins (e.g. ! 4∀1), loss of RNA) and loss of mitochondria and other remaining organelles presumably through autophagy. The integrity of the

Band3 and GPC complexes is paramount to erythrocyte functionality underscored by the haemolysis and aberrant clearance of erythrocytes in patients harbouring specific mutations found within proteins comprising these complexes.

In contrast to proper expression of vital membrane proteins and blood group antigens, we found that *in vitro* cultured CD71+/GPA+ reticulocytes from adult peripheral blood mononuclear cells (PBMCs) are functionally compromised in deformability and osmotic resistance compared to peripheral reticulocytes. This suggests that crucial membrane-cytoskeleton associations as well as membrane macro-complex assembly during *in vitro* culture is not finished or not occurring properly. Recently, we found that co-culture of erythroblasts with bone marrow stromal cell lines results in 80% CD71-/GPA+ cells indicating the presence of mature erythrocytes. We hypothesise that during erythropoiesis assembly of essential erythrocyte membrane complexes is dependent on external signals emanating from supportive feeder cells in the bone marrow.

SL-126

Track: Drug Discovery in Preclinical Research

GROWTH FACTOR MODULATION OF ERYTHROID EXPANSION *EX VIVO*

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Fine tuning growth factor response may play an important role in optimizing the numbers of red cells that may be generated *ex vivo* from discarded stem cell sources as transfusion products. It is known that hematopoietic progenitor cells from cord blood (CB) and from patients with *JAK2*V617F positive polycythemia vera (PV) generate in Human Erythroid Massive Amplification cultures (HEMA) greater numbers of erythroid cells (Erys) than progenitor cells from peripheral blood mononuclear cells from adult blood donations (discarded during leukoreduction). Proliferation of Erys *ex vivo* requires the cooperation between signaling delivered by the growth factors stem cells factor (SCF), interleukin-3 (IL-3) and erythropoietin (EPO) and the hormones dexamethasone and estradiol. In order to clarify the mechanisms underlying differences in erythroid amplification among the three sources, analysis of the signaling pathways activated in erythroid cells derived from AB, CB and PV were recently characterized at the proteomic level by Reverse Phase Protein Array (RPPA) (Hricik T. *et al.*, Am. J. Hematol. 2013). These experiments showed that AB Erys express the highest levels of CD63, a member of the Transmembrane 4 Superfamily Proteins that quenches the tyrosine kinase functions of cKIT, the receptor for SCF, and of cKIT phosphorylation at Y721 that mediates the interaction between cKIT and CD63 (Anzie *et al.*, Blood 2002; 99: 4413). Since experiments in mice demonstrated that fetal cells are more responsive to cKIT stimulation by Stem Cell Factor (SCF) than the adult cells (Bowie *et al.*, Blood 109:5043, 2007), we hypothesized that the stringency of the interaction between CD63 and cKIT may determine different levels of signaling activation and of proliferation in response to SCF in Ery from AB, CB and PV. This hypothesis was tested by determining the response to SCF of Erys obtained from an extended number of AB, CB and PV. Extensive biological (proliferation assays in the presence of pathway inhibitors), biochemical (receptor down modulation by FACS, confocal analyses and western blot analyses) and signaling (RPPA) studies were performed.

Results will be described indicating that CB Erys respond more readily to SCF (lower concentration and for longer time) than Erys from AB and PV because in these cells activation of cKIT is not blunted by interaction with CD63. Strategies to exploit cKIT/CD63 interactions to improve *ex vivo* expansion of Erys for cell therapy purposes will be discussed.

SL-99(a)

Track: Diabetes and Obesity Drug Discovery & Therapy

CONFORMATIONALLY AND GEOMETRICALLY CONSTRAINED NOVEL PPARs LIGANDS: DESIGN SYNTHESIS AND DOCKING STUDIES

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The discovery of the crucial role of Selective Peroxisome Proliferator Activated Receptor Modulators (SPPARMs) as regulators of lipid and glucose metabolism has raised interest in the development of synthetic ligands as potential tool for therapeutic intervention in Type 2 Diabetes (T2D) and Metabolic Syndrome.

We report our research work on the hypothesis that Selective Modulators of PPARÄ(SPPARÄMs) will retain the antidiabetic efficacy comparable to full PPARÄagonists while displaying reduced PPARÄmechanism based side effects, hence to provide 'Novel Antidiabetic Agents' having improved efficacy and safety profiles.

A virtual library of novel unsaturated thiazolidinedione and acyclic analog of isoxazolidinedione based PPAR ligands was designed, by the manual modification of rosiglitazone as PPARÄfull agonist and tesaglitazone and KRP 297 as PPARÃ/Ädual agonists while taking into consideration the structural features of PAT5A [1], MK 0533 [2] and compound E [3] additionally and subsequent introduction of *conformational and geometric constrains* by decreasing the length of the linker and introducing unsaturation [1, 4] to attach the hydrogen bonding parts with the phenyl moiety, as potential Selective Peroxisome Proliferator Activated Receptor$_{gamma}$ Modulators (SPPARÄMs) for the management of Type 2 Diabetes and Metabolic Syndrome.

Accordingly series of *N*-benzyl substituted benzimidazol-2-yl and indol-2-yl and also indol-3-yl linked at the *meta* position of the central phenyl ring of the benylidene moiety, through a methyleneoxy (2 atom)/oxy (1 atom)/direct (0 atom) linker, holding the required hydrogen bonding parts, which as per present design are the Thiazolidine-2,4-dione (TZD), Diethyl malonate (DEM) and Methyl acetoacetate (MAA) were selected for synthesis (Fig. **1**).

Fig. (1). Pharmacophoric requirements for SPPARÄMS?

The criterions set for classification and selection of the novel ligands *(conformationally and geometrically constrained)* for synthesis, among the designed ligands were theoretical as well as computational. The General *(Double Bond/ One Atom or Direct Linked/ Indole-3-yl and N-Benzyl etc.)* and Docking and Scoring (G Score) and the Binding Dispositions and Hydrogen Bond Interaction and distances in the active site of the proteins concerned.

Docking and Scoring Studies, of all the Designed NCEs and Selected Standard Molecules, in the active site of the proteins concerned (PPARÄ 1i7i and PPARÃ 1i7g) with Tesaglitazar (PARÃ/ÄDual Agonist) as the template were

carried out using the SurflexDock and CScore module of *SYBYL* (A TRIPOS SOFTWARE) available in our *in silico* Drug Design Laboratory.

The research work pertaining to Design, Docking and Scoring Studies and subsequent Synthesis of the selected ligands will be discussed during DDTWC 2014 to be held on June 16-19, 2014 at Boston USA.

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SL-101(a)

Track: Drug Discovery in Preclinical Research

IN OVO TESTS FOR DRUG DISCOVERY

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Since its inroduction, the chick embryo model involving the technique of chorioallontoic grafting has proved extremely valuable for the *in vivo* studies to tumor development, angiogenesis and malignant cell dissemination. The ability of the chick embryo's chorioallontoic membrane (CAM) to efficinetly support the growth of inoculated xenogenic tumor cells greatly facilitates the analysis of human tumor cell metastasis. We have developed highly sensitive and reproducible assays for monitoring the grwoth and the mtatstatic dissemination of humun tumor cells in the chick embryo. These tests are validated for 12 human tumor cells lines including various carcinomas, gliomas and melanomas as well as reference drugs currently marketed. Using these assays we can investigate the efficacy and the toxicity of new drug lead candidates in oncology. These assays can also be used to study genetically modified cell lines. The data obtained with this model are much faster, more reliable, less expensive and need only minute amounts of drug (>1,000 times less) compared to the mouse model. Our tests are applicable to any precinical anti-cancer drug discovery program. Moreover, they could be used for the early evaluation of the toxicity of any new drug candidates, i.e. not just in oncology. Altogether, our tests make the chick embryo CAM system an attractive model to reduce animal experimentation for drug discovery.

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SL-169

Track: Women's Health Drug Discovery & Therapy

THE EFFECT OF *APIUM NODIFLORUM* IN EXPERIMENTAL OSTEOPOROSIS

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Treatment of osteoporosis remains a therapeutic challenge. The effect of *Apium Nodiflorum* extract on development of experimental osteoporosis and carrageenan induced inflammation has been studied in ovariectomized osteoporotic Wistar rats. After osteoporosis verification rats were randomized and received for 8 weeks: vehicle, HPLC-standardized *Apium* extract (equal to 2.4 mg/kg Quercetin) or Genistein (2.5 mg/kg). To verify the effect of *Apium* on development of osteoporosis, bone mineral density (BMD) and content (BMC), bone histology and plasma levels of IL-6 and RANKL were measured 6 months after ovariectomy and 8 weeks after treatment with *Apium* extract or Genistein as comparator. Inflammatory hyperalgesia was induced by intraplantar injection of 1% Carrageenan. *Apium* extract and Genistein retarded development of osteoporosis (significant differences of BMC and BMD levels in drug *vs.* vehicle treated rats) and improved bone histology and histological score. *Apium* and Genistein decreased IL-6 level. Both treatments alleviated mechanical hyperalgesia, decreased exudative reaction and lowered inflammatory pain threshold.

The results suggest that *Apium* extract could be an alternative management of post-menopausal osteoporosis.

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SL-89

Track: Drug Discovery in Preclinical Research

STATE OF THE ART OF *EX VIVO* PRODUCED RED CELLS FOR TRANSFUSION

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Erythrocyte transfusion is the most common form of cellular therapy. It rapidly restores the oxygen supply to the tissues in patients suffering from sudden blood loss, reduced erythrocyte production following for instance cytotoxic cancer treatment, or in patients suffering from chronic anaemia due to congenital diseases such as sickle cell anaemia. Matching transfusions for ABO and Rh blood group antigens is in general sufficient for successful transfusion. However, over 200 additional blood group antigens are known, belonging to in total 34 blood group antigen systems. Normally patients do not generate antibodies against these antigens, and they are not used for matching donor and recipient in general transfusion practice. However, Unmatched transfusions result in alloimmunisation in 3-5% of each transfusion. Once allo-immunised, it may become very difficult to find appropriate donor erythrocytes, especially when multiple antibodies or rare combinations of antibodies are present. This problem becomes increasingly urgent in an aging population where the need increases and the donor population decreases, and in a multicultural society in which the population at risk for chronic anaemia, for instance sickle cell disease, does not match with the general donor population.

Several research institutes, mostly associated with Blood supply centres, are developing protocols to culture erythrocytes for transfusion purposes. Primary erythroblast cultures can be expanded to large numbers, and differentiated to hemoglobinised enucleated red blood cells (RBC) that express the appropriate blood group antigens. However, many challenges remain to be solved: From which source should we expand erythroblasts, how can we render the expansion phase efficient, can we regulate the expression of foetal *versus* adult hemoglobin, and can we obtain stable biconcave erythrocytes.

Even when cultured erythrocytes are only required for a small percentage of transfusions, the number of yearly transfusions is so large that the availability of erythrocytes with rare blood groups for allo-immunised patients constitute may solve a major health problem. Particularly sickle cell disease patients would benefit immediately from better matched cRBCbecause of the increased risk of stroke in SCD patients with low haemoglobin.

SL-183(a)

Track: Traditional Chinese Medicine

EFFECT OF SHU WEI DECOCTION ON THE REPAIR AND REGENERATION OF CAJAL INTERSTITIAL CELL IN RATS WITH FUNCTIONAL DYSPEPSIA

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Objective: Shu Wei decoction on functional dyspepsia (FD), the effect of Cajal interstitial cell repair and regeneration.

Methods: 72 rats were randomly divided into control groups, model groups, Shu Wei decoction of low-dose group (LDG), Shu Wei decoction in the dose group (SZG) and Shu Wei decoction in higher dose group (SGG), mosapride Group (MG). Etiology of complex models, resulting in FD deficiency of liver Qi stagnation and spleen deficiency rat model.

Result: SGG, SZG and MG compared with the model groups, more normal full ICC ultrastructure, cholinergic -ICC-SMC network basic integrity of ICC and the marked increase in the number of nerve fibers, the fluorescence intensity significantly strengthen.

Conclusion: Shu Wei decoction thus contributing to the ICC morphological repairing and functional recovery and regeneration of the ICC, keeping cholinergic-ICC-SMC integrity of the network structure, increasing neurotransmitter signal transduction and expression, and an effective treatment of FD.

SL-81

Track: Drug Discovery in Preclinical Research

UNMEET NEEDS IN TRANSFUSION MEDICINE

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The World Health Organization reported that there are marked differences in the level of access to safe blood on a global level. Economic opportunities in developed countries and expansion of businesses into emerging markets has led to population migrations that have altered the distribution of blood groups in blood donors and transfusion recipients leading to shortages of the universal type O (Rh negative) blood as well as alloimmunization to minor blood group antigens and difficulty identifying compatible blood. This talk will provide an overview of the challenges currently facing transfusion medicine and explore the potential role of *ex-vivo* expansion in providing solutions.

SL-36

Track: CNS Drug Discovery & Therapy

TRADITIONAL CHINESE MEDICINE B401 IS AMELIORATIVE AND NEUROPROTECTIVE IN R6/2 MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an incurable neurodegenerative disease characterized by abnormal motor movements, personality changes, and early death. Despite years of research, the mechanisms responsible for chronic, progressive neurodegeneration of HD remain elusive. When the western medicines are incompetent in curing these complex diseases, the approach to explore traditional

medicine systems may provide new insights or new leads. Traditional Chinese medicine is a good example of the potential approaches. Traditional Chinese medicine B401 is a well-known Taiwan-US patent formula and consists of six herbal ingredients including Ginseng (Radix Ginseng, Ren Shen), Astragalus (Radix Astragali, Huang Qi), Chinese Angelica (Radix Angelicae sinensis, Dang Gui), Rehmannia (Radix Rehmanniae, Di Huang), Ligustrum (Fructus Ligustri lucidi, Nu Zhen Zi) and Eclipta (Herba Ecliptae, Han Lian Cao). The B401 is comprised of essence herbs traditionally used to support healthy endocrine and hormone levels and the formula may also aid in supporting healthy cardiac function. This study is to elucidate its neuroprotective effect and mechanism of ameliorative effect of the syndrome of HD. We compared the lifespan and body weight of HD mice with and without oral B401 treatment. The ameliorative effect of B401 on symptom of HD mice was investigated through behavior tests including swimming test and open-field test. The neuroprotective effect s of B401 on symptom of HD mice were used through immunostaining and western blot techniques to compare mitochondrial stress-related autophagy and apoptosis in brain of HD mice. Our results showed that those HD mice with oral B401 treatment lived longer and heavier than those without oral B401 treatment. Those HD mice with oral B401 could ameliorate the typical symptom of HD and suppressed oxidative stress, inflammation, fibrosis, autophagy, and apoptosis. These results suggested that B401 possessed protective and ameliorative properties in HD mice.

SL-186(b)

Track: Traditional Chinese Medicine

THE REVIEW OF DEVELOPMENT OF CHINESE MEDICINE PREPARATION

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Chinese medicine preparations have a long history and play an important role in preventing and treating diseases in clinic of Chinese Medicine. In comparison to chemical medicines, Chinese medicine preparations have itself characteristics in the aspects of raw materials and excipients, dosage forms, preparation technologies, quality control, clinical applications and so on. In order to promote the people of different states to understand Traditional Chinese Medicine, the application and development general situation of Chinese medicine preparations will be introduced. The main contents to introduce include some famous Chinese patent medicines and some traditional and modern dosage forms, preparation technologies (such as the processing of raw material, pulverization, extraction, separation or purification, concentration and drying, formation, *etc*.), and quality evaluation methods of Chinese medicine preparations.

SL-161

Track: Diabetes and Obesity Drug Discovery & Therapy

PRIVILEGED FRAGMENT TARGET NETWORK FOR ANTI T2D DRUG DISCOVERY

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Systemic diseases, such as type 2 diabetes mellitus (T2D), involve multiple mechanisms and molecular targets. After analysing more than 50 T2D drug targets and more than 550 corresponding anti-T2D chemical agents, we have derived a set of privileged fragments (chemoyls), and constructed a chemoyl-target network that links the chemoyls with their targets (and therefore, corresponding mechanisms of action) [1]. Based upon the network, a virtual library for anti-T2D drug lead screening is constructed by recombining the chemoyls [2, 3]. By virtual screening the library, 26 compounds were synthesized/acquired, and ALR2 inhibitors with nanomolar activities were discovered [4].

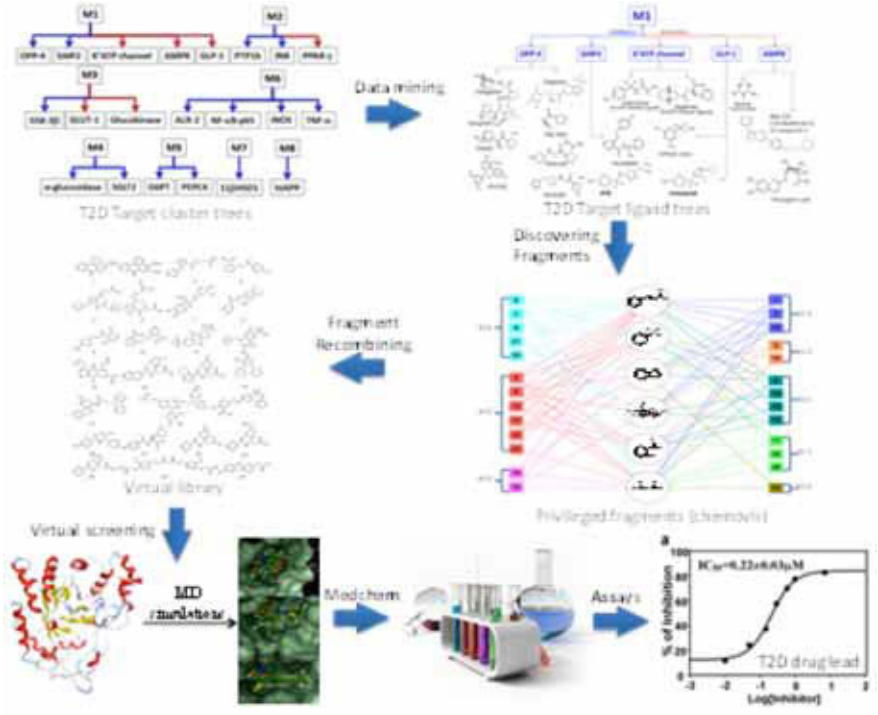

Fig. (1). Flow-chart for chemoyl-target network based anti T2D drug discovery.

ACKNOWLEDGEMENTS

This work was funded in part by the National Natural Science Foundation of China (nos. 81001372 and 81173470), the National High Technology Research and Development Program of China (863 Program) (No. 2012AA020307), and the R&D team program of Guangdong Province (NO. 2009010058).

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SL-37

Track: *In Silico Drug Design and Screening*

AN INTERESTING RELATIONSHIP BETWEEN DRUG ABSORPTION AND MELTING POINT

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The ability to predict the extent of passive intestinal drug absorption is very important for efficient lead candidate selection and development. Physicochemical-based absorption predictive models previously developed use solubility, partition coefficient and p*K*a as drug input parameters for intestinal absorption.

Alternatively, this study looks at the relationship between melting point and passive transport for poorly soluble drugs. It is based entirely on the expression derived from the General Solubility Equation (GSE) that relates melting point to the *product* of intrinsic solubility and partition coefficient. Given that the melting point of a compound is one of the first and

more reliable physical properties measured, it can be advantageously used as a guide in early drug discovery and development.

This paper elucidates the interesting relationship between the melting point and dose to the fraction absorbed of poorly soluble drugs, i.e., class II and IV compounds in the Biopharmaceutics Classification System. The newly defined melting point based absorption potential (MPbAP) parameter is successful at distinguishing 90% of the 91 drugs considered being well absorbed (FA> 0.5) or poorly absorbed. In general, lower melting compounds are more likely to be well absorbed than higher melting compounds for any given dose. The fraction absorbed for drugs with high melting temperatures is limited by the dose to a greater degree than it is for low melting compounds.

SL-131

Track: Inflammation and Immunology

BACILLUS CALMETTE-GUARIN'S PROTECTION EFFECTS AMONG CHINESE PEOPLE

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Background: Our previous study showed that the protection effect of Bacillus Calmette-Guarin (BCG) declines with time, which is supported by a following study which said "BCG vaccination protection against tuberculosis varies between populations, to an extent that cannot be attributed to chance alone". Objective to investigate BCG protection effect among Chinese people, so as to inform immunological agents development and use.

Methods: Systematic review was carried out: electronic databases searches, screening of identified studies based on inclusion and exclusion criteria, data extraction and meta-analysis were undertaken.

Results: Initial search identified 3067 articles from four Chinese databases, after title and abstract screening, 237 full-length papers were read carefully and 26 observational studies were included, among which 7 were cohort studies and 19 were case-control ones, with the total participant of 1513002 and 6951 respectively. None experimental study such as randomized clinical trial was identified. The P value of Egger's test were 0.008 for cohort studies and 0.006 for case-control studies, indicating that the available evidences were at risk of publication bias. The pooled estimate of RR for cohort studies was 0.218, with 95% confidence interval (95%CI) of 0.103-0.463, and the pooled estimate of OR for case-control studies was 0.378, with 95%CI of 0.260-0.550.

Conclusions: The available evidence shows positive and definite effect of BCG in preventing onset of tuberculosis among Chinese people, but is at risk of bias. High quality studies are encouraged worldwide to inform immunological agents development and use to cope with tuberculosis epidemiology.

SL-29

Track: Drug Delivery & Targeting

NOVEL SULPIRIDE LOADED SOLID LIPID NANOPARTICLES WITH ENHANCED INTESTINAL ABSORPTION

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Solid lipid nanoparticles (SLN), novel drug delivery carriers, can be utilized in enhancing both intestinal permeability and dissolution of poorly absorbed drugs. The aim of this work was to enhance the intestinal permeability of sulpiride (Sp) by loading into SLN. A unique ultrasonic melt-emulsification

method with minimum stress conditions was used for the preparation of SLN. Several formulation parameters were optimized, including the drug-to-lipid ratio, and the types of lipids and surfactants used. The produced SLN were evaluated for their particle size and shape, surface charge, entrapment efficiency, crystallinity of the drug and lipids, and the drug release profile. The rat everted sac intestine model was utilized to evaluate the change in intestinal permeability of Sp by loading into SLN. The method adopted allowed successful preparation of SLN with a mono-disperse particle size of 147.8–298.8 nm. Both scanning electron microscopic and atomic force microscopic images showed uniform spherical particles and confirmed the sizes determined by the light scattering technique. Combination of triglycerides with stearic acid resulted in a marked increase in zeta potential, entrapment efficiency, and drug loading; however, the particle size was increased. The type of surfactant used was critical for particle size, charge, drug loading, and entrapment efficiency. Generally, the *in vitro* release profile demonstrated by all formulations showed the common biphasic mode with a varying degree of burst release. The everted sac model showed markedly enhanced Sp permeability in the case of the SLN-loaded formulation. The *in situ* results showed a very good correlation with the *in vitro* release data.

SL-112

Track: Cancer Targeted Drug Delivery

HSP90 INHIBITOR DRUG CONJUGATES (HDCs): PROOF OF CONCEPT IN PRECLINICAL STUDIES

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One of the major challenges in cancer treatment is to selectively deliver oncology drugs directly to tumors, and thus spare normal tissues. One successful approach to meet this challenge is demonstrated by the development of Antibody Drug Conjugates (ADCs).

It has been shown in human cancer patients and mouse xenografts that heat shock protein 90 (Hsp90) is overexpressed in tumors, and inhibitors of Hsp90 are preferentially retained in tumor tissue in contrast to their rapid clearance from normal tissues. We have developed a small-molecule drug conjugate platform technology using these unique properties of Hsp90 proteins and Hsp90 inhibitors. Hsp90-Inhibitor Drug Conjugates (HDCs) offer many of the advantages of antibody-driven targeted delivery with potentially broader applicability.

To date, we have conjugated over 40 payloads representing various oncology drug categories to Hsp90 inhibitors. Conjugates with payloads like SN-38 and docetaxel have been advanced into preclinical studies and have been shown to prolong intratumoral drug exposure in mouse xenografts, reduce on-target adverse effects, and confer superior efficacy in a variety of tumor types.

In HDCs, we have created a promising platform technology which will result in many novel anticancer agents in the near future.

SL-97

Track: Drug Discovery in Preclinical Research

DEVELOPMENT OF THE LEADING HCV NS3 AND NS5A INHIBITORS ZN2007 AND ZN6818 EXCELLENT FOR CLINICAL COMBINATION THERAPY

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Background: The NS3 and NS5 of hepatitis C virus (HCV) are non-structural protein and essential for viral replication and infectivity. So far, it has been intensively studied in discovery of new HCV NS3, NS5A and NS5B inhibitors.

Results: This presentation discloses both well optimized HCV NS3 and NS5A inhibitors "ZN2007 and ZN6818". Both ZN2007 and ZN6818 had not only excellent pan-genotypic potency (e.g., EC50 for NS5A: 4-25pM for all GT-1 to GT-6, respectively), but also excellent preclinical results such as PK and safety in rats and Monkeys. There was no any side effect determined with different kinds of potential targets such as hERG, Cytochrome P450, Y93N, etc, respectively. The concentration of both compounds in liver is much higher than in plasma and other organs, and their metabolic stability is very good (once-daily dosing). There was no any death, no any serious drug-related toxicity and adverse events observed during toxicity study in rats (100-2000mg/kg/day) and Monkeys (250-1000mg/kg/day).

Conclusions: The leading NS3 and NS5A inhibitors ZN2007 and ZN6818 had been well optimized with the best-in-class potency, excellent safety and PK, which strongly support our goal to develop one of the best DAA combination therapies effectively for HCV patients in clinical trials.

Keywords: HCV Inhibitor, Potency, PK, Toxicity.

SL-72

Track: Cancer Targeted Drug Delivery

ANTI-GLIOMA AGENTS BY TARGETING MULTIPLE TUMOR METABOLIC REGULATORS

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Gliomas are the most common and malignant brain tumors. Chemotherapy has played an important role as an adjuvant in the treatment of gliomas. However, the efficacy of current drugs is limited due to serious side effect, poor drug delivery, and chemo-resistance. There is a need to develop novel anti-glioma agents with unique mechanisms for treating gliomas. Accumulating evidence demonstrates that enhanced glycolysis, glutaminolysis, and lipogenesis are prominent hallmarks in gliomas. Several important metabolic enzymes, such as hexokinase 2 (HK2), 6-phosphofructo-2-kinase/2,6-bisphosphatase 3 (PFKFB3), pyruvate kinase M2 (PKM2), lactate dehydrogenase 5 (LDH5), glutaminase (GLS), and fatty acid synthase (FASN), have been revealed to be related to the tumorigenesis of gliomas. Targeting these metabolic regulators has emerged as a promising strategy for the discovery of novel anti-glioma drug leads. In this poster, we will present three bioactive compounds with potent activity agaisnt gliomas. These bioactive agents have been demonstrated to exert their antitumor activity by targeting several key metabolic regulators of HK2, PFKFB3, PKM2, LDH5, GLS and some important oncogenes of c-Myc, Bmi-1, Notch-1, Bcl-2, Bcl-xl, and Survivin. The results suggested that these bioactive compounds could be further developed as potential therapeutics of gliomas.

Keywords: Anti-glioma agents, Tumor metabolic regulators, oncogenes, multiple targets.

SL-141

Track: CNS Drug Discovery & Therapy

PREGNENOLONE SULFATE NORMALIZES SCHIZOPHRENIA-LIKE BEHAVIORS IN DOPAMINE TRANSPORTER KNOCKOUT MICE THROUGH AKT/GSK3BETA PATHWAY

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Pregnenolone sulfate is an endogenous neurosteroid in the central nervous system, and acts as a positive allosteric modulator of NMDA receptor for its role in learning and memory. Here, we study the actions of pregnenolone sulfate using dopamine transporter knockout (DAT-KO) mice, which exhibit

endophenotypes that recapitulate certain symptoms of schizophrenia, including the psychomotor agitation, stereotypy, prepulse inhibition (PPI) deficits and cognitive impairments. We found that acute treatment of pregnenolone sulfate normalized the hyperlocomotion and stereotypic bouts, and completely rescued the prepulse inhibition deficits in DAT-KO mice. In addition, the cognitive deficits of DAT-KO mice in the novel object recognition and social transmission of food preference tests were rescued by long-term treatment of pregnenolone sulfate. We also showed that pregnenolone sulfate normalized behavioral abnormalities in MK801-treated WT mice, whereas partial rescue of MK801-induced behavioral abnormalities was observed by pregnenolone, indicating distinct mechanisms between pregnenolone sulfate and its precursor, and involvement of NMDA receptor signaling in the action of pregnenolone sulfate. Moreover, we found that acute treatment of pregnenolone sulfate, but not its precursor pregnenolone, increased the phosphorylation levels of striatal AKT and GSK3beta in DAT-KO mice, and that long-term treatment with pregnenolone sulfate increased expression levels of NR1 subunit of the NMDA receptor in hippocampus. Thus, pregnenolone sulfate was able to rescue the behavioral anomalies of DAT-KO mice through the NMDA receptor-mediated AKT/GSK3beta signaling pathway.

SL-38

Track: Cancer Targeted Drug Delivery

GANKYRIN PROMOTES COLORECTAL CARCINOGENESIS VIA TSC/MTOR PATHWAY

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Gankyrin is overexpressed in some malignancies. However its roles in the initiation step of colorectal carcinogenesis and underlying mechanisms remain largely unexplored. Herein, we showed that the expression level of Gankyrin was significantly higher in high-grade intraepithelial neoplasia and cancer tissue compared with low-grade intraepithelial neoplasia and paired-noncancerous tissues. Increased expression of Gankyrin was also observed in colorectal cancer (CRC) cell lines comparing to those in normal colon epithelial cells. Gankyrin induced anchorage-independent growth and tumorigenicity in NIH3T3 cells. Importantly, mTOR signaling was significantly activated in transformed NIH3T3 cells, both *in vitro* and *in vivo*, which was further confirmed by over-expressing Gankyrin in CRC cells lines and xenografts. Furthermore, Gankyrin regulated mTOR signaling by PI3K/AKT and AMPK independent, TSC/Rheb dependent pathway. Gankyrin over-expression accelerated TSC2 degradation. Finally, Gankyrin knockdown in a panel of CRC cells lines and xenografts delayed TSC2 degradation, increased TSC2 protein level and inhibited mTOR signaling. Together, our findings reveal a unique mechanism by which Gankyrin promotes colorectal carcinogenesis and point out Gankyrin is a potential prognostic marker and therapeutic target to improve clinical management of CRC.

SL-41

Track: Process Chemistry and Drug Manufacturing

PROCESS DEVELOPMENT OF A GCS INHIBITOR INCLUDING DEMONSTRATION OF LOSSEN REARRANGEMENT ON KILOGRAM SCALE

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Fabry disease is a rare X-linked genetic disorder that leads to the progressive accumulation of glycosphingolipids in lysosomes of a variety of cell types and tissues. A small molecule, currently in clinical studies, is under investigation as an inhibitor of glucosylceramide synthase (GCS) for potential use in Fabry disease. An early synthetic route towards this molecule, while suitable for

preparing small quantities of drug substance in Discovery, posed challenges for scale up including a problematic dialkylation protocol, the processing of azide chemistry and highly-energetic intermediates, and the need for chromatographic purifications in multiple steps. To support preclinical and clinical activities, a new 4-step synthesis was developed and used to prepared kilogram quantities of the drug substance in good overall yield. The new route features a scalable CDI-mediated Lossen rearrangement as a substitution for hazardous azide chemistry that was employed in the original route. Aspects of the new route, including process safety considerations, generation and depletion of impurites and performance on pilot-plant scale, will be described.

POSTERS

PO-13

Track: Cancer Targeted Drug Delivery

TARGETING AMPK IN HCC: A POTENTIAL ROLE FOR COMBINED ASPIRIN AND METFORMIN THERAPY

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Hepatocellular carcinoma (HCC) ranks the 2nd most common cancer among Egyptian population with a rising incidence mostly due to high prevalence of viral hepatitis and its complications. Chemotherapy is one of the palliative approaches for un-resectable tumors, but the efficacy of current HCC chemotherapy is only modest and HCC remains an unmet medical need.

AMP-activated protein kinase (AMPK), an energy sensor that plays a key role in metabolism, emerged as an attractive target molecule for cancer prevention and treatment.

Aspirin and Metformin, having an excellent and a safer therapeutic index with few side effects, were reported to have a protective effect against development of cancer. Therefore, due to their potential effect on AMPK, we investigated the possible *in vitro* anticancer activity of combined Aspirin and Metformin therapy on HepG2 cell line by MTT assay. Furthermore, we assessed AMPK, p-AMPK and mTOR protein expression levels, apoptosis induction (Caspase 3 Activity Assay), and autophagy (TEM) as molecular targets for Aspirin/Metformin in both HepG2 cell line and Egyptian patients' HCC tumor samples.

Combined treatment by Aspirin/Metformin inhibited cell growth and induced both apoptosis signaling and autophagy. These findings suggest that Aspirin/Metformin combined treatment might be a promising anticancer strategy for Egyptian HCC's patients.

PO-37

Track: Neutraceutical Drug Discovery & Therapy

EFFECTS OF FERMENTED WHEY IN TREATING BACILLARY DYSENTERY AND ON THE GASTROINTESTINAL FLORA OF APPARENTLY HEALTHY ALBINO RATS

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In this study, the effects fermented whey (FW) in treating bacillary dysentery caused by *Shigella flexneri* in albino rats and on the gastrointestinal (GIT) flora of apparently healthy albino rats (AHARs) were investigated. Prior the therapeutic assay, the growth inhibitory activity (GIA) of whey subjected to different fermentation durations at 30 + 2 ^{o}C was first investigated using agar diffusion assay on the test organism, conventional antibiotics served as control. After this, the infectious dose of the organism was determined and used to infect another set of AHARs. The infected rats were grouped into two; group one was treated with 1.0ml of the FW that exerted the highest GIA in the *in vitro* assay (FW1), once daily for 7d while group two was left untreated. The rats were observed for signs of recovery while their large intestine was subjected to histopathological examinations. For the effects of whey

on GIT flora of AHARs, another group of AHARs was fed with FW1 for 3months. At 7d intervals, their faeces were examined for microbial types and load. The *in vitro* GIA of the FWs on the test organism was superior to that of most of the antibiotics used and the administration of FW1 to infected rats caused them to recover by 72h while those not treated with FW1 started to recover by 168h. FW1 did not significantly ($p< 0.05$) affect the GIT microflora loads but only the types.

Keywords: Bacillary dysentery, fermented whey, gastrointestinal flora, histopathology, non conventional therapy, *Shigella flexneri.*

PO-20

Track: Pharmaceutical Biotechnology

UNDERSTANDING THE ROLE OF CHITOSAN BASED LONG ACTING INJECTABLE IMPLANTS FOR POLYETHYLENE GLYCOL CONJUGATED L-PHENYL ALANINE MUSTARD

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In present study, we have used L-phenyl alanine mustard also called Melphalan (ML) as a model drug, used extensively for the treatment of breast cancer. Due to its remarkable hemolytic activity, clinical application of this drug is limited. We incorporated the two synthesized PEGylated melphalan (MLPEG) conjugates *viz*. MLPEG 2000 and MLPEG 5000 separately in to the medium molecular weight chitosan (CS) based smart thermoreversible *in situ* forming injectable hydrogel.Prepared hydrogels were evaluated for gelation time, rheological behavior, drug release and stability. Although, MLPEG shows significant increase in aqueous solubility and decrease in hemolytic activity, it was loaded to hydrogel to improve dose frequency and local effect. Hydrogel comprising of CS (3.22%, w/v) and glycerophosphate disodium salt (GP) (16%, w/v) showed consistent gelation time and retard the release of drug without compromising its stability. To underline the role of GP, conjugates were loaded into CS solution with and without the GP. Remarkably, absence of GP results in rapid initial burst with nearly complete drug release within 50 hrs, while addition of GP exhibited drug release up to 100 hrs. Thus, the present study highlighted the role of CS/GP thermoreversible injectable hydrogel for successful loading of PEGylated melphalan.

Keywords: Chitosan, Glycerophosphate, thermoreversible, Injectability, Hydrogel.

PO-86

Track: Cancer Targeted Drug Delievery

ANTIPROLIFERATIVE ACTIVITY OF NOVEL THIOPHENE AND THIENOPYRIMIDINE DERIVATIVES

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A novel series of newly synthesized thiophene derivatives, ethyl-4,5-dimethyl-2-(3-(3,4,5-trimethoxyphenyl)thioureido) thiophene-3-carboxylate 3, ethyl-2-[(2-(dimethylamino)ethoxy)mercapto)methyleneamino)]-4,5-dimethyl-thiophene-3-carboxylate 9, thienopyrimidines 4, 7, 10-20, triazolothienopyrimidines 5, 6 were prepared and tested for their antiproliferative activity. The structures of the synthesized compounds were confirmed on the basis of elemental

analysis, IR, 1H-NMR, 13C-NMR and mass spectral data. The results showed that the synthesized compounds were more active on breast cancer than on colon cancer cell lines and the most potent compounds in this study are compounds 3 and 13 which exerted remarkable activity against MDA-MB-231 (breast cancer) and HT-29 (colon cancer) cell lines with IC50 values (40.68, 49.22 μM) for compound 3 and (34.04, 45.62 μM) for compound 13. Also, compounds 4-6, 9 showed a moderate activity against breast cancer cell line, while compounds 15, 19 and 20 showed no activity.

Keywords: Antiproliferative activity, thienopyrimidines, thiophenes.

PO-46

SYNTHESIS ANTITUMOR ACTIVITY OF 2-ARYL-1H-BENZO[D]IMIDAZOLE DERIVATIVES AND THEIR SPECTROSCOPIC AND X-RAY CRYSTALLOGRAPHIC STUDIES

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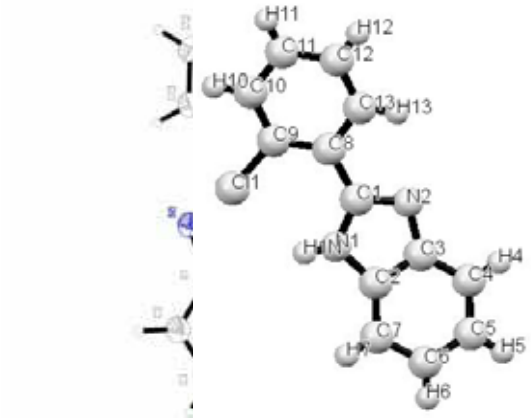
Cancer is a leading health problem in developed as well as developing countries. The enormous cancer incidence forces the researchers to search for safer and eff cient anticancer agents who have selectivity and sensitivity towards cancer cell lines. Among various types of chemical substances designated for novel antitumor agents, nitrogen-containing heterocycles are of particular interest [1-3]. Among them, benzimidazoles have been found very important group to display a variety of biological applications such as antihelmintic, antihistaminic, anticancer, antiviral, antiproliferative, antioxidant *etc*. [4]. The biological importance of benzimidazoles and its derivatives in the field of medicinal chemistry (especially in modern drug discovery) [5, 6], is attractive due to their structural resemblance and good bioisostere to the naturally occurring nucleotides, which allow them to interact with the biopolymers of the living system [7]. In this work, we report a series of benzimidazoles derivatives, synthesized from benzene-1,2-diamine and aldehydes at room temperature. The structures of the synthesized compounds have been characterized with the help of number of techniques *viz*., microanalysis and various spectroscopic studies. Additionally, structure of the reported compounds has been determined by employing single crystal X-ray diffraction measurements. The synthesized benzimidazoles derivatives were subjected to in-vitro antitumor studies against various cancer cell lines cell *viz*., Breast, Leukemia, Prostate and Lung. The results suggested our reported compounds to be good anticancer agents against these cancer cell lines.



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PO-60

Track: Chemistry

3-SUBSTITUTED-4-QUINOLONES: EFFECT OF SUBSTITUENTS ON NMR FEATURES AND THE RELATIONSHIP BETWEEN MOLECULAR DOCKING AND ANTIBACTERIAL ACTIVITY

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In the present study the NMR spectroscopic features of the synthesized compounds 1-(7-Bromo-4-hydroxyquinolin-3-yl)ethanone, Ethyl 3-[(7-chloro-4-hydroxyquinolin-3-yl)formylimino]butanoate, 1-(7-Chloro-4-hydroxyquinolin-3-yl)-3-(4-(dimethyl amino)phenyl)prop-2-en-1-one and 3-((1H-Benzo[d][1,2,3]triazol-1-yl)methyl) quinolin-4-ol were studied in DMSO-d6 with the aim of investigating the effect of substituents on the NMR parameters of basic bicyclic quinolone ring systems. For this purpose, the ^{1}HNMR & ^{13}C-one and two-dimensional NMR methods were used. The analysis of ^{1}HNMR and ^{13}CNMR, infrared and high resolution mass spectra confirmed the structures of investigated quinolones.

A molecular docking study was performed against topoisomerase II (PDB code: 2XCT) by using MOE 2012.10 and leadit 2.1.2. softwares. The synthesized compounds were evaluated for their antibacterial activity.

Keywords: Antibacterial, molecular docking, quinolone, spectroscopic features.

PO-25

Track: Inflammation and Immunology

ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF A CHITIN-BINDING PROTEIN ISOLATED FROM MORINGA OLEIFERA SEEDS (MO-CBP4) IN MICE: CYTOKINE PRODUCTION-DEPENDENT MECHANISM

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Moringa oleifera Lam. is a perennial multipurpose tree that has been successfully used in folk medicine to cure several inflammatory processes. A thermostable chitin-binding protein (14.3 kDa) was isolated from Moringa oleifera seeds and named Mo-CBP4. In this study, we tested for the antihypernociceptive activity of Mo-CBP4 administrated *via* oral by gavage (40 and 80 mg/ kg) in mice using mechanical models of hypernociception induced by carrageenan (CG, 300 µg/paw), prostaglandin E2 (PGE2, 100 ng/paw) or epinephrine (EP, 100 ng/paw). We also investigated the anti-inflammatory effect of Mo-CBP4 (10, 20 and 40 mg/Kg, v.o) on the model of zymosan- induced neutrophil migration. Oral treatment with Mo-CBP4 (40 mg/kg) inhibited the development of mechanical hypernociception induced by CG

and EP; however, no effect was observed on hypernociception induced by PGE2. The inhibition of inflammatory hypernociception by Mo-CBP4 was associated with the prevention of neutrophil recruitment to the plantar tissue of mice. In addition, Mo-CBP4 significantly inhibited the neutrophil influx in peritoneal cavity induced by zymosan. This inhibitory effect was completely prevented when protein was combined with N-acetylglucosamine, demonstrating the role of carbohydrate-binding sites. Furthermore, Mo-CBP4 reduced IL-1 and increased IL-10 levels in peritoneal fluid and serum, respectively. Our results provide information about the antinociceptive and anti-inflammatory properties of Mo-CBP4 and suggest that this glycoprotein might be potentially interesting in the development of new clinically relevant drugs for the management of painful and/or inflammatory disease.

Keywords: Hypernociception, inflammation, lectin, Moringa oleifera.

PO-63

Track: Drug Discovery in Preclinical Research

LATICIFERS PROTEINS FROM HIMATANTHUS DRASTICUS (HDLP) ACCELERATES WOUND HEALING IN MICE

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Himatanthus drasticus (Apocynaceae) has long been recognized in folk medicine as a therapeutic plant in northeastern Brazil. The latex obtained by cutting its stem bark is mixed with water and sold in public markets to treat and prevent different inflammatory disorders, cancer and wounds. Recently, our research group demonstrated that the soluble protein extracted from the latex of Himatanthus drasticus (HdLP) display antinociceptive and anti-inflammatory properties. The aim of the present study was to evaluate healing potential of HdLP in a preclinical study on mice using a cutaneous excision-punch wound model. Excisional wounds (1 cm^2) were performed with a tissue punch on the dorsal surface of mice under anesthesia and aseptic conditions. The ointment of HdLP, at concentrations of 0.5%, 1.0%, and 2.0% w/v prepared in Polyethylene glycol, was applied topically once daily for 13 days. Control animals received the ointment without HdLP in a similar form. On alternate days following the surgery, wounds were macroscopically evaluated by the presence of flogistic signs, wound area size and presence of crust. Moreover, wounds were removed for histopathological evaluation. Wounds treated with HdLP healed significantly faster than control group, as indicated by the significant improved rate of wound contraction, a higher percentage of re-epithelialized wound area and decreased time taken for epithelialization ($P<0.05$). These results were also supported by histological examinations. These findings suggest that HdLP ointment possesses a significant wound healing activity and, at the same time, additional pharmacological studies of this effect are in progress.

Keywords: Himatanthus drasticus, laticifers proteins, wound healing.

PO-17

Track: Pharmaceutical Research & Development

DEVELOPMENT OF *IN SITU* FORMING LONG ACTING IMPLANTS FOR THE DELIVERY OF PEGYLATED ALKYLATING AGENT CONJUGATE, MELPHALAN

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In the present study, we have investigated the role of thermoreversible injectable hydrogel for the successful administration of a PEGylated anticancer drug. To achieve the objective, a modified PEGylated melphalan (MLPEG) synthesized from a linear methoxy poly (ethylene glycol) (M-PEG) 2000 and 5000, Da with improved solubility was loaded to the thermosensitive Poloxamer 407 (P407) gel to produce an injectable hydrogel (MPX).As far as the safety issues are concern, conjugates at a concentration of 32 Ãg/ml after 1 h, showed low hemolysis (48.8 ± 1.5%) compared to high hemolysis (81.3 ± 0.5) for MLPEG 5000 and MLPEG 2000, respectively. Therefore, a significant decrease in hemolytic activity was found in case of MLPEG 5000 conjugate compared to MLPEG 2000. The tightening of the PEO chains due to the presence of NaCl salt reduced the initial burst release of the drug from the hydrogel and only 43% of drug released during 2 hours from MPX-CG hydrogel. Moreover, a lower diffusion coeff cient (D) for MPX-CG gelas compared to MPX-7.4 gel (4.8 X 10^{-6} *vs* 19.7 X 10^{-6} cm^2 min^{-1}, respectively) showed prolonged release of melphalan from the MPX-CG hydrogel. Administration of the prepared hydrogel *via* subcutaneous and intramuscular routes, confirms the depot formation, good syringeability and biocompatibility.

Keywords: P407, Melphalan, Thermosensitive, Diffusion coefficient, PEG.

PO-119

Track: Hot Topics in Natural Products

ANTI-INFLAMMATORY AND ANTI-OXIDATIVE EFFECTS OF GUGGULSTERONE IN HUMAN MAMMARY EPITHELIAL CELLS

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Guggulsterone (GS) [4, 17(20)-pregnadiene-3, 16-dione] is a phytosterol found naturally in the gum resin (guggulipid) of the guggul plants, such as Commiphora wightii, Commiphora mukul, Commiphora myrrh, and Balsamodendron mukul. These plants are found in India, Bangladesh, Pakistan, and Arabia. GS has been used to treat hypercholesterolemia, atherosclerosis, rheumatism, and obesity. GS has two stereoisomers, E-guggulsterone (cis-GS) and Z-guggulsterone (trans-GS). It has been reported that GS has anti-proliferative and anti-inflammatory properties. However, the underlying molecular mechanisms remain largely unresolved. In the present study, the anti-inflammatory and antioxidant effects of both GS enantiomers were evaluated in human mammary epithelial (MCF-10A) cells. To evaluate the antioxidant properties of GS, MCF-10A cells were treated with GS isomers and western blot analysis was conducted. Both isomers upregulated the expression of the antioxidant enzyme heme oxygenase-1 (HO-1) in a time and concentration dependent manners. Interestingly, cis-GS induced HO-1 expression to a greater extent than the trans-GS.

NF-E2-related factor (Nrf2), a basic-leucine zipper transcription factor, was reported to play a key role in regulating the antioxidant/electrophile responsive elements ARE/EpRE)-mediated expression of various phase-II detoxifying or antioxidant enzymes including HO-1. Gel shift assay was performed to determine whether HO-1 induction was mediated via activation of Nrf2/ARE signaling. Data show that both GS isomers activate Nrf2/ARE-DNA binding activity in a time and concentration dependent manners. Then to confirm Nrf2 activation and nuclear translocation upon treatment of

MCF10A cells with GS, immunocytochemistry was performed. Nrf2 underwent nuclear translocation in response to treatment of MCF10A cells with GS for 12 h in a concentration dependent manner. GS isomers suppressed the 12-O-tetra-decanoyl-phorbol-13-acetate (TPA)-induced expression of cyclooxygenase-2 (COX-2) in MCF10A cells. Again, cis-GS was more potent than the trans-isomer.

In another experiment, pretreatment of MCF-10A cells with cis-GS for 12 h markedly inhibited NF-kB DNA binding activity induced by TPA. These findings suggest that cis-GS may provide the cells with acquired antioxidant and anti-inflammatory defense capacity and thereby conferring protection against carcinogenesis.

Keywords: Chemoprevention, Cox-2, guggulsterone, HO-1, MCF-10A, NF-kappaB, Nrf2.

PO-41

Track: Pharmaceutical Biotechnology

A SENSOR CELL AS *IN VITRO* ALTERNATIVE TO CYTOTOXICITY EVALUATION OF NANOPARTICLES

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Nanoparticles are increasingly used as drug delivery vehicles, but their behavior inside the cells and the metabolic and immunological responses induced by these particles remain unclear. Very few reports on the toxicity of nanoparticles are available. Our study aims to construct a genetically modified cell that carries reporter gene GFP (green fluorescent protein) under the control of Bax promoter to provide functional information about the impact of cytotoxic reagents on cell physiology. The Bax is a proapoptotic member from the Bcl-2 family of proteins that are key mediators of the apoptotic response. In this work the Bax promoter DNA segment was amplified from the HUVEC (Human Umbilical Vein Endothelial Cells) genome. The PCR amplicon was digested with the restriction enzymes *PstI* and *XmaI*. The fragment containing the functional promoter region of Bax was cloned into the *PstI* and *XmaI* sites of pAcGFP1-1 (Clontech) to create pBaxp-AcGFP. To create the sensor cell, the Bax-GFP reporter plasmid will be transfected into HUVEC cell line to provide a new *in vitro* alternative to cytotoxicity evaluation of nanoparticles.

Acknowledgements: The authors are thankful to CAPES for the financial support.

Keywords: Apoptosis, bax promoter, cytotoxicity, nanoparticles, GFP, sensor cell.

PO-47

Track: Protein and Peptide Sciences

SNAKE VENOM THROMBIN-LIKE SERINE PROTEASE BIOPHARMACEUTICAL PERFORMANCE IMPROVES AFTER PEGYLATION

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PEGylation is considered one of the most successful techniques to enhance the therapeutic and biotechnological potentials of biomolecules by increasing solubility, protecting from degradation and reducing renal clearance and immunogenic and antigenic reactions. Thrombin-like serine proteases are able to convert fibrinogen into fibrin, forming an unstable clot that is easily degraded by the fibrinolytic system, thus leading to depletion of plasma fibrinogen. Therefore, these enzymes can be used to treat some vascular disorders. The purification of a serine protease from Crotalus durissus collilineatus venom (CdcV) resulted in the isolation of Collinein-1 and 2. The Collinein-1(Col-1, 29.5 kDa) was modified by site-specific PEGylation using mPEG-Mal5kDa and produced monoconjugate Col-1-PEG-Mal5kDa of molecular mass around 35 kDa. The PEGlated Col-1 showed similar effects to the native enzyme on bovine fibrinogen by cleaving preferentially AÄ chain and releasing fibrinopeptide A. Unexpected Km value was obtained for the Col-1-PEG-Mal5kDa, Km=0.47mM, against Km=0.73 mM for the native Col-1, indicating that the PEGylated enzyme has a higher affinity for TAME substrate. The values of Kcat/Km (1114 mM.min-1 for Col-1-PEG-Mal5kDa and 492 mM.min-1for native Col-1) confirm that PEGylated enzyme presents higher catalytic efficiency. These results demonstrated the relevant biopharmaceutical potential of PEGylated Col-1.

Support: FAPESP, CNPq, NAP-TOXAN-USP.

Keywords: Crotalus durissus, hemostasis, PEGylation, serine protease, snake venoms.

PO-36

Track: Regenerative Medicine and Stem Cells

EFFICIENCY OF AUTOLOGOUS BONE MARROW STEM CELLS TRANSPLANTATION AT SYSTEMIC SCLERODERMA

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At systemic scleroderma on the background of autoimmune disorder inflammatory and fibroplastic reactions are initiated, proliferative/oblitirating generalized vasculopathy and progressive fibrosis of internal organs are developed. In this regard, use of bone marrow stem cells, the central organ of immunogenesis, that are characterized to have immunomodulatory (natural suppressive activity), morphogenetic and also angiogenetic and anti-fibrotic effects, can be effective pathogenetically justified method of treatment of systemic scleroderma.

The aim of the investigation is to give clinical and morphological estimation of the effectiveness of autologous bone marrow stem cells transplantation at systemic scleroderma.

Materials and Methods: We observed 15 patients aged from 27 till 38 years, with definite diagnosis of systemic scleroderma (SSc) according to the criteria of the American College of Rheumatologists (ACR, 1980). Duration of the disease was more than 3 years. Resistance to immunosuppressive therapy (D-penicillamine, azathioprine and prednisolone) and high index of autoimmune process activity (EScSG), according to the criteria of the European Group for the Study of SSc, formed the fasis for carrying out bone marrow stem cells transplantation.

Aspiration of bone marrow from the iliac crest in quantity of 400 ml was conducted after clinical and laboratory examination of patients with SSc. Biotechnological methods allocated hematopoietic stem cell fraction. Based on the view that chronic diseases (chronic pathological stress) functional and bioregulatory activity of stem and progenitor cells of bone marrow (BM) is reduced, and the recovery of activity can be achieved by preliminary cultivation *in vitro* in culture media, eliminating inhibiting stress effects, before transplantation we carried out preliminary cultivation within 72 hours.

Hematopoietic stem cell transplantation was carried out systemically (intravenous) on an average up to 140 Ā 106 cells. Clinical efficacy was estimated by the criteria of the European Group studying SSc. Bioptic skin material of top third shin was investigated in stage of induration before and in dynamic of treatment for the morphological assessment of the effectiveness of the treatment. Preparations were stained with hematoxylin and eosine and also Masson-trichrom.

Electron- microscopic investigation of the material was performed by the standard method on electron microscope Libra 120 of Carl Zeiss firm.

After three months of stem cells transplantation, we observed a significant decrease in the density of the skin with a reduction of skin accounts from 12.9 to 8.7 points. Significant decrease in activity of SSD by EScSG from 3.9 to 2.5 points was revealed. At light-optical investigation it was observed sharp decrease of staining degree of connective tissue by Masson trichrom, density decrease of collagen bundles with the collapse of the fragments. Numbers of myofibroblasts are decreased. The most expressed fibrous tissue decrease was observed around the vessels. Thus character changed cellular composition of the perivascular localization. There were bud of sweat glands and hair follicles. Fields of new formed vessels with focal endo-and perivascular cellular infiltration was noted. Electron-microscopic process of biodegradation of fibrous tissue was shown in form of fibroclaziya and macrophages activation, inactive and destroyed forms of fibroblasts.

Thus, preculturing autologous hematopoietic stem cells of bone marrow transplantation create conditions for the regulation and inhibition of autoimmune inflammation and create conditions for the restoration of morpho-functional skin condition of patients with systemic scleroderma.

PO-115

Track: Regenerative Medicine and Stem Cells

THE IMMUNOLOGICAL AND MORPHOLOGICAL EFFECTS OF TRANSPLANTATION OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS (AHSCs) IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS (PBC)

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Background: PBC is further characterized by highly specific serum antimitochondrial autoantibodies (AMA) and autoreactive T cells, a striking female predominance, a strong genetic susceptibility. It is proposed that the AHSCs influence on the molecules of autoreactivity and then the proinflammatory cells are eliminated. Consequent generation of new native T-lymphocytes in patients is interrupted; it results to the restoration of the tolerance to autoantigens.

Aim of the Study: to estimate efficiency of application of AHSCs in the primary biliary cirrhosis.

Methods: 10 patients with primary biliary cirrhosis: 4 with the II morphological stage PBC, 2 - III stage, 2- IV stage and 2 - with overlap syndrome: PBC IIIst+AIH resistant to the standart therapy (UDCA and methotrexate) have been included to the study. The AHSCs has been realized by 2 stages: 1-aspiration of bone marrow suspension in the amount 200 ml; phenotyping of cells by cellcytometer BD; isolation of the mononuclear fraction and precultivation during 24-48 hours. 2 – Intravenous transplantation of the cells suspension. The cytokines levels have been investigated in the cell supernatant and in the blood serum by ELISA.

Results: 7 days after transplantation of AHSCs the tests have shown the decrease of ALT level from 1,930±0,300, Std. deviation (SD) 0,949, to 1,466±0,260, SD=0,821, p=0,007, of AST level from 1,926±0,415, SD=1,313 to 1,384±0,207, SD=0,655, p=0,005. The total bilirubin level decreased from 60,184±22,539 mkmol/l, SD=71,275 to 43,619±17,498, SD=55,334, p=0,005, the level of direct bilirubin decreased from 44,751±20,605, SD=65,158, to 20,074±11,447, SD=36,200, p=0,007. Serum protein 69,688±2,457, SD=7,770, to 77,465±1,172, SD=3,706, p=0,005 and serum albumin 39,712±2,853, SD=9, 0197, to 44,088±2,619, SD=8,282, p=0.028 increased. Three months later the levels of IL-10, IL-12p70, IL-17, IL-2 and IL-4 did't change significantly, IFN- level was increased (p=0,005). IL-1Ä level (p=0.006) and TNF-Ālevel (p=0,005) decreased. The SF36 results showed significant improvement. The morphological study was able to spend in 8 patients with the II, III stage PBC and with overalap syndrome. The results of morphological exams showed the decrease of the portal tructs infiltration by lymphocytes, plasmocytes, and decrease of the dystrophia of hepatocytes.

Conclusion: The application of AHSCs by immunological effect affects on the histological activity of liver. Possibly it is promising method of treatment of primary biliary cirrhosis.

PO-118

Track: CNS Drug Discovery & Therapy

BRANCHED AMPHIPHILIC PEPTIDE CAPSULES: RETENTION OF ENCAPSULATED SOLUTES AND DNA VACCINE DELIVERY VEHICLES

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The self-assembling Branched Amphiphilic Peptide Capsules (BAPCs) have properties that suggest applications in medicine. BAPCs assemble from two branched amphiphilic peptides: bis (Ac-FLIVIGSII)-K-K4 and bis (Ac-FLIVI)-K-K4. The BAPCs package solutes during assembly. BAPCs display a uniform size of ~20 nm when prepared at 4°C and they retain their size at elevated temperatures. BAPCs are resistant to detergents, proteases, chaotropes and the cell's degradative machinery. They can be dissociated by placing them in 50% ethanol allowing for the quantification of entrapped solutes. They are taken up by cells *in vitro*, concentrate in the peri-nuclear region, and persist in cells for weeks. Given the resilience of the BAPCs we encapsulated the alpha-emitting radionuclide 225Actinium. The BAPCs were able to withstand the a-particle emission and the recoil of daughter radionuclides. Recent *in vivo* studies showed that the 225Ac containing BAPCs remained in circulation for 24 h. The biodistribution of the 225Ac containing BAPCs was different than that observed with free radionuclide. In the presence of DNA, they can act as cationic nucleation centers around which DNA winds. They were used to transfect cells and stably express EGFP. Similarly, pDNA was delivered *in vivo*, as a vaccine DNA encoding the E6 and E7 oncoproteins of HPV-16. It elicited an immune response activating CD8 + T cells and provided anti-tumor protection in murine models.

Keywords: BAPCs, DNA vaccine, HPV-16, radionuclide.

PO-40

Track: Hot Topics in Natural Products

FUNGAL CHITOSAN AS CELLULITE BACTERIAL ANTI-VIRULENT AGENT

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Diabetic foot infections and ulcers are the more serious consequences of limb amputation leading to some complications among the diabetic patients that is associated of morbidity and mortality. The extensive use of antibiotics has resulted in the emergence of pan-drug resistant bacteria. The search for newer source of antibiotics is a global challenge, since many infectious agents are becoming resistant to synthetic drugs.

Fungal chitosan are used in a variety of applications in pharmaceutical preparations. *In vitro,* ten antibiotics were tested against diabetic foot cellulite bacteria. Using four fungal strains, the extracted chitosan showed maximum activity by minimum inhibitory concentrations (MIC) 2 and 4 µg/ml against Gram positive and negative, respectively. Combination of fungal chitosan (FC) with chloramphenicol (C) and ampicilin (AMP), separately, reduced the MIC value by 94% for Gram positive bacteria and 88% for Gram negative bacteria. Final formula of FC + chloramphenicol (C) + ampicilin

(AMP) showed a synergistic effect at FICI = 0.2 by a rate of killing at 8 hr. The findings suggest on the use of fungal chitosan as enhancing agent in combination with antibiotics in pharmaceutical preparations.

Keywords: Cellulite bacteria, diabetic infections, fungal chitosan, synergy.

PO-30

Track: Translational Medicine

ICTR TTRC CREATING NEW PATHWAYS FOR DRUG DISCOVERY AND DEVELOPMENT IN ACADEMIA THROUGH PARTNERSHIPS

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Translational research for novel therapeutics is globally challenged by rising research and development costs, limited new chemical entities in the pipeline, longer development time, and lack of efficacy. The Translational Technologies and Resources Core (TTRC) of the NIH CTSA-funded Institute for Clinical and Translational Research (ICTR) at UW-Madison was created to establish an infrastructure through partnerships with campus-wide expertise, other research institutions, and industry to move new drugs and therapies down the developmental path.

First, the TTRC has created a nimble network of affiliated resource facilities, specifically in the area of drug discovery and development, to address the research needs of investigators. Second, the TTRC launched three new internal funding mechanisms to meet investigators' urgent needs for funds to take their research to the next step along the developmental continuum with clear milestones and go/no-go decisions. Third, the TTRC has developed a number of outreach and educational programs to enhance the competency for investigators, students, and the community in the area of therapeutic discovery and development. In addition, ICTR and the TTRC are working with campus stakeholders to establish an integrated resource to attract interests and investments from the public and private sector, including multinational pharmaceutical companies. For more information on ICTR and TTRC please go to https://ictr.wisc.edu and https://ictr.wisc.edu/LaboratoryServices.

Keyword: Drug Discovery and Development in Academia.

PO-90

Track: CNS Drug Discovery & Therapy

ROLE OF SP1 INHIBITING DRUGS IN MODULATING APP AND BACE1 LEVELS IN HUMAN CELLS: IMPLICATION IN OF A NOVEL TARGET FOR ALZHEIMER'S DISEASE

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Aberrations in AD are believed to result, in part, from the over-production of amyloid-Ä peptide (AÄ), a product of AÄ precursor protein (APP). Expression studies suggest that dysregulation of proteins involved in AÄ production, such as APP and beta-secretase, or BACE1, may contribute to excess AÄ deposition. The rate-limiting step in the production of AÄ is the processing of APP by Ä-site APP-cleaving enzyme (BACE1). Understanding how expression of these proteins is regulated will ultimately reveal new drug targets. The transcription factor specificity protein 1 (SP1) coactivates the expression of the APP and BACE1 gene. We tested SP1-mediated regulation of APP with Mithramycin A, a selective inhibitor of SP1, and Tolfenamic acid, an inducer of SP1 degradation in human glioblastoma cells U373 and human neurosphere (NSP) cultures. NSPs were cultured in Neurocult basal media plus differentiation supplement (Stem Cell Technologies). U373 (ATCC) cells were cultured and transfected, and Western blot analysis was performed as previously described (Long *et al*., JBC-2014). Mithramycin A (Santa Cruz) and Tolfenamic acid (Sigma Aldrich) were prepared in 1 ĀM and 5 ĀM doses. After 72-hour treatment or transfection, cell viability was assessed using CTG assay (Promega), and protein lysates made. Western blot analysis reveals a significant decrease in the expression of APP in U373 and NSP treated with Mithramycin A. NSP treated with Mithramycin A also exhibit a decrease in BACE1 expression. Treatment with Tolfenamic acid, however, does not significantly decrease APP or BACE1 expression in either cell model. APP siRNA effectively knocks down APP expression in U373 and NSP cultures. BACE1 siRNA and SP1 siRNA did not significantly affect APP levels. CTG showed no significant changes in cell viability among treatment groups in U373 and NSP. We show that expression of APP is decreased after treatment with the SP1 inhibitor Mithramycin A in both U373 and human neurospheres cells. However, APP expression is not affected by treatment with Tolfenamic acid, perhaps due to the differences in the mechanisms between these SP1-inhibiting drugs. We also show that transfection with siRNAs can effectively change the expression of APP and BACE1 in both the human cells. It is important to discover whether drugs or small RNAs targeting this transcription factor could be used to effectively decrease amyloid load and possibly the symptoms of AD in patients.

Keywords: Amyloid-beta, BACE1, mithramycin A, neurosphere, siRNA, SP1, tolfenamic acid.

PO-112

Track: In-Silico Drug Design and In-Silico Screening

QSAR MODELING, VIRTUAL COMBINATORIAL GENERATION, MOLECULAR DOCKING AND EXPERIMENTAL EVALUATION OF POTENTIAL CATHEPSIN K INHIBITORS

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Cathepsins are protein degrading enzymes that have been thoroughly studied from different viewpoints, regarding their structure and function. Cat K is a member of enzyme family of lysosomal proteases. It plays an important role in many diseases including osteoporosis, obesity, diabetes, atherosclerosis and Paget"s disease which makes Cat K an interesting drug target.

The first part of our work comprised computational part including QSAR modeling, virtual combinatorial generation and molecular docking. Neural networks based QSAR model was constructed from the dataset of benzamide containing aminonitrile structures and their experimentally determined inhibition constants (Ki) to predict biological activity of previously untested compounds. Structural analogues of benzamide-containing aminonitriles were designed by virtual combinatorial chemistry approach. Molecular docking and post-docking analyses were applied to inspect docked ligands poses. Consensus results yielded new compounds with potential sensitivity and selectivity over Cat K.

The second part of the study covered experimental section. The most interesting compounds were synthesized, characterized and subjected to enzyme inhibitory assay. The binding mode of one of the most potent inhibitor was explored by X-ray crystallography, confirming our *in silico* binding model predictions.

Keywords: Cat K inhibitors, QSAR modeling, molecular docking, virtual combinatorial chemistry, synthesis, aminonitriles, drug design, biological evaluation.

PO-79

Track: Cardiovascular Drug Discovery & Therapy

CARDIOPROTECTIVE EFFECTS OF TRANS-ISOFERULIC ACID AND GALLIC ACID IN RAT HEART

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Background: Cardiac contractility can be modulated both directly by contractile myocytes and indirectly by non-contractile fibroblasts. A combination of both increased muscle contraction and excessive proliferation of non-contractile fibroblasts over a prolonged period contributes to the contractile dysfunction associated with heart failure. Treatments targeting both myocytes and fibroblasts may be useful in treating different stages of disease progression. The present study has investigated the effect of polyphenols gallic acid (GA) and trans-isoferulic acid (IFA) on contractile and proliferative parameters of the adult heart. Both of these polyphenols are known to have cardioprotective action, however confirmation of their effects upon contractile responses is required and to date, there is no information on their effects upon proliferative responses in the heart.

Methods: A bolus dose of either isoprenaline (1ĀM) (an established positive inotrope), verapamil (1ĀM) (an established negative inotrope), (GA) (10ĀM) or (IFA) (10ĀM) were added to rat left ventricular (LV) papillary muscles and atrial tissue preparations to determine the effects upon the basal contractile response. In addition, to establish the effects of GA and IFA on stimulated responses, the tissues were pretreated with GA and IFA for 15 min before stimulation with cumulative doses of isoprenaline. To determine the effects of GA and IFA on proliferation, adult rat cardiac fibroblasts were maintained in culture and treated with both polyphenols individually as well as the vehicle (methanol) over a period of 48h. A positive control (serum-containing media) and a negative control (serum-free media), were also included for reference. Live cell proliferation was monitored using an IncuCyte kinetic imaging system.

Results: Both GA and IFA significantly reduced (26 and 30%) the basal contractile response of LV papillary muscle to a similar extent (27%) as the negative inotrope verapamil. Interestingly, the effect of IFA (69.47%) upon the isoprenaline-stimulated contractile response of LV papillary muscle was not as significant as that observed for GA (43.95%). GA and IFA pre-treatment reduced the isoprenaline induced contractions in atrial tissue, but GA showed greater negative inotropism. The proliferation of adult cardiac fibroblasts was significantly inhibited by IFA throughout 48h treatment while only a slight inhibitory effect was observed at 20 to 24h with GA.

Conclusions: This work has shown that both GA and IFA can reduce cardiac contractility in atrial and ventricular preparations. For the first time we have demonstrated that these polyphenols also have an anti-proliferative effect on adult cardiac fibroblasts. Given these effects, it seems possible that both GA and IFA could be useful therapeutically in conditions where contractile dysfunction and fibrosis are prevalent e.g.cardiac hypertrophy and heart failure. Future studies on these polyphenols will focus on potential intracellular targets that may modulate their cardioprotective effects.

Keywords: Cardioprotective effect, Trans-isoferulic acid.

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PO-70

Track: Pharmaceutical Research & Development

OPTIMIZATION STUDIES TO INCREASE THE ANTIOXIDANT ACTIVITY OF SECONDARY METABOLITES OBTAINED FROM HALOTOLERANT *ASPERGILLUS TERREUS*

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The aim of this study was to optimize cultural conditions for optimum growth and bioactive secondary metabolite production by *Aspergillus terreus*, isolated from the Tuz Lake, Turkey. Fungi secondary metabolites which are isolated from saline medium are unique sources for natural antioxidants. To increase the antioxidant effect of different culture media, temperature, pH, incubation period, shaking, and various carbon and nitrogen sources on the mycelial growth and secondary metabolite production in a fixed volume of culture broth were studied. Free radical scavenger effect test was used *via* DPPH to specify antioxidant activity and total phenols were determined Folin cicoalteu methods. To do this, fungi were incubated static conditions in an antioxidant liquid medium (3% sucrose/lactose/maltose, 0.1% yeast extract, 0.1% polypeptone, 0.3% $(NH_4)SO_4$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, $7H_2O$, 0.05% KCl, and 0.001% $FeSO_4.7H_2O$), in 27, 30, 33, 37°C incubator for 7, 14, 21 days and 5%,10% and % 15 NaCl. According to these results natural antioxidants gained from fungi are very essential resources in terms of biotechnology.

Keywords: Antioxidant activity, *Halotolerant fungi*, secondary metabolite.

PO-80

Track: Drug Delivery & Targeting

THE ATRA-INDUCED DIFFERENTIATION ENHANCED WITH LOX/COX INHIBITORS: AN ANALYSIS OF GENE EXPRESSION IN NEUROBLASTOMA AND MEDULLOBLASTOMA CELLS

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Our previous findings showed that inhibitors of lipoxygenases (LOX) and cyclooxygenases (COX) have the capability of enhancing the differentiating action of ATRA. This study provides more detailed information concerning the molecular mechanisms of this phenomenon. A detailed analysis of the expression of 440 cancer-related genes was performed after the combined treatment of neuroblastoma and medulloblastoma cells with all-*trans* retinoic acid (ATRA) and LOX/COX inhibitors. Two neuroblastoma (SK-N-BE (2) and SH-SY5Y), and two medulloblastoma (Daoy and D283 Med) cell lines were chosen for this study. Caffeic acid (an inhibitor of 5-lipoxygenase) and celecoxib (an inhibitor on cyclooxygenase-2) were used in combined treatment with ATRA. The expression profiling was performed using Human Cancer Oligo GEArray membranes, and expressions of the most promising genes were verified using RT-PCR.

The expression profiling of the selected cancer-related genes clearly confirmed that the differentiating effects of ATRA should be enhanced *via* its combined administration with caffeic acid or celecoxib. Our results showed a concentration-

dependent increase in expression of genes involved in the neuronal differentiation, especially in cytoskeleton remodeling and also genes involved in cell cycle control, reparation processes and proteasome activity.

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PO-32

Track: Inflammation and Immunology

3-DEOXYSILYBIN INHIBITS LIPOPOLYSACCHARIDE-INDUCED INFLAMMATORY RESPONSE BY SUPPRESSING NF-! B ACTIVATION IN RAW264.7 MACROPHAGES

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3-deoxysilybin, also known as (–)-isosilandrin A, is a natural flavonoid of *Silybummarianum*. This study was designed to investigate the anti-inflammatory effect and the underlying molecular mechanisms of 3-deoxysilybin in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. 3-deoxysilybin dose-dependently inhibited the production of NO and expression of iNOS in LPS-stimulated RAW264.7 macrophages. 3-deoxysilybin also inhibited the production of pro-inflammatory cytokines (MCP-1, TNF-Ã, IL-6, and IL-1Ã) in LPS-stimulated RAW264.7 macrophages. Moreover, 3-deoxysilybin decreased NF-! B DNA binding activity in LPS-stimulated RAW264.7 macrophages. Furthermore, 3-deoxysilybin suppressed NF-! B activation by inhibiting degradation of I! BÃ and nuclear translocation of p65 subunit of NF-! Bin LPS-stimulated RAW264.7 macrophages. Taken together, the present study demonstrates for the first time that 3-deoxysilybin exhibits anti-inflammatory effect through the suppression of NF-! B transcriptional activation in LPS-stimulated RAW264.7 macrophages.

Keywords: 3-deoxysilybin, inflammation, NF-! B, NO, iNOS.

PO-109

Track: Drug Delivery & Targeting

HWANGRYUN-HAEDOK-TANG FERMENTED WITH *LACTOBACILLUS CASEI* SUPPRESSES OVARIECTOMY-INDUCED BONE LOSS

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Hwangryun-haedok-tang (HRT) is the common recipe in traditional Asian medicine, and microbial fermentation is used for the conventional methods for processing traditional medicine. We investigated the inhibitory effect of n-butanol fraction of HRT (HRT-BU) and fHRT (fHRT-BU) on the RANKL-induced osteoclastogenesis in bone marrow-derived macrophages. mRNA expression of osteoclastogenesis-related genes were evaluated by real-time QPCR. The activation of signaling pathways was determined by Western blot analysis. The marker compounds of HRT-BU and fHRT-BU were analyzed by HPLC. The inhibitory effect of HRT or fHRT on ovariectomy-induced bone loss were evaluated using OVX rats with orally administered HRT, fHRT (300, 1000 mg/kg), or its vehicle for 12 weeks. fHRT-BU significantly inhibited RANKL-induced osteoclastogenesis, and phosphorylation of p38, IKKÃ/Ã, and NF- Bp65 compared to HRT-BU. In addition, fHRT-BU also significantly inhibited the mRNA expression of Nf b2, TNF-Ã, NFATc1, TRAP, ATPv0d2, and cathepsin K. Furthermore, administration of fHRT had a greater effect on the increase of BMD, and improved bone microstructure of femora than that of HRT in ovariectomy rats. This study demonstrated that bacterial

fermentation enhances the inhibitory effect of HRT on osteoclastogenesis and bone loss. These results suggest that fermented HRT might have the beneficial effects on bone disease by inhibiting osteoclastogenesis.

PO-51

Track: Drug Delivery & Targeting

PROTECTIVE EFFECT OF HERBAL PRESCRIPTION KIOM-4L ON NON-ALCOHOLIC FATTY LIVER DISEASE

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Non-alcoholic fatty liver disease (NAFLD) is one of the common liver disease and major cause of worldwide chronic liver injury which can be progressed to the severe pathological stage cancer. In traditional medicine, there are several herbal prescriptions used for hundreds of years to treat liver diseases and obtain hepatoprotective effect. To develop novel hepatoprotective herbal prescription against NAFLD, we screened individual herbs and assayed multi-herb prescription KIOM-4L *in vitro*, and identified significant efficacy *in vivo* animal model. Using the cell lines HepG2 and AML12, compared viability of cells given free fatty acid (FFA) and KIOM-4Lextract. Anti-NAFLD activity was examined by NILE and Oil red staining showing significant FFA-lowering effect. Effect of KIOM-4L also identified in high-fat diet fed animals showing pathologically recovered liver conditions in autopsy observation and liver-function indexes. KIOM-4L was not affected normal body weight increase during 14 days of experiment and induced no abnormal animal behavior or clinical signs. As a result, KIOM-4L can be regarded as safe and non-toxic hapatoprotective material against NAFLD, with remaining in-depth study of elucidating functional mechanism. Furthermore, this material also can be applied for development and modernization of complementary and alternative traditional medicine.

PO-6

Track: Drug Delivery & Targeting

SURFACE-MODIFIED IMMUNO-LIPOSOMES AS CARRIERS FOR DRUG DELIVERY TO MACROPHAGES IN ATHEROSCLEROTIC LESIONS

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Purpose: Development of drug delivery systems for therapy of atherosclerosis is expected. In this study, efficacy of surface-modified liposomes as carriers for drug delivery to macrophages in atherosclerotic lesions was evaluated.

Method: Unmodified liposomes and immuno-liposomes modified with oxidized low-density lipoprotein receptor-1 (LOX-1) antibody containing fluorescence-labeled phospholipid were prepared, and their uptake by cultured macrophages (RAW264) was examined *in vitro*. The uptake of liposomes by RAW264 was evaluated by the confocal laser scanning microscopic images.

Results & Discussion: The uptake of immuno-liposomes by RAW264 was significantly higher than that of unmodified liposomes. This indicates that the endocytosis through LOX-1 contributes to enhancement of the liposomal uptake by macrophages.

Conclusion: This study shows that immuno-liposomes modified with LOX-1 antibody are useful as carriers for drug delivery to macrophages in atherosclerotic lesions.

Keywords: Immuno-liposomes, macrophages, atherosclerosis.

PO-117

Track: Diabetes and Obesity Drug Discovery & Therapy

EFFECT OF LIPIDIC SERENOA REPENS EXTRACTS IN PROSTATE HEALTH IN MALE WISTAR RAT'S COMPARISON BETWEEN OBESITY AND HORMONAL MODELS OF BENIGN PROSTATE HYPERPLASIA

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The incidence and prevalence for prostate disease is higher in obese individuals and the symptoms are increased in severity. Current pharmacological therapy has severe adverse effects, which detrimentally affect the life quality of the patients. Alternative therapies such as Serenoa repens have been proposed. We tested a lipidic Serenoa repens extract in Wistar rats with hypercaloric diet-induced obesity, with and without testosterone treatment to induce benign prostate hyperplasia. Treatment with the extract significantly decreased prostate weight in obese individuals but did not show an effect in controls. Hypercaloric diet caused stromal and epithelial hyperplasia in prostate, hormonal treatment exacerbated hyperplasia in the prostate; all these alterations were reversed by treatment. The treatment improved oxidative stress markers such as nitrite concentrations, which were significantly lower compared to groups receiving vehicle, malondialdehyde levels were significantly reduced with treatment compared to vehicle and antioxidant factors such as glutathione and superoxide dismutase levels were significantly improved by treatment with the extract. All treatments showed more significant differences in the hormone-treated groups. Our data suggests that Serenoa repens has significant antioxidant activity and can be useful in the treatment of benign prostate hyperplasia in obese patients.

Keywords: Hyperplasia oxidative stress, obesity prostate.

PO-55

Track: Protein and Peptide Sciences

ANTITUMOR EFFECTS OF AN L-AMINO ACID OXIDASE FROM *CALLOSELASMA RHODOSTOMA* SNAKE VENOM: INDUCTION OF APOPTOSIS AND EVALUATION OF THE EXPRESSION OF FAS, BAX AND BCL2 GENES IN TUMOR CELL LINES

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L-amino acid oxidases (LAAOs) from snake venoms are targets for a large number of studies due to several pathophysiological effects in which they are involved, such as induction of apoptosis, cytotoxicity, microbicide activities, among others. In the present study, we evaluated the apoptotic effects of an L-amino acid oxidase from *Calloselasma rhodostoma* snake venom, named CR-LAAO, on HL-60 e HepG2 tumor cell lines. The CR-LAAO was highly cytotoxic to HepG2 and HL-60 tumor cells and showed low toxicity on peripheral blood mononuclear cells (PBMC). In the presence of catalase, these effects were inhibited. The apoptotic/necrotic effect of CR-LAAO was assessed by flow cytometry using annexin V FITC and propidium iodide (PI) as markers. It was observed that the

protein induces apoptosis (AV+) in HepG2 cells at lower concentrations (0.1-2.5 Āg/mL) and causes apoptosis/necrosis (PI+/AV+) at higher ones (5-100 Āg/mL). CR-LAAO also induces apoptosis/necrosis (PI+/AV+) in HL-60 cells. HepG2 tumor cells treated with 1.0 and 10.8 Āg/mL of CR-LAAO expressed the pro-apoptotic Bax gene, while HL-60 cells treated with 1.7 Āg/mL expressed Fas and Bax genes. None of the strains tested expressed the anti-apoptotic Bcl2 gene. Our results suggest that CR-LAAO possesses a noticeable biotechnological potential as an antitumor agent.

Keywords: Apoptosis, gene expression, L-aminoacid oxidase, snake venom.

PO-106

Track: Protein and Peptide Sciences

THE L-AMINO ACID OXIDASE ISOLATED FROM *CALLOSELASMA RHODOSTOMA* (CR-LAAO) MODULATED APOPTOMIRS EXPRESSION IN BCR-ABL POSITIVE CELLS

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Chronic Myeloid Leukemia (CML) is a disease characterized by Bcr-Abl oncoprotein, responsible for apoptosis resistance. ApoptomiRs are noncoding RNAs that regulate cell death process and their abnormal expression has been described in CML patients. Since CR-LAAO is described as apoptosis-inducer in tumour cells, the aim of this study was to investigate whether CR-LAAO was capable of modulating apoptomiRs in Bcr-Abl positive cells. HL-60 and HL-60.Bcr-Abl cell lines were treated with 0.05; 0.25 and 0.05; 0.35 µg/mL respectively of CR-LAAO for 24 h. The apoptomiRs hsa-mir-15a, hsa-mir-16, hsa-mir-29c, hsa-let-7d expressions were assessed by real-time PCR and the reference genes (RNU24 and RNU 48) were used to normalization. The anti-apoptotic proteins targets were evaluated by western-blot. CR-LAAO increased the expression of hsa-mir-15a in HL-60.Bcr-Abl at 0.35 µg/mL (fold change: 1.84) and hsa-let-7d in HL60 (fold changes to 0.05 and 0.25 µg/mL: 14.4; 22.2) and in HL-60.Bcr-Abl (fold changes to 0.05 and 0.35 µg/mL: 2.0; 1.81). The western-blot showed that the increase in hsa-mir15 and hsa-let-7d expressions resulted in a decrease of Bcl-2 and A1 (anti-apoptotic protein targets) in HL-60.Bcr-Abl. These findings showed for the first time that CR-LAAO effect on apoptomiRs expressions can be a useful tool to improve the CML treatment.

Keywords: Apoptosis, chronic myeloid leukemia, L-amino acid oxidase, miRNA.

PO-62

Track: Drug Delivery & Targeting

ENHANCED TRANSPORT OF MATERIALS INTO DENTAL ENAMEL NANOPORES *VIA* ELECTROKINETIC FLOW

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Ability to infiltrate various molecules and resins into dental enamel is highly meaningful in dentistry, yet transport of materials into dental enamel is limited by the nanometric scale of their pores. Materials that cannot be infiltrated into enamel by diffusion only are thought of as having molecules above the critical threshold (MACT). We challenge this by reporting the use of electrokinetic flow to transport MACTs, aqueous solution with high refractive index (Thoulet's solution) and curable fluid resin infiltrant (without acid etching), significantly beyond the limits resulting from diffusion.

Volume infiltration by Thoulet's solution is increased by 5-6 fold, and resin infiltration depths ranged from 600-2000 µm, while ~10 µm depth results from diffusion after acid etching. After demineralization *in vitro* for 192 hours, demineralization occurred at non-infiltrated histological points but not at resin-infiltrated ones. These results open new avenues for the transport of materials (including drugs) in dental enamel, with potential applications in the prevention/non-invasive treatment of dental caries and tooth whitening.

Keywords: Dentistry, dental enamel, electrokinetic flow, nanofluidics.

PO-76

Track: Pharmaceutical Research & Development

CYTOTOXIC AND ANTIPROLIFERATIVE EFFECTS OF SECONDARY METABOLITES OF HALOTOLERANT PENICILLIUM SP. ON COLON ADENOCARCINOMA (CACO-2) CELLS USING XCELLIGENCE TECHNOLOGY

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Fungal secondary metabolites are receiving increasing attention due to their diverse compounds making them interesting candidates for drug discovery. Fungi have appeared as a substantial source of antioxidant compounds and they are considered to be the main secondary metabolites. *Penicillium chrysogenum* is a commonly used in industry for produce important antibiotics which are a variety of important secondary metabolites, like penicillins. In this study, we were investigated cytotoxic and antiproliferative effects of ethylacetate extract of *Penicillium chrysogenum* var. chrysogenum on the Caco2 cells. Cytotoxic effects of extract were determined by the WST-1 assay. Real-time cell analysis system was used to analyze antiproliferative effects according to change of cell index values. It was shown that extract had important cytotoxic and antiproliferative effects on Colon adenocarcinoma cells. Especially, the extract showed significant cytotoxic potential on Caco2 cells at low concentration (50 µg/ml) at 48h. Also, antiproliferative effects increased on Caco2 cells according to concentration and time depends. IC50 concentration was analyzed as 102 Ãg/mL at 72h. It was observed that extract of *Penicillium chrysogenum* var. chrysogenum has anti-cancerogenic effects on Caco2 cells, and it has potentially more pharmacological activities.

Keywords: Fungi, WST-1, Xcelligence technology, Caco2, antiproliferative.

PO-12

Track: Chemistry

SYNTHESIS, CHARACTERIZATION, AND ANTIMICROBIAL ACTIVITY OF POLY(ACRYLO-NITRILE-CO-METHYL METHACRYLATE) WITH SILVER NANOPARTICLES

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Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic-scale tailoring of materials. The main aim of this study is to biosynthesize silver nanoparticles (AgNPs) using *Trichoderma viride* (HQ438699); the metabolite of this fungus will help either in reduction of the silver nitrate-adding active materials which will be loaded on the surface of the produced AgNPs. Poly (acrylonitrile-co-methylmethacrylate) copolymer (poly (AN-co-MMA)) was grafted with the prepared AgNPs. The poly (AN-co-MMA)/AgNPs were examined against ten different pathogenic bacterial strains, and the result was compared with another four different generic antibiotics. The

produced poly (AN-co-MMA)/AgNPs showed high antibacterial activity compared with the four standard antibiotics. Moreover, the grafting of these AgNPs into the copolymer has potential application in the biomedical field.

Keywords: Antimicrobial, nanomaterials, polymer, silver nanoparticles.

PO-23

Track: Anti-Infectives

THE CLINICAL PRACTICE OF VANCOMYCIN DOSING AND MONITORING, AND FACTORS AFFECTING LEVELS AMONGST ONCOLOGY AND CARDIOLOGY PATIENTS IN QATAR: A RETROSPECTIVE ANALYSIS

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This is the first study of its kind in Qatar focuses on the assessment of current vancomycin use for Cancer and Cardiology patients among Qatar population admitted to National Centre for Cancer Care and Research (NCCCR) and Heart Hospital (HH) (the only tertiary care/ cardiology facilities in Qatar) which are 2/7 hospitals in Hamad Medical Corporation the main and largest healthcare organization in Qatar. This study is a retrospective review of the clinical practice of vancomycin which is essential to establish vancomycin guideline which will assure the standardization of practice and meets the unique needs of our patients population, and our objectives are to elucidate the factors affect vancomycin trough levels, and to describe vancomycin prescribing practices and identify targets for improvement at NCCCR and HH.

Keywords: Vancomycin, trough level, staphylococci, dosing, clinical pharmacist.

PO-113

Track: Pharmaceutical Research & Development

NF-! B PLAYS AN IMPORTANT ROLE IN PROTEASOME INHIBITION OF MLN9708 ON CACO2 HUMAN COLON CANCER CELL LINE

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The ubiquitin proteasome system regulates the degradation of the majority of intracellular proteins. Proteasome inhibition has emerged as an effective anti-cancer theraphy. Recently nuclear factor B (NF- B) regulates critical survival pathways in cells. Proteasome inhibitors block I BÄ degradation, subsequently NF- B activation. The NF- B inhibition contributes to proteasome inhibitor-induced cell death. Bortezomib is the best characterized proteasome inhibitor and has molecular effects, including inhibition of NF- B. MLN9708, and new proteasome inhibitor, is currently being evaluated in multiple phase I clinical studies for especially hematologic malignancy and some solid tumors. MLN9708 hydrolyzed to MLN2238, biologically active form, when it is exposed to aqueous solutions or plasma. In this study NF-! B and c-myc mRNA expression levels were investigated by RT-PCR method in different concentrations (1, 5, 10, 20 ĀM) of MLN2238 compared with Bortezomib on Caco2 cells. We found that depending on the increasing concentrations of MLN2238 and Bortezomib downregulated NF- B and c-myc mRNA expression at 24 h on Caco2 cell lines. The decreased activity of NF-! B through proteasome

inhibition is presumed to play an important role in apoptosis of Caco2 cells. In this study, bortezomib-like effect of MLN2238 was observed. It shows that MLN2238 might be potential agent for chemotherapy of solid tumors such as colon cancer.

Keywords: Proteasome inhibitor, MLN9708, Bortezomib, Caco2, NF-ĀB.

PO-101

Track: Drug Discovery in Preclinical Research

OPTIMIZING MOUSE MODELS OF HUMAN DISEASE TO ACCELERATE THERAPEUTIC DISCOVERY

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Substantial progress has been made in translational research, but bringing mouse discoveries into human clinics is a slow process. Mouse models should recapitulate as many aspects of the human disease as possible. Causative genetic mutations found in humans may or may not mimic disease severity and pathology in mouse models. Optimization of current models and new genetic engineering techniques are crucial to accelerating therapeutic discovery. For example, the C57BL/10ScSn-*Dmd*^{mdx}/J mouse model of Duchenne muscular dystrophy has a milder phenotype than the same mutant on the DBA/2J genetic background. Alternatively, phenotypes found in patients but lacking in the mouse model are unmasked when this mdx mutation is combined with a knockout of *Cmah*. Models for Amyotrophic Lateral Sclerosis (ALS) have had poor translational ability in the past. Mice harboring the newly discovered mutant TARDBP gene implicated in ALS develop mild neurodegeneration, but ultimately succumb to bowel blockage rather than overt neuronal disease. By changing this strain's genetic background, however, the clinically relevant neurological deficits are exposed. Through partnering with disease-based foundations connecting clinicians and researchers, we are able to optimize preclinical mouse models, for higher translatability of experimental results.

PO-69

Track: Anti-Infectives

STAPHYLOCOCCAL TWORT-LIKE PHAGES' HOMOLOGS OF BACTERIAL VIRULENCE DETERMINANTS

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Staphylococcus aureus is one of the most challenging bacterial pathogens, due to the increasing number and spread of antibiotic resistant strains. Thus, bacteriophage therapy is a promising alternative to antibiotic therapy in curing *S. aureus* infections. *S. aureus* bacteriophages that are common in therapeutic phage collections belong to the Twort-like species of *Myoviridae* family. They are obligatorily lytic, cannot transfer genetic material between bacteria by transduction and infect over 80% of strains in staphylococcal strain collections. All these phages are alike with respect to their genome organization. They share from 52 to over 99% identity at the DNA level. Previously, we characterized the genomes of seven of their representatives [1]. By definition, phages for therapeutic purposes cannot encode toxins or other bacterial virulence factors. Thus, we were surprised to find two homologs of bacterial virulence determinants among predicted products of Twort-like phages' genes. One of them, gpORF92, resembles neuraminidases that are essential colonization factors of certain bacterial pathogens. The second one, gpORF50, is similar to major staphylococcal antigens, which are required for *S. aureus* virulence and nasal carriage. Attempts to insert the PCR-amplified ORF92 in a plasmid vector resulted in the cloning of several mutants, in place of a wild-type gene, indicating the toxicity of gpORF92 protein to bacteria. The expression of cloned ORF50 in bacteria caused their lysis. Clearly, in the evolution of staphylococcal Twort-like phages the products of ORF50 and ORF92 genes gained bactericidal functions. Their homologies to bacterial virulence factors do not preclude the use of staphylococcal Twort-like phages for therapeutic purposes.

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Keywords: Bacteriophage, phage therapy, *Staphylococcus aureus*.

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PO-52

Track: Anti-Infectives

UNPREDICTED PROPERIES OF *PSEUDOMONAS AERUGINOSA* CELLS TREATED WITH BACTERIOPHAGE PS44 - A CANDIDATE FOR THERAPEUTIC APPLICATIONS

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Obligatorily lytic *Pseudomonas aeruginosa* bacteriophages are promising bactericidal agents that could be used as alternative to antibiotics in curing infections by drug-resistant *P. aeruginosa* strains [1]. We characterized one of such phages, PS44, from the therapeutic phage collection of the IIiTD, PAS, in WrocÃaw, Poland. It belongs to the *Pbunalikeviruses* genus of *Myoviridae* family, whose representatives appeared to be effective therapeutically in curing pulmonary infection and keratitis caused by *P. aeruginosa* in mice [2, 3]. All predicted products of PS44 protein-coding genes are homologous to the predicted gene products of prototypical *Pbunalikeviruses*. PS44 adsorbs quickly to its host cells, has a short propagation time, and forms clear plaques with a turbid halo on a layer of cells of its propagation strain. We isolated colonies of PS44 resistant cells upon infection of certain *P. aeruginosa* strains with PS44. They represented two or three different morphological types, depending on the strain. Surprisingly, the colonies of one type contained cells with detectable PS44 DNA, suggesting that they represent lysogens. Colonies of the second type contained cells of atypical swimming motility. Colonies of the third type were characteristic only to some strains. Their cells contained a large insertion in a gene, whose disruption results in the increase of *P. aeruginosa* virulence. However, they occasionally segregated into heterogeneous population of PS44 sensitive and PS44 resistant cells. Comparisons of genomic sequences of PS44 resistant mutants and wild-type cells are in progress to elucidate the molecular basis of PS44 resistance phenotypes. However, lysogen-like properties of certain PS44 resistant bacterial isolates makes the therapeutic applications of *Pbunalikeviruses* questionable.

This work was supported by funds from the Operational Program ‘Innovative Economy, 2007-2013’ (Project No. POIG01.03.01-02-003/08).

Keywords: Phage therapy, *Pseudomonas aeruginosa*.

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PO-29

Track: Pharmaceutical Biotechnology

BIOSUPERIORS: TrasGEX™ AND CetuGEX™ – RESULTS FROM CLINICAL PHASE I STUDIES

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Background: By establishment of the GlycoExpress toolbox (GEX) we have generated a set of glycoengineered human cell lines for the high yield production of fully human glycoproteins to optimize the glycosylation of antibodies and non-antibody biotherapeutics for improvement of the clinical efficacy and side effects.

CetuGEX™ and TrasGEX™ based on the monoclonal antibodies cetuximab (Erbitux) and trastuzumab (Herceptin) are produced in GlycoExpress cells and by this glycooptimized with respect to manifold improvement of anti-cancer activity, optimization of bioavailability, and removal of immunogenic components and broadening of the patient and indication coverage.

Besides the improvement in ADCC function, in contrast to Cetuximab, CetuGEX™ does not contain any immunogenic non-human glycan structures such as NeuGc and Galili epitope (Gal-Gal carbohydrate structures).

Single agent dose escalation studies with late stage patients with progressive disease showed for both agents strong single agent activity including various complete and partial responders as well as long lasting clinical benefit, in case of CetuGEX a Clinical Benefit Rate of 76%.

Conclusions: The glycooptimization principle was clinically proven by the fact that strong responses and clinical benefit was seen in patients who showed no benefit or were progressive with the non-glycooptimized trastuzumab or cetuximab with better side effect profile.

PO-100

Track: Drug Delivery & Targeting

SOLUBILITY ENHANCEMENT OF POORLY SOLUBLE ANTICANCER DRUG, DOCETAXEL USING SOLID DISPERSION TECHNIQUE

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Background: This study was designed to evaluate and enhance the solubility of poorly soluble drug, docetaxel through solid dispersion (SD) technique prepared using freeze drying method.

Method: Docetaxel solid dispersions were formulated with Kollidon 12PF as binary formulations and Poloxamer 188 as ternary formulations in different weight ratios. Freeze drying method was used to prepare the solid dispersions. Solubility of the solid dispersions were evaluated respectively and the optimized of drug-solubilizers ratio systems were characterized with different analytical methods like Differential scanning calorimeter (DSC), Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) to confirm the formation of complexes between drug and solubilizers.

Results: The solubility data revealed an overall improvement in solubility for all SD formulations. The ternary combination 1:5:2 gave the highest increase in solubility that is approximately 3 folds from the pure drug, suggesting the optimum drug-solubilizers ratio system. This data corresponds with the DSC and SEM analyses, which demonstrates presence of drug in amorphous state and the dispersion in the solubilizers in molecular level.

Conclusion: The solubility of the poorly soluble drug, docetaxel was enhanced through preparation of solid dispersion formulations employing freeze drying method. Solid dispersion with multiple carrier system shows better solubility compared to single carrier system.

Keywords: Docetaxel, freeze drying, solid dispersion, solubility enhancement.

PO-50

Track: Green Techniques in Medicinal Chemistry

ANTIMICROBIAL PROPERTIES OF FOUR NOVEL SYNTHESIZED TERPENOIDAL DERIVATIVES AGAINST *STAPHYLOCOCCUS EPIDERMIDIS*

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Staphylococcus epidermidis has been identified to be an acne causing bacteria in addition to *Proprionibacterium acnes*. Terpenoidal compounds have shown to have antimicrobial activity against acne causing bacteria especially *Staphylococcus epidermidis*. The present study was conducted to evaluate the antimicrobial properties of four novel synthesized terpenoidal derivatives against *Staphylococcus epidermidis*. The test compounds are terpenoidal derivative compound 5 (2-(3-methylbut-2-en-1-yl)benzenethiol), terpenoidal derivative compounds 6 (1-(2-mercapto-3-(3-methylbut-2-en-1-yl)phenylethanone), 7 (1-(2-amino-3-(3-methylbut-2-en-1-yl)phenylethanone) and 8 (1-(2,4-dihydroxy-5-(3-methylbut-2-en-1-yl)phenylethanone). The disc diffusion method used for experimentation showed that terpenoidal derivative compound 5 has significant antimicrobial activity and strong inhibitory effects against *Staphylococcus epidermidis*. The MIC of terpenoidal derivative compound 5 against *Staphylococcus epidermidis* was identified to be 4 Ãg/mL using the broth dilution assay and was comparable with standard gentamycin at 3.6Ãg/mL. Terpenoidal derivative compounds 6, 7 and 8 showed no antimicrobial or inhibitory activity against *Staphylococcus epidermidis*. This study shows that terpenoidal derivative compound 5 (2-(3-methylbut-2-en-1-yl) benzenethiol) is a potential antimicrobial agent against *Staphylococcus epidermidis* and can be used as an anti-acne treatment agent after further studies have been conducted.

PO-2

Track: Anti-Infectives

ANTICANDIDAL ACTIVITY OF SERIES OF SOME MANGANESE (II) COMPLEXES

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Metal based agents are continuously gaining increasing attention as potential drug candidates or as tools in diagnostic applications. Combination of unique intrinsic properties of metal ions and complexes, e.g. redox-properties, radioactivity, magnetism or reactivity, with the multiplicity of various organic and bioorganic ligands afforded an inconceivable number of potential molecules. The known activities of several metal ions in biology have stimulated further developments of metal-based therapeutics and diagnostics.

In this study, we examined anticandidal activity of a series of manganese (II) complexes which contain phen (1,10 phenanthroline), 2,2'-bipyridine and together with o-aba (o-aminobenzoic acid) and ba (benzoic acid) ligands {[Mn(phen)2(ClO_4)2)], [Mn(phen)3]2(ClO_4)(H_2CO_3), [Mn(2,2'-bipy)2(ClO_4)2)], [Mn(2,2'-bipy)3]2(ClO_4), [Mn2(o-

aba)2(2,2'-bipy)4]2(ClO_4) and [Mn(ba)(phen)2(H_2O)](ClO_4)2(CH_3OH)} which have been synthesized in methanol at 50 ^oC and characterized by single crystal X-ray diffraction technique. Six species of Candida (*Candida tropicalis, Candida krusei, Candida zeylanoides, Candida parapsilosis, Candida albicans and Candida globrata*) causing severe infections in humans were tested by micro broth dilution test to investigate the minimal inhibitory concentration (MIC) value of each compound. The anticandidal assessment revealed that the new compounds posses' significant activity on all Candida species tested. In comparing their MIC values with ketokenozole, complexes [Mn (phen) 2(ClO_4)2)] and [Mn (phen) 3] (ClO_4)2)2(H_2CO_3) were particularly effective. It is concluded that metal based ligands have significant importance of controlling infectionous diseases, particularly in immun supressed patients.

Keywords: Anticandidal activity, manganese complexes.

PO-3

Track: Chemistry

ON TAUTOMERISM OF SOME BENZODIAZEPINE AND RELATED ANXIOLYTIC MEDICINES

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When at least two isomers exist in mobile equilibrium with each other, tautomerism occurs [1]. Many biomolecules display prototropic tautomerism. This process affects the chemical structure of biomolecules and consequently their stability, biochemical reactivity and biological activity [2]. Ab initio and DFT quantum chemical methods give us an opportunity to investigate and predict the stabilities of all tautomeric forms in a compound having tautomerism phenomenon.

In this study, tautomerism of some well-known benzodiazepines and related anxiolytic medicines, which are Diazepam, Clonazepam, Lorazepam, Oxazepam, Chlordiazepoxide, Etizolam, were investigated in both in gas phase and aqueous solution to find the most stable forms of these medicines by using Ab initio and DFT quantum chemical methods.

Keywords: Tautomerism, Ab initio and DFT quantum, anxiolytic.

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PO-105

Track: Genomics

SUBUNITE A OF COAGULATION FACTOR XIII AS A NEW BIOMARKER IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA?

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The expression of coagulation factor XIII subunit A (FXIIIA) in leukemic B-cell progenitor lymphoblasts was shown to influence overall survival of children with acute lymphoblastic leukemia (ALL). We analyzed 3 public datasets in GEO Database (GSE47051, GSE13351 and GSE13425) of children with ALL using GeneSpring and Ingenuity Pathway Analysis softwares. Defining the expression level of the gene F13a1 encoding for FXIIIA either as low or high, patients

was separated into two groups with at least 3-fold difference in F13a1 expression level. Low F13a1 expression was prevalent among "B-other" samples, high F13a1 expression was associated with t(1;19) genetic subgroup. Eight genes were significantly down-regulated, and 1 was up-regulated in the F13a1 low expression group in all datasets, and 28 genes were dysregulated in two datasets. We identified two chromosomal loci, 19p13.3 and 16q22, where 11 and 2 genes, respectively, were dysregulated within the F13a1 low expression group. We found a network of genes participating in B cell development that were downregulated in the F13a1 low expression group. Ingenuity upstream regulator analysis predicted aberrant activation of NUPR1, and aberrant suppression of TCF3 and IKZF1 in the F13a1 low expression group, which is similar to the molecular pathology of BCR-ABL1-like ALL.

Keywords: Acute lymphoblastic leukemia, gene expression.

PO-87

Track: Chemistry

FORMULATION AND STATISTICAL OPTIMIZATION OF ALGINATE-ACACIA GUM FLOATING SYSTEM FOR ORAL DRUG DELIVERY

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During last few decades, numerous oral drug delivery systems have been investigated and developed to act as drug reservoirs from which drug molecules are able to release in sustained manner over period [1]. These sustained-release oral dosage forms prolonged also offer several advantages such as delivery of drug molecules to the target site with specificity, limiting fluctuation within the therapeutic range, reduction in side-effects, decreased dosing frequency and improved patient compliance [2]. However, the challenge in the development of such systems is not just to sustain the drug but also to prolong gastric residence of the dosage forms release, until all the drug is completely released at the desired period [3].

This work investigates the development, optimization and *in vitro* characterization of floating calcium alginate-Acacia Gum (CA-AG) beads by an ionotropic gelation method for prolonged sustained release of sodium declofenac. The effects of sodium alginate and acacia gum weight masses on drug encapsulation efficiency (DEE) of beads and cumulative drug release at 8h (R_{8h}) from beads was analyzed by 3^2 factorial designs. The swelling and degradation of the optimized beads were influenced by pH of test mediums. These beads were also characterized by Optical spectroscopy, FTIR and DSC spectroscopy for surface morphology, excipients-drug interaction analysis and theremal analysis respectively. These developed floating CA-AG beads containing drug could possibly be advantageous in terms of advanced patient compliance with reduced dosing interval.

Keywords: Acacia gum, alginate, factorial design, beads, floating system, sodium declofenac.

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PO-9

Track: Drug Delivery and Targeting

DUAL SELECTED CANCER LIGANDS IN TARGETED NANOMEDICINES

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Specific targeting of liposomal chemotherapies to one type of cancer cells using ligands from landscape phage libraries has proven successful *in vitro* and *in vivo*. To further broaden the applicability of this technology, a novel selection scheme was developed to select individual ligands that will specifically bind two different cell lines. The results of this broadened selection scheme produced numerous phage clones that target both pancreatic cancer cells (PANC-1) and lung cancer cells (CALU-3). During screening, the clones were studied for their specificity to PANC-1 and CALU-3, in additional to phenotypically normal lung cells (SAE) and serum. Based on their affinity to either CALU-3 or PANC-1 over serum, 10 phage clones were chosen for further study comparing binding strength to both cell lines over SAE cells. From this further analysis, the top 5 phage ligands were: GEFDELMTM, DHVWAEGDS, DPNWEATVG, GSLEEVSTL, and AEYGESVNA (designated by the sequence of inserted foreign peptides). The kinetics of interaction with cancer cells of the top 5 clones was studied using florescence microscopy and has shown similar binding patterns in CALU-3 and PANC-1 cells. It was shown that selected phage proteins inserted into liposomal doxorubicin increases its cytotoxicity in CALU-3 and PANC-1 cells.

Keywords: Phage display, dual selected, cancer, nanomedicines.

PO-14

Track: Drug Delivery & Targeting

THERANOSTIC PHOTOIMMUNOTOXIN INDUCES SELECTIVE CELL DEATH IN EGFR POSITIVE OVARIAN CANCER

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Photoimmunotherapy has emerged as a promising theranostic approach for both cancer monitoring and treatment due to the great selectivity and safety represented by antibody high specificity to the target cell receptor and light-dependent photosensitizer toxicity, respectively. However, there are serious challenges to be taken into consideration to translate photoimmunotherapy into clinical development with optimal pharmacokinetic, efficacy, and safety profiles. One of these challenges is the conjugation method to equip antibody with photosensitizer molecules. Traditional protein conjugation methods are efficient for preparation of photoimmunotoxins (PITox), however these methods are unsuitable for high homogeneous, standardized and pharmaceutically acceptable products. To overcome this problem, we have developed a universal and robust method for labeling antibody fragments with photosensitizer molecules using SNAP-Tag technology. In this study, highly potent photosensitizer IRDye®700DX phthalocyanine dye (IR700) was conjugated with antibody fragment (scFv-425) against epidermal growth factor receptor (EGFR) using SNAP tag technology, generating high homogeneous PITox with defined pharmacokinetic and therapeutic profiles. This PITox shows potential *in vitro* imaging and treatment properties against ovarian cancer cell lines as well as primary human tissues.

PO-68

Track: Drug Delivery & Targeting

AN INTEGRATED METHOD FOR A SYNERGISTIC HERB COMBINATIONS AND TARGETING MOLECULAR MODULES FOR TREATING EMOTION-RELATED DISEASES

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Many diseases are associated with emotions like depression, bipolar disorder, attention-deficit/hyperactivity disorders (ADHD), obsessive-compulsive disorders (OCD) and other neurotic disorders. With long history of traditional medicine in Korea, we have lots of prescriptions to treat a disease like these. However; the process of extracting meaningful data combined with current scientific knowledge has not been clearly established. Here we present a specific method to extract information from 5,000 kinds of herb-formulation in the classical text of traditional Korean medicine called <Inje-Ji (仁濟志); documents of generous salvation>. In general, herb-formulations are composed of four functional categories, which is called king, minister, assistant, envoy in metaphor, so we tried to figure out specific interaction of each herb (herb module) to exert therapeutic effect on emotion-related diseases. Using a customized method we found several herb combinations (e.g. Polygala tenuifolia and Acarus gramineus Soland) are in action under the therapeutic process. Further we included modern data bases such as Chembl, Drugbank, GeneCards and suggest that inside the herb combinations active molecular modules should be traced by linking medicinal herb DB and compound-protein interaction DB. Collectivelyintegrated method of traditional and modern DB might be a powerful engine to discover promising drug candidates for the treatment of emotion-related symptoms.

PO-56

Track: Drug Delivery & Targeting

GALLARHOIS EXERTS ITS ANTIPLATELET EFFECT BY SUPPRESSING ERK1/2 AND PLCĀ PHOSPHORYLATION

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Gallarhois and its components have various biological activities, including protective effects on liver cells as well as antimetastatic, antiplatelet, and antibacterial effects. In the present study, we identified the antiplatelet activity and possible mechanism of action of a *G. rhois* extract (GRE).We investigated the effect of GRE and its components on rabbit platelet activation, and their possible molecular mechanisms. The GRE inhibited collagen-, AA-, and thrombin-induced platelet aggregation as well as serotonin secretion, in a concentration-dependent manner. The GRE significantly inhibited the production of lipoxygenase-mediated 12-hydroxyeicosatetraenoic acid. The GRE effectively suppressed thrombin-stimulated PLCÃ3 phosphorylation and collagen-induced ERK1/2 phosphorylation, in addition, the GRE significantly restored the cAMP level, which had decreased due to collagen or thrombin. Among the components of GRE, methyl gallate inhibited the collagen-induced platelet activation through suppression of ERK phosphorylation; penta-O-galloyl- β -D-glucoside inhibited the thrombin-induced platelet activation through suppression of PLCĀphosphorylation. These results indicate that the GRE including methyl gallate and penta-O-galloyl-! -D-glucoside suppressed platelet activation by inhibiting ERK1/2 and PLCÃ3 phosphorylation.

PO-102

Track: Pharmaceutical Biotechnology

EFFECT OF EARTHWORM ACTIVE PROTEIN ON FIBROBLAST PROLIFERATION AND ITS MECHANISM

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Fibroblasts play a vital role in tissue regeneration, restoration and reconstruction of wound healing, which could secrete extracellular matrix such as collagen and growth factors to stimulate wound healing. In recent years, many researches have demonstrated that the earthworm extracts could promote wound healing. However, its mechanism remains unknown. In our study, we investigated the effects of earthworm active protein (EAP) seperated and purified from fresh earthworms on mouse embryonic fibroblast (NIH/3T3) proliferation and its mechanisms. The main research content and results are as follows.

1. The Effect of EAP on NIH3T3 Cell Proliferation and Migration

The effects of EAP in different concentrations on NIH3T3 cells were detected by the MTT and Brdu incorporation assay. The results showed that EAP could promote NIH3T3 fibroblasts proliferation by increasing DNA replication in a dose-dependent manner. The cell cycle was detected by flow cytometry and the result showed that EAP could increase the percentage of S phase fraction; the following western blotting results showed that the expression level of Cyclin D1 protein which regulates the G1/S phase test point was significantly increased. Finally, the results of cell scratch experiments and the transwell chamber showed that EAP promoted the migration of NIH3T3 cell in a dose-dependent manner.

2. Possible Mechanism of EAP Promoting NIH3T3 Cell Proliferation

Different signaling pathway inhibitors were used to study the signaling pathways involved in the process of EAP promoting NIH3T3 cell proliferation. MTT assay and Western blotting results showed that the MEK inhibitors PD98059 and U0126 could significantly inhibit NIH3T3 cell proliferation and block the EAP activated ERK phosphorylation. Flow cytometry and Western blotting results showed that U0126 could significantly block cell cycle progression and the expression levels of Cyclin D1 protein. MTT assay showed that B-Raf and Raf-1 inhibitors had no significant effect on cell proliferation. MTT and Western blotting showed that PI3K inhibitor LY294002 significantly inhibited NIH3T3 cell proliferation and suppressed the Akt and ERK phosphorylation levels, which suggested that PI3K is also involved in the EAP-induced proliferation of NIH3T3 cells.

In summary, the mechanism of EAP promoting NIH3T3 cell proliferation should be as follows: EAP elevated CyclinD1 expression by activating MEK/ERK signaling pathway, and then promoted cell cycle from G1 to S phase, finally caused the proliferation of NIH3T3 cell. PI3K signaling pathway may be the upstream of MEK/ERK signaling pathway.

Keywords: Earthworm active protein, ERK, NIH3T3 fibroblasts, PI3K, proliferation.

PO-34

Track: Inflammation and Immunology

ADJUNCTIVE LIGUSTRAZINE MAY IMPROVE OUTCOMES IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS: A RANDOMIZED, CONTROLLED, PILOT STUDY

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Objectives: Ligustrazine has been clinically used as an adjunctive therapy in active rheumatoid arthritis (RA) treatment for a long time under the guidance of Chinese medicine therapeutic principles "activating blood circulation and resolving stasis", yet the clinical outcome hasn't been proved with randomized, controlled clinical trials. The aim of this study was to assess the effectiveness of ligustrazine as adjunctive therapy to leflunomide in patients with active rheumatoid arthritis.

Methods: In this proof-of-concept, randomized, controlled study involving 54 patients with active RA, 27 patients received Leflunomide (10mg/day orally) and 27 received Leflunomide (10mg/day orally) combined with Ligustrazine (for the first 2 weeks, 160mg/day, intravenously guttae; from the 3rd week, 50mg 3 times a day orally). The primary outcome was the change in Disease Activity Score in 28 Joints (DAS28) at 24 weeks. Secondary outcomes included the percentages of subjects with 20%, 50%, and 70% improvement in disease according to the American College of Rheumatology criteria (ACR20, 50, and 70), the 20% improvements in ESR and CRP. Health-related quality of life assessments (the Health Assessment Questionnaire) and safety were also compared between the two groups.

Results: At week 24, DAS-28 was significantly reduced in each group (by 2.1±1.3 in Lef +Lig group; 1.6±1.6 in Lef group), and there was a significant difference between the 2 groups (*P=0.0456*). Significantly more patients in Lef+Lig group achieved 20% improvements of CRP (86.96%) and ESR (80.00%) than in the Lef group (57.14% and 36.36%, respectively). Non-significant difference were detected in ACR 20, 50, 70, HAQ, and safety outcomes between the two groups.

Conclusions: Ligustrazine as an adjunctive therapy can effectivly and safely reduce the DAS-28, ESR, and CRP in patients with active RA than routine leflunomide therapy. These data suggest a potentially new drug in RA therapy.

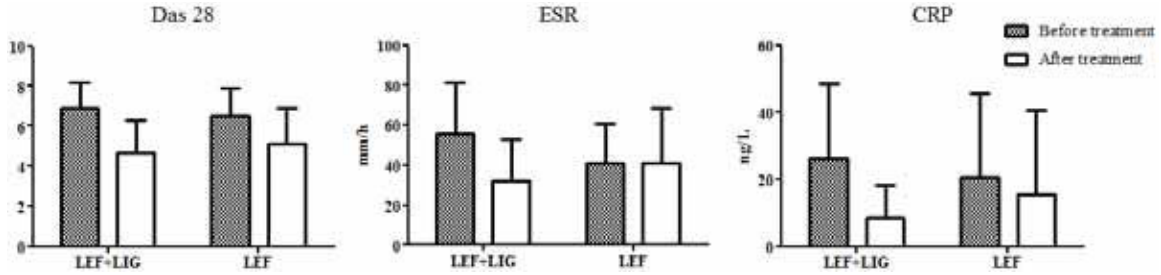

Fig. (1).

Keywords: Ligustrazine, leflunomide, rheumatoid arthritis, adjunctive therapy, randomized controlled trial.

PO-33

Track: CNS Drug Discovery & Therapy

THE COMPARISON OF ACETYLCHOLINESTERASE INHIBITORY ACTIVITY AND CYTOTOXICITY OF NEW DRUG CANDIDATES FOR ALZHEIMER'S DISEASE

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Cholinesterase inhibitors inhibiting acetylcholinesterase (AChE, EC 3.1.1.7) activity, maintain acetylcholine (ACh) level by decreasing its cleavage rate. The increasing level of ACh and duration of the neurotransmitter action leads to improvement of cholinergic transmission. Alzheimer's disease (AD) is associated with loss of cholinergic neurons in the brain and the decreased level of ACh. The important therapeutic approach in the AD therapy strategies is the inhibition of brain AChE. Nowadays the new drug discovery for therapy of neurodegenerative diseases is intensively studied. We compared the inhibition potency against cholinesterases and *in vitro* toxicity of tetrahydroaminoacridine (tacrine), 7-methoxytacrine (7-MEOTA) and adamantylamine with 8 newly prepared heterodimer derivatives. The AChE and butyrylcholinesterase inhibition activity was assessed on the recombinant human AChE by the Ellman's colorimetric method. Cytotoxicity all tested compounds was determined using the MTT assay after 24 h of incubation. Three human cell lines HepG2, SH-SY5Y, ACHN were employed. The relationship among inhibitory activity and cytotoxicity was studied.

This work was supported by the Ministry of Defence, Czech Republic - Long-term organization development plan 1011 and grant of the Ministry of Defence of the Czech Republic no. GAP303/11/1907.

Keywords: Alzheimer's disease, cholinesterase inhibitors, cytotoxicity, inhibition potency, tacrine.

PO-11

Track: Anti-Infectives

EFFECT OF AQUEOUS EXTRACT OF BARBERRY ON *PROPIONIBACTERIUM ACNES*

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Background: Acne is a dermatitis disease with world wide prevalence and *Propionibacterium acnes* is one of the agents in establishing acne. Plants have been effective role in the treatment of infectious diseases of the times past. On the other way microorganism resistance over industrial antibiotics lead to uses alternative plants barberry is full of antocianin and alkaloids, it has several application in medicine and food. So if the barberry has antibacterial effect on *Propionibacterium acnes* we can use it instead of industrial antibiotics. This study was aimed to determine the antibacterial effects of barberry on *Propionibacterium acnes in vitro.*

Methods and Materials: In this experiment study aqueous extract of barberry was examined for antibacterial activities on *Propionibacterium acnes* using the disk-diffusion (then compare the zone of inhibition of extract with antibiogram). Minimum inhibitory concentration (MIC) minimum bactericidal concentration (MBC) and serial dilution methods.

Results: Aqueous extract of barberry has an inhibitory effect on *Propionibacterium acnes*. The most inhibition zone was 23±1 mm in 200 mg/ml and the minimum inhibition zone in 50 mg/ml concentration was 7±1 mm. MIC was 25 mg/ml while MBC was 50 mg/ml.

Conclusion: The examination shows that aqueous extract of barberry has lethal effect on *Propionibacterium acnes* and we can use it as an antibacterial product in acnes treatment.

Keywords: Aqueous extract of barberry, *Propionibacterium acnes*, acnes.

PO-84

Track: Pharmaceutical Research & Development

COMPARISON OF THE CYTOTOXIC EFFECTS OF RG108 (A DNA METHYLTRANSFERASE INHIBITOR) AND TRICHOSTATIN A (A HISTONE DEACETYLASE INHIBITOR) ON PC-12 ADH CELLS

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Epigenetics is a rapidly growing field and holds great promise for a range of human diseases, including brain disorders. Recently, a role of epigenetic alterations in development of neurodegenerative diseases has been increasingly discussed. The DNA methyltransferase (DNMT) inhibitors and the histone deacetylase (HDAC) inhibitors are epigenetic drugs that hold promise to overcoming neurodegenerative disorders. RG108 is a DNMT inhibitor which inhibits the enzyme by blocking its catalytic site and Trichostatin A suppresses the activity of HDACs leading to an increase in histone acetylation. PC-12 Adh cell line was derived from rat pheochromocytoma. It is a useful model for studying cell signaling because differentiation, proliferation, and survival can all be assessed independently. To compare these two epigenetic cancer drugs cytotoxicity on PC12 Adh cells, viability of the cells was assessed by MTT assay at 24 and 48h. We found that both RG108 and Trichostatin A don't have cytotoxic effects at 24h. Trichostatin A showed cytotoxic effetcs at higher concentrations (100, 200ĀM) after 48 hours but RG108 didn't show any cytotoxic effects even in high concentrations. Subsequently, we morphologically showed that RG108 induced neurite outgrowth at 10 and 100nM concentrations according to the control group.Therefore RG108 is a potential agent for neurodegenerative diseases.

Keywords: Cytotoxicity, PC-12 Adh, Trichostatin A, RG108.

PO-18

Track: Inflammation and Immunology

MODELLING NEUROGENIC INFLAMMATION IN THE HUMAN ORAL CAVITY

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Salivary fluid from the human oral cavity can provide a novel, non-invasive model for the extravasation of leukocytes, which can be used to study neurogenic inflammation. The aim of this project was to confirm and build upon previous works. To corroborate past work volunteers were given 10% Tabasco sauce to stimulate the TRPV1 receptor. Furthermore Mustard powder stimulation was used to induce neurogenic inflammation *via* activation of the TRPA1 receptor. With the use of a combined Tabasco and Mustard solution the possibility of dual activation of TRPV1 and TRPA1 receptors was investigated.

The eleven volunteers recruited were given three different solutions a week apart: 10% Tabasco sauce, 15% Mustard powder and a combination of the two. Volunteers would swill one chosen stimulant for 30 seconds. Saliva sample collection was with a 0.9% saline wash out 1-hour pre-stimulation, immediately before stimulation and 1-hour post stimulation. Leukocytes were extracted and analysed for count and reactive oxygen species (ROS) with the use of 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) and flow cytometry.

Tabasco stimulation caused increased leukocyte counts (157%, $p<0.05$ Wilcoxon) and ROS production confirming previous findings. Mustard stimulation also caused increased leukocyte counts (209%, $p<0.05$ Wilcoxon) and ROS

production. Tabasco had an increase of 354% in Median Fluorescent Intensity (MFI) and Mustard 281%. Tabasco and Mustard combined solution did increase Leukocyte counts (186%) and ROS species (291%) however were not of statistical significance. On comparison of stimulants mustard was the strongest neuro-inflammatory stimulus for leukocyte migration with a 216% increase in count.

The human oral cavity is an important non-invasive model for studying neurogenic inflammation. Neurogenic inflammation in the oral cavity can be elicited *via* both TRPV1 and TRPA1 activation but the degree of cross-talk between these two pathways is unclear. Further work on TRPV1 agonists potentially modulating TRPA1 activity is needed.

Keywords: Inflammation, immunology, neuropathic pain.

PO-95

Track: Cancer Targeted Drug Delivery

LOSS OF TUMOR SUPRESSOR GENE (*TP53*) FUNCTIONS IN THE PATIENTS OF ORAL SQUAMOUS CELL CARCINOMA OF PAKISTAN

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Oral squamous cell carcinoma (OSCC) is the leading cause of death in the developing countries like Pakistan. The major risk factor for developing OSCC is the excessive chewing habit of *paan* (betel quid) *chaliya* (betel nut), tobacco, *niswar* (type of dipping tobacco, made from fresh tobacco leaves, calcium oxide, and wood ash) *gutka* (a preparation of crushed betel nut, tobacco and sweet or savory flavors) and *manpuri* (the powder of betel nut, tobacco and slaked lime). Mutagens can damage DNA and generate promutagenic lesions. This study aims to find out the loss of tumor supressor gene (*TP53*) functions due to mutation/polymorphism caused by genomic alteration and interaction with tobacco and its related ingredients in Pakistan.

The change of single nucleotide may be responsible for the substitution of amino acid in the p53 protein. This may result in the germ line mutation(s) of the *TP53* due to chewing habits which are involved in different steps of tumourgenesis and increasing the susceptibility of OSCC in Pakistan.

Global analysis of this signaling pathway could be evaluated more exhaustively by a mutational approach associated with an expression. Genetic profiling and molecular control of various pathways will allow more accurate diagnosis and assessment of the prognosis of oral cancers and may lead to novel approaches in early diagnosis and therapy of the disease.

Future collaboration could be beneficial for the analysis of the altered protein products and for the assessment of their three dimensional structural abnormalities to develop the safe and secure society in Pakistan.

Keywords: Missence mutations, p53 gene, oral squamous cell carcinoma.

PO-97

Track: Cancer Targeted Drug Delivery

ANTICANCER DRUGLOADED MICROCAPSULE/HYDROGEL AS DUAL-DRUG DELIVERY SYSTEM FOR CANCER THERAPY

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Drug delivery systems have a variety of systems such as particulate carriers, polymer gels, lipids etc. However, simple drug delivery systems cannot fulfill the needs of such therapy. Therefore, developing the DDDS which can control the release behavior of each drug is desired. In this study, we report an antitumor activity of the DDDS based on microcapsule/hydrogel for cancer therapy.

Doxorubicin-loaded microcapsules (Dox-Cap) were prepared using a mono-axial nozzle ultrasonic atomizer with a Dox core and a PLGA shell. The formations were prepared by mixing Dox-Cap and 5-fluorouracil-loaded Pluronic (5Fu-PL) or methoxypoly (ethylene glycol)-b-(polycaprolactone-ran-polylactide) (5Fu-MCL) solution. We measured viscosity of PL and MCL hydrogels for the evaluation of thermo-sensitive property. *In vitro* Dox and 5Fu release behavior of Dox-Cap/5Fu-hydrogels were examined at 100 rpm at body temperature. TheDox-Cap only, Dox-Cap/5Fu-hydrogels were examined for anti-proliferative effects against B16F10 cancer cells cultured *in vitro*. In addition, Dox delivery extension into cells was measured using fluorescence microscopy.

The experimental result shows that, all formulation exhibited sol-to-gel phase transition at around body temperature. The Dox and 5Fu release was controlled by PL and MCL hydrogels in which viscosity of hydrogels was an important factor. B16F10 after addition Dox-Cap only, Dox-Cap/5Fu-hydrogels, B1610 cancer cell viability decreased over a period of 3 days. In particular, Dox-Cap/hydrogels almost completely inhibited cell proliferation.

In conclusion, this work indicates that DDDS using Dox-Cap/5Fu-hydrogels can act effectively to induce long-lasting inhibition in the cancer cell growth.

PO-116

Track: Inflammation and Immunology

EXTRACTION, CHARACTERIZATION AND BIOMEDICAL APPLICATIONS OF CHITOSAN EXTRACTED FROM CRAWFISH SHELL

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The present study was undertaken to define the optimal conditions for the extraction of ultra-pure chitin and chitosan from crawfish shell wastes and fixation of chitosan into cellulose fibres. Chitin was extracted by deproteinization and demineralization steps. The optimal conditions as follows: the deproteinization was carried out by the use of 5 % NaOH at 90 ^{o}C for 18 h and protein content was measured, the demineralization was completely achieved with 10 % hydrochloric acid at ambient temperature for 2h. Chitosan was obtained by removing the acetyl group from chitin in a deacetylation process. The obtained chitin and chitosan were characterized by elemental analysis, UV-Vis spectra, SEM, ICP-OES, NMR, and X-ray diffraction. Chitosan and cellulose fibres were covalently joined by the use of citric acid as a chemical bridge. Since polyfunctional citric acid is able to “produce” ammonium groups of chitosan moiety, the prepared wound cover samples had excellent antibacterial activity against Gram-negative bacteria *Escherichia coli* (*E. coli*) and Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*). From the data of cytotoxicity of the extracted biopolymers, we could conclude that chitin and chitosan didn't show any toxicity against mouse fibroblast cell line (NIH-3T3) and keratenocytes cell line HaCaT. The healing properties of the extracted chitosan were evaluated in rats; chitosan promoted and accelerated the healing rate. We believe that the extracted chitin and chitosan can be used for different medical applications, especially in wound healing and dressing areas.

PO-59

Track: Drug Discovery in Preclinical Research

SELECTIVE INHIBITORS OF MAPK-INTERACTING KINASES (MNK1/2) EXHIBIT A LACK OF PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIP

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We identified three highly selective inhibitors with a biochemical Mnk1/2 inhibitory activity (IC_{50} values ranging between 4 and 22 nM). The compounds inhibited p-eIF4E in HCT-116 cells with an IC_{50} value of <1 uM. The objective of this study was to evaluate the pharmacokinetic-pharmacodynamic (PK-PD) relationship of these inhibitors. To that end, the compounds were dosed intraperitoneally or orally at 30 and 100 mg/kg in SCID mice bearing HCT-116 tumors. All three compounds showed significant plasma exposures (above the HCT-116 *in vitro* cellular IC50) for 4 – 8 hours post dose. The maximum inhibition of p-eIF4E in the tumor compared to vehicle treatment was about 60% (in contrast to almost complete cellular inhibition) in spite of the tumor compound concentrations being up to 65X above the p-eIF4E *in vitro* IC_{50}. The inhibition of tumor p-eIF4E did not correlate with tumor compound concentrations. Approximately 50-60% inhibition of *in vivo* tumor p-eIF4E was observed with tumor compound concentrations both significantly above the *in vitro* HCT-116 cellular p-eIF4E IC_{50} as well as in some cases significantly below the *in vitro* HCT-116 cellular p-eIF4E IC_{50}. Furthermore, a significant induction of p-Mnk was observed concurrently with inhibition of p-eIF4E both *in vitro* and *in vivo*. In conclusion, selective inhibitors of Mnk1/2 exhibited an *in vitro – in vivo* disconnect in modulation of eIF4E phosphorylation, and a lack of PK-PD relationship *in vivo*. This may be due to an incomplete understanding of Mnk related signaling pathways and feedback loops, lack of predictive *in vitro* models to determine Mnk response *in vivo*, or a lack of complete understanding of the cellular role of p-eIF4E in MNK pathway *in vivo*.

PO-78

Track: CNS Drug Discovery & Therapy

ABAD INHIBITORS/MODULATORS AS POTENTIAL AD TREATMENT STRATEGY

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Although the aetiology of Alzheimer's disease (AD) is still unknown, the build-up of intracellular amyloid Ā-peptide (AĀ) is considered to play a principal role in the pathogenesis of the disease. Amyloid-binding alcohol dehydrogenase (ABAD) is currently the most characterized AĀ-binding intracellular protein. AĀ binding to ABAD triggers a series of events leading to mitochondrial dysfunction characteristic for AD. Thus this interaction may represent a novel target for treatment strategy against AD. Our aim is focused on the blockade of the ABAD-AĀ interaction by small molecule inhibitors, analogues of benzothiazolyl urea.

Methods: Novel compounds were synthesized using standard chemical procedures. The crude products were purified by crystallization. Identity and purity of all compounds was verified by melting point, ^{1}H and ^{13}C NMR, ESI-MS and elementary analysis.

Results: A series of 28 benzothiazolyl urea analogues was synthesized. *In vitro* evaluation of their ability to inhibit ABAD showed very promising and significant results compared to standards.

Conclusions: Novel compounds, potential ABAD inhibitors were designed, synthesized and tested. Their *in vitro* ability was significant and they are matter of further investigation.

Keywords: Alzheimer's disease, ABAD, urea analogues, interaction.

PO-22

Track: Cancer Targeted Drug Delivery

HIGHLY ACTIVE AND SELECTIVE INHIBITORS OF THE BREAST CANCER RESISTANCE PROTEIN

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Cross-resistance of tumor cells to cytotoxic drugs constitutes a critical hurdle to cancer therapy. One of the most investigated mechanisms is the energy-dependent efflux of a large panel of cytotoxic agents mediated by ATP-binding cassette (ABC) transporters. Overexpression of these transmembrane efflux pumps in cancer cells alters anticancer drugs potency by significantly reducing their intracellular accumulation. One of the lately discovered efflux pump that play a major role in the efflux of anticancer drugs is the Breast Cancer Resistance Protein (BCRP/ABCG2).

One of the strategies developed to abolish tumor resistance to chemotherapy is the design of selective and nontoxic inhibitors of ABC transporters involved in cross-resistance. The therapeutical use of such inhibitors will be their combination with anticancer drugs in order to overcome the resistance. Intense investigations were conducted aiming to develop potent inhibitors of ABCG2 leading to the development of ABCG2 inhibitors, used as reversal agents in combination with anticancer drugs.

We recently identified a chromone derivative, 5-(4-bromobenzyloxy)-2-(2-(5-methoxyindolyl)ethyl-1-carbonyl)-4*H*-chromen-4-one (Fig. **1**) as a potent, selective, nontoxic and non-transported inhibitor of ABCG2-mediated drug efflux [2]. In order to study the structure-activity relationships controlling both drug efflux and ATPase activity of ABCG2, we have now synthesized and investigated a new series of chromone derivatives [3]. Herein we describe the design, the synthesis and the pharmaceutical evaluation of this new series of BCRP inhibitors.

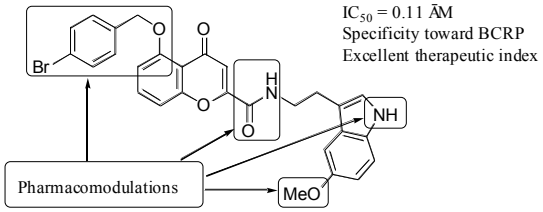

Fig. (1). Structure of a representative inhibitor of BCRP derived from chromones.

Keywords: Multidrug resistance, ABC transporters, ABCG2, inhibitors.

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PO-38

Track: CNS Drug Discovery & Therapy

MURINE URINARY BIOMARKERS PROVIDE SIGNATURES OF AD PROGRESSION AND THE THERAPEUTIC EFFECTS OF MODIFIED WEN-DAN DECOCTION

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Aims and Objectives: Urine samples potentially contain information pertaining to physiologic status, pathologic conditions and treatment efficacy, etc. We aimed to investigate whether the urine samples from transgenetic Alzheimer's disease model mice could provide biomarkers that may indicate the progression of the disease and the therapeutic effect of herbal medicine prescription, modified Wen-dan decoction, against it.

Methods: Headspace solid-phase micro-extraction and gas chromatography-mass spectrometry (HS-SPME-GC/MS) was applied to detect and identify the volatile organic components (VOCs) released from 24-hour urine samples of 3-month/5-month old Model group mice [male Prp-hAPP/hPS1 transgenetic mice, (C57BL/6J-TgN (APP/PS1) ZLFILAS)], Drug treatment group mice [male Prp-hAPP/hPS1 transgenetic mice receiving modified Wen-dan decoction treatment], and Vehicle control group mice [C57BL/6J mice].

Results: Specifically, we identified groups of VOCs that varied significantly and constantly through the progression of AD in the model group samples compared with vehicle control group. Interestingly, chemometric evaluation indicated that the amounts of some of these VOCs between drug treatment group urine samples with vehicle control group samples were similar. Subsequently, 7 VOCs were identified as potential biomarkers for mutation status confirmation, and another 4 VOCs were considered to be correlated to the progression AD and might be useful for treatment efficacy monitor.

Conclusions: Monitoring VOCs of urine samples using SPME-GC/MS is efficient to follow onset progression of AD and may be applied for non-invasive investigation of the treatment efficacy.

ACKNOWLEDGEMENTS

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Keywords: Alzheimer's disease, biomarker, SPME-GC/MS, urine, volatile organic components.

PO-107

Track: Process Chemistry and Drug Manufacturing

INNOVATIVE METHOD FOR PREPARATION OF LIPOSOMES

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Encapsulation/micro-encapsulation is a technique that is widely used in pharmaceutical, cosmetic and textile industries. For per-oral administration, liposomes whose membrane is composed of lecithin

(phosphatidylcholine) are very popular. Lecithin is an amphiphilic surfactant of plant (soy, rape) or animal (egg yolk) origin. The technology of preparation of lecithin liposomes for pharmaceutical purposes is problematic due to the use of organic solvents in their preparation because these organic solvents cannot be completely removed from the formed liposomes. We describe a new method for the preparation of capsules which is based on a purely aqueous system and finally using a spray dryer to prepare the "dry" capsules. For example, liposome of sodium chloride and a food supplement called mummy liposome were prepared with this technique. The prepared liposomes were visualized by Electron Scanning Microscope (EMS), their size was determined by dynamic light scattering (Zeta-Sizer).

Keywords: Encapsulation/micro-encapsulation, liposomes.

PO-7

Track: Drug Delivery & Targeting

SYNTHESIS OF PAMAM MEGAMER MI (GN-GM) FOR GENE CARRYING AND DRUG DELIVERY

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Dendrimer synthesis strategies now provide virtual control of macromolecular nanostructures as a function of size and surface/interior functionality. These strategies involve the covalent assembly of hierarchical components reactive monomers, branch cells or dendrons around atomic or molecular cores according to divergent/convergent dendritic branching principles.Combinon of two or more dendrimers led us to new macromolecule which called as Megamers. The Megamers is constricted by two specific parts so called as core and shell. Core and shell could be the same or different dendrimer. In this study Gn and Gm of PAMAM dendrimers with various generation and - NH_2 and - COOH end groups have been synthesized and formed as megamers. This product has the ability to carry drugs and genes, also particularly drug delivery. The Megamer have been characterized by HNMR, CNMR, GPC, ATR-FTIR analytical instrument.

Keywords: Megamer, PAMAM dendrimers, drug delivery, gene carrying.

PO-49

Track: CNS Drug Discovery & Therapy

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL ISOQUINOLINE COMPOUNDS AS POTENTIAL ANTIDEPRESSANT AGENTS

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The World Health Organization (WHO) report has predicted that major depression will become a key cause of illness-induced disability by the year 2020, second only to ischemic heart diseases. Despite of a large number of antidepressant drugs commercially available, there are many issues lead to risks of depression therapy. Consequently there is a desirable need to find new chemical entities as potential antidepressant candidates with novel underlying mechanism, which may lead to a more advantageous benefit-risk balance. In this study, a series of novel isoquinoline compounds were designed, synthesized and screened for their antidepressant-like activities *in vitro* and *in vivo*. The values for two descriptors (ClogP, tPSA) of the blood-brain barrier (BBB) were calculated for early assessment of their central nervous

system (CNS) drug-likeness. Several compounds demonstrated potential protective effects on corticosterone-induced lesion of PC12 cells. Three promising compounds were further evaluated for their *in vivo* effects by force swim test (FST) and open field test (OFT) in C57 mice models. The FST results showed that compounds 40 remarkably reduce the immobility time of the mice, much more efficacious than Agomelatine and comparable to Fluoxetine. The OFT results showed that mice treated with compound 40 travelled a longer distance than those treated with Agomelatine or Fluoxetine, displaying a better general locomotor acitivity. Relevant of cellular and molecular studies were also conducted and compound was found to up-regulate mRNA of BNDF *in vitro*, which present us a possible underlying mechanism of the active compound 40 as a potential antidepressant agent.

PO-96

Track: Inflammation and Immunology

THE STUDY OF ANTI-VIRAL EFFECTS OF THE NOVEL HERBAL MEDICINE KIOM-C

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The aim of this study is development of novel herbal medicines. To evaluate the anti-viral effect of KIOM-C, we examined the antiviral activity against human influenza viruses (H1N1 and H5N1) *in vitro* experiments. We also investigated the anti-viral effect of KIOM-C *in vivo* experiments using H1N1-infected mouse and ferret models. Furthermore, to enhance the convenience for taking, we developed the formulation of KIOM-C, which had more efficacy than that of previous formulation. Additionally, KIOM-C exhibited antiviral efficacy against Enterovirus 71(EV71), Newcastle disease virus (NDV) and Vesicular stomatitis virus (VSV). We already completed the safety tests including acute toxicity, reverse mutation test, micronucleus test, and chromosome aberration test. KIOM-C has also anti-viral efficacy against PCVAD (porcine circovirus associated disease) and are commercialized as feed additives for pig.

PO-28

Track: Inflammation and Immunology

EFFECTIVENESS OF THE NOVEL HERBAL MEDICINE, KIOM-MA128 ON THE TREATMENT OF ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a chronic inflammatory skin disease caused by cutaneous hyperreactivity to environmental triggers.We developed the novel herbal medicine, KIOM-MA, and its Lactobacillus-fermented product, KIOM-MA128, for the treatment of AD. To evaluate their oral administration efficacy on AD, BALB/c mice AD-induced by Ovalbumin and aluminum hydroxide were treated with KIOM-MA, KIOM-MA128, and dexamethasone. Next, using NC/Nga mice treated with 2, 4-dinitrochlorobenzene, we compared their application effectiveness with Elidel cream® containing 1% pimecrolimus. KIOM-MA128 reduced major clinical signs of AD including erythema/darkening, edema/papulation, excoriations, lichenification/prurigo, and dryness. KIOM-MA128 significantly decreased IgE level in the plasma and reduced scratching behavior, skin severity in the mouse models. After the safety of KIOM-MA128 was verified in Good Laboratory Practice system, we tested the

therapeutic effect of KIOM-MA128 on AD patientsthrough the analyses of Eczema Area and Severity Index score, Transepidermal Water Loss, Erythema index. KIOM-MA128 significantly improved AD symptoms at 4 weeks post-treatment. To elucidate the molecular mechanism of KIOM-MA128 effect, we investigated the effect of KIOM-MA128 on inflammatory mediators in RAW 264.7 cells. KIOM-MA128 decreased the production of nitric oxide and proinflammatory factors by LPS stimulation. Taken together, our results suggest KIOM-MA128 has potential as therapeutic reagent for AD.

PO-82

Track: Drug Discovery in Preclinical Research

SELECTIVE ANTI-BREAST CANCER ACTIVITY OF NEO-TANSHIONLACTONE THOROUGH INHIBITION OF GLYCOLYSIS

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Neo-tanshionlactone was isolated from Tanshen, the rhizome of Salvia miltiorrhiza Bunge, with selective antibreast cancer activity. Cell proliferation assay showed that neo-tanshionlactone is active against both Estrogen Receptor positive (ER+) breast cancer cell line MCF-7 and human Epidermal Growth Factor Receptor 2 overexpressing (HER2+) breast cancer cell line SK-BR-3, but was inactive against the triple-negative breast cancer cell lines MDA MB-231 as well as the none breast cancer cell line LNCaP. The selectivity of neo-tanshionlactone against breast cancer cell lines was confirmed by apoptosis assay and clony formation assay. Which implies neo-tanshionlactone could be a potential targeted therapy reagent against both ER+ and HER2+ breast cancer. To unveiling the mechanism of action of neo-tanshionlactone's selectivity, we constructed the PCR-array consisting of major pathways in breast cancer initiation, development and metastasis. It was showing that Pyruvate kinase M2 (PKM2) is significantly down regulated upon neo-tanshionlactone treatment in the two response cell lines while not in MDA MB-231 and LNCaP cells. Western blot analysis also showed decreased protein level of PKM2 and this was further confirmed by accumulation of its substrate phosphoenolpyruvic acid (PEP) as well as decreased intracellular level of pyruvate. Since increased aerobic glycolysis is one of the hallmarks of cancer cells and PKM2 play important role in this pathway, we reasoned that both ER+ and HER2+ breast cancer cells may be addicted to aerobic glycolysis for their survival and neo-tanshionlactone exert its selective activity against these cells by inhibiting this pathway.

Keywords: Neo-tanshionlactone, breast cancer, PKM2.

PO-65

Track: In-Silico Drug Design and In-Silico Screening

COMBINING QSAR MODELS WITH A LIGAND-BASED PHARMACOPHORE MODEL FOR THE EVALUATION OF NEW POTENTIAL ANTIOXIDANTS

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Free radicals are highly reactive chemical species that can cause oxidative stress, which can result in damage to biological macromolecules and increases the risk of various diseases. Norbadione A (a pulvinic acid derivative) was found to display a very good antioxidant activity and thus represents a promising starting point in search for new antioxidants.

We have developed predictive QSAR models, based on data from *in vitro* studies, where

the antioxidative activity of pulvinic acid and structurally related coumarine derivatives was evaluated. Multiple linear regression (MLR), counter-propagation artificial neural networks (CP-ANN) and support vector machines (SVM) were used for the modeling. All models have been developed in accordance with the OECD, including the determination of validation parameters and the assessment of the applicability domains. The models with satisfactory parameters were further used to predict the activity of 239 new compounds, bearing a vinylog fragment.

Parallel to this a ligand-based pharmacophore model was constructed on the basis of most active pulvinic acid derivatives and validated using a ROC curve. Pharmacophore screening was employed and the most promising 271 compounds were chosen for evaluation with the QSAR models.

The predictions from QSAR models are regarded as valid only for the compounds that lie inside the applicability domains, and we found out the use of a pharmacophore model for selecting the screening molecules decreased the percentage of structural outliers in comparison with using a substructure search, especially for SVM and MLR models.

Keywords: Antioxidants, pharmacophore model, QSAR model.

PO-39

Track: Drug Delivery & Targeting

STUDIES ON THE APPLICABILITY OF NATURAL POLYMER, *TERMINALIA CATAPPAGUM* IN THE DESIGN OF MUCOADHESIVE DRUG DELIVERY SYSTEM

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Background: The present investigation targets the applicability of natural polymer, *Terminalia Catappa* (TC) gumin the design of mucoadhesive drug delivery system using a model drug, propranolol HCl.

Methods: TC gum was evaluated for all physicochemical properties. Direct compression method was employed for the preparation of mucoadhesive tablets of propranolol HCl (80 mg) using different concentrations of TC gum. The tablets were evaluated for all tableting properties, *ex vivo* bioadhesive strength, *ex vivo* residence time and *in vitro* dissolution studies. FTIR studies were conducted to study the drug polymer interaction.

Results: Results showed that TC gum is a free flowing material and having high swelling index (3250 %). All the tablet formulations were as per the specifications of the official compendia of tablets. The bioadhesive strength and *ex vivo* residence time values were between 4.5 to 10.2 dyne/cm^2 and 8 to 16 h respectively. Formulation containing drug to TC gum ratio (1:2.5) was selected as an optimized formulation based upon its mucoadhesive strength and the drug retardation. FTIR studies proven that there was no interaction between the drug and polymer.

Conclusion: Based on the above positive results, it can be conclude that TC gum is a suitable polymer for the mucoadhesive drug delivery systems.

PO-103

Track: Regenerative Medicine

BIODEGRADABLE AMINO ACID-BASED POLYMERIC MICROPARTICLES FOR IMPROVED FUNCTIONAL RECOVERY IN STEM CELL THERAPY AFTER STROKE

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No long term effective treatments are currently available for functional recovery after stroke. Although cell therapy is a promising strategy [1-4], it's still incomplete as cell survival, differentiation and restoration of lost tissue are minimal.

New developments, such as the biomimetic approach of using the scaffolds/microparticles loaded with different bio-active factors, may improve the control of cell proliferation, survival, migration, differentiation and engraftment *in vivo* [5-7].

Microparticles (MP), loaded with growth factors BM4 and wnt3a were prepared by double emulsion solvent evaporation technique, using originally developed biodegradable poly(esteramide) 4F4 [8]. Particles with spherical shape and porous surface, in the size range of 5-50μm were monitoring by scanning electron microscopy. After 4 months transplantation of MP in rodent brain, no inflammation was observed.

Preliminary data indicated, that growth factors can successfully release from MP and affect on cells differentiation *in vitro*.

Overall, biomaterials can direct the differentiation of stem cells for regenerative medicine applications.

Keywords: Microparticles, stem cell therapy, stroke.

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PO-4

Track: In-Silico Drug Design and In-Silico Screening

THE INFLUENCE OF *MYCOBACTERIUM TUBERCULOSIS* GYRB MUTANTS ON 6-FLUOROQUINOLONES RESISTANCE DEGREE: *IN SILICO* MUTAGENESIS AND STRUCTURE-BASED EVALUATION

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Despite the therapeutic success of 6-fluoroquinolone (6-FQ) antibacterials in tuberculosis chemotherapy, the daily reports related to development of different forms of quinolone-caused "acquired resistance" in *Mycobacterium tuberculosis* are becoming rather frequent. Alongside the extensively reported mutations targeting predominantly the quinolone resistance-determining region (QRDR) of the gyrA subunit, some recent studies are pointing out the emergence of several gyrB point mutations additionally contributing to the *M. tuberculosis* resistance. To clarify the influence of gyrB alterations on 6-FQs resistance, *in silico* mutagenesis and structure-based methodology were proficiently employed. Three *M. tuberculosis* gyrB mutant models (N473Tmod, T474Pmod, and E475Vmod) based on the lately confirmed structural data were constructed. The established mutant models were subsequently employed as a starting point for performing structure-based calculations on a set of 145 6-FQs with experimentally-determined biological activity values, while their resistance profiles (capability for identification of active/inactive 6-FQs) were

assessed relative to that of the wild-type (WT) model. This profiling methodology propound the following order of resistance degree for our models (T474Pmod < E475Vmod < N473Tmod < WT) that was additionally confirmed by molecular docking of a set of pre-selected 48 combinatorially-generated 6-FQ hits. Moreover, we identified several attractive, synthetically feasible substructural fragments that could promote the development of novel 6-FQs with possible enhanced anti-mycobacterial activity against various *M. tuberculosis* gyrB mutant strains.

Keywords: Tuberculosis; 6-Fluoroquinolones; DNA gyrase; gyrB mutants; *in silico* mutagenesis; Structure-based design.

PO-104

Track: CNS Drug Discovery and Therapy

FORMULATION AND EVALUATION OF DILTIAZEM SUSTAINED RELEASE TABLETS

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Sustained releases tablets of Diltiazem hydrochloride were formulated by employing hydroxypropyl methylcellulose (HPMC K100 M) and the sustained release behaviour of the fabricated tablets was investigated. Sustained release matrix tablets containing 120 mg Diltiazem hydrochloride were developed using different drug: polymer (HPMC K100 M) ratios. Tablets were prepared by wet granulation technique. Formulation was optimized on the basis of acceptable tablet properties and *in vitro* drug release. The resulting formulation produced robust tablets with optimum hardness, consistent weight uniformity and low friability. All tablets but one exhibited gradual and near-complete sustained release for Diltiazem hydrochloride (96-100%) at the end of 24 h. The results of dissolution studies indicated that formulation B5 (drug to polymer 1:1.25) was found to be most successful as it exhibits drug release pattern very close to theoretical release profile. A decrease in release kinetics of the drug was observed on increasing polymer ratio.

Keywords: Diltiazem hydrochloride, hydroxypropyl methylcellulose (HPMC K100 M), sustained releases tablets.

PO-10

Track: Pharmaceutical Biotechnology

DRUG LEADS ISOLATED FROM MARINE NATURAL SOURCES FOR SELECTIVE CANCER THERAPY

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Compounds derived from natural sources as marine algae are well known to possess an important therapeutic potential. New Caledonia's lagoon is home to an extremely rich marine biodiversity which remains relatively unexplored. Because of their originality with respect to depth and particular localization (extreme environment), two red algae species found only in the South Pacific Ocean were collected and for the first time studied for their anti-proliferative potential.

Following automated extractions (Dionex) of algal samples with solvents of different polarities, crude extracts were screened for their anti-proliferative properties by MTT cytotoxic assay on several human cancer cell lines including orphan and pediatric malignancies.

We have uncovered potent and cell-selective *in vitro* anticancer activity of algal crude extracts.

The most promising extracts were run through HPLC to isolate and purify active molecules. 1H NMR analyses showed that the isolated compounds are mainly bioactive lipids and aromatics. Molecular characterization by GC-MS is on going.

Our findings may lead to the discovery of new molecules that could be promising candidate leads for the development of innovative new medicines for the treatment of cancer, especially for malignancies with unmet medical needs.

PO-83

Track: Hot Topics in Natural Products

DISCOVERY OF SPONGE-DERIVED CYTOTOXIC METABOLITES *VIA* DOWN-REGULATING THE LEVEL OF Ā-CATENIN

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Deregulation of Wnt/! -catenin signaling promotes the development of a broad range of human cancers, and is thus a potential target for the development of anticancer agents. In our ongoing efforts targeting discovery of sponge-derived anticancer metabolites, we have isolated a series of sesquiterpenoids and evaluated their anticancer potential. Herein we suggest the sponge-derived sesquiterpenoids as a new chemical scaffold to suppress Ā-catenin response transcription *via* degradation of Ā-catenin, and thus exerting anti-proliferative activity on myeloma and colon cancer cells.

Keywords: Wnt/! -catenin signaling; anticancer metabolites; sponge-derived sesquiterpenoids.

PO-16

Track: Anti-Cancer Discovery & Therapy

ANTIOXIDANT AND PHYTOCHEMICAL SCREENING OF *PHILENOPTERA VIOLACEA* AND *XANTHOCERCIS ZAMBESIACA* LEAF, FLOWER & TWIG EXTRACTS

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The aim of this study was to investigate the antioxidant activity and phytochemical contituents of *Philenoptera violacea and Xanthocercis zambesiaca* leaf, flower & twig extracts. The scavenging activities *Philenoptera violacea and Xanthocercis zambesiaca* extracts were determined by 2, 2- diphenyl-1-picrylhydrazyl (DPPH) assay and were compared with standard antioxidant (ascorbic acid). *Xanthocercis zambesiaca* showed moderate (50%) free radical scavenging activity, while *Philenoptera violacea* extract had low free radical scavenging activity at the concentration of 2.5 mg/ml when compared to ascorbic acid. Qualitative phytochemical analysis of these plant extracts confirmed the presence of tannins, flavonoids, steroids, terpenoids, alkaloids and cardiac glycosides from *Philenoptera violacea* extract, while *Xanthocercis zambesiaca* extract showed the presence of flavonoids, saponnins, terpenoids and glycosides. Our results indicate that, *Xanthocercis zambesiaca* extract is a weak source of antioxidant and *Philenoptera violacea* extract has a number of phytochemical compounds.

Keywords: *Philenoptera violacea*, *Xanthocercis zambesiaca,* phytochemical constituents, Antioxidant activity.

PO-67

Track: Pharmaceutical Biotechnology

A NOVEL HERBAL MEDICINE KIOM-MA EXERTS ANTI-INFLAMMATORY EFFECT IN LPS-STIMULATED RAW 264.7 MACROPHAGE CELLS

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KIOM-MA was recently reported as a novel herbal medicine effective for atopic dermatitis and asthma. In this study, we have demonstrated the inhibitory effect of KIOM-MA on pro-inflammatory mediator produced in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. KIOM-MA significantly inhibited the expression of inducible nitric oxidesynthase (iNOS) and cyclooxygenase (COX)-2 as well as nitric oxide (NO) and prostaglandin (PG) E2. Consistent with inhibitory effect on PGE_2, KIOM-MA suppresses LPS-induced migration of macrophages and gelatinase activity, expression of matrix metalloprotease (MMP)-9 in a dose-dependent manner. Additionally, KIOM-MA showed strong suppressive effect on inflammatory cytokines production such as tumor necrosis factor (TNF)-Ā andinterleukin (IL)-6. We also found that KIOM-MA inhibits the activation of nuclear factor (NF)- B and represses the activity of extracellular signal-regulated kinase (ERK), p38 and c-Jun NH_2-terminal kinase (JNK) mitogen-activated protein kinases (MAPKs). Taken together, we elucidated the mechanism of anti-inflammatory effect of KIOM-MA using RAW 264.7 cells stimulated by LPS.

PO-58

Track: Pharmaceutical Biotechnology

A NOVEL HERBAL MEDICINE, KIOM-C, SUPPRESSES THE METASTATIC POTENTIAL AND INDUCES CELL DEATH OF HIGHLY MALIGNANT TUMOR CELLS

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KIOM-C, a novel herbal formula, was reported to be effective for treating pigs suffering from porcine circovirus-associated disease. In addition, administration of KIOM-C promoted clearance of influenza virus *via* production of antiviral cytokines, such as TNF-Ā and IFN-Ä In this study, we observed inhibitory properties of KIOM-C in migrationand invasion. Matrix metalloproteinase-9 (MMP-9) activity in HT1080 cells was dose-dependently decreased by KIOM-C treatment *via* suppression of NF- B activation. Daily oral administration of KIOM-C at 170 and 510 mg/kg efficiently blocked lung metastasis of B16F10 cells in C57BL/6J mice. Following KIOM-C treatment (500 and 1000 μg/ml), the extent of caspase-3 activation, PARP cleavage, Beclin-1 expression, and LC3-II conversion was remarkably up-regulated. In particular, the JNK-specific inhibitor SP600125 blocked KIOM-C-induced ROS generation and CHOP expression almost completely, which consequently almost completely rescued cell death, indicating that JNK activation plays a critical role in KIOM-C-induced cell death. In addition, we also observed that daily oral administration of KIOM-C at 85 and 170 mg/kg efficiently suppressed the tumorigenic growth of HT1080 cells in athymic nude mice, without systemic toxicity. Collectively, our results suggest that KIOM-C is a potential therapeutic formula that is useful as a safe herbal medicine for controlling metastatic malignant cancer.

PO-110

Track: Women's Health Drug Discovery & Therapy

TRANSIENT FETAL GOITER AND HYPOTHYROIDISM CAUSED BY MATERNAL POTASSIUM IODINE

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Excessive iodine intake causes goiter and hypothyroidism, so called Wolff-Chaikoff effect. Fetal goiter caused by maternal excessive Potassium Iodide (PI) often causes tracheal compression and neurodevelopment complications. Here we report a successful management of fetal goiter, followed by fetal thyroid volume using 3-dimentional (3D) ultrasonography. A 25-year-old nullipara had been treated for Graves' disease from 14-year-old. At 14 weeks of gestation, medication was changed from propylthiouracil (PTU) to PI (50mg/day). Then, PI dose gradually decreased to 10mg until 32 weeks of gestation. Just then, her fetal goiter was pointed out and admitted to our hospital.

Transverse diameter was 40mm and its volume was 15mm^3. Nuchal hyperextension and cardiomegaly (cardio thoracic area ratio CTAR; 0.55) were observed. Fetal blood sampling date showed hypothyroidism (TSH; 5.39 μIU/mL, FT3; 2.0 pg/mL, FT4; 1.22 ng/dL, TSH receptor antibody; 0.6 IU/L). Just after stopped PI intake, fetal goiter and cardiomegaly rapidly improved. At 37 weeks of gestation, thyroid was 30 mm in diameter, 11 mm^3 in volume, and CTAR was 0.35. The newborn showed normal thyroid function and neurodevelopment 1 year after birth. Measurement of fetal thyroid volume by 3D must be a good tool of following fetal goiter non-invasively.

PO-35

Track: Others - Experimental Pharmacology (Neuromuscular Junction)

THE FACILITATORY EFFECT OF *CASEARIA SYLVESTRIS* SW. FRACTIONS ON THE FUNCTION OF MAMMALIAN AND AVIAN SKELETOMOTOR APPARATUS

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The facilitatory effect of apolar to polar fractions of *Casearia sylvestris* Sw. on the function of mammalian (mouse phrenic nerve diaphragm, PND) and avian (chick biventer cervicis, BC) skeletomotor apparatus was investigated, using the forces elicited by indirect nerve *versus* direct muscle stimulation and traditional antagonists as pharmacological tools. Only methanol (MeOH fraction) and ethyl acetate (EtOAc) fractions, but not hexane and dichloromethane, exhibited facilitatory effect on PND. Due the outcome of these fractions, MeOH fraction was chosen for further assays. The study was addressed to the following targets, where facilitation could occur: presynaptic sites, on axons or at the nervous terminal; post synaptic sites, at cholinergic receptor, sarcolemma or T-tubule; and at cleft synaptic, on acetylcholinesterase enzyme. MeOH fraction produced a facilitation of indirect twitch responses of the mouse PND preparation, but not in curarized (d-tubocurarine, d-Tc) preparations directly stimulated (30V). MeOH fraction was not able to competing against dantrolene. In curarized preparations, MeOH fraction showed a dual response, sometimes antagonizing as neostigmine does, sometimes failing completely. Using 3,4-diaminopyridine (3,4-DAP) that also is able to replace d-Tc from nicotinic receptors, MeOH fraction decreased the twitches amplitude. Under high-frequency tetanus (40 Hz) MeOH fraction increased the tetanic tension. Using BC preparation MeOH fraction did not have any changes on contractures induced by exogenous acetylcholine (ACh) and potassium chloride (KCl) addition. Taken these

results together we can suggest that the site of action of MF is at the pre synaptic level, but the exact local remains to be clear.

Keywords: *Casearia sylvestris*, chick biventer cervicis, "guaçatonga", mouse phrenic nerve-diaphragm preparation, neuromuscular junction.

PO-99

Track: Drug Delivery & Targeting

DESIGN AND DEVELOPMENT OF A NOVEL GRAPHENE OXIDE BASED HYDROGEL BIOCOMPOSITES WITH ANTI-HYPERGLYCEMIC ACTIVITY

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The pursuit for improved targeted drug delivery systems has led to the development of highly improved biomaterials with enhanced biocompatibility and biodegradability properties. Hydrogels are of particular interest for drug delivery applications due to their ability to address targeted drug delivery. Hydrogel biocomposites containing a combination of thermally reduced graphene oxide, natural and synthetic polymers were prepared followed by pH swelling analysis and drug release studies at selected pH values. Drugs that exhibit anti-hyperglycemic activity were loaded onto the hydrogel biocomposites in selected ratios. *In vitro* analysis was performed against 3T3-L1 pre adipocytes cell lines. The biocomposites were further characterized by Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM) and RAMAN.

Keywords: Acylamide, drug release, hydrogels, pH swelling, polymerization.

PO-43

Track: Women's Health Drug Discovery & Therapy

CLINICAL PRESENTATION AND TREATMENT PATTERN OF BREAST CANCER: A SINGLE CENTRE STUDY

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Objectives: To describe the clinical presentation of breast cancer in terms of tumor staging, estrogen and progesterone receptor status, HER-2 status and to describe the management / treatment pattern of breast cancer patients in terms of chemotherapy, hormone therapy, radiation therapy and surgery.

Methods: A retrospective, single-centric study of breast cancer in duration of December 2012 to April 2013 at Hemato Oncology Clinic, Vedanta Institute of Medical Sciences, Ahmedabad, Gujarat, India. Data were recorded for different parameters like age group, menopausal status, tumor staging, hormone receptor status and treatment given to the patients.

Results: A total of 215 cases were recorded including 212 female patients and 03 male patients who had undergone treatment at the above mentioned hospital and in whom follow up data of at least 4 months available. Among them 59.06% patients were in age group of 41 to 60 years. 22.79% patients were in age group less than 40 years. Number of patients were in premenopausal and post menopausal were almost equal with 43.87% patients were premenopausal. Majority of patients were in stage II and stage III (78.71%). Triple negative patients were 21.39% while patients with triple positive were 18.60%. Patients with hormone receptor positive were 57.67% and 63.25% patients had undergone surgical treatment at Hemato Oncology Clinic,

among them only 9.56% had undergone breast conserving surgery. Only 12.09% of patients required reduction in their chemotherapy schedule due to toxicity and other side effects.

Conclusion: The patients in our study were relatively younger. The number of patients with hormone receptor positive was less. Majority of patients were in very advanced stage of cancer. Breast conserving surgery was offered to less number of patients. Most patients received chemotherapy after surgery. Majority of them were treated with adjuvant chemotherapy and most of them were able to tolerate chemotherapy without dose reduction.

Keywords: Breast cancer, chemotherapy, hormone receptor, radiation therapy.

PO-44

Track: Cancer Targeted Drug Delivery

IN VITRO SCREENING FOR ANTICANCER AND ANTIFUNGAL ACTIVITY OF VARIOUS EXTRACTS OF *ALSTONIA SCHOLARIS*

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Purpose: The purpose of the study is to evaluate the anticancer and antifungal activity of *Alstonia Scholaris* leaf extracts.

Methods: Four solvent extracts which Methanolic, Chloroform, Acetone and Petroleum ether of *Alstonia Scholaris* leaves were investigated for anticancer activity against cancer cell lines (MCF-7, Hela, HEp-2, MDA-MB 468 and NCI-H522) and normal cell line (HEK-293) by MTT assay. Six fungal strains *Aspergillus fumigatus*, *Candida albicans*, *Candida parapsilosis*, *Aspergillus flavus*, and *Candida tropicalis* were used to test antifungal activity by NCCLS method.

Results: Among four extracts tested, methanolic extract has found concentrated dependent activity against all cell line (HeLa: IC50 22.87 µg/ml, MCF-7: IC50 85.77 µg/ml, MDA-MB-468: IC50 96.71 µg/ml, NCI-H522: IC50 71.90 µg/ml and Hep-2: IC50 23.28 µg/ml) significant to methotrexate except HEK-293 normal cell line which has no activity. Methanolic extract showed most potent activity against *Aspergillus fumigatus* (IC50 31.13 µg/ml) and *Aspergillus flavus* (IC50 47.33 µg/ml) more then Amphotericin B (IC50 22.45 µg/ml and 46.87 µg/ml respectively). Chloroform extract also shown significant activity against *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis* (IC50 value 61.80 µg/ml, 67.62 µg/ml, and 89.11 µg/ml respectively).

Conclusion: The study confirms the anticancer and antifungal activities of *Alstonia scholaris* leaves extracted using methanol, and is therefore, a potential drug that requires further studies and development.

PO-71

Track: Hot Topics in Medicinal Chemistry

MITOCHONDRIA-TARGETED ANTIOXIDANT SKQ1 INHIBITS AHR-DEPENDENT GENES IN RETINA OF OXYS RATS WITH AMD-LIKE RETHINOPATY

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Mitochondria-targeted antioxidant SkQ1 (cationic plastoquinone derivative SkQ1 (10-(6'-plastoquinonyl) decyltriphenylphosphonium) is a novel medicine which is positioned as retarding

the development of age-related diseases and aging and even be a component of Visomitin eye drops. Recently it was shown that SkQ1 reduces clinic signs of retinopathy in senescence-accelerated OXYS rats [1]. The OXYS strain was developed from Wistar stock by selection for their susceptibility to the cataractogenic effect of galactose and now it is avowed animal model for human age-related macular degeneration (AMD) [2]. OXYS rats develop retinopathy similar to the dry form of human AMD based on clinical symptoms, morphology and some molecular changes. This study aims to investigate whether SkQ1 affects on transcriptional activity of aryl hydrocarbon receptor (AhR) and AhR-dependent genes: cytochrome P4501 (CYP1A1, CYP1A2 and CYP1B1. AhR called a candidate gene of involvement in AMD pathogenesis in association study examined human genetic polymorphisms [3] and later in AhRÄ/Ä mice were shown its potential role in the AMD pathogenesis [4].

To study the effects of SkQ1 on gene expression 250 nmol SkQ1 per kg of body weight per day were added to the feed of OXYS and Wistar (without signs of disease) rats between ages 1.5 and 3 months. The control groups of rats did not receive SkQ1. mRNA expression of AhR and AhR-dependent genes were studied in the retinas of the control and SkQ1-treated OXYS and Wistar rats by real-time PCR. Statistical analyses were performed using the STATISTICA software package using two-way ANOVA and Newman-Keuls post-hoc test.

Our study showed that no difference in AhR mRNA level in the retina of OXYS and Wistar rats. At the same time retinal CYP1A1 mRNA level was twice lower in OXYS compared to Wistar and CYP1A2 and CYP1B1 mRNA level was 2-fold and 1.7-fold ($p<0.05$) respectively higher in OXYS compared to Wistar. SkQ1 supplementation caused decrease of AhR mRNA level in OXYS 1.9-fold ($p<0.05$) both in Wistar 1.7-fold ($p<0.05$). SkQ1 supplementation caused decrease only CYP1A1 mRNA level 2-fold ($p<0.05$) in retina of Wistar rats whereas CYP1A1 mRNA level in retina of OXYS rats, by contrast, remained unchanged. In retina of OXYS rats CYP1A2 and CYP1B1 mRNA level was decreased 2.2-fold ($p<0.05$) and. 1.7-fold ($p<0.05$) respectively under SkQ1 supplementation.

Thus, these results allow suggesting that CYP1A2 and CYP1B1 are pathogenetic components in retina of OXYS rats with AMD-like retinopathy and targets of SkQ1 as therapeutic agent.

Supported by the Russian Foundation for Basic Research (project No. 12-04-01352-)

Keywords: Age-related macular degeneration, aryl hydrocarbon receptor, mitochondria-targeted antioxidant SkQ1, OXYS rats.

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PO-73

Track: CNS Drug Discovery and Therapy

SOD1 AGGREGATE LOAD CHARACTERIZATION AND TARGETING BY SMALL MOLECULES IN A SOD1 MOUSE MODEL OF ALS

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Mutations in the gene encoding human superoxide dismutase (SOD1) cause approximately 20% of familial cases of amyotrophic lateral sclerosis (ALS). SOD1 positive aggregates have been identified by immunohistochemistry in post mortem spinal cord tissues of patients with both familial and sporadic ALS. Transgenic mouse models that over-express mutant or wild-type (WT) SOD1 have been developed which recapitulate many elements of human ALS pathology and symptomatology. Here we optimized previously published SOD1 aggregate filter retardation assay to study SOD1 aggregate load in the spinal cord of B6-SJL-G93A-SOD1 mice. We used the assay to determine if there is a correlation

between SOD1 positive aggregate load and mouse age or mouse symptomatic disease state. Finally, we used SOD1 aggregate load as a pharmacodynamic endpoint for efficacy of small molecules designed to reduce SOD1 misfolding and subsequent aggregation.

PO-98

Track: Innovative Drug Discovery and Nanotechnology

THE POTENTIAL OF NEW CHLORINE FOR PHOTODYNAMIC THERAPY

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Photodynamic therapy has been established in several countries as alternative or adjuvant therapy for the treatment of various malignant and non-malignant diseases. This therapy involves the incorporation of a photosensitizer (PS) that is activated by light and form reactive oxygen species leading to tumor cell death by apoptosis or necrosis. A derivative of chlorine (CHL-TRISMA) was synthesized in Brazil to improve the hidrofilicity and decrease the aggregation. The aim of this study was to characterize the cytotoxic effects of this new molecule compared to its chlorine source (CHL) and hypericin (HY). The accumulation of the PSs inside the cells was evaluated; the MTT assay was used to determine the live cell and apoptosis/necrosis as investigated by fluorescence microscopy in HEp-2 and HeLa cell lines. The chlorines presented higher accumulation for the two cell lines comparing to hypericin. After 1 and 2h of incubation, the photosensitizers showed the same accumulation in both cell lines. The CHL-TRISMA seems to accumulate more than CHL after 8h in both cell lines. With 2h of incubation it was obtained a better cytotoxicity for CHL-TRISMA by about 10 times compared to CHL for both cell lines. With 16h of incubation time, the better efficiency for CHL-TRISMA was kept about 10 times for the proposed new photosensitizer compared to CHL in both cell lines too. Comparing the IC_{50} of the photosensitizers, it has been found greater cytotoxic effect caused by the CHL-TRISMA than by the original CHL, probably because of the addition of the solubilizing group. Fluorescence microscopy images were obtained after photodynamic treatment at 630 nm using staining cells with ethidium bromide and acridine orange. Increasing concentrations there was a raise in the proportion of cell death as expected. For incubation time of 2 h, the efficiency of the new PS was higher, since the concentration used was 10 times lower compared to CHL and HY. Under these conditions it was possible to achieve cell death around 99% at the highest concentrations used. CHL-TRISMA showed 50% of apoptosis at 0.92 ĀM. This percentage of apoptosis was higher than for CHL (22 %) and HY (23 %) at the highest concentrations. Thus, in 2 h of incubation is observed that CHL-TRISMA induces more apoptosis than necrosis compared with the others PSs that induce more necrosis. With 16h of incubation, a higher percentage of cell death occurs by necrosis. We conclude that the newly synthesized chlorine has great potential to kill tumor cells in photodynamic PDT.

Financial Support: FAPESP, CAPES, and CNPq.

PO-1

Track: Hot Topics in HIV Research

EFFECTS OF *CHELIDONIUM MAJUS* ON METABOLIC ABNORMALITIES IN AIDS PATIENTS

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Introduction: Highly Active Antiretroviral Therapy (HAART) increased survival of AIDS patients. HAART-associated major toxic effects: neuropathy, myopathy, pancreatitis, hepatic steatosis, lactic acidosis, lipoatrophy, metabolic complications (fat redistribution, insulin resistance and hyperlipidemia). *Chelidonium majus* exhibit apoptotic activity, antioxidant and hepatic-protective effects.

Objective: Current study evaluated the effect of *Chelidonium majus* on metabolic abnormalities in AIDS patients. Material and methods: *Chelidonium majus* was diluted in 1x1012 alcohol/water 8%. AIDS patients with metabolic abnormalities were divided into two groups: Group I comprised patients who received a drug once a day, by a sublingual dose of 10 drops, for four months. Group II consisted of patients who received a placebo once a day at the same dosage. Clinical evaluation was performed and the serum cholesterol, triglycerides, hepatic enzymes (AST and ALT) was assessed by specific methods for each month to the end of treatment. Results were analyzed with GraphPad Prism using Student´s t test.

Results: Participated in the study 60 patients who had metabolic abnormalities. All were using HAART for at least five years. Had high levels of cholesterol and / or triglycerides 26 (44, 8%) patients and elevated liver enzymes 34 (58, 6%). After treatment showed improvement in cholesterol and triglycerides 22/26 patients an in liver enzyme levels 20/34.

Conclusion: The mechanisms whereby antiretroviral drugs alter adipose function have been partly deciphered. Lipodystrophic adipose tissue presents the inflammatory state that can influence the liver and muscles, leading to metabolic alterations. *Chelidonium majus* at the concentration used could be decreasing liver damage and improving metabolic abnormalities in AIDS patients.

Keywords: Metabolic abnormalities, AIDS, *Chelidonium majus*.

PO-61

Track: Drug Delivery and Targeting

SMA-RL71 AS A NOVEL NANOMEDICINE FOR TRIPLE NEGATIVE BREAST CANCER

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Triple negative breast cancer (TNBC) is a subtype of breast cancer characterized by the lack of expression of three keys proteins known to respond to targeted therapies, namely the estrogen receptor (ER), progesterone receptor and Her2. We have previously synthesized a suite of second generation curcumin derivatives of which RL71 had the highest potency toward TNBC cell lines. We also improved this cytotoxicity by encapsulating it in styrene maleic acid (SMA) micelles. In this study we have used a xenograft model of TNBC to examine the efficacy of SMA-RL71. Female SCID mice (6-7 weeks old) were implanted with MDA-Mb-213 cells and left to form palpable tumors. Mice were then randomized into treatment groups which received iv injections of RL71 (10 m/kg), SMA-RL71 (10 mg/kg) or vehicle control on days 1 and 8. Tumor volume was measure 3 times a week for 31 days using electronic calipers. The results showed that the free drug RL71 was unable to alter tumor growth compared to control. However, tumor growth curves for the mice treated with SMARL71 diverged 18 days after the first dose and

remained significantly smaller for the remainder of the study. At necropsy the weight of the tumors were 50% smaller in the SMA-RL71 treatment groups compared to control. All mice gained 2 – 2.5 g of body weight during the studay and plasma alanine aminotransferase activity in all treatment groups was in the normal range. This study demonstrates that SMA miceller formulation of the novel curcumin derivative RL71 is a safe and efficacious method of drug delivery. In order to develop this drug formulation further we will examine its efficacy in models of metastatic TNBC.

PO-24

Track: Proteomics & Bioinformatics

A NETWORK-BASED DRUG-REPOSITIONING APPROACH USING RELATIVE SCALING AND PARAMETERIZED SEGREGATION FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurological disorder in which the death of brain cells causes memory loss and cognitive decline. This study presents a novel supervised inference method for drug repositioning of AD. There are very few algorithms for drug repositioning which deal with analysis of target protein in diseasome as well as in interactome. In this large scale data analysis with human interactome we propose a network-based approach which refines the drugs and their target proteins separately to extract best possible candidates for repositioning. The target refinement scales proteins according to their topological significance in a global scale than in a local scale. The drug refinement uses parameterized segregation of drug-targets with a novel measure namely the diseasome efficiency score of the drugs to find out their significance in the diseasome. Both the refinements have given sets of best probable drugs and drug-targets for AD. These drugs were proposed as the most significant repositioning candidates for AD. The results of our work will provide insight into the drugs known to work in other diseases to treat AD as well.

Keywords: Alzheimer's disease, drug repositioning, drug-target network, protein-protein interaction network.

PO-31

Track: Innovative Drug Discovery and Nanotechnology

TARGETED THERAPEUTICS BASED ON MOLECULAR COMPUTING: TECHNIQUE FOR TARGETED THERAPEUTICS BY USING MOLECULAR CASCADES ON CELL SURFACE

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Diseases and traits, such as many cancers and autoimmune diseases, can be addressed through interactions with matching multi-input molecular networks or molecular automata. We merge recently developed concepts from molecular robotics and molecular computing with advances in oligonucleotide and protein chemistry in order to create synthetic molecular automata capable of operating on cell and tissue surfaces. Our approach to evaluate presence of specific marker on the cell-surface will be broadly applicable in all diseases that require selective elimination of specific cell type, as best exemplified by hematopoietic malignancies.

We demonstrated that a chemical reaction cascade could be engineered to depend on the presence or absence of the particular cell. Specifically we showed that components of chemical reaction cascades could be coupled to antibodies targeting cell membrane surface markers (CDs) in a way that precisely defined subpopulations of cells that displayed all of these constituents in close proximity, thus enabling cascades to proceed to completion. Cell-enabled reaction cascades

represent molecular automata, or collection of molecules that execute their programs based on inputs from cell surface. Our automata condense information about interactions of cell with multiple antibodies into a unified answer related to the identity of cells.

Keywords: Molecular computing, surface markers, targeted drugs.

PO-114

Track: Hot Topics in Natural Products

NON-SPECIFIC SIRTUIN INHIBITION AS A MECHANISM OF CYTOTOXICITY FOR GINKGOLIC ACIDS AND URUSHIOLS

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Gingkolic acids found in Ginkgo biloba L. leaves have been pointed out for their genotoxic, cytotoxic, immunotoxic and allergenic potential. For these reasons, the phytopharmaceutical Gingko extract (EGb 761) has been standardized to contain less than 5 ppm gingkolic acids [1]. Accordingly, urushiols are largely present in Anacardiaceae plant species and are known for inducing allergic reactions. Ginkgolic acids and urushiols are natural alkylphenols structurally related to anacardic acids, also known for their cytotoxic potential. However, their cytotoxic mechanisms have not been completely elucidated so far. Considering our previously published *in silico* data, suggesting a sirtuin inhibition by anacardic acids [2], we hypothesized that gingkolic acids and urushiols are able to inhibit sirtuins, partially contributing to their toxic potential. We herein demonstrated that selected gingkolic acids and urushiols were able to inhibit human SIRT1 and SIRT2 *in vitro*. Their inhibitory activity and their cytotoxic profile on HEK and HeLa cells was shown to be comparable to that of the non-specific inhibitor sirtinol, contrary to EX527, a selective SIRT1 inhibitor that exhibits less cytotoxicity for both cell lines.These results, supported by *in silico* data, suggest a non-specific sirtuin inhibition as a new mechanism of cytotoxicity for ginkgolic acids and urushiols.

Keywords: Cytotoxicity, gingkolic acid, molecular modeling, sirtuin inhibition, urushiol.

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PO-8

Track: Innovative Drug Discovery and Nanotechnology

DEVELOPMENT OF REDOX THERAPY FOR PERIODONTAL DISEASE USING REDOX INJECTABLE GEL

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Reactive oxygen species (ROS) are considered to cause various disorders if they are produced in excess and become uncontrollable in the body. However, redox nanoparticle that effectively eliminates excess ROS has been developed and reported to be effective for the treatment of various systemic disorders. This particle is expected to be developed into drugs due to its high cost-performance, regional specificity, and few adverse effects. Therefore, to develop redox therapy for periodontitis,

we designed novel Redox Injectable Gel (RIG), which is formed after disintegration of the nano-assembled flower micelle at 37°C, allowing nitroxide radicals to locally act as a specific ROS scavenger, and evaluated inhibitory effects of RIG on Porphyromonas gingivalis (Pg)-induced bone loss in rat experimental periodontitis model, which has been reported to be related to ROS. In this study, alveolar bone loss was imaged by Micro-computerized tomography (Micro-CT), the gingival blood flow was measured using laser Doppler flowmetry, and osteoclasts were evaluated with TRAP staining. These results suggest that RIG result in inhibition of Pg-induced bone loss, increase in gingival blood flow, and decrease in the number of osteoclasts. It can be anticipated as a novel therapeutic drug in the treatment on Pg-induced periodontitis.

Keywords: Periodontal disease, redox injectable gel.

PO-15

Track: Cancer Targeted Drug Delievery

INTERACTION BETWEEN WHEY PROTEIN NANOPARTICLES AND FATTY ACIDS

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The cytotoxicity of formulated nanoparticle complexes of different fatty acids (oleic, eliedic, Cis-vaccenic, Trans-vaccenic, and linolenic acids) in the presence or absence of whey protein isolate (WPI) was investigated in this study. Nanoparticle complexes formed with WPI was examined for surface tension, circular dichroism (CD), turbidity, isothermal titration calorimetry (ITC) and Cytotoxic activity. Surface tension values were decreased with adding fatty acid to WPI. This would indicate that WPI can bind greater amount of fatty acid. Cis-fatty acids such as oleic, cis-vaccenic and linolenic caused higher decrease in the surface tension of WPI nanoparticles than that of trans-fatty acids (eledic and trans-vaccenic acids). The tertiary structure of protein (WPI) was lost and changed from fold to unfold after binding with fatty acids. The changes in protein structure would be correlated to exhibit a cytotoxic activity to tumor cells. All formed protein WPI/fatty acid complexes presented lower turbidity measurements compared to the fatty acid only at same concentration. The turbidity values for nanocomplexes of WPI/fatty acids were lower confirming higher ability in binding fatty acids. All nano complexes formed of WPI/fatty acids exhibited a cytotoxic ability as a lysis in erythrocytes. The cytotoxic activity of WPI/fatty acid complexes was almost as found with Ā-LA complexes. Nanocomplexes can be formed from WPI with good cytotoxic effect to tumer cells using cis-vaccenic and linolenic fatty acids comparable to oleic acid. It was a new interesting observation being that the nanocomplexes formed of WPI with fatty acids has a comparable cytotoxcisty to that of Ā-LA and Ä-lg and can be used in tumor therapy.

Keywords: Nanoparticles, whey protein isolate, fatty acids, surface tension, circular dichroism, turbidity, cytotoxicity.

PO-88

Track: Protein and Peptide Sciences

MICROBICIDE POTENTIAL OF CR-LAAO, AN L-AMINO ACID OXIDASE FROM *CALLOSELASMA RHODOSTOMA* SNAKE VENOM

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Background: Some proteins and peptides isolated from snake venoms have been shown to possess antimicrobial effects against various species of bacteria and fungi, including some drug-resistant strains. These effects make them potential candidates for use as microbicide agent. In the present study, we evaluated the bactericidal effect of an L-amino acid oxidase from *Calloselasma rhodostoma* snake venom, named CR-LAAO, on standard and resistant strains of *Staphylococcus aureus* and *Escherichia coli*.

Results: CR-LAAO showed bactericidal effects against *S. aureus* and *E. coli* strains. After 6 h of incubation with the toxin, minimum inhibitory concentrations (MICs) for standard *S. aureus*, *S. aureus* DT, *S. aureus* 56, and standard *E. coli*, *E. coli* IAL 45 and *E. coli* JF239 were respectively: 0.2; 0.78; 0.39; 7.8; 31.25 and 125 Ãg/mL, while after 24 h of incubation, MICs increased to 0.78; 1.6; 1.6; 31.25; 125 and >500 Ãg/mL, respectively. The effects of CR-LAAO on the bacteria walls was evaluated by transmission electron microscopy, showing the destruction of the cell wall, the inner membrane detachment and the formation of electron-dense granules in the bacterial cytoplasm.

Conclusion: Our results suggest that CR-LAAO can provide important information for the development of therapeutic strategies with directed action, such as more effective antimicrobial agents.

Keywords: Bactericidal activity, L-aminoacid oxidase, snake venom, transmission electron microscopy.

PO-81

Track: Pharmaceutical Research & Development

OPTIMIZATION OF CROSSLINKED SODIUM ALGINATE POLYMYXIN B SULPHATE-LOADED SLN BY FACTORIAL DESIGN APPROACH TO ENHANCE THE ADHESION IN BUCCAL MUCOSA

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This study reports the production of innovative drug delivery systems intended for sustained release of hydrophobic drugs. Polymyxin B sulphate (PLX) is known for its cationic charge, being therefore an obstacle for its efficient loading into Solid Lipid Nanoparticles (SLN). To overcome this limitation, PLX was too cross-linked with sodium alginate (SA), producing a complex with three different ratios 1:1, 1:2 and 1:3 SA/PLX. The complexes SA/PLX were characterized for their encapsulation efficiency (EE), and loading capacity (CC) for PLX, degree of swelling, rheological characterization, minimal inhibitory concentrations (MIC) and scanning electron microscopy (SEM) analysis. The produced SLN formulations were optimized by factorial design to evaluate the optimum parameters of the production by High Pressure Homogenization (HPH). Results showed that SA/PLX (1:1) was the best formulation reaching EE, CC and degree of swelling, respectively 99.08%±1.2; 0.99g ±0.10 and 212.07±5.84. SA solution showed the behavior of concentrate polymer solutions and SA/PLX exhibited gel-like dynamic mechanical spectra. MIC showed SA/PLX and

had the same MIC as the native PLX at all time points. Optimized SLN were produced applying 500 bar pressure and 5 passages. The EE, diameter, polydispersity index and zeta potential were, respectively, 82.7%±5.5; 439.5 nm±20.42, 0.241±0.050 and -34.8 mV±0.55. These results indicate that SLN might be a promising delivery system to enhance the adhesion of PLX in buccal mucosa.

Keywords: Polymyxin, solid lipid nanoparticle, high pressure homogenization, crosslinked.

PO-26

Track: Drug Discovery in Preclinical Research

ELABORATE LIGAND-BASED MODELING AND SUBSEQUENT SYNTHETIC EXPLORATION UNVEIL NEW NANOMOLAR CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II INHIBITORY LEADS

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Ca2+/calmodulin-dependent protein kinase II (CaMKII) has been recently implicated in cardiovascular diseases and hypertension prompting several attempts to discover and optimize new CaMKIId inhibitors.Towards this end we explored the pharmacophoric space of 88 CaMKIId inhibitors using nine diverse sets of inhibitors to identify high quality pharmacophores. Subsequently, genetic algorithm and multiple linear regression analysis were employed to select an optimal combination of pharmacophoric models and 2D physicochemical descriptors capable of accessing self-consistent quantitative structure-activity relationship (QSAR) of optimal predictive potential (r272 = 0:70, F = 18.19, r2LOO = 0:71, r2PRESS against 16 external test inhibitors = 0.60). Three orthogonal pharmacophores emerged in the QSAR equation suggesting the existence of at least three binding modes accessible to ligands within CaMKIId binding pocket. Receiver operating characteristic (ROC) curves analysis established the validity of QSAR-selected pharmacophores. We employed the pharmacophoric models and associated QSAR equation to screen the national cancer institute (NCI) list of compounds. *In silico* screening identified nanomolar and low micromolar inhibitors. The most potent hits exhibited IC50 values of 20 and 82 nM. The best pharmacophoric model (Hypo8/31) was employed to guide the synthesis of novel triazine-based CaMKIId inhibitors, of which the most potent illustrated an IC50 value of 154 nM against CaMKIId.

Keywords: CaMKIId, *in silico* screening, pharmacophore modeling, Triazine.

PO-94

Track: Drug Discovery in Preclinical Research

ELABORATE LIGAND-BASED MODELING UNVEILED NEW SELECTIVE POTENT SMOOTHENED ANTAGONISTS

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Abnormal reactivation of the Higdgehog pathway (Hh) after developmental stage is implicated in many cancers and neurodegenerative disorders. As a transducer of Hh signaling, the GPCR-like receptor Smoothened (Smo) can be considered as a valuable target for disruption of unregulated Hh signaling. Therefore, in the interest of finding new Smo inhibitors we applied a computational workflow based on tandem pharmacophore-QSAR modeling. Initially, exhaustive pharmacophore modeling was employed to explore the structural requirements for potent Smo inhibitors employing 190

known Smo ligands. Subsequently, genetic function algorithm (GFA) coupled with multiple linear regression (MLR) analyses were employed to search for self-consistent and predictive QSAR models based on optimal combinations of pharmacophores and physicochemical descriptors. n = 152, r2152 = 0.65, r2LOO = 0.6203, F = 280.141, r2PRESS (38) = 0.45. Successful pharmacophores were complemented with exclusion spheres to optimize their receiver operating characteristic curve (ROC) profiles. Optimal QSAR models and their associated pharmacophore hypotheses were validated by identification and experimental evaluation of several new promising Smo inhibitory leads retrieved from the National Cancer Institute (NCI) structural database. The cytotoxic activity of these compounds was evaluated against breast cancer (MCF-7), Human Embryonic Kidney 293 (HEK-293) and normal fibroblast cell lines by a cell viability assay. The most active hits illustrated selective cytotoxic activities against the Smo-expressing HEK-293 with IC50 values ranging from 1.22 to 7.32 ĀM.

Keywords: Pharmacophore modeling, QSAR, smoothened.

PO-42

Track: Cancer Targeted Drug Delievery

LACTOFERRIN-DOXORUBICIN CONJUGATES IMPROVE THE DOXORUBICIN RETENTION AND CYTOTOXICITY IN CELLS BY REDUCING P-GLYCOPROTEIN EXPRESSION

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Doxorubicin, a potent cytotoxic drug used for chemotherapy, is plagued by the emergence of multi drug resistance in cancers. Lactoferrin, a multifunctional, iron-binding 76-80 kDa milk glycoprotein was found not only to be cytotoxic to cancer cells but also to reduce P-gp expression in DU145 and drug resistant OVCAR-3 cells at 20 nM. The amine group (-NH_2) of doxorubicin was conjugated to carboxyl (-COO) groups of the bovine lactoferrin (bLf) molecule using carbodiimide reaction to develop a protein-drug conjugate. Conjugation was confirmed using FTIR spectroscopy. The LD50 concentration of pure doxorubicin was reduced from 60 ĀM to 10 ĀM in the case of conjugates. Confocal microscopy showed increased accumulation of doxorubicin in the nucleus when administered as a lacto-dox conjugate. A significantly increased retention of doxorubicin of up to 24 hours*in vitro* was seen in the case of bLf-dox conjugates, which was attributed to the decreased P-gp expression, assessed by both immunofluorescence and Western blotting. In 3D culture, the bLf-dox conjugates reduced spheroid diameter significantly compared to pure dox which was near ineffective. The study findings indicate that lactoferrin, with its high cell penetrating ability and capacity to reduce P-gp expression, is an appealing molecule to overcome the drug resistance to doxorubicin.

PO-19

Track: Pharmaceutical Biotechnology

USE OF THE DENDRITIC ARCHITECTURE FOR DESIGNING ANTIOXIDANTS WITHOUT PRO-OXIDANT ACTIONS

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Popular naturally occurring antioxidants such as vitamins C and E and quercetin have been reported to be beneficial as well as harmful for human health. They are known to neutralize harmful free radicals, thereby preventing cellular damage. However, during inflammation, release of excess metal ions such as iron, react with antioxidants to generate new free radicals. This pro-oxidant action of antioxidants may be one reason for their controversial role in human health and disease. In this presentation, we discuss how the use of the dendritic architecture led to the synthesis of antioxidants with potent free radical scavenging properties and lack of pro-oxidant actions. The dendritic architecture, with its flexible branches, is well known to be an effective structure for sequestering metal ions. We have designed and synthesized several antioxidant dendrimers. Their synthesis, radical scavenging and pro-oxidant actions as well as protection of DNA and human lipoproteins against free radicals will be presented.

PO-89

Track: Pharmaceutical Biotechnology

DENDRIMER ELECTROPHORESIS

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Electrophoresis is a workhorse technique in proteomics. It is relatively inexpensive, uses disposable materials, offers high-resolution separation and is extremely versatile in its ability to separate any water-soluble material that can be induced to possess a charge. In this presentation, we discuss the applications of native polyacrylamide gel electrophoresis for separation and characterization of various dendritic macromolecules such as polyamidoamine dendrimers, L-DOPA dendrimers and antioxidant dendrimers. The use of isoelectric focusing electrophoresis in determining the isoelectric points of dendrimers will also be discussed.

PO-21

Track: Cardiovascular Drug Discovery & Therapy

MOLECULAR DYNAMIC SIMULATIONS ON THE REDOX-DEPENDENT CONFORMATIONAL CHANGES IN INTERDOMAIN ELECTRON TRANSFER IN HUMAN INDUCIBLE NITRIC OXIDE SYNTHASE

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Nitric oxide (NO) is critical to numerous physiological functions but also contributes to the severity of diseases such as cancer, stroke and hypertension. Nitric oxide synthase (NOS) enzyme is responsible for biosynthesis of NO through interdomain electron transfer (IET) processes. The interdomain electron transfer (IET) from FMN to heme is essential to NOS catalysis, and is under

stringent control. However, the molecular mechanism underlying the FMN-heme IET process remains unclear. In the present study, molecular dynamic simulations were carried out on a model of an oxygenase/FMN (oxyFMN) construct of human inducible NOS (iNOS). Our results indicate redox-dependent conformational changes that affect the distance between the heme and FMN centers. Moreover, specific residues important in the interdomain FMN/heme docking were identified on the FMN, heme and CaM domains. Residues involved in the heme/CaM interactions were identified as well. The predictions of the key interacting sites are well supported by experimental data in literature. The new mechanistic information might facilitate bio-rational development of new selective and direct activators or inhibitors for these clinically important enzymes, in order to provide better therapeutic interventions.

Keywords: Nitric oxide synthase (NOS), molecular dynamic simulation, interdomain electron transfer (IET), conformational change.

PO-27

Track: Hot Topics in Natural Products

SEPARATION & IDENTIFICATION OF NEW MARKER COMPOUNDS FROM BUTANOL FRACTION OF METHANOLIC EXTRACT OF *BASELLA ALBA*

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This study is an attempt to isolate new compounds from *Basellaalba* which isa coveted multipurpose herb that is cultivated as an ornate plant, used as a vegetable and to treat various ailments. It is widely used in Ayurvedic system of medicine for treating fever, dysentery, tenesmus, gonorrhoea and balanitis. Its medicinal uses are justified due to its many pharmacological activities especially antifertility, anti-inflammatory, antioxidant, antibacterial, anti-depressant, anti-cancer and antiulcer activity. Comparatively a few compounds have been isolated from this green variety of spinach like Ã-sitosterol, stigmasterol, quercetin and kaempferol. In the present study, the methanol extract of the aerial parts of the plant was partitioned using butanol, chloroform and water. The butanol fraction was subjected to column chromatography using silica gel and Diaion HP-20 to isolate and purify the phytoconstituents which can be termed as markers. Two compounds were isolated andfound to be flavanone glycoside vitexin and its derivative, vitexin-7-Ã-D-glucopyranoside respectively. Structures of these compounds were confirmed by Mass, NMR and IR spectroscopic studies.

PO-54

Track: Enabling Technologies

DEPLOYMENT OF FLOW CYTOMETRY BASED HIGH-THROUGHPUT ASSAYS FOR WHOLE BLOOD SYSTEMS IN DRUG DISCOVERY SCREENING

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Translational *in vitro-in vivo* correlation of novel chemical and biological entities remains critical challenge for drug discovery. While recombinant and primary cell lines remain essential high throughput (HT) tools for understanding mechanism of action of molecules, functional activity in primary, physiologically relevant cells pertaining to disease state provide important information to advance molecules from *in vitro* studies to clinical trials. In addition, multiplex flow cytometry analysis in complex cellular context can provide in-depth detection of cell surface and intracellular markers. Furthermore, molecule activity in whole blood serves as a surrogate determination for pre-clinical and clinical dose prediction due to binding to serum components. This study describes whole blood-based assays

quantifying immune responses in B and T lymphocytes using flow cytometry read-outs. Advanced lab automation coupled with HT-flow cytometry enabled us to implement robust IC50 potency determinations for cellular activation markers and intracellular pSTAT levels in response to small molecules, thus making these assays critical for compound selection with preferred mechanism of action.

Keywords: Drug discovery, flow cytometry, whole blood, B lymphocytes, T lymphocytes, high throughput, immunology, screening, lab automation.

PO-5

Track: Diabetes and Obesity Drug Discovery & Therapy

SERUM PARAOXONASE (PON155) POLYMORPHISM AND PON1 ACTIVITY IN PATIENTS WITH DIFFERENT DEGREE OF OBESITY

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Obesity is a component of metabolic syndrome (MS). Paraoxonase (PON1) participates in the degradation of hydrogen peroxide. PON activity is strongly dependent on the genetic polymorphisms. The leucine at position 55 is associated with 30% increase of enzyme activity in comparison with the form of Met55.

PON1 activity and PON155 polymorphism was analyzed in the blood of patients with: MS, early form of MS and metabolically obese but normal weight (MONW). They were divided into 5 subgroups based on BMI value.

In the study group the most common disorder was obesity I° (particularly among men) and overweight (more often among women). Genotype $PON1_{55Leu/Leu}$ implicated higher paraoxonase activity in blood serum than $PON1_{55Met/Met}$. $PON1_{55Met/Met}$ genotype was not found either in MONW or in control group and was associated with low paraoxonase activity. There were no significant differences in PON1 activity between the three groups of obesity. The activity of this enzyme was lower in patients with III° of obesity in comparison with patients with normal BMI and overweight.

The lowest values and the most beneficial anthropometric parameters were found among $PON1_{55Leu/Leu}$ patients (MONW, normal BMI). $PON1_{55Leu/Met}$ showed visceral obesity (EMS, II° obesity). The highest values of body mass descriptors were found in $PON155Met_{/Met}$ patients (MS, III° obesity).

The redox systems' disruption was seen in advanced stage of diseases and largely depends on $PON1_{55}$ genotype. These abnormalities are not included in metabolic syndrome diagnostic criteria. This knowledge may be useful in the implementation of metabolic disorders prevention, particularly in MONW persons.

PO-74

Track: Drug Delivery & Targeting

THE ASSESSMENT OF FLUIDITY AND STRUCTURAL ARRANGEMENT OF ULTRADEFORMABLE LIPOSOMES WITH TERPENES USING ELECTRON SPIN RESONANCE SPECTROSCOPY

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This study aims to investigate the influence of liposomal components (Terpenes as skin penetration enhancer and Tween 20 as terpene solubilizer) on phospholipid bilayer fluidity of ultradeformable liposomes with terpenes (UL with terpenes). The liposomal fluidity was assessed by electron spin resonance (ESR) spectroscopy. The free radical spin labels, 5-doxyl stearic acid (5-DSA) and 16-doxyl stearic acid (16-DSA) were used as detectors for phospholipid bilayer fluidity near polar head group (hydrophilic region) and hydrocarbon chain (hydrophobic region), respectively. Addition of Tween 20 resulted in the increase of liposome fluidity near polar head group whereas terpenes increased the liposome fluidity near hydrocarbon chain of phospholipid bilayer. It was, therefore, suggested that Tween 20 inserted its molecule near polar head group (hydrophilic region) while terpenes localized its molecule near hydrocarbon chain (hydrophobic region) of phospholipid bilayer. The effect of different type and amount of terpenes loaded in UL on liposomal fluidity was also investigated. Increasing the amount of terpenes in UL led to the increase of liposomal fluidity near the hydrocarbon chain of phospholipid bilayer. It was found that this effect, however, depended on type of selected terpenes. Different type of terpenes loaded in UL also affected the liposomal fluidity levels.

Keywords: Electron spin resonance spectroscopy, terpenes, ultradeformable liposomes, fluidity.

PO-45

Track: Anti-Cancer Discovery & Therapy

BIOLOGY AND PATHOLOGY OF CHEMOTHERAPEUTIC TREATMENT OF GASTRIC SCIRRHOUS CANCER

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The prognosis of patients with scirrhous gastric carcinoma still remains very poor; although chemotherapy for other gastric cancers has improved, with good results being obtained in Japan. To develop new therapeutic approaches based on characteristic biologic features of scirrhous cancer cells, we sought to examine mechanisms underlying the cytotoxicity of various cancer drugs toward a scirrhous cancer cell line, HSC-39, *in vitro*.

In generally, current treatment for scirrhous gastric cancer is based systemic therapy with an oral fluoroupyrimidine (i.e. S-1 or capecitabin), showed a goodresponse rate in combination with Cisplatin or Docetaxel in clinical treatment. S-1 plus Cisplatin is a standard treatment in Japan. Because the superior to S-1plus Cisplatin compared with S-1 alone was demonstrated in SPRITS trail. *In vitro,* 5-FU effectively and dose-dependently induced apoptosis in the HSC-39 cells; whereas Adriamycin and CPT-11induced necrosis and/or apo-necrosis single-handed.

These results suggest that combination treatment with a fluoropyrimidine such as 5-FUalong with other chemotherapeutics is a reasonable chemotherapy for scirrhous gastric carcinoma.

PO-77

Track: Drug Metabolism

ACETAMINOPHEN TOXICITY - ESTIMATION OF A NEW POSSIBLE MECHANISM OF CELL IMPAIRMENT

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Acetaminophen is a safe drug at therapeutic doses. However, after overdose, it can cause cell impairment that may lead even to acute liver or kidney failure. Although the toxic mechanism has been studied for 40 years, some principles of toxicity remain unknown. In addition, a number of clinical studies focused on occurrence of acetaminophen toxicity at therapeutic doses have been published and some rather unexplainable reports from animal studies have appeared as well. Therefore, it is obvious that some parts contributing acetaminophen toxicity likely remain to be found. The aim of our work was to test our hypothesis about possible pathological role of an acetaminophen metabolite which is synthesized trough the detoxification process in cell and its potential toxicity has not been estimated at all. We synthesized and purified the molecule and estimated its toxic effect in cells. We tested the changes in cellular viability (WST-1), redox state (glutathione levels, ROS production) and morphological changes after treatment of human proximal tubular cells with the acetaminophen metabolite. We found that the tested compound was able to induce significant cellular impairment. At millimolar levels, the decrease of cellular viability was by 90 % after 6 and 24 hours of treatment. As well, we proved that mitochondrial function was damaged significantly. We conclude, that also the final products of acetaminophen metabolism should be considered in the evaluation and understanding of acetaminophen toxicity mechanism. This project was supported by the grants NT/14320-3/2013 and CZ.1.05/3.1.00/10.0217 of the Czech Republic.

Keywords: Acetaminophen, acetaminophen toxicity, liver failure.

PO-57

Track: Hot Topics in Natural Products

ASSESSING THE EFFECTIVENESS OF FITOSCAR® VERSUS COLLAGENASE OINTMENT IN THE TREATMENT OF VENOUS LEG ULCERS: A CLINICAL TRIAL

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The aim of this study was to evaluate the effectiveness of Fitoscar® as a therapeutic option in the treatment of varicose ulcers compared with Collagenase ointment. A randomized clinical trial was performed on all patients admitted to a hospital in southern Brazil for vascular surgery, from May 2012 to September 2013. The randomized trial consisted of 16 patients in the Fitoscar® group and 14 patients in the Collagenase group. Sociodemographic variables and comorbidities were evaluated, and the dependent variable was measured every week using a millimeter ruler to determine the exact diameter of the wounds and make a photographic record. The Revised Photographic Wound Assessment Tool (revPWAT) was used to assess the reduction in size and improvement of wound healing. Both Fitoscar® and Collagenase groups had a significant reduction in the wound areas ($p<0.001$ and $p=0.001$, respectively). No statistically significant differences between the groups were observed, either in the mean reduction of the ulcerated areas, or in the PWAT scores. The results suggest that both drugs might be equally effective; however, there was a trend towards a longer hospitalization in the Fitoscar® group ($p=0.053$). In conclusion, Fitoscar® is as effective as Collagenase for this purpose; however, further investigation is needed to confirm these findings.

PO-85

Track: Neurology

HYPOTHALAMIC PHOSPHOLIPIDS (LIPOSOMES FORTE):THERAPEUTIC POTENTIAL IN VASCULAR DEMENTIA

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There is evidence that different classes of lipids can significantly improve the formation and activity of neural networks. Nanoliposomes, derived from natural sources, promises to be a very effective drug carriers. Phospholipids liposomes have been proposed for the treatment of Alzheimer's dementia only in recent years. The effect of phospholipids liposomes on cognitive function in vascular dementia is unknown. The present study was aimed at evaluating the efficacy of Liposome-Forte in the complex treatment of cognitive impairment in patients with vascular dementia.

Patients with ischemic stroke and transient ischemic attacks in their anamnesis were observed, used a modified Hachinski scale. The Mini Mental State Examination (MMSE) and Barrow Neurological Institute Screen for Higher Cerebral Functions (BNIS) were administered to determine the degree of cognitive impairment while the RivermeadExtended Activities of Daily Living Questionnaire (READLQ) was utilized to measure quality of life. Comparative analysis of the results achieved has shown that Liposome Forte supplementary intake improves cognitive function significantly. Indicators evidenced an improvement of 16.1% (Ă<0.05) over the baseline period. The results of this study point to the positive impact of Liposome Forte supplementary administration on cognitive function in patients with vascular dementia.

PO-48

Track: Pharmaceutical Research and Development

ENANTIOSELECTIVE DETERMINATION OF METHADONE AND ITS MAIN METABOLITE IN SERUM AS AN EFFECTIVE TOOL FOR OPTIMIZING DOSE IN METHADONE TREATMENT PROGRAMME

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Methadone is commonly used in the treatment of opioid dependence. The drug is usually administered orally as a racemate, although the (R) - enantiomer possesses the main pharmacological effect. Due to a high interindividual variability in the stereoselective metabolism, the evaluation of each enantiomer separately is required for therapeutic drug monitoring of the patients to improve efficiency and safety of the treatment.

The relationship between administered dose and serum level of methadone enantiomers andstereoselective profile of methadone metabolism was evaluated.

Chiral LC-MS method for quantification methadone and EDDP enantiomers was developed. The analysis serum sample in 82 patients on long term-methadone maintenance programme was realized. The range of daily methadone dose was

20-225 mg. A significant correlation (Pearson, r = 0, 72, p< 0, 01) between administered dose and serum concentration of (*R*)-methadone was found. There was no marked correlation of dose with racemic methadone. The ratio of R/S EDDP indicated which patientswere very fast metabolizers.

PO-53

Track: Traditional Chinese Medicine

IDENTIFICATION OF NEUROPROTECTIVE EFFECT COMPONENTS IN THE ESSENTIAL OIL OF *RHIZOME ACORI GRAMINEI* BASED ON THE COMPOSITION-ACTIVITY RELATIONSHIP ANALYSIS

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Objectives and Aim: Rhizome Acori Graminei (*RAG*) is a traditional Chinese medicine that has been used to prevent dementia through out history. Research indicated the essential oil extracted from *RAG* as an active fraction with neuroprotective effects. In this study, we aimed to explore active components with the neuroprotective effects from the essential oil of *RAG* based on the GC-MS analysis and cellular assay data analysis.

Method: Gas chromatography-mass spectrometry (GC/MS) was applied to detect and identify the volatile organic components (VOCs) of the essential oil that steam distillation extracted from 10 *RAG* samples collected from various area of south-east China. Fifteen main components were identified as characteristic compounds of *RAG* essential oil. Their relative amounts were represented using the relative peak area. Then SRB assay was applied to evaluate the viability of neuro-2a cells pre-treated with Beta-Amyloid peptide fragment 25-35 and with the *RAG* essential oil intervention. The correlation between characteristic compounds relative peak area and neuroprotective effect was analyzed using bivariate correlation analysis (SPSS software v18.0), and then the active compounds with significant correlation were identified based on Pearson Correlation Coefficient.

Results: Consequently, there were 5 characteristic peaks (corresponding components: ∀-Asarone, #-Asarone, Methyl-isoeugenol, Shyobunone and ! -Asarone) shown significant correlation to the neuroprotective effects.

Conclusions: The *RAG* essential oil samples have shown moderate neuroprotective effects against the cytotoxicity induced by Beta-Amyloid peptide fragment 25-35. According to our study, this protective effect might be mainly benefited by the activity of five key components in the essential oil. Further research based on cellular experiments and animal model has to be carried out for activity confirmation and mechanism exploration.

Keywords: Components, composition-activity relationship, essential oil, rhizome acori graminei, volatile organic.

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PO-72

Track: Pharmaceutical Research & Development

SIMULTANEOUS DETECTION OF SIX FLAVONOIDS BY HPLC-MS/MS FOR *IN VITRO* PHARMACOKINETICS STUDY OF TOTAL FLAVONOIDS OF ASTRAGALUS IN CACO-2 CELL LINE

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Radix Astragali, the dry roots of Astragalus mongholicus Bge. is the most popular Traditional Chinese Medicinal herb that has been used clinically in China for centuries to treat various diseases. Series of studies have shown that the total flavonoids of Astragalus (TFA), the major active components in Radix Astragali, have antitumor, antimutagenic, inhibition of atherosclerosis, antioxidant, improving immune function, lowering blood pressure and other biological effects. In this study, we want to investigate absorption mechanism of TFA. Simultaneous determination of 6 flavonoids (including Formononetin, calycosin 7-o-glucoside, Quercetin, Isoliquiritigenin, Kaempferol, Genistein) was performed on an Agilent 1290 Infinity LC system coupled to an Agilent 6460 triple quadrupole MS/MS. The method was fully validated with respect to linearity (r2 >0.999), sensitivity, precision, and accuracy (RSD below 8.06%). And the developed method was successful used for *In vitro* TFA absorption study in Caco2 monolayers model. We found that formononetin, isoliquiritigenin, kaempferol, and genistein have related high transcellular absorption with Papp values around 2 x 10-5, while calycosin 7-o-glucoside and quercetin have moderate transcellular absorption with Papp values 6.6 x 10-6 and 2.8 x 10-6, respectively. The efflux ratio for calycosin 7-o-glucoside was 2.5 indicated that calycosin 7-o-glucoside may undergo active efflux.

Keywords: Caco-2 cell line, flavonoids of astragalus, pharmacokinetics.

PO-111

Track: Cancer Targeted Drug Delivery

MULTIMODAL THERAPY OF A STAGE IV PROSTATE CANCER PATIENT WITH EXTENSIVE LUNG AND BONE METASTASES– A CASE REPORT

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A polymer conjugated drug, poly (hydroxypropylmetacrylamide)-pirarubicin conjugate (P-THP), developed in our laboratories was capable to achieve cancer selective delivery in mice based on the EPR effect [1]. Here we report a successful treatment of advanced stage IV prostate cancer treated with P-THP who had multiple metastatic pleural tumor nodules as well as multiple bone metastasis. In April 2013, a 55-year-old male was diagnosed stage IV prostate cancer, since then received endocrine therapy as well as LH-RH analogue, but these treatments found ineffective as judge by PSA value and CT-imaging. Thus, proton beam radiotherapy for the primary prostate cancer was undertaken, but the primary tumor remained also unremarkable.

To control massive metastatic of tumor nodules in the lung, a newly developed P-THP was tested with consent of the patient and IRB. The result was remarkable yielding complete remission of multiple metastatic nodules in the lung, as well as significant regression of bone metastasis in 3 months. Decrease of PSA from 102 ng/ml to 0.662 ng/ml in two weeks after iv dosing; all these effects may be attributable to the P-THP chemotherapy. So far the doses of P-THP given ivranged from 30mg to 75mg free THP equivalent dose / 70kg body wt at every two to three weeks, which did no show any remarkable sign of toxicity, including that of parental drug (pirarubicin) such as cardiacas well as hematological suppression. QOL has been excellent through out the period of P-THP treatment until now. These results warrant further application of P-THP in different tumor types.We have no disclosures and received no financial support.

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PO-108

Track: Drug Discovery in Preclinical Research

A LEADING HCV NS5A INHIBITOR ZN6818 WITH PAN-GENOTYPIC PICOMOLAR POTENCY AND EXCELLENT SAFETY

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Background: The NS5A of hepatitis C virus (HCV) is a non-structural protein and be essential for viral replication and infectivity. So far, it has been intensively studied in the discovery of new HCV inhibitors.

Results: This presentation discloses a novel optimized HCV NS5A inhibitor ZN6818. The ZN6818 had not only outstanding pan-genotypic picomolar potency (EC50: picomolar potency, 4-25pM for all GT-1 to GT-6, respectively), but also excellent PK and safety in rats. There was no any side effect determined with different kinds of potential targets such as hERG, Y93N, Cytochrome P450, etc, respectively. Its metabolic stability is very good, and could be formulated as once-daily dosing tablet. Regarding the safety issue, there was no any death, no any serious drug-related toxicity and adverse events observed during toxicity study in rats (100-2000mg/kg/day, respectively).

Conclusions: The NS5A inhibitor ZN6818 had all pan-genotypic picomolar potency, excellent safety and PK, which appeared much better than other reported NS5A inhibitors. Our goal is to develop a global leading anti-HCV DAA combination therapy with our optimized NS5A inhibitor ZN6818 and another best-in-class NS3 inhibitor ZN2007 in clinical studies.

Keywords: HCV NS5A inhibitor, PK, potency, toxicity.

PO-64

Track: CNS Drug Discovery & Therapy

HISTAMINE INDUCES UPREGULATED EXPRESSION OF HISTAMINE RECEPTORS AND INCREASES RELEASE OF INFLAMMATORY MEDIATORS FROM MICROGLIA

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Histamine is a potent mediator of inflammation and a regulator of innate and adaptive immune responses. However, the influence of histamine on microglia, the resident immune cells in the brain, remains uninvestigated. In the present study, we found that microglia can constitutively express all

four histamine receptors (H1R, H2R, H3R and H4R), and the expression of H1R and H4R can be selectively upregulated in primary cultured microglia in a dose dependent manner by histamine. Histamine can also dose dependently stimulate microglia activation and subsequently production of pro-inflammatory factors TNF- alpha and IL-6. The antagonists of H1R and H4R but not H2R and H3R reduced histamine-induced TNF- alpha and IL-6 production and MAPK and PI3K/AKT pathway activation, and mitochondrial membrane potential loss in microglia, suggesting that the actions of histamine are *via* H1R and H4R. On the other hand, inhibitors of JNK, p38 or PI3K suppressed histamine-induced TNF-alpha and IL-6 release from microglia. Histamine also activated NF- kappa B, and ammonium pyrrolidinedithiocarbamate, an inhibitor of NF-kappa B, reduced histamne-induced TNF- alpha and IL-6 release. In summary, the present study identifies the expression of histamine receptors on microglia. We also demonstrate that histamine induced TNF- alpha and IL-6 release from activated microglia *via* H1R and H4R-MAPK and PI3K/AKT-NF-kappa B signaling pathway, which will deepen the understanding of microglia-mediated neuroinflammatory symptoms of chronic neurodegenerative disease.

Keywords: Histamine, histamine receptors, inflammatory factors, microglia activation, neuroinflammation.

PO-120

Track: Cancer Targeted Drug Delivery

ANTI-GLIOMA AGENTS BY TARGETING MULTIPLE TUMOR METABOLIC REGULATORS

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Gliomas are the most common and malignant brain tumors. Chemotherapy has played an important role as an adjuvant in the treating gliomas. However, the efficacy of current drugs is limited due to serious side effect, poor drug delivery, and chemo-resistance. There is therefore a need to develop novel anti-glioma agents with unique mechanisms for treating gliomas. Accumulating evidence demonstrates that enhanced glycolysis, glutaminolysis, and lipogenesis are prominent hallmarks in gliomas. Several important metabolic enzymes, such as hexokinase 2 (HK2), 6-phosphofructo-2-kinase/2,6-bisphosphatase 3 (PFKFB3), pyruvate kinase M2 (PKM2), lactate dehydrogenase 5 (LDH5), glutaminase (GLS), and fatty acid synthase (FASN), have been revealed to be related to tumorigenesis of gliomas. These regulators manipulate the important glycolytic, glutaminolytic and lipogenetic pathways used by tumor cells to generate energy substance ATP and biosynthetic precursors that are required for the synthesis of biomacromolecules, leading to facilitate the proliferation of glioma cells. Therefore, targeting metabolic regulators has been emerged as a promising strategy for the discovery of novel anti-glioma drug leads. In this poster, we will present three bioactive compounds with potent activity against gliomas. These bioactive agents have been demonstrated to exert their antitumor activity by targeting several key metabolic regulators of HK2, PFKFB3, PKM2, LDH5, GLS and some important oncogenes of c-Myc, Bmi-1, Notch-1, Bcl-2, Bcl-xl, and Survivin. The results suggested that these bioactive compounds could be further developed as potential therapeutics of gliomas.

Keywords: Anti-glioma agents, multiple targets, oncogenes, tumor metabolic regulators.

PO-75

Track: Drug Discovery in Preclinical Research

N (6)-ISOPENTENYLADENOSINE AS REGULATOR OF EPIGENETIC MODIFICATIONS IN CANCER

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N (6)-isopentenyladenosine (iPA), a naturally occurring modified nucleoside characterized by an isopentenyl chain, shows a direct anti-tumor activity against several cancers, although the precise mechanism of action in inhibiting cancer cell proliferation remains to be clarified.

In this study, we investigated the anti-proliferative effect of iPA in colorectal cancer (CRC) cell lines (DLD1, SW620, HCT116), studying the cell cycle deregulation analyzed by flow cytometry. In order to identify the molecular mechanisms of the compound, we determined iPA-direct protein partners through fishing for partners gel free approach in DLD1 cells and the attention was focused on histone H2B, the only protein identified both in cytosolic and nuclear extracts.

Since H2B is modified *in vivo* by the attachment of ubiquitin, ubiquitinated H2B levels have been detected, through Western Blot analysis after iPA exposure, showing an interesting modulation. In agreement with the role of mono-ubiquitinated H2B, in CRC cell lines, iPA also regulates histone H3 Lys-4 and Lys-79 methylation, revealing the ability of iPA to control "H2B-H3 histone cross-talk" involved in the transcriptional regulation. The preliminary results seem to suggest that the anti-proliferative effect of iPA could arise from epigenetic regulation of genes involved in cell survival.

Keywords: Cancer, H2B-H3 histone cross-talk, N (6)-isopentenyladenosine.

PLENARY LECTURES

PL-55

OBESITY DUE TO ADENOVIRUS 36 INFECTION: CLINICAL PRESENTATION AND MECHANISMS

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The concept of obesity due to virus infection is now well accepted. Early studies in animals showed that canine distemper virus, Rous-7 virus, Borna virus, and scrapie agents caused obesity by damaging areas of the brain, predominantly the hypothalamus. Adenoviruses are particularly interesting as they appear to cause obesity *via* peripheral mechanisms and do not appear to cause brain damage. SMAM-1, an avian adenovirus, causes obesity in chickens and one study showed that humans with antibodies to SMAM-1 were heavier than uninfected individuals. The most exciting work has been done on human adenoviruses. Adenovirus 36 (Adv36) was the first human adenovirus reported to cause obesity in animals. Multiple studies have been done in humans associating Adv36 with human obesity as described below. Adv37 and Adv5 were shown to cause obesity in animals (chickens and mice respectively), but do not appear to be associated with human obesity. Adv36 has been administered to chickens, mice, rats, and monkeys, causing obesity in 60%-90% of lower animals and 100% of monkeys. Multiple studies in humans from across the world show that about 30% of obese humans and about 15%-20% of non-obese humans have been infected with Adv36. A large majority of studies show that prior Adv36 infection is associated in some way with obesity and this effect is strongest in children. Two meta-analyses show that Adv36 is associated with human obesity, with Odds Ratios of 1.9 and 1.6 in infected *vs* uninfected individuals. One meta-analysis showed that in children the Odds Ratio for obesity was 1.95 for infected *vs* uninfected. There is disagreement about the effects of Adv36 on metabolic variables. Animal studies show a consistent effect on serum lipids, with a paradoxical reduction of serum cholesterol and triglycerides in infected *vs* uninfected. In humans, some studies show lower serum cholesterol and/or triglycerides, whereas others show no effect or higher serum lipids in infected *vs* uninfected. Children's studies generally show higher levels of lipids with Adv36 infection. There also is some disagreement on the effects of Adv36 on glucose metabolism. Some studies have shown no effect of Adv36 on serum glucose and insulin, but most show that serum insulin levels and insulin resistance by HOMA are lower in animals or humans infected with Adv36. Again this is a paradoxical effect since both lipids and glucose metabolism would be expected to be worse with obesity. Mice that were made mildly diabetic with streptozocin, then infected with Adv36 actually had a decrease in serum glucose compared to uninfected mice. The mechanisms of Adv36 induced obesity and the effects on glucose metabolism appear to be due to direct action of the virus on peripheral cells. With the initial virus infection, there is an intense viremia that results in infection of most organs of the body. Seven months after experimental Adv36 infection in monkeys, viral DNA, but not live virus, could be recovered from brain, lung, liver, muscle, and adipose tissue. With initial infection of cells by Adv36, the viral DNA travels to the host nucleus and initiates a series of actions. In upper respiratory epithelium, the virus turns off apoptotic protective mechanisms, allowing the virus to rapidly replicate and make large numbers of copies of itself. Eventually the infected cell dies and releases the viral particles into the bloodstream where they travel throughout the body. In differentiated cells of most organs, the virus does not appear to replicate, or does so slowly. In multiple cell types the virus increases the number of glucose transporters in the cell membrane *via* stimulation of the Ras pathway. Glucose transport into cells is facilitated, thus lowering the need for insulin for glucose transport and giving the appearance of increased insulin sensitivity. Once glucose enters the cell, it is transformed into fatty acids by Adv36 induced stimulation of fatty acid synthase (FAS). FAS is the final step in production of fatty acids from glucose. Multiple cell types, including adipocytes, show increased stores of lipids. PPAR-Āis stimulated by Adv36, which results in differentiation of human adult stem cells into adipocytes. All of the actions are due to the viral gene, early gene 4, open reading frame 1 (E4orf1). If E4orf1 is blocked with siRNA, the effects of the virus are blocked. Transfecting the E4orf1 gene into cells, usually by lentivirus, results in the spectrum of Adv36 effects. Currently there are no antiviral agents that can be used to treat Adv36 infection or block its effects. Research is ongoing to identify active agents against Adv36. The long term solution for this disease will be final development of a vaccine which has been shown to produce antibodies in animals and block the obesity effect in mice.

Scope of the Topic: Adv36 causes obesity in multiple animal species. About 30% of obese people and about 15% of non-obese people are infected. Children show a stronger correlation of Adv36 with obesity. Adv36 improves glucose metabolism and reduces the effects of diabetes. Anti-virals and a vaccine are under development.

CONFLICT OF INTEREST

Richard Atkinson is the owner of Obetech, LLC. This company provides assays for adenoviruses that produce obesity and has several patents and patent applications regarding virus-induced obesity including diagnostic assays, vaccine, and antiviral agents.

PL-155

NATURAL PRODUCT BASED PHARMACOPHORES - *WHAT ARE THE MOST PRODUCTIVE APPROACHES?*

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From centuries natural products are serving as excellent sources of human medicines, and only about 200 years ago scientific progress allowed drug research to adopt its present shape. Today almost 50% of modern therapeutic agents are either natural products or their derivatives. The initial systematic drugs studied were derived from plants, some are still being used today, such as morphine, salicylic acid, quinine, digitoxin and pilocarpine. In spite of the successful outcomes from natural products as a rich source for drug leads, the pharmaceutical industries have abandoned or deemphasized natural product based drug discovery in favor of combinatorial libraries of synthetic compounds. However, this approach was eventually found to be less effective to improve the success rate in new drug discovery. Therefore, pharmaceutical R&D is facing an unprecedented decline in identification of new molecular entities (NME) against prevailing and emerging diseases. The change in the strategy of target identification may provide more useful start-points. The more productive approach is the phenotypic i.e. target–naive screening approach which can identify initial hits that can be useful start points for further development. Since the last two decades, we have been working in natural product-based drug discovery by using the phenotypic approach. This has led to the discoveries of several NMEs.

Epilepsy is among the leading neurological disorders in the world. About 50 million people worldwide have epilepsy with almost 90% of these people being in developing countries. Epilepsy is non-curable disease, but can be controlled and managed with medications. Unfortunately most of anticonvulsant drugs available in the market are synthetic in nature, and associated with severe side effects and have to be used whole life to control the seizures. Through extensive studies on medicinal plants of family Ranunculaceae, anticonvulsant natural products, isoxylitones, were discovered from medicinal plants *Delphinium denudatum*, and as well as in non alkaloidal aqueous extracts of *Aconitum cochleare*, *Aconitum laeve*, and *Delphinium nordhagenii*. *Delphinium denudatum* was found to exhibit a good anticonvulsant activity in *in vivo* animal models of epilepsy. Bioassay-guided isolation studies on the roots of this plant, afforded a non-toxic and non-alkaloidal aqueous extract, which exhibited strong anticonvulsant activities in *in vivo* animal models of epilepsy, such as MEST test, scPTZ, scBIC, scPTX, and scSTN tests. Further purification of aqueous extract led to isolation of a strongly anticonvulsant isomeric mixture of *E/Z* isoxylitones which were then synthesized to investigate their anticonvulsant activities in *in vitro* and *in vivo* models. Studies have shown a potent anti-epileptogenic activity of isoxylitones in scPTZ-induced kindling model in mice and they also found to affect some of the underlining molecular changes that are induced following the seizures. These compounds were also subjected to various toxicological studies and compound did not exhibit LD_{50} up to the dose of 1,000 mg/kg and were found to be harmless to the model animals with higher potency as antiepileptic compounds than the currently available drugs.

Multidrug resistance (MDR) is a challenging problem for the healthcare sector. It is very common in most important pathogens, such as vancomycin-resistant *Enterococci* and *Staphylococcus aureus*. Exposure and inappropriate use of the antibiotics is the major cause of MDR, both in developed and developing regions. We have been focusing our efforts on the discovery of natural and synthetic compounds, active against MDR bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* (resistant to over 20 antibiotics). About 1400 fully characterized natural and synthetic compounds were evaluated by high throughput screening against MDR *S. aureus* and *P. aeruginosa*. We have discovered some potent, reproducible and highly active MDR inhibitors of flavonoids, monoterpenes, sesquiterpenes, quinolones, thiourea and organometallic derivatives. Mechanism-based studies on selected compounds of both synthetic

and natural origin were also carried out to assess the compound-induced effects on membrane potential, efflux pump inhibition, etc. We also studied the reversal of multidrug resistance by using the MDR inhibitors, which boost the activity of existing antibiotics.

During this plenary presentation, underlying philosophy and approach of our research on cost-effective discovery of lead molecules at the interface of chemistry and biology will be discussed.

PL-1

ADVANCES IN CLINICAL APPLICATIONS OF SYNTHETIC THYMOSINS IN TREATING LIFE-THREATENING DISEASES

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Significant biochemical and clinical advances in the preparation and use of two of the synthetic thymosins in clinical medicine have occurred. Thymosin Ā$_1$ (TĀ$_1$) and Thymosin Ä$_4$ (TÄ$_4$) have been synthesized by solid-phase methodology and have reached the clinic. TĀ$_1$ (Trade name Zadaxin) is approved in 35 countries for the treatment of hepatitis B and C, and as an immune stimulant and adjuvant. The most recent reports of clinical trials with TĀ$_1$ are pointing to important, hitherto unrecognized, applications in a number of diseases and disorders, including severe sepsis, acute respiratory distress syndrome, peritonitis, acute cytomegalovirus infection, TB, and lung infections in critically ill patients. It is also emerging as a promising chemo-protective agent in patients undergoing chemotherapy and in the treatment of late stage melanoma in combination with chemotherapy. TÄ$_4$ is the first of the synthesized Ä-thymosins to reach the clinic. Many of its activities directly affect the repair and regeneration cascade following injury. For example, TÄ$_4$ guides progenitor stem cells from the outer layer of the heart to repair tissue sites within the heart after a heart attack and stimulates oligodendrogenesis in the brain. TÄ$_4$ also has been found to protect cells and tissues from further damage and to reduce apoptosis, inflammation, and microbial growth. In experimental studies in mice, rats, rabbits and pigs, TÄ$_4$'s activities centering around wound healing have provided the scientific foundation for ongoing and projected human trials (phase I and II) in the treatment of eye injuries, dermal wounds, repair of heart following and acute myocardial infarction and in the brain following stroke, trauma or neurological diseases such as multiple sclerosis, and peripheral neuropathies. In the results of two early phase II trials in patients with dry eye, TÄ$_4$ was found to significantly improve several signs and symptoms of dry eye, as well as to show positive trends in other outcome measures. The availability of a number of synthetic thymosin peptides like TĀ$_1$ and TÄ$_4$ has significantly accelerated animal experimentation in the field and is helping researchers to consider a number of new and novel clinical applications. In recently publishes studies, TÄ$_4$ has been shown to be an effective promoter of corneal healing in patients with chronic, medically unresponsive, non-healing corneal defects related to loss of corneal innervation primarily associated with diabetes and neurotrophic keratitis due to herpes zoster, and in patients with moderate to severe dry eye secondary to GVH.

PL-2

LIPID-MODIFYING THERAPIES AND CARDIOVASCULAR PREVENTION: 2014 UPDATE

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The field of lipid management and cardiovascular prevention continues to move rapidly ahead. In the past year, a major development has been the release of controversial new guidelines from the American College of Cardiology and the American Heart Association on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults. These guidelines include important modifications in risk assessment designed to better estimate risk in women and African Americans. A radically new feature is the elimination of low-density lipoprotein cholesterol targets, which have been replaced by recommendations regarding the intensity of therapy in four groups of individuals that can benefit from statin treatment.

Data regarding the relative efficacy and safety of statins and other lipid-lowering drugs, including niacin, resins, fibrates, and ezetimibe, continue to accumulate. Recently introduced agents, including the microsomal triglyceride transfer protein inhibitor lomitapide and the antisense therapeutic mipomersen, have been approved for the treatment of homozygous familial hypercholesterolemia for more than a year in the United States, with recently published studies providing additional information on their use in this population of very high-risk individuals. Cholesteryl ester transfer protein inhibitors, anti-inflammatory agents, and drugs targeting PCSK9 are advancing rapidly in clinical development and have the potential to address the residual cardiovascular risk that persists even with optimal statin therapy.

PL-105

MECHANISMS OF BARIATRIC SURGERY: IT'S IN THE CHEMISTRY, NOT THE PHYSICS

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Bariatric surgical procedures, including Roux-en Y gastric bypass (RYGB), vertical sleeve gastrectomy (VSG) and others, are the most effective treatments for obesity, with the vast majority of weight lost as fat. In patients with type 2 diabetes, these operations lead to full remission at one year in a substantial portion of patients, an effect that appears to be out of proportion to the associated weight loss alone. Although long considered to act through mechanical restriction of food intake and malabsorption of ingested nutrients, recent clinical observations suggest that both RYGB and VSG exert their therapeutic effects by altering the physiological regulation of energy balance and glucose homeostasis. To explore the mechanisms of action of these procedures, we have developed rat and mouse models of these operations and examined their effects on food intake, food preference, energy expenditure and glucose homeostasis. In both rats and mice, RYGB induces weight loss by decreasing food intake and increasing resting energy expenditure. The alterations in food intake are associated with decreased appetitive drive and altered food preferences. Late after surgery, these animals exhibit a change in preference from a high-fat, high-sugar diet to a normal chow diet. The RYGB-associated increase in energy expenditure results from stimulation of diet-induced thermogenesis, suggesting that this operation works in part by enhancing the normal thermogenic response to ingested nutrients. Like human patients, animals with type 2 diabetes exhibit a dramatic improvement in fasting blood glucose and glucose tolerance after both RYGB and VSG. These changes are associated with increased hepatic insulin sensitivity as well as improved pancreatic Ā-cell function and glucose-induced insulin secretion. The molecular mechanisms of these profound effects are just beginning to be understood. Although these operations are associated with elevated postprandial concentrations of GLP-1 and decreased circulating ghrelin, genetic deletion studies demonstrate that signaling by these hormones is not required for their effectiveness. Rather, the underlying mechanisms appear to include alteration of the intestinal microbiota, enhanced glucose transport into small intestinal enterocytes, bile acid signaling through the nuclear receptor FXR, and neural signaling through the melanocortin type 4 receptor. Dissection of the myriad physiological effects of these operations and identification of the cellular and molecular mechanisms underlying their effects will facilitate the development of

novel, less invasive therapies for obesity, diabetes and related metabolic disorders that reproduce the profound and durable benefits of these operations, without the need for the surgery itself.

PL-3

IMPORTANT ROLES OF MicroRNA IN NEURODEGENERATIVE DISEASES

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Non-coding RNAs play essential roles in neurodegenerative disorders (Salta and Strooper, 2012). MicroRNAs (miRNAs) are an abundant class of small RNAs that mediate potent inhibitory effects on global gene expression. Recent advances in molecular methods allow us to study the contribution of these miRNAs to gene expression in neurodegenerative diseases, such as Alzheimer's disease (AD), which is the most prevalent form of dementia. One of the major hallmarks of AD is the presence of amyloid-Ā (AĀ) peptide plaques. Neurochemically, AD is believed to result from the misregulation of the production or clearance of AĀ. The rate-limiting step in AĀ production of is the cleavage of the amyloid- Ā precursor protein (APP) by a Ā-secretase or Ā-site APP-cleaving enzyme (BACE1). Expression studies suggest that dysregulation of proteins involved in AĀ production, such as APP and BACE1, may contribute to excess AĀ deposition. Elucidating how expression of these proteins is regulated will ultimately reveal new drug targets. Our aim is to utilize the novel approach of studying the regulation of these gene products by miRNAs. Here we review miRNA-mediated regulation of APP and BACE1. Using multiple bioinformatic tools and a series of functional studies in neuronal and glial cultures, we reported specific microRNA species regulate APP levels, such as miR-101 and miR-153 (Long, Ray and Lahiri, *J. Biol. Chem.,* 2012). We have recently reported the discovery of novel BACE1-specific miRNAs (Long, Ray and Lahiri, *J. Biol. Chem.,* 2014). Briefly, we prepared a chimeric BACE1 3'-UTR reporter construct by inserting the 3.9 kb BACE1 3'-UTR downstream of a reporter *Renilla* luciferase gene and then delivered the reporter construct along with several miRNAs predicted to target the BACE1 3'-UTR into human cell lines. Several "hits" (e.g. miR-298, miR-339-5p) resulted in reduced reporter expression. We further validated the reporter expression data for miR-339-5p by Western analysis of native BACE1 levels, which were significantly reduced following miR-339-5p delivery, with a significant reduction in potentially toxic AĀ levels. Delivery of miR-339-5p mimic also significantly inhibited expression of BACE1 protein in human primary brain cultures. Finally, miR-339-5p levels were found to be significantly reduced in brain specimens isolated from AD patients as compared to age-matched controls. Therefore, miR-339-5p regulates BACE1 expression in human brain cells and is most likely dysregulated in at least a subset of AD patients. These results reveal a novel regulatory interaction between two important AD-related genes (APP and BACE1) and specific endogenously expressed miRNA species. These novel miRNAs are likely to serve as novel therapeutic targets for AD.

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PL-56

THE BIGGER IS BETTER IN THE CANCER TARGETING DRUGS: THE EPR EFFECT FOR THE PRIMARY AND METASTATIC TUMORS FOR TREATMENT AND IMAGING, AND BEYOND

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The enhanced vascular permeability and retention (EPR) effect is more universal tumor targeting mechanism for most solid tumors using macromolecular biocompatible drug conjugates or nanoparticles than so called molecular target drugs or antibody conjugated drugs. It utilizes vascular permeability of tumor vasculatures, which is uniquely different from

the blood vasculature of normal tissues. This difference is not only seen in the anatomical architecture, but also seen in the production of vascular mediators excreted by the tumor tissue. Normal tissue surrounding the tumor tissue may be affected by these permeability factors, such as nitric oxide, bradykinin, vascular endothelial growth factors (VEGF), prostaglandins, and others [1].

Enhanced vascular permeability means extravasation of macromolecules including plasma proteins and other macromolecules (nanoparticles) or lipidic particles into interstitium of the solid tumors. Then what would happen to those nanoparticles. In the normal tissues, little extravasation of nanoparticle would occur usually. However, if it does occur as in the inflamed tissue, their clearance will be *via* the lymphatic system or reticuloendothelial system. One should recall that the lymphatic system is the most preferred site for cancer metastasis. Cancer cells traverse lymphotropically into the lymphatic system then to the regional lymph node, where they would propagate and become lymphatic metastatic nodules. This means once the nano-drugs are leaked out of blood vessels near the tumor tissue they will be targeted to the metastatic lymph nodes. Therefore, such nano-drugs may be advantageous candidates as an anti-lymphatic metastasis [2]. It should be noted, however, that larger lymph metastatic tumor nodules are fed *via* the neovasculature (blood vessels) [3], not by the lymphatic system that is also the subject of EPR effect [1]. Whichever the tumor feeding system, i.e. *via* the lymphatic or blood vessels, nanoparticles are therefore more advantageous than low MW drugs.

The EPR effect can be augmented to 2-3 fold by infusing permeability modulator such as nitric oxide generators (e.g. nitroglycerin), or by elevating the blood pressure, e.g. from 110 to 150 mmHg using angiotensin II. These tactics were found useful also in human clinic [1,5]. Further, angiotensin I-converting enzyme (ACE)-inhibitor, such as enalapril, would inhibit degradation of bradykinin (BK). As a consequence, the level of BK in tumor would increase, which will result in 2-3 fold enhancement of EPR effect (nano-drug delivery to tumor). None of these methods cause adverse effect [1].

Further, some hypovascular tumors, such as pancreatic and prostate cancer, metastatic cancer in the liver, and also tumors with heterogeneous EPR effect, that show least EPR effect and less cancer drug delivery, may become more tumor accessible by utilizing these tactics.

Metastatic tumors in experimental tumor model also exhibit the EPR effect, of which data will be presented. Consequently, polymer (styrene-co-maleic acid, SMA) conjugated pirarubicin (a derivative of doxorubicin (DOX), 4'-O-tetrahydropyranyl doxorubicin) at 20mg/kg of free THP equiv. iv dose only once at day 20 eradicated all lung metastatic tumor by day 50.

To best utilize the EPR effect we have recently synthesized another polymer conjugated pirarubicin using HPMA (hydroxypropylmethacrylamide) polymer [P-THP], which is designed to release THP at tumor site, namely by its unique environmental low pH and hydrolytic enzymes of tumor. P-THP, which accumulate more in tumor selectively by EPR effect, released free THP from the conjugates near in tumor tissue will be taken up into the tumor cells about ～ 100 times faster than free DOX. Thus cytotoxicity is seen only at tumor site. This EPR driven tumor selective accumulation is seen even 48 hr to 72 hr after iv infusion, where no normal organs showed drug accumulation after 24 hr.

Preliminary results of compassionate use of this P-THP conjugate for stage IV patients of end stage prostate cancer and lung cancer in a hospice indicate promising results, and no indication of toxicity at effective dose, 30～50 mg/1.8m^2 of free THP equivalent.

Photodynamic therapy (PDT) has been known for more than a century; which has longer history than cancer chemotherapy, and in PDT, it requires photosensitizers (PSs). The examples of commonly used PSs are Photofrin® and Laserphyrin®, not nano-drugs, and they emit fluorescence and generate oxygen radicals when illuminated at excitable light. The problem of these PSs is that they do not accumulate in the tumor selectively; rather they are distributed throughout the body evenly, but more preferably in the liver. And they are quickly excreted into the bile and then into the feces. Another problem is that current PDT uses He/Ne or YAG laser. However, they do not fully utilize excitation wavelength of PSs due to insufficient spectral fitting.

We have developed PS conjugated with HPMA polymer, SMA polymer, or PEG, and PS of our choice is zinc protoporphyrin (ZnPP), which have multiple cancer suppressing mechanisms other than generating oxygen radical upon light excitation (by blue fluorescent light, xenon light of normal endoscopic light). Spontaneously formed breast cancer in rats *in vivo* (autochthonous) and other cancers were completely eradicated by once or twice light irradiation after only one iv infusion of this polymer ZnPP conjugates at 20mg/kg. No toxicity was seen.

In conclusion, there are definitely advantageous aspects of nanodrugs as described above, however, sophistication and wide knowledge of tumor physiology, biology and biochemistry should be fully incorporated [4]. Augmentation of tumor delivery, in low EPR or heterogeneous tumor, will be possible to make nanodrugs more accessible to tumor 2-3

fold. We have demonstrated this in clinic using SMANCS in Lipiodol which was infused arterially and by elevating the blood pressure, for highly advanced stage IV patients, or with NO generating agents, and they found incredibly effective [5].

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PL-103

POWER OF MOLECULAR PATHOLOGICAL EPIDEMIOLOGY (MPE) APPROACH TO DISCOVER DISEASE BIOMARKERS FOR PRECISION MEDICINE

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This lecture introduces "Molecular Pathological Epidemiology (MPE)" (= Molecular Pathology + Epidemiology)" (Ogino *et al*. J Natl Cancer Inst 2010; Ogino *et al*. Nat Rev Clin Oncol 2011; Field *et al*. JAMA 2013; etc.) as simply as possible. Any given human disease represents fundamentally heterogeneous process, as implicated by the "Unique Disease Principle" (Ogino *et al*. Expert Rev Mol Diagn 2012; Ogino *et al*. Mod Pathol 2013). MPE dissects complex interplay between environmental, dietary and lifestyle factors, molecular pathogenic alterations, and disease occurrence and progression. MPE is a logical next step of genome-wide association studies ["GWAS-MPE Approach" (Ogino *et al*. Gut 2011)]. MPE has proved itself to be a promising approach to identify biomarkers for precision medicine (Chan *et al*. NEJM 2007; Liao *et al*. NEJM 2012; Nishihara *et al*. NEJM 2013, etc.). It is increasingly possible to design MPE database worldwide using routine molecular testing data, as molecular pathology testing is becoming routine clinical practice. It is essential to build large-scale population-based databases including medication use, lifestyle factors, molecular pathology, and clinical outcome. Such databases can generate novel information on potential chemopreventive or therapeutic benefits of drugs, which can be further tested by experimental models and clinical trials. Because disease heterogeneity is a ubiquitous phenomenon, the MPE paradigm should become routine to advance epidemiology in the 21st century, and move us towards personalized prevention and treatment.

PL-154

NEW CONCEPTS IN THE PATHOPHYSIOLOGY OF TYPE 2 DIABETES: INSIGHTS FROM HUMAN STUDIES

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Type 2 diabetes (T2D) is a major public health problem worldwide. However, the underlying molecular defects that confer T2D risk remain unknown. While diabetes risk factors are well-recognized, including family history, abnormal intrauterine environment, obesity, inactivity, and aging, the underlying defects that predispose to diabetes remain unknown. Insulin resistance, particularly in muscle, is an important contributor to T2D pathophysiology, occurring years before reduced insulin secretion or glucose intolerance, and also predicts T2D. Therefore, understanding molecular mechanisms underlying insulin resistance is essential to develop new approaches for prevention and treatment of T2D.

The association of insulin resistance and risk for T2D with obesity and inactivity indicates an important, and potentially pathogenic, link between fuel and energy homeostasis and the emergence of metabolic disease. Given the central role for mitochondria in fuel metabolism, alterations in cellular mitochondrial oxidative function may contribute to the pathogenesis of T2D. Consistent with this hypothesis, analysis of genomic, metabolomics, and *in vivo* data has demonstrated evidence for alterations in oxidative metabolism in individuals at risk for T2D.

This presentation will focus on pathways altered in tissues of humans with established T2D, as well as those with insulin resistance and genetic risk, which we have identified in unbiased analyses of human tissue samples obtained from patients across the spectrum of insulin sensitivity and risk for type 2 diabetes. One such pathway is marked by overexpression of genes regulated by serum response factor (SRF), its coactivator MKL1, and its upstream regulator ABRA (actin-binding Rho-activated protein, also known as STARS). Importantly, ABRA is the top-ranking differentially expressed gene in muscle of humans with T2D, and its expression correlates with insulin resistance. Our recent studies in both animal and cellular models suggest that the ABRA-MKL1-SRF pathway is a very potent regulator of metabolism.

PL-153

MULTIFUNCTIONAL PHARMACEUTICAL NANOPREPARATIONS

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The use of pharmaceutical nanocarriers, including liposomes and polymeric micelles, for the delivery of a broad variety of both soluble and poorly soluble pharmaceuticals is our days a well-established paradigm for enhancing the *in vivo* efficiency of many drugs. The next challenge now is to develop a new generation of nanopharmaceuticals making those multifunctional and stimuli-responsive. In other words, such nanocarriers, depending on the specific requirements, can circulate long; target the site of the disease via both non-specific and/or specific mechanisms, such as enhanced permeability and retention effect (EPR) and ligand-mediated recognition; respond local stimuli characteristic of the pathological site by, for example, releasing an entrapped drug or deleting a protective coating to facilitate the contact between drug-loaded nanocarriers and target cells; provide an enhanced intracellular delivery of an entrapped drug; and even target individual organelles inside cells. Additionally, these carriers can include contrast moieties to follow their real-time biodistribution and target accumulation. Among new developments to be considered are: drug- or DNA-loaded delivery systems additionally decorated with cell-penetrating peptides for the enhanced intracellular delivery; “smart” multifunctional drug delivery systems, which can reveal/expose temporarily hidden functions under the action of certain local stimuli characteristic for

the pathological zone; new means for controlled delivery and release of siRNA; organelle-targeted nanocarriers; nanocarriers co-loaded with siRNA and chemotherapeutics to treat multidrug resistant cancers; and theranostic nanopreparations.

PL-57

EGFR-TYROSINE KINASE INHIBITOR AGENTS AND NEUROGENIC INFLAMMATION ASSOCIATED WITH HYPOMAGNESEMIA

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Background: Erlotinib (ERL, Tarceva™), approved as a first line treatment, maintenance treatment, and 2nd- or 3rd-line treatment for advanced-stage non-small cell lung cancer, is a reversible tyrosine kinase inhibitor targeting the EGFR receptor (EGFR) which is up-regulated in the majority of lung, colorectal and head and neck cancers. However, EGFR activation is also required for active epithelial Mg-absorption/re-absorption mediated by the transient receptor potential melastatin 6 (TRPM6) channel in the kidney and colon. We determined whether prolonged treatment with ERL causes hypomagnesemia, oxidative stress and cardiac dysfunction in rats, and if blockade of the neurokinin-1 (substance P [SP]) receptor is protective.

Methods and Results: ERL was administered in the diet (~10 mg/kg/day) to normomagnesemic rats for 9 wks. Plasma magnesium in ERL-treated rats decreased gradually after 10 days and became significant between 3-9 weeks: -9% at wk 3, -13% at wk 5, -16% at wk 7 and -26% at wk 9. Moderate but significant increases in plasma substance P (SP) were detected at week 3 (+27%) and week 9 (+25%). At the end of 9 weeks, neutrophils isolated from whole blood exhibited a 3-fold higher basal, and a 2-fold higher stimulated (by PMA) superoxide generating activity. Concomitantly, total plasma 8-isoprostance content, a marker of systemic lipid peroxidation, rose significantly to 210%. The effect of SP receptor blockade was assessed by dietary administration of Emend™ (as aprepitant, a SP receptor antagonist, ~ 2mg/kg/day).

Emend mildly (NS) attenuated (up to 35%) ERL-induced hypomagnesemia, but significantly attenuated SP increases, neutrophil activation and 8-isoprostane elevation. Echocardiography revealed significant decreases in left ventricular ejection fraction (LVEF: -11%) and % fractional shortening (%FS: -17%) after 7 weeks, indicative of systolic dysfunction, and significant reduction (-17.5%) in mitral valve E/A ratio at week 9, indicative of diastolic dysfunction.

Concomitantly, left ventricular posterior wall thinning accurred consistent with early sign of dilated cardiomyopathy. Treatment with Emend completely prevented both systolic and diastolic dysfunction and partially attenuated anatomical changes caused by ERL treatment.

Conclusion: Since hypomagnesemia alone can cause neurogenic inflammation, our study suggested that chronic ERL treatment induced moderate but progressive hypomagnesemia, which in turn triggered SP-mediated oxidative inflammation and significant levels of cardiac dysfunction. Our study also demonstrated, for the first time, that administration of a clinically used SP receptor blocker, Emend, effectively prevented the ERL-induced systemic oxidative stress and cardiac dysfunction.

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PL-104

IMPORTANCE OF HUMORAL IMMUNITY IN *M. TUBERCULOSIS* INFECTION

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Mycobacterium tuberculosis (Mtb) infection is a major world public health problem. One third of the world's population is thought to have latent tuberculosis, a condition where individuals are infected by the intracellular bacteria without active disease but are at risk for reactivation, if their immune system fails. Here, we discuss the role of non-specific inflammatory responses mediated by cytokines and chemokines in response to interaction of innate receptors expressed in macrophages and dendritic cells (DCs). We also review current information regarding the importance of cytokines in the development of protective T cell mediated responses to Mtb and their influence in the formation and stability maintenance of granuloma. Will discuss novel insights of the mechanisms of failure of Mtb control, including the immune dys-regulation induced by the treatment with biologic drugs in different autoimmune diseases. Future functional studies focused in the mechanisms involved in the early control of Mtb infection and the interplay with host innate and acquired immunity may be helpful to understand the pathogenesis of TB particularly the role of humoral immunity and IL-17 in latent tuberculosis. A possible new vaccine could be based on booster immunizations with tuberculin together with Ag-85B-ESAT-6.

Most important is the fact that although cellular immunity is very important in the pathogenesis of active and latent tuberculosis, will discuss evidence that humoral immunity is also important. In this regard, recent evidence (Comas *et al*., Nature Genetics 2010) demonstrated that conserved genes of *M. tuberculosis* coding the epitopes that induce T cell dependent are naturally selected; mycobacteria benefits from the immune response. Also, cellular immunity deos not control drug resistant strains of MTB. Therefore, it is possible that more efforts should be made to study humoral immune responses in MTB infections particularly comparing individuals that had been BCG vaccinated with those without and comparing the immune responses in relation to the socioeconomic class, the pro-inflammatory state related to the microbiome.

KEYNOTE LECTURES

KNL-60

Track: Cancer Targeted Drug Delivery

TARGETING RESISTANT CANCER WITH SPECIFIC DRUG-EFFLUX INHIBITORS AND APOPTOSIS INDUCERS

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Overexpressed ABC ("ATP-binding cassette") transporters are involved in multidrug-resistant tumors by pumping anticancer drugs out of the cells. For early discovered ABCB1/"P-glycoprotein", third-generation drug-efflux inhibitors are under clinical development.

For more recently identified ABCG2/"breast cancer resistance protein", we have screened different series of flavonoids and derivatives, such as flavones, rotenoids and acridones, and more recently chalcones [1, 2] and chromones [3, 4], as inhibitors of mitoxantrone efflux from transfected HEK293 human cells and as chemosensitizers of cell proliferation, to establish 3D-Quantitative Structure-Activity Relationships. Two types of selective, non-competitive, inhibitors have been characterized, either inhibiting or stimulating the basal ATPase activity. The most potent inhibitor is indeed efficient *in vivo* on *SCID* mice, xenografted with human ABCG2-transfected cells, by chemosensitizing tumor growth to the drug-substrate irinotecan [5]. These selective inhibitors constitute good drug candidates, with low intrinsic toxicity, as sensitizers of cell proliferation to conventional chemotherapeutics.

The "Multidrug Resistance Protein 1" ABCC1 is able to catalyze the efflux of either glutathione conjugates or free glutathione together with hydrophobic substrate drugs. We have identified modulators such as verapamil [6, 7] mimicking substrates and inducing a fast and massive efflux of intracellular glutathione from ABCC1-overexpressing cells, leading to a selective cell death through apoptosis called "collateral sensitivity". Since verapamil is known for its cadiotoxic effects, we investigated other types of modulators such as xanthones [8], flavones and flavonoid dimers. Glutathione efflux appeared to be necessary, but not sufficient alone, to trigger apoptosis, indicating the contribution of other partner(s) or signaling pathway(s). Such apoptosis inducers may constitute a new type of anticancer drugs operating through an original strategy aimed at selectively targeting and eliminating multidrug-resistant tumors overexpressing the ABCC1 transporter [9].

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KNL-58

Track: Drug Discovery in Preclinical Research

ANTI-VZV VACCINE AS ANTI-HERPES THERAPY

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The phylogenetic tree of the members of the family *Herpesviridae* shows a close relationship between *Human herpesvirus*- (HHV-) 3 also known as *Varicella zostervirus* (VZV), and HHV-1 and HHV-2. The possibility of using the anti-VZV (anti-HHV-3) vaccine against orobuccal HHV-1 and genital HHV-2 was suggested.

A prospective study was conducted from January 2005 through January 2011. Twenty-four patients afflicted with HHV-1 and HHV-2 recurrences over a period of several years averaging 5–8 recurrences per year, agreed to receive the anti-VZV vaccine. They were compared with 26 non vaccinated patients presenting HHV recurrences 2–5 times a year. All 50 patients were checked by serological testing for anti-HHV-1, anti-HHV-2, and anti-VZV antibodies.

From 2005 through 2011, for all 24 anti-VZV vaccinated patients, the number of herpes relapses fell to 0 and was correlated with an increased anti-VZV antibody level and clinical recovery of all patients, whereas no improvement was observed for the 26 non vaccinated herpes patients.

A defective anti-VZV genetic immune power in these patients was suggested in correlation with a significant increase of anti-VZV serological antibody levels among the vaccinated patients ($P < 0.001$) and with the clinical recovery of all these herpes patients. As suggested recently [1], an increase in anti-HHV-3 antibodies with the anti-HHV-3 (anti-VZV) vaccine boosts HHV-1/HHV-2 defence against recurrent herpes diseases.

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KNL-75

Track: Chemistry

HEPARIN-BASED THERAPEUTICS WITH IMPROVED PROPERTIES

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Heparin, a highly sulfated polysaccharide anticoagulant, commands worldwide market of ~$7B. Currently, heparin and related low molecular weight heparins (LMWHs, such as enoxaparin, tinzheparin, dalteparin), require an extraction from pig intestine. The ultra-low molecular weight heparin (ULMWH) fondaparinux, a chemically synthesized pentasaccharide, is expensive and has limited clinical applications. We have developed an efficient chemoenzymatic synthesis of heparin, LMWH, and ULMWH. Here, we show our efforts to prepare low molecular weight heparin with improved pharmacological properties (*i.e.,* defined pharmacokinetics, liver clearance and protamine reversibility) using chemoenzymatic synthesis. These improved properties might expand the use of LMWH in patients with compromised renal function. Our approach relies on chemical synthesis and transformations relying on biosynthetic enzymes,

including heparosan synthases, sulfotransferases and epimerase. Chemoenzymatic synthesis is reliable over a wide range of scales and should be useful in both research and pharmaceutical applications. Furthermore, this biotechnological process should allow the preparation of this critical drug under cGMP. This controlled technological process should afford improved products and help prevent the introduction of impurities, contaminants and adulterants, possible through less highly regulated processes as illustrated by the 2008 heparin contamination crisis.

KNL-71

Track: Chemistry

MODERN METHOD FOR COMPONENT ANALYSIS AND IDENTIFICATION OF SUBSTANCE USING THE THz SIGNAL WITH BROAD SPECTRUM AND MODERN ASSESSMENT CRITERIA

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In the past decade, the THz Time-Domain Spectroscopy has been widely used in problems of the detection and identification of explosives, drugs and other dangerous chemical and biological substances. THz-TDS technology is based on the analysis of frequency spectrum of THz radiation passed through or reflected from the substance. However, there is an essential disadvantage, which makes difficulties for reliable identification in many cases using this technology. For example, some dangerous materials have THz Fourier spectra, which is similar to spectra of ordinary harmless materials or have not pronounced absorption frequencies. We proposed method, named as SDA-method, which is free from this disadvantage. It allows to analyze the dynamics of many spectral lines simultaneously and to obtain the spectrogram - the unique 2D THz signature of the substance. Early, the SDA-method was successfully applied for identification of explosives, including those hidden under opaque covers; substances in compound media; the mixture of substances with similar Fourier spectra in GHz and THz range of frequencies. We also showed the possibility to use the spectrogram for the detection and identification by the reflected THz signal.

In this report, the SDA-method is applied for the detection and identification of illicit drugs - methamphetamine (MA), methylenedioxyamphetamine (MDA), 3, 4-methylenedioxymetham-phetamine (MDMA) and Ketamine by the transmitted THz signal. The drugs MA, MDA and MDMA have similar Fourier spectra in the range 0.1-3.0 THz, and their spectra do not have obvious absorption characteristics. However, we show that there is possibility to identify these drugs if the special criteria are used for the probability assessment for substances analyzing.

KNL-172

Track: Anti-Infectives

TECHNOLOGIES FOR NEW AND IMPROVED VACCINES

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Vaccines are without a doubt the most successful of mankind's medical interventions. However, despite more than two centuries of effective use of vaccines, many substantial challenges remain. These include: 1) improvement of existing but suboptimal vaccines (e.g., tuberculosis, influenza), 2) discovery and development of new vaccines against targets to address large unmet medical needs (e.g., HIV, malaria, cancer), and 3) rapidly responding to new pathogens (e.g., newly emerging

microbes, bioweapons). Recent advancements have demonstrated proof of concept for active immunization in the treatment of cancers. Taking full advantage will require the application of new technologies and paradigms in the areas of tumor antigen identification and optimization, novel potent and safe adjuvants, and enhanced vaccine delivery systems.

KNL-84

Track: Structural Biology

ALLOSTERIC INHIBITION OF PROTEIN-PROTEIN INTERACTIONS IN TRANSLATION INITIATION FOR DESIGN OF ANTI-TUMOR AGENTS

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Eukaryotic translation is regulated by features of the mRNA 5'UTR and by the concentrations and state of initiation factors. The eIF4E protein recruits the small ribosomal subunit to the 5' end of the mRNA *via* interactions with eIF4G and eIF3. Elevated concentrations of eIF4E have been found in several forms of cancer. The activity of eIF4E is regulated by the 4E-binding proteins (4EBPs), which are targets of kinase in signaling pathways and are validated tumor suppressors. We hypothesized that weakening the interaction of eIF4E with eIF4G would selectively reduce the translation of oncogenes. Using high-throughput screening we discovered small molecules that inhibit the eIF4E/eIF4G interaction. The inhibitors termed 4EGIs displace eIF4G from eIF4E but do not affect the interaction with 4EBP-1. Binding of the initial hit compounds and analogs to eIF4E were studied with NMR, X-ray crystallography and other biophysical techniques. The compounds and analogs were tested in *in vitro* and *in vivo* assays. Indeed, the molecules discovered exhibit activity against melanoma, breast, lung, prostate cancer and acute myelogenous leukemia (AML). The lead compounds inhibit eIF4E/eIF4G interactions in xenograft tumors in mice and reduce tumor growth. 4EGI-1 inhibits tumor expression of oncogenic proteins such as cyclin E, cyclin D1, c-myc and Bcl-2. Intra-peritoneal treatment with 4EGI-1 did not exhibit toxic effects in mice. Recently we determined high-resolution structures of eIF4E/inhibitor complexes and discovered that the inhibitors act by an allosteric mechanism. Furthermore, we find enhanced activity of 4EGI-1 against breast cancer stem cells at hypoxic conditions, such as found in solid tumors.

KNL-88

Track: Inflammation and Immunology

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) DNA BLOCKS IN NONAGERIANS AND CENTENARIANS OF MEXICO: ROLE OF SOCIOECONOMIC CLASS

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The knowledge of genetic fixity, that is the use of frequency of DNA Blocks within the MHC (short arm of human chromosome 6) to measure population genetic diversity, should be taken into account in studies of longevity. Genetic admixture and control of infections explains the increasing incidence of autoimmune diseases in Europe (mixture of MHC autoimmune genes, MHCA with non-MHC autoimmune genes, NMHCA). And, in Mexicans not only MHCA are important in autoimmune disease but together with other genes (NMHCA genes) and the socioeconomic class of cohorts are markers of longevity. In this report, using the known DNA blocks of HLA in genetically admixed Mexicans, we found that genes within the class II region, present in autoimmune diseases in developed countries contribute to the increased frequency of class II DNA blocks (DRB!*15:01, DQB!*06:02, DRB*01:01 and *102, DQB1*05:01 in high socioeconomic class and DRB1*04:04, DQB1*03:02 and DRB1*06:03, DQB1*03:01 in low socioeconomic class in nonagenarians and centenarians in Mexicans. Also. the decrease of the added frequencies of Class I DNA blocks contributed by absence of the class I block B*07:02, C*07:02 is important in the low socioeconomic class. This finding explains the higher frequency of the ligands of group 2 of HLA-C of NK receptors (KIRs) which are important in the longevity of the low socioeconomic class. Therefore, our results are consistent with the concept that 'cohort morbidity phenotype' represents inflammatory processes that persist from early age into adult life producing different genetic effects related to the socioeconomic class of young controls compared with groups of genetically admixed nonagenarians and centenarians of Mexico.

INVITED LECTURES

IL-7

Track: Medical Biotechnology

INTEGRATIVE SYNTHETIC BIOLOGY: MAKING BIOLOGY PREDICTABLE FOR ACCURATE DISEASE DIAGNOSIS AND RELIABLE TREATMENT

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Until recently, reductionism has been the dominant scientific approach; however, the contributions made by this singular approach to science have also caused delays in better elucidation and solutions to scientific phenomena applied to other related fields, including medicine. Most scientific advances were made in predictable fields, such as physics, chemistry, and also in engineering, bringing benefits to the development of medical technology after 1980. Yet, unpredictability in biology and related fields such as medicine prevails, demonstrating a need for a multi-dimensional and integrative scientific approach, such as integrative synthetic biology.

Integrative synthetic biology helps to make biology and related fields such as medicine predictable, thus reducing unintended consequences that are common in biological processes, such as genetic investigation. Similarly, in related fields like medicine, the diagnosis and treatment of disease will be more accurate and reliable. The impact of integrative synthetic biology is maximized in medical areas, such as disease diagnostic and treatment, including gene therapy. The synthetic biology approach - an application of integrative biology - has highlighted the importance of integrative biology for the solution of problems, as well as the implementation of scientific and technological research in various fields including medicine, life sciences, biotechnology, environmental science, food and agriculture, energy, and space research. Furthermore, integrating computational modeling, along with the fundamental and systematic fitting approach of engineering, to biological and related sciences, would contribute to reducing the unpredictability of biology and related areas such as medicine. Perhaps, this biological unpredictability is due to the lack of attention and/or understanding of biological processes at the atomic level.

Conventional biology is mainly approached at the cellular level, and in a few instances, only at the molecular level. Fortunately, integrative synthetic biology reminds us that DNA is made up of hundreds of atoms; thus, DNA and related biological functions, such as transcription and translation, are influenced by the same scientific principles and/or physical laws, including electric and magnetic fields that affect the atoms. This suggests that disease could be a result of a biological process initiated and carried out at the atomic level. The measurement of biological processes at the atomic level has always been challenging; so, the integrative synthetic biology approach can be a good tool to offset such difficulty; however, while using integrative synthetic biology, we must make sure to consider the importance of cellular physiology in our experimental designs, since physiology relates to the autonomous principle of life. If we look at DNA as made up of hundreds of atoms, we are now able to assemble different genetic parts and construct genetic sensors for disease diagnosis and therapy. Construction of genetic sensors is suitable for attaining tangible results in disease diagnosis because genetic sensors require less number of genetic parts, shorter base pair sequences, and vectors with lower number of copies.

Currently, I have been able to apply interactive synthetic biology by developing with other colleagues, very sensitive DNA sensors to diagnose early diabetes in patients - sensors can detect both very low to higher concentrations of glucose (0-500 mg/dL)(Patent pending). Similarly, I designed with a colleague, a genetic device to diagnose Alzheimer's by using mineral ions under *in vitro* conditions (patent pending). In other research, with another colleague, we were able to construct an anti-UV plasmid to protect human and animal skin and fruits against damage by UV radiation (patent pending). The anti-UV DNA could also be used to protect astronauts in space missions, as well as future space travelers. Despite the advantages shown by integrative synthetic biology, there is still much progress to be made, especially at the chromosomal and genomic level.

IL-59

Track: Medical Biotechnology

INSULIN AND LYSPRO INSULIN: WHAT IS COMMON AND DIFFERENT IN THEIR BEHAVIOUR?

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There are a few insulin analogues with different pharmacokinetic characteristics, in particular the onset and duration of action. As the duration of action may be connected with the duration of the lag-phase, the challenge is to consider the process of amyloid formation for different analogs of insulin. One of them is LysPro insulin. The behavior of LysPro insulin in the process of amyloid formation has not been studied in detail yet. To quantitatively investigate the differences between the two samples in the aggregation reaction and estimate the difference in the lag-time, we used thioflavin T fluorescence assay, electron microscopy, X-ray diffraction methods, and theoretical modeling. Kinetic experimental data for both insulin and LysPro insulin samples demonstrated the increasing of the lag-time for LysPro insulin at low concentrations of monomers, particularly at 2 and 4 mg/ml, which corresponds to the pharmaceutical concentration. The obtained analytical solution and computer modeling allow us to determine the size of the nucleus from the experimentally obtained concentration dependences of the relationship between the lag-time and the time of growth of amyloid fibrils. In the case of both insulin and LysPro insulin, this relationship is independent of the protein concentration. According to the developed theory, this means that the size of the nucleus corresponds to one monomer in both insulin and LysPro insulin [1-2].

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IL-86

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.

SCIENCE-DRIVEN TECHNOLOGY DEVELOPMENT: BALANCING TUMORICIDAL *VS* TUMORIGENIC (YIN AND YANG) PROPERTIES OF IMMUNE SURVEILLANCE AS CORRECT TARGET FOR CANCER RESEARCH

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Ongoing controversies and misunderstandings of the role that inflammation plays in cancer have been extremely costly for taxpayers and cancer patients. A reason for repeated failed (90% ± 5) outcomes of clinical trials is excessive investment by decision makers (establishment, insiders) in cancer community on 'molecular false-flagging' of too many genetic mutations in the chaotic molecular landscape of site-specific cancers that are used for 'targeted' therapies or 'personalized' medicine. Analyses of data on our 'accidental' discoveries in 1980s on models of acute and chronic inflammatory diseases demonstrated at least three developmental stages of immune dysfunction and response interactions between resident and recruited cells in conjunctival-associated lymphoid tissues (CATLs): (a), acute phase-, activation and degranulation of mast cells (MCs); (b), intermediate phase-, down-regulation phenomenon, exhaustion of MCs, heavy eosinophils (Eos) infiltrations into epithelia and goblet cells (GCs) and neovascularization; and (c), chronic phase-, induction of lymphoid hyperplasia, activated macrophages (M! s), increased (irregular size) B and plasma cells, loss of integrity of lymphoid tissue capsular membrane, follicular and germinal center formation, increased ratios of local IgG1/IgG2, epithelial thickening (growth) and/or thinning (necrosis) and angiogenesis. These data are perhaps the first and only evidence for a direct association between inflammation and identifiable phases of immune dysfunction in the

direction of tumorigenesis. Stimuli-induced induction of activation, recruitment and infiltration of Eos and !∀ s (TAMs) in tissues whose principal resident immune cells are MCs, GCs, and lymphocytes perhaps play crucial synergistic roles in enhancing growth promoting capacities of host toward multistep tumorigenesis. Recently, unresolved (chronic) inflammation was defined as loss of balance between two tightly regulated and biologically opposing arms of acute inflammation (Yin–Yang) or immune surveillance. Chronic inflammation, in all likelihood, is a common denominator in initiation and progression of nearly all age-associated neurodegenerative and autoimmune diseases and site-specific cancers. Systematic studies of interactions between resident and recruited cells should provide key information in understanding early events in the loss of immune surveillance that would help making informed decisions in science-driven technology development for balancing inherent properties of immunity (Yin-Yang), particularly during aging process, and for effective disease prevention and therapeutic approaches and accurate risk assessment formulations toward improving public health.

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IL-82

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.

SIMULTANEOUS TARGETING OF CANCER CELL PROLIFERATION AND IDENTITY SWITCHING

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The war on cancer has been going on for more than a century and so far cancer is winning it. One reason for this failure is that most if not all therapies discovered so far are directed against one aspect of cancer and that is cell proliferation. However, recent findings from our laboratory and others have shown that cancer cells possess another very important characteristic which is their ability to switch identity, though a cellular process called Epithelial-Mesenchymal Transition (EMT). The ability of cancer cells to undergo EMT is critical because it allows them to adapt to therapy and also to escape from it. We have shown that many of the genes expressed in the epithelial state are reduced or even absent in the mesenchymal state and *vice versa*, making it difficult to target individual genes without knowing their fate in cells that are susceptible to EMT. On the basis of this, one needs not only to inhibit proliferation but also to deny cancer cells from switching identity in order to achieve superior efficacy. To this end, research from our laboratory has led to the identification of small molecules capable of simultaneously inhibiting cancer cell proliferation and their ability to undergo EMT. Mechanistically, these compounds activate signaling pathways leading to degradation of cyclins and transcriptions factors implicated in cell growth and EMT respectively. As a result of this, our compounds exerted strong anti-cancer activity *in vitro* and in animal tumor models. Altogether these findings shed light on a new type of anti-cancer drug candidates to simultaneously inhibit cancer cell proliferation and deny them identity switching. It is anticipated that this type of compounds will have superior efficacy than current cancer therapeutics.

Keywords: Cancer Cell Proliferation, Epithelial-Mesenchymal Transition and Identity Switching.

SESSION LECTURES

SL-109

Track: Marine Biotechnology

BACTERIOCIN FROM *PSEUDOMONAS PUTIDA* FStm2 ISOLATED FROM SHARK SKIN AND ITS POTENTIAL AS AN ANTI-BIOFILM

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A study was conducted to isolate and identify marine bacteria with potential to produce antimicrobial peptides. In addition the study also aimed to characterize the activity of the antimicrobial peptide bacteriocin against biofilm forming bacteria. One isolate obtained from shark skin and given the code FStm2 was identified as *Pseudomonas putida* based on biochemical tests, hemolysin assay, API 20NE test, Biolog test, 16S ribosomal RNA and gyrB gene sequences, scanning electron microscopy (SEM) and fatty acid analysis. Treatment with some enzymes showed that only proteinase K reduced the activity of the bacteriocin against the indicator bacterium Serratia marcescens ATCC13880. The bacteriocin was inactive at temperatures >80^{o}C and was active over the range of pH 3-9. The TSB medium was the best for bacteriocin production. Minimum inhibitory concentration (MIC) assay, minimum bactericidal concentration (MBC) assay, mode of action and observation by transmission electron mikroscopy (TEM) all indicated that the action of the bacteriocin against *S. marcescens* ATCC13880 was bactericidal. The molecular weight of the bacteriocin was ~32 kDa. Bacteriocin at a concentration of Ā16 Äg/ml was not toxic to Vero cells and at the MBC concentration of 1.25 Äg/ml did not cause hemolysis of red blood cells. Profile analysis of this bacteriocin by MALDI-TOF (MS/MS) showed no match against any of the known bacteriocins which suggested that it is a novel bacteriocin. *De novo* peptide sequencing produced a partial amino acid sequence containing CHWRHLNLSTGK which again did not match with any of the known Pseudomonas or other bacteria bacteriocins. The bacteriocin was active against biofilm forming bacteria Burkholderia cepacia strain UC(A)a3, UC(A)a5 and UC(A)b8 and Staphylococcus hominis strain UC(A)b1 isolated from urinary catheter. Observation of MBEC assay kit pegs by SEM showed that the biofilm layer decreased together with the decrease in biofilm bacteria density after treatment with bacteriocin. Confocal laser microscopy showed that bactericidal effect on biofilm forming bacteria was markedly higher at bacteriocin concentration of 20 Äg/ml compared to at 10 Äg/ml. Biofilm thickness of *B. cepacia, S. hominis* and *S. marcescens* in foley catheter was reduced after treatment with the bacteriocin. This bacteriocin has very good potential to be developed as an antibacterial agent especially against bacteria that are resistant toward commonly-used antibiotics.

SL-53

Track: Plant and Environment: Transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae; genomics-assisted breeding

CYANIDE IN THE MINING INDUSTRY: THE CHEMISTRY OF APPLICATION, ENVIRONMENTAL CHALLENGES AND CURRENT STATE OF AFFAIRS

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Cyanides compounds contain monovalent combining group of carbon and nitrogen referred to as the cyano group (CN). They are produced by some groups of bacteria, fungi and algae and are found in some plant species such as cassava, lima beans and almond in small amounts. Cyanides breaks down some heavy metals and may form complexes with such metals. Such complexes usually very stable even under acidic conditions. Cyanides are used in gold and silver mining worldwide but its potential toxicity in the environmanet has been of concern. Although cyanides can be degraded by several processes, its impact on the environemt and toxicity to plants and animals is still widely debated. Biological degradation of cyanide is an environmentally friendly process, which has been commercially used at gold mining operations. This paper reviews the application of cyanide in the mining industry, the chemistry of the processes, the environmental challenges they present, the successes and challenges encountered in the remediation practices, and possible new strategies for treating these coumpounds in the mining industry.

Keywords: Cyanide, cyano group, mining industry.

SL-43

Track: Plant and Environment: Transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae; genomics-assisted breeding.

DEVELOPMENT OF REFERENCE GENETIC FINGERPRINTS FOR CONSERVATION OF THREATENED MEDICINAL PLANTS IN ARID ENVIRONMENTS

I. VARIATION IN ESSENTIAL OIL COMPONENTS IN RELATION TO GENETIC DIVERSITY IN ACHILLEA FRAGRANTISSIMA POPULATIONS IN SINAI, EGYPT

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Medicinal plants in the arid environments are exposed to serious threats due to abiotic stress imposed by drought and heavy human impacts such as uncontrolled collection, overgrazing, mining and quarrying. Inclusion of wild plant conservation strategies ensures sustainable use of medicinal plants for future options. *Achillea fragrantissima* is a medicinal plant used for the treatment of cough and as aromatic bitter stomachic, anthelmintic and hypoglycaemic treatments. The fresh floral parts of *Achillea fragrantissima* contain essential oil; many of the oil components were identified. The plant is threatened by over collection for herbal medicine in Egypt and other arid countries in the Middle East. However, no studies are available on the conservation genetics and propagation of this plant for conservation. In this study, DNA fingerprinting has been applied for documenting genetic diversity and GC/MS has been used to identify the major components of the essential oil in leaves and flowering shoots of different populations growing in Sinai, Egypt. The genetic diversity among the examined populations of *A. fragrantissima* was estimated based on variation in morphological traits and polymorphism in DNA finger printing as revealed in ISSR profiles and expressed in a number of trees using the NTSYS-pc and CAP software programs. Higher diversity was found in populations growing in the mountainous sites compared to valleys at lower elevations. The populations growing in mountains were also found to possess more essential oil, while plants in the lower valleys were found to contain more essential oil's components. The presence and distribution of major components of the essential oil was assigned to specific ISSR markers in some of the examined populations. My talk deals with the conservation strategies for wild medicinal plants in arid environments and involves presentation of results on *A. fragrantissima*.

Keywords: Medicinal plants, arid environment, *Achillea fragrantissima*, genetic diversity, molecular markers, essential oil, Egypt.

SL-79

Track: Other Areas: Food; marine; bio-safety; systems biology, clinical research/clinical trials; bioethics; nanobiotechnology.

NEGATIVE RESISTANCE IN DNA NANOWIRES

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Recently, DNA has increasingly interested in the potential technological applications that not directly related to the coding for functional proteins that is the expressed form of genetic information. One of the most interesting applications of DNA is related to the construction of nanostructures of high complexity, design of functional nanostructures in nanoelectronical devices, nanosensors and nanocercuits. In this field, DNA is of fundamental interest to the development of DNA-based molecular technologies, as it possesses ideal structural and molecular recognition properties for use in self-assembling nanodevices with a definite molecular architecture. Also, the robust, one-dimensional flexible structure of DNA can be used to design electronic devices, serving as a wire, transistor switch, or rectifier depending on its electronic properties. In order to understand the mechanism of the charge transport along DNA sequences, numerous studies have been carried out. Theoretical methods often concentrated on electronic states and conduction through DNA. In this regard, One of the models that have been recently introduced, with coupling of charge with nonlinear dynamical Peyrard-Bishop-Dauxois (PBD) model forms the Peyrard–Bishop–Holstein (PBH) model, describes more accurately the

coupled structural and electronic aspects of DNA. The highly nonlinear nature of the PBH model implies the possibility of applying the nonlinear dynamics and chaos theory concepts to study its dynamics. In this regard,the Lyapunov exponent is one of the most popular concepts of the nonlinear dynamics to measure how stable the systems are. On the other hand, Landauer resistance is related to Lyapunov exponent via the transmission coefficient of the system. Then, chaos theory tools could open the new horizons in understanding the charge transfer mechanism in DNA. The obtained results express the negative resistance behaviors in DNA in some temperatures ranges. Also, by applying the external electrical field, DNA shows detected phenomena in some regions of the amplitude and frequency of the field. This phenomena, is commonly employed in the fields of low-power memory or logic devices. Now, we determine the critical limit of external field which leads to instability of system. Also, we could characterize the temperature ranges that DNA shows an anomalous behavior. On the other hand, we could study the variation of electrical current through the DNA. These results confirm the negative resistance phenomena detected in experimental studies and gives insight into determining the ranges of temperature and external electrical field to design the nanodevices.

Keywords: Charge transfer in DNA, Negative resistance, Chaos theory, Mean Lyapunov exponent, Landauer resistance.

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SL-117

Track: Marine Biotechnology: Environment Applications of Marine Biotechnology; Marine Natural Products; Bioproducts and Bioactive Compounds; Marine Microbiology and Biodiversity; Marine-based Drug Discovery & Development; Genomics and Proteomics of Marine Organisms; Aquatic Microbial Ecology

DIVERSITY OF *LISTERIA MONOCYTOGENES* ISOLATES OF MARINE ORIGIN STUDIED BY SEROTYPING AND PULSED- FIELD GEL ELECTROPHORESIS

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The microbiological analysis of 1025 marine samples, among which 345 of sea water, 337 of shellfish and 343 of sediments collected from January 2000 through December 2002 from 18 shellfish sites in the Atlantic littoral part of mid-west of morocco / region of Agadir, has permitted to isolate 143 strains of listeria spp (L. monocytogenes : 38, L. innocua : 109, L. ivanovii :1). The total incidence of Listeria spp in the littoral environment was 5.3%. L monocytogenes was isolated in 13 of 345 sea water samples, in 7 of 343 samples of sediments and in 12 of 337 shellfish samples. The phenotypical characterization of the 38 strains of L. monocytogenes has shown that all the strains belonged only to two chemotypes according to API-Listeria classification (see Methods): the 2510 (8 strains) ĀMan negative haemolytic and the 6510 which is majority (30 strains) ĀMan positive, among which 8 strains are non haemolytic. The serotyping has revealed that all the strains of L. monocytogenes belonged to 1/2 serogroup. The serotype 1/2b was clearly prevalent (78.9%), the rest of isolates were serotype 1/2a, exclusively formed by non haemolytic strains. The molecular typing of listeria monocytogenes strains by DNA macrorestriction method revealed the existence of 6 different pulsotypes by using the restriction enzymes AscI and ApaI. The pulsotype II is dominant (42%) followed by the pulsotypes I (21%) representing the strains of serotype 1/2a, and the pulsotypes IV (10.5%), III (7.9%), and VI (2.6%). The pulsotype VI is only represented by one strain, isolated from a shellfish. The analysis by compartment shows that this distribution is not the same. Whereas the pulsotype II remains predominant in sea water (43.8%) and the shellfish (60%), in sediments, it is the pulsotype IV that dominate. In this last compartment, all pulsotypes are represented except for the rare pulsotype VI. The pulsotype I comprises the 8 isolates belonging to serotype 1/2 a, which all are devoid of haemolytic activity. The Analysis of macrorestriction profiles with bionumerics software shows that the pulsotypes I are genetically distant from the other pulsotypes. The atypical serotype 1/2b (! Mannoside negative) comprises some strains of the pulsotypes III, IV and V.

SL-128

Track: Industrial and Manufacturing

WHAT IS C1?

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What is C1? A robust and versatile fungal platform for gene discovery, expression and the production of enzymes and other proteins Based on the *Myceliophthora Thermophila* fungus, a soil-borne saprophyte. Developed over the past two decades through UV-induced mutation and other bioengineering methods, C1addresses the critical bottlenecks of protein discovery, development, scale-up and commercialization. The technology enables new product introduction with less time, cost and risk. Broad platform capabilities have been validated through 17 years of commercial-scale production (up to 150,000 liters) and 15 years of product sales and partnerships.

SL-69

Track: Other Areas: Food; marine; bio-safety; systems biology; clinical research/clinical trials; bioethics; nanobiotechnology

COMPOUNDS WITH POTENT ANALGESIC AND ANTIINFLAMMATORY POTENTIALS FROM *STEREOSPERMUM KUNTHIANUM* (BIGNONIACEAE) USED TRADITIONALLY IN THE MANAGEMENT OF PAINFUL AND INFLAMMATORY CONDITIONS IN WEST AFRICA

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Stereospermum kunthianum (Bignoniaceae) is a woody shrub of the Sudano-Guinea savannah regions of Africa where the plant parts are used to treat various ailments including inflammatory conditions (rheumatoid arthritis) and pain [1].

The bioactivity guided isolation technique [2] led to the isolation of Stereospermiside, Stereospermin and Stereostin from Stereospermum kunthianum stem bark. The compounds were characterized and identified using NMR (1D and 2D), IR, MS-(HRSMS, ESI) and UV spectroscopic methods [3].

The analgesic and antiinflammatory activities of the isolated compounds were studied using the Randall-Selitto and formalin pain tests [4, 5]. At the dose of 20 mg/kg, Stereostin, Stereospermin ($p<0.0001$) and Stereospermiside ($p<0.05$) significantly increased the carrageenan-induced pain threshold compared to the distilled water treated animals. Similarly, at the same doses the three compounds significantly ($p<0.0001$) inhibited both phases of formalin pain test with a more pronounced effect on the second phase than in the first phase. The results obtained show that the compounds from Stereospermum kunthianum possess analgesic and antiinflammatory activities. This paper reports for the first time the isolation and the biological activity of the compounds from *Stereospermum kunthianum* stem bark.

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SL-54

Track: Plant and Environment: Transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae; genomics-assisted breeding.

MULTIPLEX GENOME EDITING OF NATURALLY COMPETENT MICROBES FOR ACCELERATED EVOLUTION

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Editing bacterial genomes is an essential tool in research and synthetic biology applications. We have developed a method for Multiplex Genome Editing by Natural Transformation (MuGENT). MuGENT allows for "accelerated evolution" and is based on the co-transformation of unlinked genetic markers in naturally competent microorganisms. We found that MuGENT allows for scarless genome editing at unprecedentedly high frequencies of ~50%. Since MuGENT does not require selection at edited loci in cis, output mutant pools are highly complex, where strains have any number and combination of the multiplexed genome edits. We demonstrate the utility of this technique in metabolic and phenotypic engineering by optimizing natural transformation in *Vibrio cholerae*. This was accomplished by combinatorially editing the genome via gene deletions, promoter swaps and by tuning translation initiation of five genes involved in the process of natural competence and transformation. Using MuGENT, we isolated a genetically edited strain with a 30-fold improvement in natural transformation. We also demonstrate the efficacy of this technique in *Streptococcus pneumoniae* and highlight the potential for MuGENT to be used in multiplex genetic interaction analysis. Thus, MuGENT is a broadly applicable platform for accelerated evolution and genetic interaction studies in diverse naturally competent species.

Keywords: MuGENT, *Vibrio cholerae* and *Streptococcus pneumoniae.*

SL-113

Track: Marine Biotechnology

THERAPEUTIC EFFECTS OF NUTRACEUTICALS FROM THE RED *MICROALGA PORPHYRIDIUM SP*.

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The biomass of the red microalgae comprises a unique combination of functional sulfated polysaccharides (including dietary fibers), polyunsaturated fatty acids, zeaxanthine, vitamins, minerals, and proteins. Since red microalgal products are believed to have potential as health foods, a series of feeding experiments was performed on mice and rats to elucidate the toxicity, nutritional value, metabolic and morphological effects, and mechanism of action of the algal products. The algal cells were added to the diets of the rodents in amounts of 5–10%.

The main findings of the feeding experiments were:

Significant improvement in cholesterol metabolism: reduction in total serum cholesterol, triglyceride and hepatic cholesterol levels.

Increase in HDL/LDL ratio, Fecal excretion of neutral sterols and bile acids.

Metabolic changes: Gastrointestinal transit time lowered significantly in biomass-fed rats whereas serum and mucosal cholecystokinin (CCK) levels lower and hepatic HMG-CoA reductase significantly increased in polysaccharide-fed rats.

Morphological changes: Small intestine and colon length significantly increased, mucosa and muscularis cross-sectional area of the jejunum increased and hypertrophy of the muscularis layer have seen in polysaccharide-fed rats.

These results suggest that red microalga can be used as potent hypocholesterolemic agents at low concentrations in the diet. The unique combination in the algal biomass of sulfated polysaccharides and unsaturated fatty acids (n-3) is thus of

special value. The encouraging results indicate that we should pursue the development of red microalgae as novel nutraceuticals.

SL-186

Track: Industrial and Manufacturing

EXTRACTION OF NOVEL NATURAL COLOURANTS FROM OPUNTIA FRUITS USING ATHERMAL PROCESSES

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Colours are one of the important aesthetic properties of food and colouring of foods has been an old age practice. This has increased several folds with the invention of synthetic colourants mainly due to its stability and colouring ability. Though synthetic colours dominate the market, even among the permitted synthetic colourants, some of them may be toxic, carcinogenic or may cause severe damage to vital organs. This has given rise to a strong interest in natural colouring alternatives. In the present investigation, extraction of novel water-soluble natural colourant, betalains from Opuntia fruits was attempted using ultrasonication and homogenization. Under optimum process conditions, ultrasonication exhibited greater extractability of 1.2 g/L betacyanin with 5 fold purity compared with homogenization (betacyanin extractability 0.96 g/L; purity 3.96 fold). However, the extractability of antioxidant activity (72%), ascorbic acid (328 mg/L), total phenol content (1.97 g/L) and total carbohydrates (161 g/L) obtained by ultrasonication were similar to homogenization. The extracted betalains was quantified spectrophotometrically at 536 nm and characterized by TLC and HPLC. The results suggested the suitability of ultrasonication for the extraction of novel water-soluble natural colorant, betalains from Optunia fruits with enhanced antioxidant activity.

SL-80

Track: Other Areas: Food; marine; bio-safety; systems biology, clinical research/clinical trials; bioethics; nanobiotechnology.

BIODIVERSITY OF RHIZOBIAL STRAINS FROM FABA BEAN (*VICIA FABA*)

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Rhizobium is the most well-known species of a group of bacteria that acts as the primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of nodules where the nitrogen fixation takes place. Eight rhizobial strains were isolated from healthy faba bean roots growing in different geographic areas in north Egypt. The native strains were presumptively identified as *Rhizobium leguminosarum* biovar viciae. They were tested against antibiotics resistance and growth on different carbon source as biochemical parameters. Also, Rl. 2 and Rl. 10 were tolerating to high NaCl concentrations and their plasmid profiles contained additional large plasmid with molecular weight about 23 kb. A relationship between salt tolerance and extra plasmid was indicated. Analysis of similarity among rhizobial strains by using the RAPD-PCR technique showed a high level of genetic polymorphism, grouping the rhizobial strains into two different clusters. These clusters reflexed the similarity among genotype of strains independent of their geographic locations. By using 16S rDNA specific amplification of the three highest salt tolerant strains as well as those of type strains belonging to Rhizobium leguminosarum biovar viciae. The 16S rDNA sequences of the strains were determined and were aligned and compared with the 16S rDNA sequences of other members of the family Rhizobiaceae available in the Gene Bank database. The obtained dendogram indicated that the 16S gene could be used as a diagnostic molecular marker for strains belonging to the bv. viciae.

SL-98

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.

STRUCTURAL PECULIARITIES OF FLAVONOIDS INFLUENCE ANTI-ANGIOGENIC, CYTOTOXIC AND ANTIOXIDANT EFFECTS: EXPERIMENTAL AND *IN SILICO* ANALYSIS

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Structural diversity of flavonoids and biological activities has remained an important discourse in the mainstream of flavonoid research. In the present studies different class of flavonoids such as flavones, flavanones and flavanolols were evaluated for their antiangiogenic, cytotoxic and antioxidant activities. The anti-angiogenic activity was evaluated using *in vivo* chorioallantoic membrane model (CAM), antioxidant potential and kinetics of free radical scavenging activity was determined using DPPH (2, 2-diphenyl-1-picryl hydrazine) and superoxide anion radical (SOR) scavenging assays, while cytotoxicity against selected cancer cell lines was carried out using MTT cell viability assay. The physicochemical properties/quantum chemical discriptors of the selected flavonoids were calculated using BioMed CAChe 6.1.10 drug. The selected flavonoids were docked *in silico* onto the proangiogenic peptides such as vascular endothelial growth factor (VEGF), hypoxia inducible factor (HIF-1Ā), and vascular endothelial growth factor receptor-2 (VEGFR2) and others from human origin. The results of the present investigation are discussed in the mainstream of structure activity relationship which may be useful in translating flavonoids as therapeutic molecules targeting angiogenesis.

Keywords: Flavonoids, antiangiogenic agents, antioxidants, cytotoxicity.

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SL-61

Track: Other Areas: Food; marine; bio-safety; systems biology, clinical research/clinical trials; bioethics; nanobiotechnology

CLINICAL EVALUATION OF *STELLERA CHAMAEJASME L.* IN NON-SMALL CELL LUNG CANCER (NSCLC)

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Cases of patients with non-small cell lung cancer, palliative chemotherapy were administered. Months later, multiple metastatic lesions had been found by CT and the level of CEA elevated, none of those patients received the treatment of HER1/EGFR-TK inhibitor Erlotinib target treatment due to their economic carrying capacity. Alternatively, we administered Chinese herbal medicine (*Stellera Chamaejasme L,* a kind of widespread herbal plant in the north of China) to help them to recover from the poor condition. After taking this specific Chinese herbal plant for 2 months, the tumor marker (CEA, CYFRA21-1) dramatically decreased with the result to the normal range. Most residual metastatic

sites reduced according to CT imaging, and the patient felt free from the complaint of plural discomfort. The quality of life has been greatly improved, we managed to have prolonged the PFS (Progression-Free-Survival) and TTP (Time-to-Progression) from the onset to date. In the course of this individual treatment, we evaluated significance of Chinese herbal medicine in the treatment of lung cancer. *Stellera Chamaejasme L* might be an additional choice with its better benefits and tolerability in the treatment of advanced non-small cell lung cancer.

SL-90

Track: Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.

THERAPY RESPONSE OF DECOCTION FOR REMOVING BLOOD STASIS IN ADVANCED CEREBRAL TUMOR OF GLIOMA：A RETROSPECTIVE OBSERVATIONAL CLINICAL TRIAL

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Aiming at starting the ball rolling and contributing humble effort to promote Chinese traditional medicine (CTM), we performed the present study to assess the therapy response of Chinese herbal decoction of Removing Blood Stasis compared to conventional therapy on critical ill patients of advanced cerebral tumor of glioma in ICU. Methods: A total

of 6 patients (1 female and 5 males) of glioma were included in this retrospective observational clinical trial. We administered Chinese medicine (*Decoction of Removing Blood Stasis*, mixed with a variety of effective herbal components) to help them to recover from poor condition. In the meantime, conventional treatment of surgical resection and radio-chemotherapy was applied in cases compared. Results: In 3 cases of CTM, after taking the Chinese herbal decoction for months, most residual intracranial tumor mass reduced according to MRI/CT imaging, and the patient felt free from the complaint of discomfort. The quality of life has been greatly improved, we managed to have prolonged the PFS (Progression-Free-Survival) and TTP (Time-to-Progression) from the onset to date. While in 3 cases compared, conventional treatment of surgical resection and radio-chemotherapy were not able to decrease the metastatic lesions, and the patient's condition worsen more. We failed in having prolonged the PFS and TTP in the compared cases of conventional treatment. Conclusions: Chinese medicine considers human body as a dynamic platform in which all organs are correlative and bind each other. Our report suggested that Decoction of Removing Blood Stasis might be an additional choice with better benefits and tolerability in the treatment of glioma.

Keywords: Glioma, therapy response, Chinese medicine.

SL-53(a)

Track: Business Development: Strategic alliances; partnering trends; product opportunities; growth; business models and strategies; licensing; merger and acquisitions; outsourcing; venture capital and financing; intellectual property.

DEVELOPING ALLIANCE CAPABILITY IN BIOTECH SMEs.

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The objective of this paper is to study alliance capability development and diffusion, investigating the organizational learning processes that underlie alliance knowledge/expertise acquisition and absorption within small and medium-sized firms. Analyzing the primary data collected during 27 interviews with CEOs and R&D managers of French biotech SMEs, we identified the internal process of expertise development and informal knowledge diffusion within these firms. The development of the alliance capability is based on a dual process of inter- and intra-organizational learning. There are few firm founders who have a previous experience in alliance formation and management. They learn progressively, during their interaction with more experienced alliance partners, and/or by trial and error. This knowledge remains highly personalized in small firms, being controlled exclusively by the managers. The level of formalization/codification of the alliance capability is almost non-existent. The intra-organizational learning process is based on a personal transmission and sharing of knowledge. As the firm grows, the number and the complexity of alliances become more important, forcing the owner-manager to transmit the alliance management knowledge to a specialized manager (usually the R&D or the alliance manager). These findings provide the basis for a series of theoretical and practical implications.

SL-171

Track: Regenerative Medicine: Stem cells, gene therapy, tissue engineering, cell based therapy, cell cultivation.

COLLAGEN AND HYALURONAN FOR REGENERATIVE MEDICINE

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Collagen and hyaluronan are important biomaterials as well as extracellular matrices of the bodies. Collagen is a major scaffold as well as structural protein of connective tissues and possesses 25% to 35% of the whole-body protein content. Hyaluronan plays key roles during embryonic developments and affects cell migration, proliferation, differentiation, and tissue regeneration. Since 1990, we first proposed the function of hyaluronan in conjunction with collagen matrix and have series of studies and publications followed. We found that a scarless wound healing can be exerted from right combination of both. These results provide new insights into the potential of artificial matrix in tissue engineering and imply good potential for future improved applications in wound treatments.

We further combined hyaluronan, collagen and hydroxyapatite to make a series of HC_HP composite microspheres and investigate an optimal composition of HC_HP composite to promote osteogenesis of mesenchymal stem cells. The aim of the study was to evaluate the HC_HP composite microspheres for osteogenesis of PDMSC (placenta derived mesenchymal stem cells). As high concentration of hydroxyapatite affects cell adhesion, our result demonstrated that HC5HP was best to promote the osteogensis of PDMSC even in the absence of induction medium through enhancing calcium deposition. The expressions of Runx2 and Osterix in osteoblast proliferation, Collagen type 1 and Alkaline phosphatase in matrix maturation and Osteopontin and Osteocalcin in mineralization during osteogenesis were in chronological order. An early shift and an increase in the expression of osteogenic markers were observed in the PDMSC cultured on HC_HP microspheres obtained by the results of real-time polymerase chain reaction (PCR).

SL-11

Track: Medical Biotechnology

TRENDS IN DIAGNOSTIC BIOCHIP DEVELOPMENT

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Technological advancements in biochips for diagnosis and prevention lead to improved healthcare cost containment with a decreasing birth rate and an aging population. Biochips have been attracting attention as a tool for improving healthcare costs. There are technological, standardization-related, ethical and societal problems in biochip development. For biochip market expansion, in addition to technological problems, it is necessary to overcome social, institutional, marketing and economic problems all together.

It is expected that the application of biochip technologies will facilitate not only 'super' early diagnosis of diseases and disease prevention based on the diagnosis, but also early treatment.

References

Ichiishi E. Expert Rev Mol Diagn. 2013 May;13(4):331-7.

SL-91

Track: Medical Biotechnology

KINETIC AND THERMODYNAMIC PROPERTIES OF BOVINE ALDOSE 1-EPIMERASE AND ITS APPLICATION IN GLUCOSE DIAGNOSTIC KIT

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Aldose 1-epimerase or mutarotase is the enzyme that responsible for carbohydrate metabolism and converts the alpha anomer into beta anomer of glucose. This enzyme was extracted from bovine kidney cortex. Crude enzyme exhibited the activity of 14.92 U mL^{-1} with specific activity of 0.153 U mg^{-1} proteins. The enzyme activity and specific activity was increased to 53.75 UmL^{-1} 4.981 Umg^{-1} respectively after 38-60% ammonium sulfate precipitation and it was further increased to 73.27 UmL^{-1} and 11.67 Umg^{-1} when subjected to diethylaminoethyl (DEAE) cellulose chromatography. Further purification was carried out by passing it through Sephadex G-150 column and observed increase in activity 79.26 UmL^{-1} with 19.55 Umg^{-1} specific activity. The optimum pH and temperature were recorded as 8.5 and 37 ^{o}C respectively. Different stabilizers (glycerol, sodium benzoate, sodium citrate) were used to study their effect on stability of enzyme. The V_m and K_m of native and stabilized Aldose 1-epimerase were derived from the Lineweaver Burke plot. Thermodynamic parameters namely E_a, Ā*H**, Ā*S** and Ā*G** for conversion of Ä-D-glucose to -D-glucose with aldose 1-epimerase were also studied. Different serum samples were checked for comparing the efficacy of indigenously prepared kits containing native (IKN), stabilized enzyme (IKS) and standard kit (SK). We found that indigenous kit with native enzyme (IKN) and standard kit (SK) exhibited similar results. The sensitivity of glucose kit with native enzyme (IKN) was measured as 0.5-1 mg dL^{-1} comparable with other kits reported in literature but indigenous kit with stabilized enzyme (IKS) showed better results and sensitivity.

Keywords: Aldose 1-epimerase, Stabilized, thermodynamics, entropy, enthalpy, kinetics, glucose estimation.

SL-77

Track: Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology

STUDIES ON MOLECULAR CHARACTERIZATION AND EPIDEMIOLOGY OF *STAPHYLOCOCCUS AUREUS* FROM CLINICAL ISOLATES

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The widespread use of antibiotics in the 1950s induced the predominance of ! -lactamase-producing resistant strains. The emergence of antibiotic-resistant strains of *S. aureus* is now considered to be a major problem in most hospitals. Data from the centers for disease control and prevention indicate that throughout the United States there has been an increase in the frequency of methicillin-resistant *S. aureus* (MRSA) strains resistant to multiple antibiotics in both large and small hospitals. Recent studies suggest that the infection due to MRSA is not only hospital-acquired but community acquired as well. MRSA now represent a global problem. Some large outbreaks have been reported from different parts of the world, where it had caused severe infections including septicemia, endocarditis and meningitis. In the 1980s, due to the widespread occurrence of MRSA, empiric therapy for staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health-care institutions. The emergence of high levels of penicillin resistance followed by development and spread of strains resistant to the semi synthetic penicillins (methicillin, nafcillin and oxacillin), macrolides, tetracyclins, and amino glycosides has made therapy of Staphylococcal disease a global challenge.

Therefore the present investigation was carried out to study the distribution rate of staphylococci and for molecular characterization and epidemiology of *Staphylococcus aureus* from clinical isolates. Clinical samples were collected and tested for resistance to various antibiotics. The overall distribution rate of staphylococci was 91.90%.the highest distribution rate was observed in anterior nares. However, the highest distribution rate in clinical samples was in urine followed by blood, pus, sputum and CSF. The overall isolation rate of MRSA among *Staphylococcus aureus* isolates was found to be 50.93%. *Staphylococcus aureus* isolates distribution rate of MRSA was highest in blood followed by pus, sputum, urine, csf, and forearm. The incidence of MSSA from the various samples was 49.07%. The overall isolation rate VRSA among *Staphylococcus aureus* isolates were found to be 1.63% and the distribution of VRSA was highest in pus, the highest rate distribution of *Staphylococcus aureus* nasal carriage among clinical samples was seen among the adults, followed by children. The highest rate distribution of *Staphylococcus aureus* nasal carriage among hospital personnel was seen among the nurses followed by attenders and doctors. The distribution rate of *Staphylococcus aureus* major carriage among the healthy individuals was showed the highest rate of *Staphylococcus aureus* was in adults followed by children the results obtained from the antibiogram for 380 isolates from infected blood, pus, urine and sputum against 14 different antibiotics belongs to different class of antibiotics showed the increase in rate of resistance against the antibiotics. the examination of the strains with primers for the van a, b and c genes revealed that 4 VRSA strains in this study were vancomycin-resistant because of the presence of van a gene, only one showed van b gene but van c gene could not be detected in the isolates of VRSA strains. The present study demonstrates for the first time emergence of VRSA from this part of the country and indicates the prevalence of the antibiotic resistance. Vancomycin resistance in staphylococcal species is beginning to emerge as a clinical threat, yet the attention it has received and serves to underscore the seriousness of the problem. A better understanding of these issues will be a key to helping the prevention and treatment of these infections in the future.

Keywords: *Staphylococcus aureus,* methicillin-resistant *S. aureus* (MRSA) and penicillin resistance.

SL-35

Track: Plant and Environment: Transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae; genomics-assisted breeding.

IMIDACLOPRID INDUCED INTOXICATION AND IT'S BIOREMEDIATION BY SOIL ISOLATES

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Various insecticides to protect crops against insects have been used over the last four decades. Most insecticides were applied by spraying in large quantities, thus inducing pollution of air, soils and waters. Imidacloprid (I-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine), is a chloronicotinyl insecticide and it is used to control biting and sucking insects. It is classified as a toxic, hazardous. In the present study, soil isolates *Escherichia coli*, *Brevundimonas* Sp. MJ 15 and *Bacillus weihenstephanensis* were exposed to imidacloprid of the concentrations ranging from 10 -7 M to 10 -3 M for a period of 96 hrs. Biochemical parameter, enzyme activities, protein profiling, genes sequencing and biodegradation was evaluated at regular intervals of 24, 48, 72 and 96 hrs. The results revealed that treatment with graded dose and duration of imidacloprid caused a significant decrease in all the biochemical parameters i.e., DNA, RNA, proteins and glucose content with significant increase in enzyme activities in stress enzymes studied i.e., superoxide dismutage, catalage and glutathione peroxidase genes but there was no significant increase was observed in amylase and protease activity in *Escherichia coli, Brevundimonas* Sp. MJ 15 and *Bacillus weihenstephanensis*. There were many proteins expressed in common with the stress induced by imidacloprid in *Escherichia coli*, *Brevundimonas* Sp. MJ 15 and *Bacillus weihenstephanensis* which may play a major role in the stability of the cells under different stress. The present study on degradation of imidacloprid analyzed by HPLC revealed that the imidacloprid was degraded significantly in all the treated groups when compared with that of their corresponding controls by *Escherichia coli, Brevundimonas* Sp. MJ 15 and *Bacillus weihenstephanensis* and a metabolite 6-chloronicotinic acid was detected in *Brevundimonas* Sp. MJ 15 and *Bacillus weihenstephanensis*. The plasmid isolation and curing studies revealed that both plasmid and chromosomal genes were involved in the bioremediation of imidacloprid by *Escherichia coli, Brevundimonas* sp. MJ 15 and *Bacillus weihenstephanensis*. Hence, present investigation revealed that imidacloprid induces toxic effects on the soil isolates and the degradation a study indicates these isolates can be used for removal of imidacloprid from the pesticide contaminated sites.

Keywords: Imidacloprid, biodegradation, *Escherichia coli, Brevundimonas* sp. MJ 15 and *Bacillus weihenstephanensis*.

SL-19

Track: Medical Biotechnology

RESISTIN, VISFATIN, ADIPONECTIN, LEPTIN AND RISK OF BREAST CANCER IN PRE AND POST-MENOPAUSAL SAUDI FEMALES, THEIR POSSIBLE DIAGNOSTIC AND PREDICTIVE IMPLICATIONS AS NOVEL BIOMARKERS

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The mechanisms of obesity-induced breast carcinogenesis are not clear. One hypothesis is that high levels of biologically active substances produced by fat cells (adipokines) could promote breast cancer development. The aim of this study was investigate correlation of resistin, visfatin, adiponectin and leptin, with breast cancer risk in pre and postmenopausal females and to evaluate the potential diagnostic, predicting role of studied adipokines and their relation to different clinicopathological features of breast cancer. Within the period from 2011to 2013, total of 82 breast cancer patients were enrolled in this study, all were newly diagnosed and histologically-confirmed breast cancer with no prior surgical, chemotherapy or radiotherapy. The healthy control subjects (n= 68) were age and BMI matched with breast cancer group. Both groups were subdivided according to menopausal status into post and premenopausal subgroups. For all subjects: resistin, visfatin, adiponectin and leptin were determined by ELISA. We found no significant difference

between premenopausal breast cancer patients and premenopausal controls for leptin, adiponectin, resistin and visfatin (*P*=0.228, 0.59,0.52 and 0.85 respectively) meanwhile we found significantly higher levels of leptin, resistin and visfatin (*P*=0.000) in postmenopausal breast cancer patients than postmenopausal normal controls. Serum adiponectin levels were significantly lower in postmenopausal breast cancer patients than respective controls (*P*=0.000). Only in postmenopausal subgroups leptin levels were positively correlated with TNM, Tumor size, LN metastasis and histological grading (r=0.338, P=0.038; r=0.241, P=0.029; r=–0.820, P=0.000 andr=–0.762, P=0.000). Serum resistin levels also were positively correlated with TNM, Tumor size, LN metastasis and histological grading (r=0.395, P=0.014; r=0.429, P=0.007; r=0.575, P=0.000 and r=0.509, P=0.000). Serum visfatin showed positive correlations with TNM, Tumor size and LN metastasis (r=0.406, P=0.000; r=0.348, P=0.028 and r=0.529, P=0.005). On the other hand serum adiponectin levels correlated negatively with all clinicopathological ($p<0.05$) features. Multivariate Logistic regression analysisin postmenopausal subgroupsrevealed that BMI, TG, adiponectin, leptin, visfatin and resistin were risk factors for breast cancer, and their OR were 0.370 (95% CI: 1.004- 4.278, P=0.049), 6.011 (95% CI: 1.823-15.673, p = 0.004), 0.423 (95% CI: 0.132 -0.692, P=.005), 0.244 (95% CI: 1.381- 3.587, P=.001), 1.089 (95% CI, 1.062–1.116; P = 0.001), 0.513 (95% CI: 3.051-22.790, P=.000), respectively. In addition, resistin, leptin and visfatin were considered independent risk factors for LN metastasis. OR for them were 2.203; (95% CI: 4.091–20.791, P = 0.002), 0.742 (95% CI: 1.504–2.921, P=0.003), 1.080; (95% CI: 1.056–1.105; P = 0.003) respectively. ROC analyses of leptin, resistin and visfatin levels between the cases and controlsin postmenopausal females revealed thatthe best cut-off point for serum leptin levels was 17.5 ng/mL. (ROC AUC=0.795; 95% CI, 0.724–0.866). The best cut-off point for serum resistin levels was 21.3 µg /mL. (ROC AUC=0. .875; 95% CI, 0.821–0.928). Meanwhile the best cut-off point for serum visfatin levels was 12.2 ng/mL. (ROC AUC=0.724; 95% CI,0 .643–0.804). In conclusion our results seemed to suggest that the serum resistin, leptin, adiponectin and visfatin levels could be considered risk factors for postmenopausal breast cancer and may provide a potential link correlating with TNM, Tumor size, LN metastasis and histological grading in postmenopausal breast cancer and promising to be novel biomarkers of diagnostic value and independent risk factors for LN metastasis in postmenopausal breast cancer.

SL-150

Track: Medical Biotechnology

OVERCOME MULTIDRUG RESISTANCE IN HUMAN CANCER BY NANOPARTICLES

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Cancer is a major cause of mortality worldwide. Many of clinical chemotherapy is not effective because the development of multidrug resistance mechanisms. Multi-drug resistance (MDR) in cancer refers to the capacity of cancer cells to survive or become resistant to treatment of a wide variety of drugs. These mechanisms include: decreased uptake of drugs, reduced intracellular drug accumulation by activation of the efflux transporters, modifications in cellular pathways by altering cell cycle checkpoints, increased metabolism of drugs, induced emergency response genes to impair apoptotic pathways, and altered DNA repair mechanisms. P-glycoprotein (P-gp) is the most common membrane transporter used in MDR. Clinical success has also been limited due to tissues regarding safety, once one of the most common strategies against MDR is the development of ATP-binding cassette (ABC) transporter inhibitors, which are poorly effective and specific, increasing the toxicity associated with chemotherapy. Therefore, there is an urgent need for more effective and valuable cancer therapeutics, in order to, creates more selective chemotherapeutic agent toward the cancerous cells. Nanotechnology, especially cancer nanotechnology offers a wealth of safety and innovative tools to treat and diagnose cancer. Nanoparticles (NPs) areusually produced to deliver and enhance the drug accumulation inside the cancer cells, using both active and passive targeting.

SL-148

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering

MODE OF ACTION OF POTASSIUM SALT OF 2-THIOXO-4-HYDROXYCOUMARIN [3, 4-B] PYRIMIDINE AND 9-BROMO-2-THIOXO-HYDROXYCOUMARIN [3, 4-B] PYRIMIDINE AGAINST EHRLICH ASCITES CARCINOMA CELLS

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Coumarin and pyrimidine derivatives have attracted intense interest in recent years because they have anti-tumor, antioxidant activities, and induce apoptosis. Our study aims to evaluate the antitumor and anti-oxidant activities of new Coumarin derivatives: Potassium salt of 2-thioxo-4-hydroxycoumarin [3, 4-b] pyrimidine and 9-bromo-2-thioxo-hydroxycoumarin [3, 4-b] pyrimidine against *in vivo* tumor model. Coumarino [3, 4-b] pyrimidine -2-thioles (4 a,b) were prepared and their cytotoxicity were determined. The compounds [3a & 3b] exhibited a significant anti-oxidant activity towards Ehrlich ascites carcinoma (EAC) cells by reduction the MDA by 30.3% & 54.9% and NO concentration by 19.4% & 39.6% ($p<0.001$), respectively; compared to the positive control group. Whereas significantly increase in the CAT activity by 150%, 700%, respectively; and SOD activity by 102.9% and 379.9%, ($p<0.001$) respectively; in [3a & 3b] treated groups compared to the positive control group. Anticancer agent kills tumors at least partially through induction of apoptosis. Furthermore, the treatments with 3a and 3b showed a significantly increase in Caspase-3 activity by 85.9% and 269.23%, ($p<0.001$), respectively; and cytochrome c by 85.9% and 269.23%, ($p<0.001$), respectively; compared positive control group. The synthesized compounds have potent antioxidant activity and good inducer for apoptosis by induction of caspase-3 and releasing of cytochrome c.

Keywords: Coumarins, Pyrimidine, Ehrlich ascites carcinoma cells, apoptosis.

SL-65

Track: Other Areas: Food; marine; bio-safety; systems biology, clinical research/clinical trials; bioethics; nanobiotechnology.

CRYOSURGERY OF BRAIN TUMORS

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We use the method of stereotactic cryosurgery in the treatment of brain tumors. Method is based on the local freezing of tumors using cryoprobe. For this purpose we use cryodevise developed in our Institute. This devise works with solid carbon dioxide, with the temperature of a cryoprobe about -79 ^{o}C. Cryoprobs form the area of cryodestruction of pathological tissues of the brain with the volume of 0.2-6 cm^3. Pointing cryoprobe on intracerebral target performs with two stereotactic systems - POANIC and NIZAN, developed in our Institute. Preparation and planning of the operations carried out with the help of modern CT, MRI, PET. Tumors with a volume of up to 20 cm^3 are total destroing by the cryosurgery. Larger tumors are frozen by the several stages. If the tumor is large in size with the area of increased proliferation revealed by PET, thus cryosurgery is only in this zone performed.

Operation method showed the following benefits:

1. You can accurately plan the size and shape of the proposed destruction of the tumor tissue.;

2. Operations of stereotactic cryosurgery can be performed through the one ore two small holes in patient' skull, with minimal surgical trauma;

3. The method does not have a cumulative effect, and thus can be used repeatedly in one patient;

4. The method can be used in combination with other methods of local impact (for example, radiosurgery);

5. Application of cryomethod with the freezing temperature of about -79 ^{o}C ensures the safety of the most part of large blood vessels in the impact zone.

The method allows to carry out surgical operations on the deep brain tumors that were previously considered inoperable. For 15 years the method we use more than 200 operations have been carried out, showing its high effectiveness in terms of survival and preservation of patients' quality of life.

SL-15

Track: Medical Biotechnology

SUPER-RESOLUTION FLUORESCENCE NANOSCOPIC IMAGING OF AMYLOID AGGREGATES

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Fluorescence microscopy has a unique advantage of being non-destructive and highly sensitive in imaging biological systems *in vivo*. In comparison with electron microscopy, however, it suffers from low spatial resolution that results from the optical diffraction limit. In studying such problems as protein aggregation in particular, a sub-diffraction imaging technique is highly required because the protein aggregates are only tens of nm in size, well below the diffraction limit of ~250 nm. We developed a new optical nanoscopic technique that is applicable to protein imaging and applied it to amyloid aggregation. We demonstrate that we can achieve a spatial resolution of ~10 nm FWHM with his technique, which is the highest resolution so far for optical imaging of amyloid proteins. We demonstrate that this new method enables resolving the structural features of the oligomeric and fibrillar forms of amyloid.

SL-47

Plant and Environment; Other Areas: Genourbanology (System ecology and population genetics).

THEORETICAL AND PRACTICAL RESULTS OF A NEW SCIENTIFIC DIRECTION-GENOURBANOLOGY

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Genourbanology (the synthesis of system ecology and population genetics) is a new scientific and practical school of thought, founded and developed by the authors [1]. The task of genourbanology consists in studying the genetic parameters and laws of stability conservation of ecosystems and ecosystem restoration in anthropogenic and, in particular, urbanized landscapes. Within the frame of genourbanology a certain methodology of assessing the condition and duration of populations in urbanized ecosystems was worked out. The state of the gene pool of the living organisms (model species) populations whose natural habitat is the fragmented landscape of Moscow and the Moscow Region (36 populations, including 21 town populations and 13 isoenzyme loci) was assessed. A sharp decline in genetic biodiversity (up to 70%) in town isolates was found. The comparative analysis of natural populations of plants and cultivated forests (i.e. Norway spruce *Picea abies* (L.) Karst.) in the Moscow Region (4 populations, of which 2 are nominally primary, 24 isoenzyme loci) was carried out. An authentic correlation between the state of the gene pool of the populations of spruce and the damage caused by the bark beetle (Ips typographus (L.) was established. The results of the research enabled to develop and test the ecological-genetic concept and strategy of biodiversity conservation in urbanized landscapes.

Keywords: geneourbanology, population, gene pool, polymorphic loci, isoenzymes, urbanized landscape.

Reference

[1] Makeeva V. M., Belokon M.M., Smurov A.V. Genourbanology as the Basis for Stable Biodiversity and Ecosystem Conservation under Global Urbanization. Biology Bulletin Reviews, Vol. 3, No. 4, Ä 261-273.

SL-144

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering

DESIGN AND CONSTRUCTION OF A NOVEL DNA VACCINE AGAINST HEPATITIS E VIRUS

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Introduction: Hepatitis E has been considered to be a travel-associated, acute, and fulminant hepatitis in Asia, Africa and Mexico with 70000 deaths annually worldwide although sporadic cases of chronic and acute hepatitis have been found in the USA and, Europe. Since HEV grows poorly in cell culture, recombinant proteins expressed in a variety of systems is the main principal for the vaccine preparation against hepatitis E virus.

Objective: The aim of our study was to design, optimize and construct a novel DNA vaccine using PVAX plasmid containing 60 KD(aa112- 660) truncated ORF2 capsid of the hepatitis E virus.

Materials and Methods: The different optimized recombinant plasmids consisting with or without flic (flagellin), tPAsp and PADRA sequences were constructed. The Hela, HEK 293 and CHO cells were transfected with different recombinant expression plasmids then determined by RT-PCR and IFA. Three doses of 100Ãg of the different constructed DNA vaccine with or without adjuvant were injected intramuscularly in 20 groups of mice (each group 8 mice) at 0,3 and 6 weeks. The homural responses including IgG1, IgG2! and IgG2∀ were evaluated in the each immunized mice. In addition to specific truncated ORF2 cytokines assay including IL-4, IL-10, IFN and IL-12 with IFN ELIspot test and lymphocyte proliferation assay was carried out for each immunized groups of mice.

Results: All the immunized mice with different constructed DNA vaccines against HEV showed both homural response and very good IFN , IL-12 ELIspot test responses.

Conclusion: this study shows truncated ORF2 DNA vaccines with genetical-microbial fliC adjuvant was found better humoral and cellular immune responses.

Keywords: DNA vaccine, Hepatitis E Virus, Truncated ORF2, Flagellin adjuvant.

SL-102

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.

DNA-BASED VACCINE ENCODING THE PARASITE ENZYME PHOSPHOLIPASE (PLA2) CONFERS PROTECTION IN MICE CHALLENGED WITH *TRYPANOSOMA BRUCEI BRUCEI*

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A 1.344kb PLA2 gene was amplified from the genomic DNA and cDNA) of BSF of *T. b. brucei* (Federe isolates) and the sequence of this gene has been assigned accession number JN603736 by GenBank. Data mining analysis of the sequence indicated 99 % similarity with PLA2 genes of *T. brucei* (TREU927; Gene ID: Tb 3661014 Tb09.211.3650; XM 822413.1) and *T. brucei* gambiense (DAL972; FN554972.1). The translated PLA2-like gene has 447 amino acids with significant homology with PLA2 protein sequence from *T. brucei* (TREU 927), *T. b. gambiense* (DAL972), *T. cruzi, L. major*, and PAF-AH. The PLA2 gene was cloned into pMal-c2E vector and transformed into competent *E. coli* (DH5Ä) and BL21 DE3 cells for expression. While the expression of recombinant PLA2 in *E. coli* was unsuccessful, native PLA2 was purified to apparent homogeneity. Our prime-double

boost immunization approach showed some level of trypano-protective activity in challenged mice. This was evident by the reduction in parasitaemia load, increase in survival time of immunized mice post-infection and the effect of immunizations on hematological indices. The significance of these findings in relation to the etiology of anemia and the vaccine potential of PLA2 is discussed in this report.

Keywords: Parasite enzyme Phospholipase, PLA2 and *Trypanosoma brucei brucei*.

SL-146

Track: Medical Biotechnology

APPLICATION OF AGRICULTURAL RESIDUES AS PHARMACEUTICAL EXCIPIENT

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Pharmaceutical drugs usually contain an active material and other components which are termed excipients. The number and types of excipients used in a drug formulation depend on the desired properties of the drug and the intended use. These excipients are used as thickeners, gelling, bulking and water retention agents, fillers, binders, disintegrants, lubricant and/or glidant, flavours and sweeteners. Tropical countries are home to many agricultural plants, most of which grow with little or no artificial inputs and large amount of wastes and residues are generated annually. Traditionally, farmers harvest grains and burn or otherwise dispose the residues (stalks, husk, etc.). Burning agricultural residues causes environmental problems such as air pollution, soil erosion, and decrease soil biological activity. Therefore, conversion of agricultural residues into pharmaceutical excipients will not only prevent pollution which adversely affect human and environmental health, but also become economically profitable for farmers who can earn a second income from the sale. Attention has now been focused on the development of pharmaceutical excipients from agricultural residues. Some of these materials have been modified by both physical and chemical methods to extend their properties. For instance, microcrystalline cellulose, a very important directly compressible excipient widely used in pharmaceutical formulations have been prepared from agricultural residues, namely, textile waste, rice straw, cotton stalks, maize cobbs and groundnut husk. There is however the need to fully develop these excipients from agricultural residues to meet pharmacopoeial standards.

SL-101

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.

PROTECTIVE EFFECT OF ETHANOL LEAF EXTRACT OF COMBRETUM ZENKERI ON LIVER FUNCTIONS OF RATS FOLLOWING BENZO(A) PYRENE EXPOSURE

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This study investigated the possible protective effect of ethanol leaf extract of *Combretum zenkeri* on liver functions of rats exposed to Benzo(a)pyrene, a known polycyclic aromatic hydrocarbon (PAH) which elicits oxidant, inflammatory and carcinogenic effects. Acute toxicity analysis produced no lethality even at high doses. In this study, albino male rats were divided into 5 groups, the first group served as normal control, the second was treated intraperitoneally (i.p) with 200 mg/kg Benzo(a)pyrene only once. The third and the forth groups were exposed to 200mg/kg BaP (i.p) and 400 mg/kg ethanol leaf extract of *C. zenkeri* by gavage at alternate timing patterns and the fifth group was treated orally with 400mg/kg plant extract only and the treatment was for five weeks. Blood samples were used for biochemical analyses and liver tissues for histology. The malondialdehyde, total bilrubin, concentrations and ALT and AST activity of groups exposed to Benzo(a)pyrene without treatment were significantly higher ($p<0.05$) compared to

those treated with leaf extract before or after Benzo(a)pyrene exposure. Also, the GSH, total protein and albumin concentrations of group exposed to Benzo(a)pyrene without treatment were significantly lower ($p<0.05$) compared to the normal control and those treated with leaf extract together with Benzo(a)pyrene exposure. Histological slides of liver tissues also showed the potency of plant extract to repair damaged tissues. Results from this study showed that ethanol leaf extract of *Combretum zenkeri* has hepatoprotective and antioxidant effects against Benzo(a)pyrene induced toxicity in rats.

Keywords: Benzo(a)pyrene, *Combretum zenkeri*, hepatoprotection, antioxidation.

SL-167

Track: Plant and Environment: Transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae; genomics-assisted breeding.

BIOACTIVE, NUTRITIONAL AND HEAVY METAL CONSTITUENTS OF SOME EDIBLE MUSHROOMS FOUND IN ABIA STATE OF NIGERIA

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The phytocemical, mineral, proximate and heavy metals compositions of six edible and non-edible species of mushrooms were investigated. Fully fleshy mushrooms were used for the analysis. On the averagely, the bioactive constituents of the mushrooms were as follows Alkaloids 0.12 ± 0.02 – 1.01 ± 03 %, Tannins 0.44 ± 0.09 – 1.38 ± 0.6,). Phenols,(0.13 ± 0.01 – 0.26± 0.00, Saponins 0.14 ± 0.03 – 0.32 ± 0.04%, Flavonoids 0.08 ± 0.02 – 0.34 ± 0.02%. The result of proximate composition indicated that the mushroom contained (5.17 ± 0.06 – 12.28 ± 0.16% protein, 0.16 ± 0.02 – 0.67 ± 0.02% fats, 1.06 ± 0.03 – 8.49 ± 0.03 % fibre, (62.06 ± 0.52 – 80.01 ± 4.71% and carbohydrate. The mineral composition of the mushrooms were as follows, calcium 81.49 ± 2.32 - .914 ± 2.32mg/100g, Magnesium(8 ± 1.39-24 ± 2.40mg/100g, Potassium 64.54 ± 0.43 – 164.54 ± 1.23 mg/100g, sodium 9.47 ± 0.12 – 30.97 ± 0.16 mg/100g, and Phosphorus 22.19 ± 0.57-53.2± 0.44 mg/100g. Heavy metals concentration indicated Cadmium 0.7-0.94ppm. Zinc 27.82 – 70.98ppm. Lead 0.66 – 2.86ppm and Copper 1.8-22.32ppm. The result obtained indicates that the mushrooms are of good sources of phytochemicals, proximate and minerals needed for maintenance of good health and can also be exploited in manufacture of drugs. Heavy metals obtained indicate that when consume intentionally in high content may cause liver, kidney damage and even death.

Keywords: Bioactive, heavy metals, mushroom, nutritive.

SL-94

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering

MANIPULATION OF GLUCOSE LEVEL COULD HAVE CANCER PREVENTIVE AND/OR THERAPEUTIC VALUE: THE CASES OF SPONTANEOUS REGRESSION OF CANCER-HYPOTHESES

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The ketogenic diet, diet with very low carbohydrate contests is under investigation in several clinical trials as the potential anticancer treatment, alone or in combination with chemotherapy or radiotherapy. Preliminary results are promising. Similarly, the well-known cases of spontaneous regression of cancer could be explained by the simple changes in the glucose concentration.

Keywords: Glucose level, ketogenic diet and spontaneous regression.

SL-132

Track: Industrial and Manufacturing

ONE AIM-SEVERAL GOALS: FROM STEM CELL TO THE BIOFUEL PRODUCTION. THE NEW BACKGROUND METHOD FOR ZERO WASTE AND HIGH EFFICIENCY BIODIESEL PRODUCTION FROM MICROALGAE *CHLORELLA VULGARIS*

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This invention is a background method for many branches of biotechnology and medicine, such as acceleration of biomaterial growth, cell therapy, transplantation, regenerative medicine, the growing of artificial organs, in surgery and traumatology, but also in the recovery of rare and endangered species and biofuel production.

Generally speaking, biodiesel is an alternative or additive to standard diesel fuel that is made from biological ingredients instead of petroleum (or crude oil). Biodiesel is usually made from plant oils or animal fat through a series of chemical reactions. It is both non-toxic and renewable. Because biodiesel essentially comes from plants and animals, the sources can be replenished through farming and recycling. But biofuels increase total costs and they are very expensive. To decrease such high costs we could *de novo* activate microalgae Chlorella vulgaris grows in 9 times for produce an alternative renewable source of oil known as biodisel. More than that this microalgae biomass did not decrease after 36h of growing. We've made this activation procedure due to the our Patent: E.V. Orlova, E.I. Majewsky, V.K. Klubkov "Method of directed docking of human stem cells activation and mitotic processes regulation", 2012. Thus was obtained the very competitive (compared with oil and even more so with hard oil (Urals)) technology for biodiesel production. This technology is biosafety and environment friendly. From microalgae biomass, biofuel could be produced quickly and in large volumes, and biosafety waste products could use as a tool for rehabilitation of tsunami or radiation- affected soils.

The authors express their appreciation to "MedProFarm Ltd." for sponsoring this invention.

SL-174

Track: Medical Biotechnology

FLUORESCENCE IMAGING USING QUANTUM DOTS AND SMALL MOLECULES FOR THE DETECTION OF HUMAN SERUM PROTEINS AFTER PAGE

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Polyacrylamide gel electrophoresis (PAGE) is an important technique for the separation of biological samples. It is highly desirable for developing new methods for protein detections. To improve the sensitivity for protein detections after native PAGE, we have introduced several fluorescent probes for protein imaging. For example, a series of small organic molecules were synthesized and subsequently utilized as fluorescent bio-probes in protein detection [1]. In addition to the biocompatible and environmentally-friendly carbon QDs and silicon QDs performed excellently in protein imaging. Furthermore, colloidal Au and Ag nanoparticles were indicated to producing greatly enhanced fluorescence emission after binding to the in-gel proteins with nearly no backgrounds, while no signal was generated in the absence of either proteins or gels [2,3]. As demonstrated, these fluorescence imaging methods are much simple, fast and sensitive, showing potentials in clinical diagnosis.

Acknowledgements

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SL-159

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering

THE CONTRIBUTION OF CHINESE HAMSTER GENOMICS TO INDUSTRIAL BIOTECHNOLOGY

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Nearly 70% of all recombinant protein therapeutics are currently produced by employing the synthesis potential of Chinese hamster ovary (CHO) cell lines. Therefore it is obvious to push the genomic analysis of the Chinese hamster (CH) as well as of CHO cell lines. In this talk an overview will be presented summarizing recent results of CH and CHO genomics. The transcriptome of the CHO-K1 cell line was analyzed by high throughput sequencing. Altogether, the sequences of more than 29,000 transcripts were obtained and roughly 13,000 of them could be annotated [1]. Further on, the genome sequence of the Chinese hamster (CH) was established by sequencing sorted chromosomes resulting in a total genome size of 2.33 Gb. It could be shown that the sequences of individual hamster and mouse chromosomes are closely related [2]. As a next step transcription start sites (TSS) were mapped in the CH genome sequence by RNA sequencing of 5' ends of CHO transcripts. More than 6500 transcription start sites could be identified. The location of TSS allowed the detailed analysis of CH promoter motifs. The results presented will contribute significantly to CHO based production processes in industrial biotechnology. In particular, production processes will be monitored by CHO specific microarrays [3] and production cell lines will be optimized by making use of selected promoters for gene expression.

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SL-177

Track: Medical Biotechnology

IDENTIFICATION OF LEISHMANIA SPECIES CAUSING CUTANEOUS LEISHMANIASIS USING REAL-TIME PCR IN IRAQ

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A total of 60 suspected cutaneous leishmaniasis (CL) cases were diagnosed during the period from December, 2013 to February,2014 with females representing 67% of the cases. The incidence rate with CL was 88.3% by using Real-Time PCR. Fourty - two (70%) of isolates from different patients were typed as *L. major* and 11 (18.3%) of isolates were typed as *L.tropica* while 7 (11.7%) of cases were gave negative results. The present investigation revealed that the highest number of patients 24 (40 %) was in age group (10 and less) year. Clinically, Our results showed that 26(43%) of CL patients were had single lesion and 34(57%) had multiple lesions, most of them 45(75%) in arm. The highest incidence of disease 39(65%) was observed in rural areas, and the lowest incidence rate 21(35%) was in urban areas. The statistical analyses were carried out with Minitab version.

Keywords: Cutaneous leishmaniasis, Real-time PCR, human.

SL-51

Track: Plant and Environment: Transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae; genomics-assisted breeding.

BIOSURFACTANT ACTIVITY AND ASPHALTENE DEGRADATION BY *BACILLUS CEREUS*

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A bacterial isolate from oily sludge sample identified as *Bacillus cereus*, was able to produce a growth-associated biosurfactant using molasses as cheap substrate. This strain degraded asphaltene as sole carbon source up to 40% after 60 days. The surface tension reduction to level 30.2 mN/m was achieved by *B. cereus* under optimum conditions in flask. The optimum values of carbon-to-nitrogen (C:N), pH and temperature for biosurfactant production were determined as 30:1, 7.3 and 29 °C using response surface methodology (RSM). The maximum emulsification activity in the culture broth was 53% after 48 h using kerosene at 25 °C. The asphaltene degradation was obeyed Tessier kinetic model, while the biosurfactant production using molasses was fit to Contois growth kinetic model (R2=0.962) and the maximum specific growth rate (Ãmax), saturation constant (Ks) and the yield of biomass per substrate (Yx/s) were determined to be 0.145 h^{-1}, 1.83 gL-1 and 0.428 g g^{-1}, respectively.

SL-121

Track: Industrial and Manufacturing

GREEN SYNTHESIS OF CORE-SHELL Fe_3O_4-AU NANOCOMPOSITE FOR BIOMEDICAL APPLICATIONS

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The design of the surface characteristics of magnetic nanoparticles (MNPs) is very important because they can offer nanostructures with desired biological or catalytic reactivity. Biopolymer stabilized core-shell nanoparticles have often great potential for biotechnological and biomedical applications such as bioseparation, wastewater treatment, remediation, drug delivery, spinal damage repairing, MRI bioimaging and biodetection.

In this research, supermagnetic Fe_3O_4 nanoparticles were synthesized using co-precipitation method and the average size of nanoparticles were determined up to 9.8 nm. The surface modification of magnetic nanoparticles was carried out using $HAuCl_4$. $4H_2O$. Gold was reduced on the surface of magnetic nanoparticles using glucose as reducing agents and nanoparticles were stabilized using cross linked biopolymer. Magnetic core-shell nanoparticles with an average size of 14.9 nm in diameter, including both optical characteristics of gold and the magnetic properties of Fe_3O_4 were prepared. The morphology and structure of the modified nanoparticles were characterized using electron microscopy (TEM), X-ray diffraction (X-ray), FT-IR and atomic force microscopy (AFM). The effect of various parameters including the amount of cross linker, pH and temperature on the equilibrium water content (EWC %) of the formed nanocomposites was evaluated. Briefly, a novel strategy for synthesis of core-shell gold coated magnetic nanocomposites is explained with emphasizing on their biomedical applications.

SL-136

Track: Industrial and Manufacturing

PRODUCTION OF S-ADENOSYLHOMOCYSTEINE BY COUPLING THE THERMOSTABLE ENZYMES FROM *THERMOTOGA MARITIMA*

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S-Adenosylhomocysteine (SAH) is an effective sedative, a good sleep modulator, and a new anticonvulsant. SAH can be synthesized from adenosine and homocysteine by using microbial S-adenosylhomocysteine hydrolase (SAHase). The extremely thermostable SAHase and lactate dehydrogenase (LDH) from *Thermotoga maritima* were successfully overexpressed in *Escherichia coli*, and easily purified by heat treatments. The SAHase exhibited the highest activity at 85^{o}C and pH 8.0 with a specific activity of 6.2 U/mg when NAD concentration was 1 mM. However, optimal SAHase reaction conditions shifted to 100^{o}C and pH 11.2, and its specific activity increased to 36.8 U/mg after NAD concentration was raised to 8 mM. Biosynthesis of SAH at 85^{o}C largely increased the adenosine solubility which was a limiting factor for improving the titer of product. At 85^{o}C and pH 8.0, 24 μmol of SAH was obtained when 0.5 mg of SAHase was applied to a 10 ml reaction mixture. The SAH production was further increased to 153 μmol by adding LDH and pyruvate into the reaction mixture for NAD regeneration. Therefore, extremely thermostable enzymes SAHase and LDH from *T. maritima* form an efficient NAD consumption and regeneration system for SAH biosynthesis. This method has great potential for industrial-scale enzymatic production of SAH.

SL-87

Track: Other Areas: Food; marine; bio-safety; systems biology, clinical research/clinical trials; bioethics; nanobiotechnology

DENDRIMER ELECTROPHORESIS

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Electrophoresis is a workhorse technique in proteomics. It is relatively inexpensive, uses disposable materials, offers high-resolution separation and is extremely versatile in its ability to separate any water-soluble material that can be induced to possess a charge. Dendrimers such as polyamidoamine (PAMAM) dendrimers are becoming increasingly popular in various biomedical applications such as imaging and drug delivery. In this presentation, we discuss the applications of native polyacrylamide gel electrophoresis for separation and characterization of various dendritic macromolecules such as polyamidoamine dendrimers, L-DOPA dendrimers and antioxidant dendrimers. The use of isoelectric focusing electrophoresis in determining the isoelectric points of dendrimers will also be discussed.

SL-163

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.

EMPLOYMENT OF PROBIOTICS AND PCF BIOTECHNOLOGY AS AN IDEAL CRISES MANAGEMENT TOOL FOR CURRENT HEALTH PROBLEMS

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At present, probiotics, paraprobiotics, and probiotical cell fragments (PCFs) deserve to be used in food, beverages, and pharmaceutical products as health restorative ingredients. By increasing their respective amounts in either upper air passage or gut, they consistently exert health benefits to the host and regulate local immune reactions without negative side effects or digestive complications. Chronic diseases that can be simply treated include common allergies (food allergy, bronchitis, hay fever, and asthma), celiac disease, diabetes and low-grade inflammations, colonic cancer, and Alzheimer's diseases. Other daily discomforts such as depression, general cognitive decline, memory loss, chronic pain, and general muscle fatigue appeared to have been influenced by these target-specific probiotical formulations. A combination of different probiotic strains demonstrated positive results in 85.5% cases of chronic tonsillitis and 90% in maxilloethmoidal sinusitis. PCFs of several lactobacilli and bifidobacteria demonstrated strong immune balancing activities, anti-tumor, anti-inflammation, anti-mutagenic activities, anti-allergy, detoxification, and radioprotective potency. The combined effects in this review enable selective probiotics and PCFs as a practical tool box for clinical application in the prevention and adjunctive treatment of current health problems in the human history.

Keywords: Allergy, current diseases, gut, probiotical cell fragments (PCFs), probiotics, upper air passage.

SL-73

Track: Other Areas: Food; marine; bio-safety; systems biology, clinical research/clinical trials; bioethics; nanobiotechnology.

SYNTHESIS OF SILVER NANOPARTICLES-DECORATED GRAPHENE OXIDE NANOCOMPOSITES IN SUPERCRITICAL CO_2 AND THEIR ANTIBACTERIAL ACTIVITY

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Nanocomposites of silver nanoparticles on graphene oxide (GO/Ag) was prepared via a facile and environmentally friendly route in the presence of supercritical carbon dioxide (scCO2). Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and energy-dispersive X-ray analysis (EDS) revealed that GO was coated by silver nanoparticles under the optimized experimental condition. In the nanocomposite, the Ag nanoparticles on the surface of GO were predominantly spherical in shape with excellent dispersion. UV-visible spectrum of the nanocomposite presented a surface plasmon resonance vibration band at around 450 nm. X-ray diffraction analysis showed that the nanoparticles were of a face centered cubic structure. Experimental results showed that the GO/Ag nanocomposite exhibited excellent antibacterial activity towards *Escherichia coli* (*E. coli*) due to the synergistic effect of GO and Ag nanoparticles.

SL-31

Track: Medical Biotechnology

REHABILITATION OF HUMAN ENAMEL USING A NEW BIOMATERIAL

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The remineralization and rehabilitation capability of human enamel, the solidest tissue in the body, is basically limited. The new discoveries in biotechnology and nanomedicine appealing new prospective, like the use of new biomaterials, which promote approach to support enamel remineralization. Here we elaborated and investigated a new peptide amphiphile (PA-DENT03). The therapeutic and remineralization capacities of this bioactive amphiphile (0.5%) were evaluated using directtooth application in children with demineralization troubles (n=63; ages=6 -15 years)during eight weeks. Three study groups where established;the first treated with PA-DENT03, the second with fluoride and the third was the control group. Three weeks after PA-DENT03application, we identifieddevelopment of enamel in all participants (n=21), while in the second group (n=21) the remineralization was observed after three months (p=0,001). This is the first time that this peptide amphiphile has been used in demineralization pathologies and we report that this triggered formation of structured minerals validate a pathway for developing biomaterials for management of dental caries and bone diseases.

SL-180

Track: Medical Biotechnology

FABRICATION AND CHARACTERIZATION OF SILK/FORSTERITE/NANOHYDROXY-APATITE COMPOSITES FOR TISSUE ENGINEERING APPLICATIONS

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Silk fibroin (SF) has been used as an in-access material for tissue regeneration purposes [1]. However, a number of problems arise regarding the use of SF in bone tissue engineering applications. The most important problem is the lack of bioactivity of SF and poor mechanical strength of porous SF. To solve this problem, an important strategy is to combine SF with inorganic materials so that the resulting hybrid materials would possess improved mechanical and biological properties. In recent years, some Si-Mg containing bioceramics have been of interest in the development of bone implant materials [2].

It is well known that bones are the products of natural minerals, which are mainly composed of inorganic hydroxyapatite (HA) nanocrystals and collagen [3]. In continuation of our ongoing research for the synthesis of composite scaffolds [5], the current research focused on the synthesis of silk fibroin/forsterite/nanohydroxyapatite composite scaffolds prepared by the freeze-drying technique. The X-ray diffraction (XRD) and scanning electron microscopy (SEM), techniques was used to investigate the microstructure and morphology of the composite. In addition, the mechanical properties, e.g. nano-hardness, elastic modulus and fracture toughness of composites were determined.

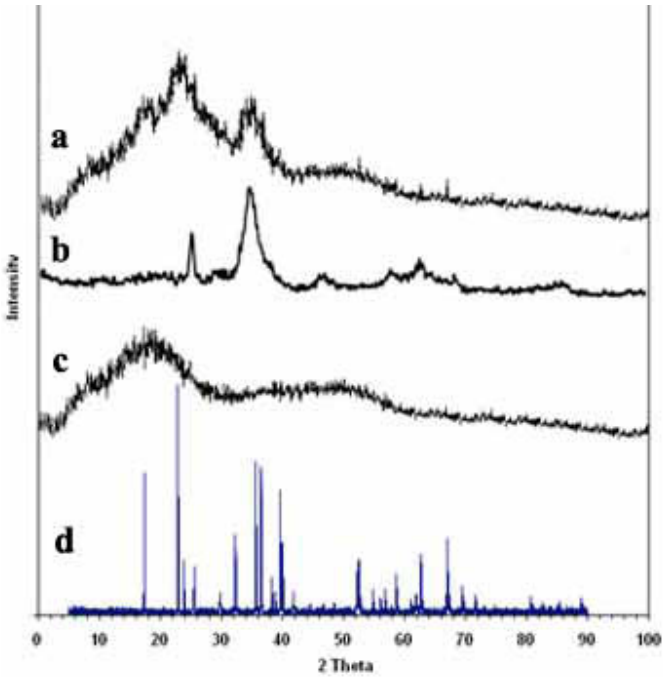

Fig. (1). XRD patterns of (**a**) SF/forsterite/nanohydroxyapatite composite (**b**) nanohydroxyapatite (**c**) pure SF and (**d**) pure forsterite.

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SL-27

Track: Medical Biotechnology

NOVEL APPROACHES TO BIOFUNCTIONALIZE ORTHOPAEDIC BIOMATERIALS: HELPING IMPLANTS HELP THEMSELVES

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Orthopaedic implant technology is based on the development and use of biomaterials in the living body. These mostly inert materials (e.g. polymers and metal alloys) are subject to risks of failure, often due to poor integration with host tissue or to susceptibility to bacterial infections. For orthopaedic implants to succeed in their desired functions and outcomes in the body, it is now realized that interactions between the constituent biomaterials and living cells and tissues, both of the human host as well as of pathogens such as bacteria, can play crucial roles. This presentation will review research strategies and discuss new approaches to modify implant biomaterials in ways that can enhance their success and longevity, particularly with regards to biocompatibility and resistance to microbial infection. Our research group has been investigating and developing novel modes to functionalize inert biomaterials with molecules that confer or enhance bioactivities, such as osseointegration, resistance to microbial adhesion and dampening of fibrous encapsulation. The presentation will include specific examples of the novel strategies in the evolution of orthopaedic biomaterials, including techniques of conferring multiple functionalities through surface biomolecular modifications of metals, as well as via nanoparticulate incorporation in polymeric materials.

SL-23

Track: Medical Biotechnology.

MEDICAL BIO-NANOTECHNOLOGY: KINASES AS BIOMEDICAL TARGETS AGAINST INFECTIOUS DISEASES: MALARIA AND TRYPANOSOMIASIS: INFLUENCE OF AG NANOPARTICLES

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The fight against infectious diseases can be manifested by considering not only differences, in structure between specific biomedical targets (enzymes) in the human host and the parasite, but whether they indeed contain the enzyme at all. In this study 3 kinases were investigated. 1) Arginine kinase *[TbAK]* that is absent from the mammalian host but is critical to the survival of *Trypanosomabrucei* (trypanosomiasis). 2) Thiazole kinase *[PfThzK]* an enzyme that is crucial in the biosynthesis of vitamin B_1 in malarial parasite *(Plasmodium falciparum)* yet does not exist in humans. 3) Hexokinase a ubiquitous glycolytic enzyme that is present in humans, *T. brucei [TbHK]* and *P. falciparum [PfHK]* but differs in structure. Consequently specific inhibition of any of these enzymes may lead to an effective treatment for the infectious disease. The His-tagged enzymes [TbAK; PfThzK] werecloned from respective parasitic genomic DNA, expressed in *Escherichia coli* BL21 DE3 cells and purified on a Ni-affinity column and by FPLC on a Superdex 200 HR. The enzymes had, respectively, specific activities of 2.92 and 0.2 Āmol.min^{-1}.mg.protein^{-1}, molecular mass of 40 and 34 kDa, temperature optima of 30 ^{o}C and 37 °C, pH optima of 7.8 and 7.5,K_m of 2.94 and 1.44 mM and V_{max} of 0.16 and 0.08 µmol.ml^{-1}.min^{-1}. Ag nanoparticles (5-7 nm) were synthesized by $NaBH_4$/tannic acid, and characterized by Uv-vis spectroscopy and TEM. The interaction of the enzymes with these nanoparticles was non-competitive with 75 % decrease in activity; Ki = 1.5 nM (TbAK) and 89% decrease, Ki = 6.45 uM (PfThzK). Fluorescence quenching, thermodynamic analysis and FRET show that there is only one binding site. *Tb*HK and human glucokinase (*h*GCK) wereover-expressed containing a 6 histidine-tag in *E. coli* BL21 (DE3) cells containing the pLysS-pRARE2 plasmid. *Tb*HK exhibited thermal stability between 30-55 °C and pH stability between 7.5 and 8.5, while the *h*GCK was thermally stable between 30 and 40°C and stable between pH 7.0 and 8.0. Kinetic studies revealed that *Tb*HK have K_m of 39 ĀM and V_{max} of 4 Āmol.ml^{-1}.min^{-1} while *h*GCK exhibits a K_m of 4.5 mM and a V_{max} of 0.225 Āmol.ml^{-1}.min^{-1}, There was selective inhibition of TbHK (over hGCK) when Ag-nanoparticles (100 nM, 6 nm) were interacted with the enzymes with 68% and 15% inhibition respectively. A mechanism for these inhibitions and interaction by the nanoparticles is proposed to be through Cysresidues strategically positioned about 3-4 Å from the respective reactive sites. The highly selective inhibition observed between *Tb*HK and *h*GCK and inhibition of TbAK and PfThzK may be used in development of novel anti-trypanosomal and/or anti-malarial drugs.

SL-124

Track: Industrial and Manufacturing

DEVELOPMENT OF CAMELINA OIL INTO BIOFUEL: REQUIREMENT OF METABOLIC ENGINEERING OF TERPENES

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Camelina (Camelina sativa) is an emerging biofuel crop. Its seeds contain 35-43% oil content. Its oil products have been recently tested for jets and showed to be effective as current fossil jetfuel products. The development of camelina oil into jetfuel requires additives such as deoxygenated monoterpenes. Unfortunately, camelina only produces a trace level of monoterpenes, the amount of which needs to be largely increased to reach the requirement of jetfuel. In our report, we will show metabolic engineering of monoterpenes in camelina plants. We have used a synthetic approach to introduce enhanced pathways to produce monoterpenes and other terpenes. Synthesized bottleneck genes of the terpene pathway have been transformed into camelina plants. The level of monoterpenes and other terpenes have been significantly increased in transgenic plants. Our data show a promising approach to increase terpenes for the development of camelina oil into jetfuel. This research is funded by ARPA-E.

SL-183

Track: Medical Biotechnology

SENSITIVE COLORIMETRIC METHOD FOR METHYLATION ANALYSIS OF p16/CDKN2 PROMOTER THROUGH HYPERBRANCHED ROLLING CIRCLE AMPLIFICATION

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Background

DNA methylation is a hallmark of the epigenetic regulation of gene expression. Highly efficient DNA methylation analysis is an emergent demand for early cancer detection. We have developed a simple, fast, sensitive and economical assay for DNA methylation analysis by combining hyperbranched rolling circle amplification (HRCA) with enzyme-based colorimetric detection.

Methods

The assay is carried out on a DNA capture probe modified 96-cell microplate with 4 steps, including target recognition, methylation-sensitive endonuclease digestion, isothermal HRCA, and colorimetric readout. With this method the methylation of p16/CDKN2 promoter in cell-free DNA of 72 breast cancer patients and 43 healthy individuals has been examined.

Results

The proposed DNA methylation assay could be accomplished within 2.5 h The strategy exhibited excellent detection specificity and showed a log-linear response to methylated DNA from 100 fM to 10 nM. The p16/CDKN2 promoter was methylated in 57 (79.2%) of 72 patients with breast cancer, and 4 (9.3%) of 43 healthy individuals. The median concentrations of methylated p16/CDKN2 promoter for the cancer and healthy groups were 1.2Ā1012 and 5.5Ā1011 copies/L, respectively ($P < 0.0001$, Wilcoxon test). There was no significant association between the methylated p16/CDKN2 promoter concentration and clinical parameters, such as age, menopausal, grade, histology grade, stage and lymph node metastasis of the patients.

Conclusions

The colorimetric DNA methylation analysis coupling with an isothermal HRCA-based signal enhancement allowed efficient methylation detection with simplicity, rapidness, low cost and high sensitivity, showing great promise for application in early diagnosis of methylation-related diseases as well as high-throughput analysis.

SL-39

Track: Plant and Environment: Transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae; genomics-assisted breeding

ENVIRONMENTAL IMPROVEMENT IN THE MARICULTURE WATERS: A CASE STUDY OF SEAWEED *GRACILARIA LEMANEIFORMIS* BIOREMEDIATION IN CHINA

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Over the past 10 years, the large-scale cultivation of seaweed Gracilaria expanded rapidly in Guangdong, Fujian, Shandong and other Chinese coastal waters. The production of Gracilaria has increased from 50,536 tons (t, dry weight) in 2003 to 114,722 t in 2010. The production of the seaweed ranks only behind the kelps (Laminaria and Undaria) in China. Nanao, Shantou City, Guangdong Province is an area in southern China which is seeing a rapid increase in production of cultivated G. lemaneiformis. The farmed area has seen a 11,538-fold (from 0.13 ha in 2000 to 1500 ha in 2010) increase. From the lab

scale study to the field seaweed cultivation practice, it has been documented that cultivation of Gracilaria is environmentally beneficial. It contributes to de-eutrophication, harmful algal blooms control, healthy mariculture systems maintenance, and CO2 sink. Gracilaria can significantly remediate and improve water environments. Gracilaria cultivation provides a new approach to coastal environmental improvement in China and the world.

Keywords: *Gracilaria cultivation,* Mariculture, Bioremediation, Environmental improvement, Chinese coastal waters.

SL-140

Track: Pharmaceutical Biotechnology: Biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering

ANTITUMOR ACTIVITY OF BALANITOSIDE EXTRACTED FROM BALANITES AEGYPTIACA FRUIT

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The current study aimed at investigating the antitumor efficacy of balanitoside extracted from Balanites aegyptiaca fruit against Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice. The extracted balanitoside was proved by chemical analysis and LD50 of balanitoside was determined. Then, mice were injected intraperitoneally with balanitoside (10mg /kg b.wt) before and after EAC inoculation, to achieve preventive and therapeutic effects daily, for 9 days. The effects of balanitoside on the count of EAC cells and life span prolongation were studied; malondialdehyde (MDA), nitric oxide (NO) levels as well as catalase (CAT) and caspase 3 activities were estimated. Cytological studies on EAC cells and histopathological examination of liver tissue were carried out. Treatment with balanitoside decreased EAC cell count for preventive and therapeutic groups. MDA and NO levels were decreased in liver and serum in preventive and therapeutic groups compared to positive control group. While CAT activity was increased in liver and plasma of preventive and therapeutic groups in comparison with positive control group. Caspase 3 activity in EAC cells, was increased in preventive and therapeutic groups in comparison with positive control group. Survivin expression in liver was decreased in preventive and therapeutic groups in comparison with positive control group. The present work indicates that balanitoside isolated from fruit extract of Balanites aegyptiaca may possess significant antitumor and antioxidant activity *in vivo*.

Keywords: Balanites aegyptiaca, EAC, Antitumor activity, Lipid peroxidation, Catalase, Nitric oxide, Apoptosis.

POSTERS

PO-71

Track: Medical Biotechnology

A METHOD FOR FAST ASSESSMENT OF OP/CB EXPOSURE IN THE JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA) USING COMBINED ESTERASES ENZYME ACTIVITY AS BIOMARKERS

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The aims of this study were to investigate the presence of different esterase activities in plasma and liver for Japanese quail and to combine determination of both carboxylesterase and cholinesterase as biochemical biomarker in order to identify the effects of carbamate and organophosphate compounds exposure. Carboxylesterase exhibits larger sensitivity to carbamate and organophosphate compounds than to cholinesterase and is present at higher levels. This permitted nature and distribution of carboxylesterase or cholinesterase to be measured. One predominant toxicological form of enzyme level constant in its patterns of motivation and inhibition with cholinesterase was identified in plasma with an apparent Michaelis constant for butyrylthiocholine iodide of 0. 394ÃmM. Carboxylesterase activity in liver was considered by its preferential hydrolysis of the S-phenyl thioacetate. A concentration dependent decrease of carboxylesterase and cholinesterase has demonstrated during *in vitro* incubation of malathion, parathion, and trichlorfon in the range 0. 125-2ÃmM, while with methomyl was in the range 0. 25-4ÃmM. When quail (n=15) was exposed orally for 48 h to concentrations of carbamate or organophosphate compounds of 3-200Ãmg/kg, the percentage inhibition of cholinesterase was in each case larger than that of carboxylesterase and reached statistical significance ($P<0.05$) at lower concentrations.

Keywords: Cholinesterase; quail, organophosphate; carbamate.

PO-35

Track: Medical Biotechnology

THE ANTIBACTERIAL ACTIVITY OF ETHANOLIC LEAF EXTRACTS OF SIX SENNA SPECIES

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Six medicinally important Senna species were studied in order to provide complementary data obtained from foliar epidermal features, phytochemical contents, antimicrobial properties and DNA fingerprinting so as to aid easy recognition of the Senna species for pharmacognostic researches. This study was carried out in order to eliminate adulteration of these medicinally important Senna species by misidentification. The species studied were *S. alata, S. obtusifolia, S. siamea, S. hirsuta, S. occidentalis* and *S. polyphylla*. The result of foliar epidermal investigation revealed the variation patterns in the epidermal morphology of the six Senna species investigated. The epidermal cell shape varied from polygonal and irregular to sinuous on the lower and upper epidermis of the six Senna species investigated. *S. alata, S. occidentalis, S. obtusifola* and *S. siamea*, epidermal cell shape were mostly polygonal, while that of *S. hirsuta* and *S. polyphylla* were irregular in shape on the lower epidermis. The lowest stomatal frequency of 11. 5% and 29. 6% were seen on the upper and lower epidermis of *S. siamea* and *S. obtusifolia* respectively. The highest stomatal frequency of 19. 8% and 58. 3% were observed on upper and lower epidermis of *S. obtusifolia* and *S. alata* respectively. Trichomes were seen in all species. The uniformity in most of the epidermal features shows the close affinities that exist among these species and the naturalness of the Senna species in general. *S. alata* contained the highest alkaloid content (1. 16 mg/l), followed by *S. hirsuta* (1. 03 mg/l) and the least was in Senna occidentalis (0. 17 mg/l). This was the same trend of *S. alata* having higher phytochemical constituents except for phytates were the highest was recorded for *S. siamea* (0. 36 mg/l). The study on antimicrobial activities show that ethanolic extracts of the six Senna species possess antimicrobial activity against human pathogens used in this study, *Escherichis coli*, *Klebssiella pneumonia*, *Staphylococcus aureus, Streptococcus pneumonia* and *Salmonella typhi*. The antimicrobial activity of *S. alata* was more pronounced at higher concentration than at lower concentration in the species of Senna investigated. This is in conformity to the phytochemical content result, where *S. alata* had the highest

phytochemical content. The antimicrobial activity of ethanolic extract of Senna alata was favourably compared with the standard drug-ciprofloxacin. From the RAPD analysis, a dendogram was generated from the detection of polymorphic fragments in the six primer sequences that was analysed, the dendrogram showed that all the species are at least 62% similar. *S. alata* and *S. hirsuta* were 95% genetically identical and far from other accessions in terms of similarity. This is common in speciation. The RAPD analysis led to a clear distinction between *S. alata* and *S. hirsuta* and other species. The result of the phytochemical and antimicrobial studies have revealed the potency of the six Senna species as active antimicrobial agents, while the result of the epidermal and molecular studies aid easy recognition of these medicinally important Senna species for pharmacognostic researches.

Keywords: Antibacterial, Senna Species, Leaf Extracts, Minimun inhibitory concentration, zone of inhibition.

PO-73

Track: Plant and Environment

DEVELOPMENT OF STORED INSECT FREE WHEAT (*TRITICUM AESTIVUM L.*) *VIA* ENGINEERED METABOLOME

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Insect infestations are major factors for post harvest loss of grain quantity and quality. Preventing, or at least slowing, the stored product infestation is important in maintaining wheat's quality and marketable value. The avidin protein causes mortality in many species of stored cereals insects by preventing the absorption of dietary biotin. Synthetic *Gallus gallus avidin* gene was introduced into spring wheat (*Triticuma aestivum* L.) cv. Giza 168 using the biolistic bombardment. The construct contained the synthetic *avidin* gene and the liberty herbicide resistance (*bar*) gene. The putative transgenic plantlets were tested for the liberty herbicide resistance or susceptibility by leaf painting. The polymerase chain reaction (PCR) along with the southern blotting have showed the integration of the *avidin* gene in the putative transgenics genome, and the semi-quantitative reverse transcription PCR (RT-PCR) has confirmed its transcription. The presence of the avidin protein in the grains of the T_1 and T_2 plants was confirmed using SDS-PAGE, dot blot and western blotting. The bioassay experiments on the red flour beetle (*Tribolium castaneum*) have showed 100% mortality of insects that were fed the transgenic wheat flour and intact grains in their diet compared to 0% mortality for insects that were fed a non-transgenic flour and intact grains control diet.

Keywords: Stored cereals insects; Post harvest loss; avidin; *Triticum aestivum; Tribolium castaneum.*

PO-72

Track: Regenerative Medicine

CONTINUOUS AND DELAYED PHOTOHEMOLYSIS SENSITIZED WITH METHYLENE BLUE AND IRON OXIDE NANOPARTICLES (Fe_3O_4)

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This research present the sensitization of methylene blue (MB), a photodynamic therapy photosensitizer which showed phototoxicity for many tumor cells *in vitro* incorporated with iron oxide nanoparticles (Fe_2O_3), which offer magnificent interaction both inside and outside the surface of biomolecules to bring about a radical change in cancer treatment and diagnosis, together with red blood cells (RBC's). The study investigated the sensitization of continuous photohemolysis (CPH) for MB with and without iron oxide, delayed photohemolysis (DPH) at room temperature, DPH at different irradiation temperature (Tirr) and at different incubation temperature (Tinc) for the same irradiation time (Tirr).

Gompertz function is apply as an appropriate model to fit the collected experimental data for CPH and DPH with minimum errors. Fractional photohemolysis ratio (a) and fractional photohemolysis rate (b) of this model and relative steepness of the curves for the photohemolysis were measured for a series of sensitizer concentrations and DPH irradiation times. The power dependence found to be greater than one for DPH and less than one for CPH. Our results indicate the relative steepness for CPH and DPH are almost independent on MB and MB with iron oxide concentrations and at different Tirr and Tinc. In addition, the parameter b is independent of iron oxide concentration while the parameter a decreases with increasing iron oxide concentration. In conclusion, CPH and DFH process are much lower in the presences of methylene blue.

Keywords: Iron oxide nanoparticle, Methylene Blue, Photohemolysis.

PO-80

Track: Plant and Environment

INFLUENCE OF SALICYLIC ACID AND POTASSIUM NITRATE ON GROWTH, OSMOLYTES AND PHYSIOLOGICAL ACTIVITIES OF SALT AND DROUGHT-STRESSED BARLEY (*HORDEUM VULGARE*)

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In respect to the previous study (Fayez and Bazaid, 2014), we are continuing evaluation the influence of salinity and drought stresses on barley cultivars. The local cultivar of barley (Hordeum vulgare L.) plants was subjected to three levels of NaCl (50, 100 and 150 mM), three levels of water deficit (80, 70 and 50 % soil water content (SWC). The highest levels of NaCl (150 mM) and water deficit (50 % SWC) were chosen for the interaction treatments with 50 ĀM or 10 mM KNO3 (150 mM NaCl + 50 ĀM SA, 150 mM NaCl + 10 mM KNO_3, 50 % SWC + 50 ĀM SA and 50 % SWC + 10 mM KNO3) for two weeks. Shoot fresh weight, leaf photosynthetic pigments and K+ contents were decreased with increasing salt and water deficit while proline leaf soluble carbohydrate and protein, malondialdehyde (MDA), total phenolic compounds and Na+ contents were increased. Application of 50 ĀM SA or 10 mM KNO_3 to plants treated with 150 mM NaCl) or 50 % SWC increased shoot growth and total leaf photosynthetic pigments. Due to the interaction treatments, SA and KNO3 increased proline and K+ contents of 150 mM NaCl treated plants. In contrast, soluble carbohydrate, malondialdehyde (MDA), total phenolic compound and Na+ contents of leaves were decreased. Interaction of water deficit of 50 % SWC + 50 mM SA treated barley caused reduction in phenolic compounds, Na+ and K+ contents while proline, carbohydrate, protein and MDA contents were approximately unaffected compared with those of 150 mM NaCl treated barley. Interaction of water deficit of 50 % SWC + 10 KNO_3 caused reduction in leaf proline, MDA and Na+ contents. Leaf soluble carbohydrate and K+ contents increased due to interaction of 50 % SWC + 10 mM KNO_3 compared to those of 50 % SWC treated barley. From the obtained results, it can be concluded that the carbohydrate in salt treated barley plays a significant role in the osmotic adjustment. Addition of SA or KNO_3 changed the physiological activities and metabolism of barley towards increasing tolerance against salt and water deficit stresses.

Keywords: Hordeum vulgare; Metabolites; Photosynthetic pigments; Salt stress; Water.

PO-47

Track: Medical Biotechnology

DETECTION OF HIV PROTEINS IN URINARY EXOSOMES

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Exosomes are extracellular vesicles, 30-100nm in size, and are found in most body fluids including blood, urine, milk, saliva and amniotic fluid. We found urinary exosomes isolated from HIV infected individuals contained HIV proteins that could be a potential diagnostic tool. Mass spectrometry identified HIV proteins, *Nef*, *Gag*, *Vif* and *Pol* present in urinary exosomes of 111 HIV infected patients, while 9 HIV negative individuals had no detectable HIV proteins present. An ELISA protocol was developed to detect HIV urinary exosomal proteins using snowdrop lectin coated 96 well plates. Using this method, we confirmed the 111 HIV positive patients from 9 HIV negative individuals. A significant difference was found between the optical densities of HIV positive patients and negative controls, (p=0. 0001). Receiver operating characteristic (ROC) curve analysis showed a sensitivity of 95. 5% and an area of 0. 94, indicating the validity of our assay as a potential diagnostic. This assay can detect HIV positive patients from HIV negative controls. Our urinary exosome test would prevent the transmission of HIV through needle sticks in healthcare workers and would be culturally acceptable in parts of the world where blood collection is considered objectionable.

PO-41

Track: Other - Molecular Biology

CHAETOCIN: NEW WARRIOR OF DEDIFFERENTIATION

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Induced pluripotent stem cells (iPSCs) offer great promise as tools for basic biomedical research, disease modeling and drug screening. We aimed at elucidating the molecular mechanisms iPSCs generation by somatic cell reprogramming. Specifically, we are interested in identifying the role of chromatin modifying enzymes (CMEs) play during reprogramming. The aim of this study is to analyse the role of one of these CMEs, namely the histone H3K9 methyl-transferase Suv39H1, on somatic cell reprogramming and develop a method to increase the efficiency of iPSCs generation by suppressing Suv39H1 function by small molecules. To examine the role of Suv39H1 in reprogramming, we utilised Chaetocin to suppress this methyltransferase in human fibroblasts while reprogramming these cells with ectopic expression of embryonic transcription factors (Oct4, Sox2, Klf4, and c-Myc –OSKM). After reprogramming of human fibroblasts, colonies were stained with Tra-1-60 antibodies to identify iPSCs. iPSC colonies were then counted using ImageJ. Chaetocin treated cells generated significantly more iPSC colonies than control cells indicating that suppression of Suv39H1 increases reprogramming efficiency. The results of the study will contribute to our understanding of the chromatin-based epigenetic mechanisms of reprogramming, improve efficiency of iPSCs generation, and eliminate or replace one or more reprogramming factors with chemical compounds.

PO-85

Track: Others - Nanobiotechnology

EFFECT OF FIVE ACIDS TOWARD THE CONTROLLING ASPECT RATIO OF GOLD NONORODS IN SEEDED GROWTH METHODS SYNTHESIS

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Different Gold nano structures such as spherical, hollow spherical, nano cage, nano shells, nano rods, show promise in variety of biomedical application such as diagnosis and therapy due to their optical properties. Gold nanorods fond especial place between them. Actually unique local surface Plasmon resonance of GNR that itself depended on to aspect ratio (long/with) of it determine the situation of medical application. Seed growth methods provide enormous boon to scientist for use of advantage of AuNR shape dependent optical property. in this study we investigated the effects of five acid including Sulphuric acid, phosphoric acid, hydrochloric acid, nitric acid, and acetic acid for changing and controlling of gold nanorod aspect ratio. The results compared with control sample that it was normal protocol free of acid additive, for this comparison we used visualization of color changing, Uv/vis spectophotometry and Transmition electron microscopy studies. Result shown that for having good nanorods with suitable aspect ratio pH changing usually is necessary and even in same pH the source of pH adjusting could affect the final products with attention the challenge of reproducibility of GNR synthesis we suggest that every researchers during optimization of low materials give more attention to pH role and source.

Keywords: Gold nano rods, aspect ratio, seeded growth, pH.

PO- 98

Track: Plant & Environment

CONTINENTAL PHYLOGEOGRAPHY OF ELDANA SACCHARINA WALKER: TANGIBLE GEOGRAPHIC POPULATIONS AND UNRELIABLE HOST PLANT ASSOCIATED GENETIC DIFFERENTIATION

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Ecological studies suggest that the indigenous sugarcane borer, Eldana saccharina, is either represented by a group of biotypes or cryptic species which are behaviourally different and morphologically similar. Identifying the factors that are responsible for behavioural variation is critical in the management of Eldana saccharina. Studies on geographic isolation and host plant associated genetic differentiation are key steps to develop and to improve monitoring and biological control strategies. We examined the effect of geographic isolation and host plant associated genetic differentiation on Eldana saccharina. Base-pair differences in the cytochrome oxidase I (COI) gene were used to characterise haplotype diversity and phylogenetic relationships of the different populations. There were 54 haplotypes among the 87 sequenced individuals from different localities in 13 African countries and eight host plant species. Genetic analyses revealed no detectable genetic differentiation between populations from different hosts. However, there was strong evidence of variation in genetic composition between populations of the pest from geographic regions. The results revealed that E. saccharina populations are separated into four major units corresponding to the West/Central Africa, Rift Valley and two southern African populations. Geographical features such as the Rift Valley and large water bodies on the continent seem to have a considerable impact on the genetic diversity in *E. saccharina*.

Keywords: Phylogeography, *Eldana saccharina,* mitochondrial DNA, sugarcane, indigenous host plants.

PO- 95

Track: Plant and Environment

DO SHORT FIBER CELLS OF UPLAND AND PIMA COTTON SHOW DIFFERENT LEVELS OF DNA METHYLATION?

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Within the 52 cotton species, two allopolyploid species; Gossypium hirsutum and G. barbadense are most cultivated in the world. Allopolyploid cotton species are believed to derive from a single allopolyploidization event that combined the Old World A genome with the New World D genome in an A genome cytoplasm. In this research DNA cytosine level differences of WRKY protein gene in the short fiber cells of TM-1 (G. hirsutum) and Pima 3-79 (G. barbadense) were studied using bisulfite sequencing technique. Analysis of 323 nucleotides of WRKY protein gene segment revealed that there were 14 CG, 11 CHG and 26 CHH patterns in the studied region. Of 629 analyzed positions in 25 day of post anthesis (DPA) short fiber cells of Pima 3-79, 53 (8.43%) were methylated while 576 were not methylated (91.57%). Of 752 analyzed positions in 25 DPA short fiber cells of TM-1 101 (13.43%) were methylated while 651 were not methylated (86.57%). The level of cytosine methylations within and between pollen and fuzz fiber cells of the two species was not statistically significant. However, the level of methylations between short fiber cells of the two species were statistically significant at the $p<0.0001$. Significant methylation differences in the short fiber cells of the two species might have resulted from the physiological age, the DNA sequence differences or species specific. Further studies are in progress using more loci and samples to reveal whether there are different level of DNA methylation levels between the short fiber cells of Upland and Pima cotton.

Keywords: *Gossypium* spp., Epigenetics, Bisulfite sequencing.

PO-19

Track: Industrial and Manufacturing

ROBUST YEAST FOR THE PRODUCTION OF BIOETHANOL FROM STEAM-EXPLODED SUGARCANE BAGASSE

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Robust yeast with high inhibitor, temperature and osmotic tolerance remain a critical requirement for the sustainable production of lignocellulosic bioethanol.

In this work, grape marc was selected as extreme environment to search for innately robust yeast because of its limited nutrients, exposure to solar radiation, temperature fluctuations and ethanol content. Forty newly isolated *Saccharomyces cerevisiae* strains gave high ethanol yields at 40 °C when inoculated in minimal media at high sugar concentrations. Moreover, the isolates showed distinct inhibitor-tolerance in defined broth supplemented with increasing levels of single inhibitors or a cocktail of inhibitory compounds. Both fermentative abilities and inhibitor resistance were greater than those exhibited by industrial and commercial *S. cerevisiae* benchmark yeast.

The isolate Fm17, exhibiting the most promising phenotype, was then evaluated to ferment liquor from steam-exploded sugarcane bagasse, having high concentrations of weak acids, furans and aldehydes. The selected strain produced high alcohol levels with an ethanol yield equal to 89% of the theoretical. This work demonstrated that yeast with high multiple stress tolerance can be obtained from unconventional ecological niches, such as grape marc. The selected yeast represents a promising platform to develop robust engineered strains suitable for the one-step processing of biomass into ethanol.

Keywords: Bio-ethanol, robust yeast, inhibitor-tolerance, sugarcane bagasse.

PO- 100

Track: *Plant and Environment*

NEUROPROTECTIVE EFFECT OF *ORIGANUM GLANDULOSUM* EXTRACT AND IDENTIFICATION OF ITS ACTIVE CONSTITUENTS

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Origanum glandulosum Desf is an endemic flavoring herb widely distributed in North Africa region and commonly used as spice and in traditional medicine. This oregano specie is rich in essential oils but little is known about is phenolic composition. In the present, crude ethyl acetate extract of *O. glandulosum* was preparated in order to isolate and investigate neuroprotective compounds through the inhibition Amyloid-Ā peptide (AĀ) aggregation. The ethyl acetate extract showed significant anti-aggregative activity. Three major compounds of the extract were isolated: Rosmarinic acid, two new cyclolignans Globoidnan A and B. Rosmarinic acid and Globoidnan A showed significant anti-aggregative activity against AĀ aggregation (IC_{50} 7.0 and 12.0 μM, respectively). In conclusion, the results revealed that *O. glandulosum* extract and its active compounds could have neuroprotective potential.

Keywords: *Origanum glandulosum*, peptide (AĀ) aggregation, neuroprotective activity, polyphenol, LC-NMR.

PO-75

Track: Marine Biotechnology

NATURAL QUORUM SENSING INHIBITORS FROM PREVIOUSLY UNCULTURED MARINE BACTERIA

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The marine world is a diverse environment that covers almost 70% of the earth"s surface and contains approximately 75% of all living organisms. Despite this, the microbial constituent of that environment has only been explored to a limited extent. Recent studies have shown that marine microorganisms provide an exciting emerging resource for the discovery of new classes of bioactive agents that may have therapeutic effects. Marine bacteria whether from the water column or marine surfaces, display a variety of communication strategies that allow them to grow and survive. Quorum sensing induction/quorum sensing (QS) inhibition strategies are important examples of these communication strategies. Indeed these strategies allow the existence of dense populations of microorganisms embedded in and on mucus layers of many living marine organisms. In this study we undertook to detect, isolate and characterize new QS inhibition materials from stony coral associated bacteria (cultured and previously uncultured). The main method used in this study included a novel culturing technique developed in our lab, employing polymerically encoated agar spheres in which previously unculturable microorganisms are entrapped. The membrane coat enables molecules to diffuse in and out of the sphere without losing the encapsulated cells, allowing the growth of the encapsulated bacteria in different environments and under different conditions. Mucus samples were collected from the surface layer of the stony coral *Favia* sp., as well as from the adjacent water column, from the of the Inter-University Institute for Marine Science in the Gulf of Eilat (291510N, 341940E), at depths of 3-7 m. The samples were diluted, and aliquots were encapsulated in our polymeric coated spheres and returned to the reef for 6-8 weeks of *in-situ* incubation. The spheres were then collected and the encapsulated organisms were tested for their QS inhibition or induction abilities using the bioreporters strains of Chromobacterium violaceum CV026, Agrobacterium tumefaciens KYC55 and *Escherichia coli* K802NR. The bacteria from the spheres that were found to have inhibition abilities were diluted and encapsulated again for further *in-situ* incubation, and the QS inhibition potential was retested to show reproducibility. The bacterial consortia from the spheres were characterized molecularly, to identify the bacterial population that may cause the QS inhibition. The dominant bacterial families found to produce QS inhibitors were Vibrionaceae and Alteromonadaceae. The active samples were

subjected to further physical analysis by Maldi-TOF. A putative QS molecule (active compound) with a precursor m/z 371 was detected and its molecular structure is currently being elucidated.

Keywords: Quorum sensing, unculturable bacteria, biofilm, coral, marine environment.

PO-7

Track: Medical Biotechnology

IN VIVO HETEROTOPIC BONE FORMATION AROUND A PERIPHERAL VESSEL BUNDLE USING DIFFERENT RATIOS OF RHBMP-2 AND TG-VEGF IN A FIBRIN MATRIX

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Aims

To investigate *in vivo* heterotopic bone formation within a dimensionally stable membrane wrapped around a vessel bundle filled a fibrin matrix loaded with recombinant human bone morphogenic protein-2 (rhBMP-2) and transgenic vascular endothelial growth factor (TG-VEGF).

Methods

Twelve New Zealand white rabbits were randomly allocated to four groups, A, B, C and D. In a standardized procedure under general anesthesia an Inion® membrane was wrapped around the femoral vessel bundle. A fibrin matrix with different ratios of rhBMP-2 and TG-VEGF was injected around the vessels within the membrane tube. In group A, the control group, the matrix was loaded with rhBMP-2only; in group B the ratio of rhBMP-2 to TG-VEGF was equal to 3 to 1, in group C 5 to 1, and in group D 10 to 1. The animals were sacrificed eight weeks postoperatively; harvest of the membrane tube with the attached surrounding soft tissue was performed. Specimens were subjected to micro-computed tomography (micro-CT), histologically examined and immunohistochemically stained. The latter examination provided microvessel density (MVD) evaluation, a measurement for angiogenesis between the vessels and the membrane tube.

Results

Uneventful intra- and postoperative course until the scheduled date of sacrifice. Within the membrane tube cylindrical heterotopic bone formed around the vessels, prevented from skeletal muscle contact due to the membrane. Compared to groups B and C, increased heterotopic bone volume was detected in group D. Anti-CD31 antibody immunohistochemical staining detected MVD in all groups, however with increased tendency in groups B and C with 120. 4±23. 3, 125. 8±14. 7 vessels/mm^2, respectively.

Conclusions

RhBMP-2 and TG-VEGF in a fibrin matrix lead to *in vivo* heterotopic bone formation around vessels within a dimensionally stable membrane tube that prevents direct contact to surrounding skeletal muscle. Whereas a rhBMP-2 to TG-VEGF ratio of 10 to 1 favored osteogenesis, angiogenesis with neo-vessel sprouting together with osteogenesis was found in lower ratios.

Keywords: Recombinant human bone morphogenetic protein-2 (rhBMP-2), transglutaminase vascular endothelial growth factor (TG-VEGF), heterotopic, bone, vessel, formation, angiogenesis, *in vivo*, animal trial.

PO-14

Track: Medical Biotechnology

SURVIVIN-DIRECTED MOLECULAR BEACON AS POTENTIAL THERANOSTIC AGENT IN MELANOMA CELLS

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Survivin is an inhibitor of apoptosis overexpressed in chemoresistant tumors. In this study, we investigated the anticancer theranostic properties of a molecular beacon-oligodeoxynucleotide (MB-ODN) that targets survivin mRNA in human malignant melanoma cells. The fluorescence signal of the lipofectamine-delivered MB-ODN in A375 cells was evaluated by confocal microscopy and compared to that obtained in normal cells. Survivin mRNA and protein expression were analyzed by real-time PCR and western blot, respectively. Apoptosis was assessed by internucleosomal DNA fragmentation, dissipation of mitochondrial membrane potential (Ā m) and nuclear staining with DAPI. Transfection of MB-ODN into A375 cells generated a high signal intensity from the cytoplasm, while no signal was detected in the extracellular environment and in survivin-negative cells (i.e., human monocytes). In A375 cells, MB-ODN treatment time dependently decreased survivin mRNA and protein expression with the maximum effect reached at 72 h (-94±1. 6 and -90. 1±1. 8%, respectively), compared to control. Treatment with MB-ODN for 48 h induced a significant (P<0. 001) variation in Ā m, accumulation of histone-complexed DNA fragments in the cytoplasmic fraction and nuclear condensation. MB-ODN also enhanced the proapoptotic effect induced by docetaxel and cisplatin. In conclusion, our findings provide evidence of a novel potential anticancer strategy for simultaneous imaging and targeted therapy.

Keywords: Survivin, molecular beacon, melanoma, cancer detection, drug resistance, targeted therapy.

PO-20

Track: Industrial and Manufacturing

REDUCTION OF CELL LYSATE VISCOSITY BY CLONING *STAPHYLOCOCCUS AUREUS* NUCLEASE GENE IN POLYHYDROXYALKANOATES PRODUCING BACTERIA

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Downstream processing may significantly affect the price of polyhydroxyalkanoates (PHA); hence, the development of an economical and efficient recovery processes is needed.

During PHA production, the high viscosity of the PHA-synthesizing bacterial cell lysate, which is due to the high content of nucleic acids, could represent a technological problem. In order to reduce viscosity during this stage, a nuclease enzyme could be used.

The aim of this work was to introduce in highly efficient PHA producing selected bacteria (*Cupriavidus necator* DSM 545 and *Pseudomonas oleovorans* DSM 1045) the nuclease gene (nuc), encoding for a staphylococcal extracellular thermo-stable nuclease (SNase), deriving from *Staphylococcus aureus*. Since plasmid pNuc, containing the nuc gene, is unable to replicate in *E. coli*, an amplified fragment of 700 bp was obtained and cloned in a broad host range plasmid. Once transferred into *E. coli* the nuclease activity was tested on chloroform-permeabilized cells incubated with Ä-phage DNA, and found to be efficiently expressed and therefore suitable for subsequent cloning purposes. After conjugation of the obtained plasmid into *C. necator* DSM 545 and *P. oleovorans* DSM 1045 nuclease activity and PHA production were analyzed.

The results obtained indicated that nuc gene can proficiently be expressed in the two recipient strains without significant limitations of PHA production.

Keywords: PHA, nuclease, biopolymers, bacteria.

PO-22

Track: Medical Biotechnology

DEVELOPMENT OF A NOVEL STRUCTURED NANOFIBROUS NERVE GUIDANCE CONDUIT FOR NERVE REGENERATION

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Conventional nerve guidance conduits (NGC) are tubular in structure and have limited cross sectional surface area available for axonal contact, which in turn adversely affects nerve regeneration. We have developed a novel biodegradable spiral structured NGC with inner aligned nanofibers, using polycaprolactone (PCL), which provided necessary cues to promote neurite outgrowth and served as a bridge to guide regenerating axons and support cell infiltration. For this study, we investigated the physicochemical properties of the spiral structured nanofibrous conduit and cellular responses to determine the suitability of applying the nerve conduit for nerve regeneration. This investigation focused on characterizing the nerve conduit in terms of morphology, porosity, mechanical properties, degradation, and attachment and proliferation of Schwann cells. We observed that the incorporation of spiral layers in the lumen of the tubular conduit provided significantly greater surface area for nerve regeneration while improving the transport features (nutrient and metabolic waste removal). Morphological characterizations were carried out to elucidate nanofiber alignment, spiral structure and presence of interconnected macro-pores. These scaffolds showed improved pore properties as compared to control tubular conduits. The improved transport features of the spiral scaffolds allow for better nutrient exchange to take place for ensuring healthy nerve regeneration. We further optimized the scaffold mechanical properties to achieve mechanical properties in the range of native tissue by altering the material composition. High tensile strength will guarantee a mechanically strong conduit that can hold suture well during cooptation and preserve the suture after surgery, and most importantly can avoid the collapse and twisting of the conduits *in vivo*. While the presence of inner aligned fibers and outer fibers may influence the mass of the conduits, it did not cause a significant difference in degradation compared to other groups. The PCL conduits degrade at a slow rate, and this is suitable for peripheral nerve regeneration since a critical gap length may take up to one year for complete recovery. The preliminary *in vitro* cellular studies showed that the attachment and proliferation of Schwann cells in the spiral structured nanofibrous conduits were significantly enhanced as compared to those in the tubular control. Additionally, the nanofibers provided a better cue for neurite guidance. The presence of nanofibers on the surface of conduits mimics the extracellular matrix and guides the unidirectional alignment of cells. The novel biodegradable spiral structured nanofibrous nerve conduit can alleviate several drawbacks experienced by several grafts used in the clinics for NGCs and has the potential to be clinically applied for peripheral nerve regeneration.

Acknowledgement:

The work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Peer Reviewed Orthopaedic Research Program under Award No. (W81XWH-13-1-0320).

Keywords: Pirepheral Nerve Injury, Nerve Regeneration, Nerve Guidance Conduit, Nano-biotechnology, Electrospinning Fibers.

PO-61

Track: Medical Biotechnology

3D IMAGING OF NATIVE AND REGENERATIVE NERVES USING FOCUSED ION BEAM SEM

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Peripheral nerve injury is a leading cause of lifelong disability in those that sustain an extensive amount of nerve damage. Current treatment for nerve injuries where a nerve gap does occur is limited to autografts (gold standard),

allografts and various nerve guidance conduits (NGCs). Previous studies have utilized electron microscopy in order to characterize the structure of native nerves as well as to both qualitatively and quantitatively analyze the extent to which NGCs have aided in promoting nerve regeneration. These studies focus primarily on using the scanning electron microscope (SEM) to obtain high-resolution 2D images of *in vitro* and *in vivo* morphology. However, 2D SEM images can not provide detailed information regarding how well the NGC can affect the 3D nerve regeneration. To improve the visualization and qualitative analysis of the 3-D morphology of both native and regenerated nerves, this study uses the combination of focused ion beam (FIB) processing with SEM imaging. The largely automated data acquisition process collects a series of 2D serial images, which can subsequently be computationally rendered into 3D image data sets to visualize the extent of nerve regeneration.

We investigated a rat sciatic nerve model. A segment of a rat's sciatic nerve was removed from the right hind leg creating the nerve gap of 10 mm. The NGC was a nanofibrous tube prepared by electrospinning of polycaprolactone. The NGC and the autografts were implanted into the nerve gap and explanted after 6 weeks. Native nerves were also extracted for analysis. The samples from the three groups were fixed in an epoxy resin, and 2D serial images were obtained by the automated FIB-SEM slice and view technique. Each image was collected at 4000x magnification with a slice thickness of 20 nm. A commercially available software system, Avizo®, was used to generate 3D renderings from the sets of serial SEM images. These 3D imaging results showed that the autograft group had a significantly greater myelin thickness than that of the tubular NGC group indicating more effective nerve regeneration. Both groups had myelin thicknesses significantly less than that of the native nerve. The average thicknesses for the native nerve, autograft, and tubular NGC are 2178 ± 165 nm, 800 ± 95 nm, and 511 ± 162 nm respectively. Moreover, thick myelin and well-aligned nerve axons were observed in the native healthy nerves. The myelin was thinner, and the axon alignment was not as strongly oriented in one direction in the autograft sample as compared to the native nerve. Mature myelination was almost completely absent in the tubular NGC group, and the nerve axons in this group had minimum alignment.

Schwann cells (SCs) are essential for proper nerve regeneration. The autograft group displayed a larger extent of regeneration as is evident by the complete wrapping of SCs along the regenerated axons. This is likely a result of the grafted tissue providing a bridge onto which the SCs can migrate along. Once the axons have fully regrown, SCs differentiate to a myelinating phenotype and wrap around the axons forming a myelin sheath. The completely myelinated axon is a fully mature and regenerated nerve. The autograft is still insufficient in promoting regeneration to the state and condition of an undamaged native nerve though this is most likely due to the shortness of the period of this study (6 weeks). The tubular NGC group displayed the most limited extent of regeneration. This is likely due to the absence of internal support structures and aligned fibers that act as a guide on which SCs can adhere to. The lack of structural cues in the tubular group resulted in a random orientation of the SCs in which the cells migrate in different directions and cannot properly and effectively proliferate. As such, there was a complete lack of fully myelinated mature axons in the tubular group. Regenerating nerve axons were only partially myelinated which can lead to poor signal transduction along these nerves. Importantly, the combination of FIB and SEM provides a powerful tool to collect morphological data in 3 D that gives insight into the nerve regeneration mechanisms as a function of the natural or synthetic scaffolding method.

Acknowledgement

This work was partly supported by NIH-R15 NS074404.

PO-17

Track: Plant and Environment

PHYTOREMEDIATION POTENTIAL OF ELATERIOSPERMUM TAPOS BLUME IN BIORETENTION SYSTEMS

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Bioretention systems are excellent stormwater management tools for the removal of nonpoint source pollutants in stormwater runoff from urban catchments before they enter and pollute our waterways. Plant selection for bioretention systems are a critical feature to ensure the pollutant removal

effectiveness. Plants are important not only for nutrient removal but also to maintain the porosity of the filter media. Tree species have great phytoremediation potential because of their large biomass and extensive root system. In addition, it is beneficial to plant native species to make such bioretention systems multifunctional - improving the quality of stormwater, maintaining the permeability of the filter media, as well as supporting local biodiversity. Pot studies using Elateriospermum tapos Blume tree saplings were used to understand the phytoremediation potential of this native species to Singapore. The saplings were planted in a standard potting mix with a top soil:compost:sand ratio of 3:2:7. Pots of soil were used as controls for the effect of the soil. A concentration of 10mg/L nitrate and 2mg/L phosphate was used to irrigate the plants to represent the highest range found in stormwater in Singapore. Control plants were irrigated with tap water. The chlorophyll fluorescence data showed that the plants were not stressed by these concentrations, with the Fv:Fm at an average of 0. 83 over 7 weeks. Pots vegetated with Elateriospermum tapos Blume tree saplings had a nitrate removal of 7. 099 - 8. 796mg compared to barren pots which had a lower nitrate removal of 5. 819 - 8. 357mg. Furthermore, the nitrate removal of barren pots was decreasing over time, compared to the nitrate removal of vegetated pots which increased over time. Total Kjeldahl Nitrogen (TKN) analysis of the dried plant parts (leaves, stems, roots) showed that TKN concentration was significantly higher in the leaves of plants irrigated with 10mg/L nitrate and 2mg/L phosphate, 15. 673mgN/gDW compared to 13. 173mgN/gDW in control plants. This indicated a conversion of excess nitrates absorbed by the plant into the leaf biomass. Phosphate removal in vegetated pots was only 2. 745 - 2. 813mg compared to barren pots which showed phosphate removal of 2. 745 - 3. 500mg. Although phosphate removal by the plants was not observed, the Total Phosphorus (TP) concentration in plants irrigated with 10mg/L nitrate and 2mg/L phosphate was significantly higher in the stems, 0. 500mgP/gDW compared to 0. 368mgP/gDW in control plants. The accumulation of TKN and TP in the above-ground biomass of the plants is an advantageous trait as such biomass can be removed from the system and is unlikely to return such excess nutrients to the filter media. This study provided evidence that Elateriospermum tapos Blume tree saplings has the potential to phytoremediate the nitrate and phosphate found in urban stormwater and will be a suitable tree species to be planted in bioretention systems.

Keywords: Bio-retention systems, phytoremediation, nitrate, native, tree, Elateriospermum tapos.

PO-93

Track: Medical Biotechnology

METABOLOMICS AND THE POTENTAL FOR DISCOVERY IN STUDIES OF LONGEVITY AND AGE-RELATED DISEASES

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Alterations in metabolism are hypothesized to influence lifespan and the propensity for successful aging, but data in humans have been lacking. Recent technical advances have allowed for the application of metabolomic profiling in population-based studies. The ability to conduct high-throughput profiling in large clinical cohorts presents unique opportunities and challenges. In this case study, we present the example of an effort to identify metabolomic markers associated with longevity and aging phenotypes in a large cohort of men and women who have been living in the community and followed longitudinally over several decades. To investigate the biochemical pathways associated with longevity in this human cohort, we applied high-throughput metabolite profiling to plasma samples collected from participants who had the chance to attain age 80 during the follow-up period. We also studied cardiovascular and mortality endpoints in relation to metabolite profiling data available at baseline. In multivariable analyses adjusting for traditional risk factors, we observed that higher levels of select citric acid cycle intermediates and bile acides were associated with lower odds of reaching age 80 and higher odds of cardiovascular disease and death. Interestingly, higher levels of citric acid cycle intermediates, but not the bile acids, were also associated with ideal cardiovascular health at baseline. Importantly, no metabolites related to longevity were also associated with the risk of developing cancer in this cohort. This case study is presented in the context of the emerging literature of metabolomics investigations of age-related diseases in humans. Lessons learned, future directions, and potential implications for clinical practice will be discussed.

PO-67

Track: Regenerative Medicine

PHYSICAL EXERCISE AND ENRICHED ENVIRONMENT ENHANCE NEUROACTIVE GENE EXPRESSION AND SYNAPTIC PLASTICITY IN ADULT BRAIN

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Objective

Housing animals in enriched environment (EE) with physical exercise enhances behavioral function. However, the mechanism underlying functional improvement and the changes in gene expression patterns have yet to be elucidated. We attempted to investigate the mechanisms associated with exposure to EE by evaluating gene expression.

Methods

Six week-old CD-1® (ICR) mice were housed in standard cages (SC) or EE comprising a running wheel, novel objects, and social interaction for 2 months (n=16 each). In these mice, motor and cognitive performances were evaluated using rotarod test and passive avoidance test. Gene expression was also investigated in isolated hemispheres of brain using microarray and gene set enrichment analysis (GSEA) (n=3 each).

Results

In behavioral assessment, EE significantly enhanced rotarod performance. At 8 weeks after treatment, rotarod latency of EE mice was shown to be significantly increased to 162. 0±23. 1 sec for constant speed (t=2. 448, p=0. 017) and to 179. 4±17. 6 sec for accelerating speed (t=2. 974, p=0. 004), compared with rotarod latency of SC mice (84. 1±21. 8 sec, 113. 7±13. 4 sec). In addition, EE significantly enhanced short-term working memory when passive avoidance tests were performed. EE-induced improvement of retention test 30 min after aversive stimulus relative to those of pre-treatment (Ä121. 4±43. 3 sec) was significantly evident after post-treatment 8 weeks compared with SC (Ä-30. 4±46. 5 sec) (t=-2. 388, p=0. 028). Microarray analysis revealed that genes associated with neuronal activity were significantly altered by EE in the brain (p<0. 05 by Fisher's exact test). Among the genes, EE significantly increase Drd1 (dopamine receptor D1A), Ppp1r1b (protein phosphatase 1r1b), P2ry12 (purinergic receptor P2Y), Pdyn (prodynorphin), and Oxt (oxytocin). On the other hand, the drastic decrease was observed in Slc6a3 (dopamine transporter) as well as Slc6a4 (serotonin transporter), raising the possibility that presynaptic reuptake of these neurotransmitters might be reduced by EE. GSEA showed that genes involved in synaptic transmission and postsynaptic signal transduction were globally upregulated, while those associated with reuptake by presynaptic neurotransmitter transporters were downregulated. Particularly, both microarray and GSEA demonstrated that EE increased opioid signaling, acetylcholine release cycle, postsynaptic neurotransmitter receptors, but decreased Na+/ClĀ-dependent neurotransmitter transporters including dopamine transporter Slc6a3 in the brain.

Conclusion

Physical exercise and EE enhanced motor and cognitive function through neuroactive gene expression and synaptic plasticity as efficient use of neurotransmitters similar to a neuropharmacologic treatment.

Acknowledgement

This study was supported by a grant from National Research Foundation (2010-0020408).

PO-9

Track: Pharmaceutical Biotechnology

ANTIHYPERTENSION EFFECTS OF PROTOCATECHUIC ACID AND DERIVATIVE COMPOUNDS ISOLATED FROM PINUS DENSIFLORA

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Lately, important and useful methologies came up to aid of drug design. This is the case of Combinatorial Chemistry and, microbiological or enzymatic conversion of biological active compounds.

Surely these tools will also contribute to plan new ACE inhibitors.

The extract of Pinus densiflora (red pine) has long been used as a nourishing tonic drug and medicine around Asia. It was separated several fraction using repeated silica gel column/HPLC. We purified PCA (Protocatechuic acid) from ethyl acetate-soluble fraction and SA from butanol-soluble fraction. PCA (Protocatechuic acid) and (shikimic acid) isolated from extract of red pine have effects of antioxidant and fibrinolytic activty.

We also found that PCA and SA possess antihypertension activity using ACE (angiotensin-converting enzyme) inhibition assay by HPLC. We tested antihypertension effects of PCA and SA derivatives for principles of antihypertension. Among PCA and SA derivatives, benzoic acid, 3-hydroxybenzoic acid, trimesic acid, salicylic acid, 1, 3, 5-Trihydroxybenzene dehydrate and cyclohexane-1, 2, 4, 5-tetracarboxylic acid showed antihypertension antoan activity

Keywords: Angiotensin-Converting Enzyme, Pinus densiflora, Antihypertension, Protocatechuic Acid, Shikimic Acid.

PO-4

Track: Pharmaceutical Biotechnology

THROMBOLYTIC, ANTICOAGULANT ACTIVITIES OF PROTOCATECHUIC ACID AND DERIVATIVE COMPOUNDS ISOLATED FROM PINUS DENSIFLORA

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Fibrinogen and fibrin play an important role as extrinsic coagulation in blood clotting. Fibrin generated thrombosis, cardiovascular and cerebral hemorrhage disease through intrinsic coagulation in blood vessel. Pinus densiflora Sieb. et Zucc has long been used to manufacture beverages and medicines. We found that Protocatechuic acid (PCA) has fibrinolytic activity in ethylacetate layer from Pinus densiflora. Lysis mechanism of the PCA purified from pine needle extract was investigated through *in vitro* system. These compounds strongly lyse fibrin clots formation, which was determined by measuring turbidity. The protocatechuic acid derivative compounds (PDCs) revealed according to structure and fibrinolysis activity. Aspirin also known as acetylsalicylic acid (ASA), is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication. At low doses aspirin, assist in preventing heart attacks, strokes, and blood clot formation in people at high risk of developing blood clots. The main undesirable side effects of aspirin taken by orally are gastrointestinal ulcers, stomach bleeding, and tinnitus, which usually occur with higher doses. Aspirin is no longer used for children and adolescents to control flu-like symptoms, symptoms of chickenpox or other viral illnesses because of the risk of Reye's syndrome. We suggested that fibrinolysis is possible with non-enzymologically activators based only on PDCs. Our work demonstrates that PDCs can activate fibrinolysis effectively and carboxylate-based fibrinolysis medicine could be developed.

Keywords: Anticoagulant, Thrombolytic, Protocatechuic acid, Pinus densiflora.

PO-10

Track: Pharmaceutical Biotechnology

ANTIHYPERTENSIVE ACTIVITY OF COMPOUNDS ISOLATED FROM NEEDLES OF PINUS DENSIFLORA

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The Angiotensin converting enzyme (ACE) is the key enzyme catalyzes angiotensin I to Angiotensin II in renin-angiotensin system. Angiotensin I produces aldosterone in combination with angiotensin II receptor, which increases blood pressure through absorption of Na+. To investigate the antihypertensive compounds from Pinus densiflora (red

pine) needles, Water, ethyl acetate- and n-butyl alcohol-soluble fractions from red pine needles were screened for the inhibitory activity against ACE. The most potent ACE inhibitory activity was detected in the ethyl acetate alcohol-soluble fractions (5 mg/mL). After the purification of ACE inhibitor compounds with column chromatography, antihypertensive activity was determined by measuring Revers Phase-HPLC. We obtained two active compounds, dehydroabietic and communic acids. To measure quantitative analysis of antihypertensive activity, we analyzed the quantities of hippuric acid using RP-HPLC. DHA (3 mM) and communic acid (3 mM) inhibited production hippuric acid in N-Hippuryl-His-Leu. We determined that DHA and communic acid separated from the red pine needles are new ACE inhibitors.

Keywords: Renin-angiotensin system, Angiotensin converting enzyme, Dehydroabietic acid, Communic acid.

PO-84

Track: Pharmaceutical Biotechnology

UNSUPERVISED CELL SEGMENTATION USING STATISTICAL ACTIVE CONTOUR MODEL

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The pathological examination using traditional biopsy requires invasive tissue removal from a living subject, followed by time-consuming and complicated procedures to determine the diagnosis of the disease. In recent years, non-invasive *in vivo* virtual biopsy, which provides comprehensive scanning tissue images without pain, is promising. In this paper, we propose a new cell segmentation algorithm based on the segmented nuclei from watershed-based approach, to process plenty of *in vivo* virtual biopsy images provided by healthy individuals. The proposed approach includes cautious identification of the nuclei position by considering both staining intensity and shape information, which enhance the accuracy of nuclear recognition. The outer cytoplasmic boundary is extracted based on the proposed statistical pressure snake, which is an optimal parameter setting snake driven by a pressure force that measuring the local statistics similarity between the snake contour movement and the image data of cytoplasm. The new snake model overcomes the well-known drawbacks of initialization and parameterization. Experimental results show that the aforementioned algorithm has high accuracy for cell segmentation and has ability to process large amounts of tissue images. Moreover, the evaluation of Nuclear-to-Cytoplasmic ratio (NC ratio) is significant for detection of skin disease with abnormal NC ratio in clinical diagnosis.

PO-53

Track: Other Area: Food

STABILITY OF *YARROWIA LIPOLYTICA* HYDROLASES DURING STORAGE

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The aim of the research was to evaluate stability of yeast *Y. lipolytica* hydrolytic enzyme (proteolytic and lipolytic) preparations during storage.

The enzymes were isolated from submerged cultures of yeast, conducted in bioreactor in media enriched with casein and fat industry waste products (added in different ratio) at pH 3. 5 for obtaining the aspartic protease and pH 7. 5 for production of serine protease.

After 48-hours the cultures were centrifuged at 4^{o}C. The resulted supernatants, containing extracellular enzymes, were concentrated with the use of 18 kDa membrane. While the yeast biomasses from acid and alkaline cultures were washed with Sorensen buffer pH 7. 0 and sonificated at 4^{o}C for 15 minutes for isolation of intracellular hydrolases. The obtained

enzyme preparations were as follows: alkaline extracellular (1), acidic extracellular (2), alkaline intracellular (3), acidic intracellular (4) and enzymatic cocktail (5) - preparation (1) and (3) mixed at the ratio 1:1 (v:v).

The stability of the enzymatic preparations (1-5) were analyzed by their proteolytic and lipolytic activity determinations during 4^{o}C and -20^{o}C storage. The stability of enzymatic cocktail (5) was also analyzed during storage at temp. 4^{o}C in buffers of pH 6. 5 and 7. 0 without or with addition of NaCl in concentrations of 2,5; 5,0 i 10%. In all preparations the endopeptidase activity was determined against casein or hemoglobin and lipolytica activity against p-NA –butyrate. The intracellular activity was analyzed against substrate Leu-pNA.

It was shown that the 28 days-long storage at temp. 4^{o}C of *Y. lipolytica* enzyme preparations: (1) and (2) caused the 57-59% decrease of activity of both aspartic and serine proteases. The most labile enzyme activity during storage under conditions used in experiments was the extracellular lipase activity of preparation (1) obtained from yeast culture performed at pH 7. 5. The loss of its activity was 81%. The addition of NaCl to enzyme preparations stabilized the activity of serine protease and the accompanying it lipase. The salt, independent from its concentration, preserved proteolytic and lipolytic activity in 100% and 85-95%, respectively. Freezing and followed thawing of all tested enzymatic preparations without any crioprotectants brought up the loss of all activities from 47 to 66%.

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Keywords: *Yarrowia lioplytica,* hydrolases, storage stability.

PO-54

Track: Pharmaceutical Biotechnology

PRODUCTION OF *GALLUS GALLUS* VITELLOGENIN II- DERIVED PROTEIN (YGP40) IN PROKARYOTIC EXPRESSION SYSTEM

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The embryogenesis of birds, unlike mammals, takes place outside the mother's organism. Thus, the whole egg, in particular the egg's yolk, has to provide the embryo with vital nutrients, that is with proteins, lipids, carbohydrates, vitamins and other elements. The growing organism also requires to be immunologically protected against external pathogens. One of the major egg yolk protein is vitellogenin II - a precursor of main egg yolk proteins: lipovitellin, phosvitin and 40 kDa glycoprotein (YGP40). After expression and release into the bloodstream from hepatocytes, vitellogenin II is internalized by growing oocyte and proteolytically cleaved by cathepsin- D into its derivatives. Above suggest the sex- dependant role of this protein. Nevertheless, recent data showed that vitellogenin, besides a female- specific yolk- protein formation, exhibits pleiotropic functions, related with the host defense reactions, as the primary immune response.

The YGP40 sequence is released from the C-terminal fragment of hen's vitellogenin II. The data obtained by Polanowski *et al.* (2013), showed that this small protein is a source of several peptides with immunomodulatory and immunoregulatory activities. This complex, named yolkin, consists of low molecular weight peptides (1-35 kDa) and expresses immunostimulating properties similar to mammalian colostrinin. In this context, vitellogenin reveals a new function, which may play an important role in the innate immune system of the developing embryo. Likewise colostrinin, yolkin could also have a potential application in neurodegenerative diseases treatment.

The natural source of yolkin is the laying hens egg yolk. The applied method allowed to isolate heterogenous group of YGP40- derived peptides, alongside IgY purification. Nevertheless, the purification yield is low and time- consuming. It takes about 4 - 5 days to obtain no more than 1,7 mg of yolkin from one egg yolk. For this reasons, in our studies we focused on heterologous expression of YGP40 gene in *Escherichia coli* BL21 as a host. The YGP40 coding sequence was back translated, synthesized and cloned into expression vectors: pQE80L and pET32b and introduced to *E. coli* BL21 expression cells.

Keywords: vitellogenin II, YGP40 expression & purification.

PO-70

Track: Medical Biotechnology

P16 HYPERMETHYLATION: A BIOMARKER FOR ESOPHAGEAL CANCER IN NORTH EAST INDIA

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Objective

North East India has the highest incidence of esophageal cancer with a very poor prognosis. The molecular mechanisms of esophageal cancer susceptibility in this part of India have not been fully understood. There is a need for identification of molecular biomarkers to screen people at risk of esophageal cancer for early detection of cancer. p16 is an essential G1 cell cycle regulatory gene whose loss of function is associated with carcinogenesis. Therefore we conducted this study to determine the prevalence of p16 gene methylation in patients with esophageal cancer to assess the feasibility of using gene methylation as a biomarker.

Method

A total of 50 newly diagnosed esophageal cancer cases along with 50 age-sex matched controls were included in this study. p16 methylation status was determined by methylation specific PCR.

Results

Aberrant promoter methylation of the p16 gene was detected in 46 of 50 (92%) esophageal cancer cases. p16 hypermethylation was found more in moderately differentiated grade of cancer compared to well differentiated.

Conclusion

Thus p16 hypermethylation can be used as a biomarker for esophageal cancer development in high incidence region of North East India.

Keywords: p16 hypermethylation; Esophageal Cancer; Biomarker; North East India.

PO-90

Track: Plant and Environment

THE OPTIMIZATION OF DILUTE ACID AND STEAM-EXPLOSION PRETREATMENT AND ENZYMATIC HYDROLYSIS TO INCREASE BIOETHANOL YIELD FROM TURKISH WHEAT STRAW AND CORN STOVER

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The aim of this study is to compare the physical and chemical characteristic of wheat straw and corn stover to evaluate the conversion efficiency of lignocellulosic feedstock to bioethanol. The wet chemistry analysis method was used to determine the compositional variation of corn stover and wheat straw samples obtained from the Black Sea and Marmara Region of Turkey in the year 2012, respectively. The major components of corn stover and wheat straw reported as average on % dry weight whole biomass basis were glucan; 31. 5 and 33. 2, xylan; 11. 2 and 10. 9, galactan; 1. 1 and 1. 0, arabinan; 2. 0 and 3. 2, lignin (corrected for protein); 16. 0 and 17. 9, starch; 1. 7 and 1. 5 and protein; 5. 3 and 2. 4, respectively.

The main goal of this research is to increase fermentable sugars' yield through high-efficiency pretreatment technology from lignocellulosic biomass. The selected feedstocks-wheat/rice straw and corn stover- are potential feedstocks for production of bioethanol due to their high carbohydrate content (Ã50%) of nearly 37% cellulose and high annual production rate with almost 3. 5 and 4. 1 million tons in Turkey, respectively. The modified dilute acid and steam-explosion was used as pretreatment technology to increase fermentable sugars yields. Effects of reaction time,

temperature and acid concentration on hydrolysis of cellulose and hemicellulose in the selected feedstocks and total sugar yields were studied. Optimum pretreatment parameters and enzymatic hydrolysis conditions for converting the selected feedstocks into fermentable sugars were identified. The material was immersed in an aqueous solution containing 0. 2 to 1. 5 % w/w H2SO4. Different pretreatment conditions are investigated such as 160 to 200 °C for 2, 5 and 10 min. The prehydrolysis and the subsequent SSF-runs were performed using the whole slurry from the pretreatment for different enzyme dose. The temperature of the slurry was 32°C during enzymatic hydrolysis and fermentation. Samples are withdrawn after 0, 2, 4, 8, 24, 28, 32, 48, 72 and 96 h of the SSF, and analyzed for bioethanol, glucose, cellobiose, glycerol, acetic acid and lactic acid.

Keywords: Agricultural residues, bioethanol, wheat straw, corn stover.

PO-87

Track: Regenerative Medicine

PRECLINICAL ASSESSMENT FOR SCORPION TOXIN-SPECIFIC NANOBODY EFFICACY TO NEUTRALIZE AND PROTECT AGAINST SCORPION ENVENOMING

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Accidents involving scorpion stings represent a real medical emergency in many regions around the world. Children under the age of 15 are the most targets.

Toxicity is mainly due to small Na channel specific toxins (7 kDa MW) responsible of lethal effects. Conventional treatment based on horse polyclonal antibody fragment of 100 kDa MW is of limited efficacy. We therefore developed Nanobody-based agents (Nb of 15,000 Da MW) that offer advantages due to their small size, high affinity and specificity, and robustness, matching the size of the toxins. The Nbs correspond to the variable domain VHH of camel-specific Heavy-Chain only antibodies.

A panel of Nanobodies with sub-nanomolar affinity to AahI' and AahII toxins from *Androctonus australis hector* venom, were isolated using phage display technology. Retrieving, NbAahII10 and NbAahI'F12 exhibit a toxin neutralising capacity never reached before with other antibody fragments. A bispecific construct NbF12-10 was engineered by joining these best neutralising Nbs. Here in, we successfully revealed first that NbAahII10 targets a distinct epitope determined by Surface Plasmon Resonance measurement (SPR). Interestingly, we demonstrated that the maximally humanized version of NbAahII10 Cys/Ser maintains its high affinity and neutralizing capacity for the antigen. More interestingly, we demonstrated the ability of the purified bispecific NbF12-10 to neutralise the total venom in murine model systems that mimic the natural envenoming, under conditions where current horse derived Fab'2 fails. Indeed The NbF12-10 was still fully protective in mice with severe signs of envenoming were treated a few minutes before untreated mice died. In addition, the pharmacokinetics of the bispecific NbF12-10 supports a fast biodistribution to various tissues, a rapid renal clearance and a reduced damage in lung and cardiac tissues of envenomed mice. In conclusion, we suggest that this NbF12-10 and humanized variant immunotherapeutics possesse all properties to replace the current horse derived serotherapeutic in the near future.

Ben Abderrazek *et al.*, *Biochem. J,* 2009; Hmila *et al.*, FASEB, 2010; Ben Abderrazek *et al.*, *PEDS*, 2011; Hmila *et al.*, Toxicol Appl Pharmacol. 2012; Bouhaouala-Zahar *et al.*, *IA-DT*, 2011

Keywords: Scorpion toxin, recombinant Nanobody, affinity maturation, humanization, immunotherapy.

PO-86

Track: Regenerative Medicine

PIVL: A NEW SERINE PROTEASE INHIBITOR FROM *MACROVIPERA LEBETINA TRANSMEDITERRANEA* VENOM, IMPAIRS MOTILITY OF HUMAN GLIOBLASTOMA CELLS

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Glioblastoma, the most malignant subtype of glioma, is associated with very poor survival even with multi-modality therapy integrating surgery, radiation therapy, and chemotherapy. Moreover, the modification of the cell surface expression of various integrins has been associated with progression of glioblastoma. Thus, we reported here that PIVL, a novel Kunitz-type serine proteinase inhibitor from snake venom, displays integrin inhibitory activity without being cytotoxic. Also, we show that PIVL is able to dose-dependently inhibit the adhesion, migration and invasion of human glioblastoma U87 cells. Our results show that PIVL impairs the function of ĀvÄ3 and to a lesser extent, the activity of ĀvÄ6, ĀvÄ5, Ā1Ä1 and Ā5Ä1 integrins. Interestingly, we demonstrate that the ^{41}RGN^{43} motif of PIVL is likely responsible for its anti-cancer effect. Using time lapse videomicroscopy, we found that PIVL significantly reduced U87 cells motility and affected cell directionality persistence by 68%. These findings reveal novel pharmacological effects for a Kunitz-type serine proteinase inhibitor.

PO-88

Track: Regenerative Medicine

SUBTYPE-SELECTIVE ACTIVATION OF K(v)7 CHANNELS BY AaTXKÄ$_{2-64}$, A NOVEL TOXIN VARIANT FROM THE ANDROCTONUS AUSTRALIS SCORPION VENOM.

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K(v)7. 4 channel subunits are expressed in central auditory pathways and in inner ear sensory hair cells and skeletal and smooth muscle cells. Openers of K(v)7. 4 channels have been suggested to improve hearing loss, systemic or pulmonary arterial hypertension, urinary incontinence, gastrointestinal and neuropsychiatric diseases, and skeletal muscle disorders. Scorpion venoms are a large source of peptides active on K^+ channels. Therefore, we have optimized a combined purification/screening procedure to identify specific modulator(s) of K(v)7. 4 channels from the venom of the North African scorpion Androctonus australis (Aa). We report the isolation and functional characterization of AaTXKÄ$_{2-64}$, a novel variant of AaTXKÄ$_{1-64}$, in a high-performance liquid chromatography fraction from Aa venom (named P8), which acts as the first peptide activator of K(v)7. 4 channels. In particular, in both Xenopus oocytes and mammalian Chinese hamster ovary cells, AaTXKÄ$_{2-64}$, but not AaTXKÄ$_{1-64}$, hyperpolarized the threshold voltage of current activation and increased the maximal currents of heterologously expressed K(v)7. 4 channels. AaTXKÄ$_{2-64}$ also activated K(v)7. 3, K(v)7. 2/3, and K(v)7. 5/3 channels, whereas homomeric K(v)1. 1, K(v)7. 1, and K(v)7. 2 channels were unaffected. We anticipate that these results may prove useful in unraveling the novel biologic roles of AaTXKÄ$_{2-64}$-sensitive K(v)7 channels and developing novel pharmacologic tools that allow subtype-selective targeting of K(v)7 channels.

PO-27

Track: Other Areas

STUDY ON ANTIFUNGAL FATTY ACID POTASSIUM AGAINST DERMATOPHYTOSIS

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Fatty acid is main component of soap, and it is used as many industrial material. Dermatophytosis (Tinea) is fungal infection that can infect the scalp, glabrous skin, and nails. The treatments of Tinea need antifungal medication and good hygiene environment. Moreover, generally recovery takes long time. The effective antifungal medication and infection prevention, and the creation of antifungal medication with high safety are required. In this study was focused on the antifungal effect of fatty acids potassium salts. We investigated the antifungal effect against Tinea. Using the nine fatty acids, butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), oleic (C18:1), linolenic (C18:2), linoleic (C18:3), were dissolved in KOH solution to a concentration of 175 mM and pH 10. 5. The antifungal method, the spore suspension (3. 0 Ā104 spores/ ml) was mixed with sample of fatty acid potassium (final concentration of 175 mM). The results show that C6K, C8K, C10K, C12K was the most inhibit 4-log unit (99. 99 %) of the fatty acids potassium incubated time for 10 min. C12K mixed with medium-chain fatty acids potassium or long-chain fatty acids potassium. C12K was 4-log unit reduction when mixing medium-chain fatty acids salt; however, had no effect even mixed with C18:1K(concentration 17. 5- 130 mM).

Keywords: Fatty acid potassium salts, Dermatophytosis, antifungal.

PO-6

Track: Medical Biotechnology

THE LONG TERM EFFECTS OF RADIOACTIVE PHOSPHOROUS SYNOVIORTHESIS ON HEMOPHILIC ARTHROPATHY

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Background

Radioactive synoviorthesis carried out by injection of radioactive materials into the joint that has been known as a successful alternative treatment to invasive surgical synovectomy. This study was designed to evaluate short-term and longterm results and complications of radioactive synovectomy of hemophilic arthropathy using radioactive phosphorus.

Methods and Materials

This study was conducted on 40 patients with hemophilic arthropathy. After obtaining clotting factors, the intra-articular injections of radioactive phosphorus was done. Thirteen patients were evaluated during 36 months (short-term followup) and 27 patients were followed up for more than 36 months (long-term followup). Patients were evaluated for hemarthrosis, factor consumption per month, joint range of motion (ROM) and clinical and Radiological involvement grade.

Results

The patients mean age was 22. 9 ± 6. 6 and there were 38 men and 2 women. Patients were followed up for maximum 7 years. Consumption of clotting factors was significantly reduced in the short term follow up of patients ($p < 0.5$), but there was no significant difference in the long term follow-up ($p < 0.05$). ROM decreased significantly in the long term follow up ($p < 0.05$). Radiologic evaluation showed significantly increased involvement in their joints ($p < 0.05$).

Conclusion

Using radioactive synoviorthesis led in decreased consumption of clotting factors and the hemarthrosis incidence in short term but it did not have significant impact on clinical situation (ROM) and radiological findings of hemophilic patients in long term follow-up.

PO-45

Track: Industrial and Manufacturing

OPPORTUNITIES IN THE CULTIVATION OF NON-SPORULATING FILAMENTOUS FUNGI BY MORPHOLOGY CONTROL

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Biotechnological production of bulk and fine chemicals by filamentous fungi are today an expanding industry. Morphology of non-sporulating production strains has been poorly studied compared to sporulating ones as e. g. *Aspergillus species*. Requirements, especially for inoculum maintenance and pre-culture setup, differ much from spore-forming fungi. In particular, morphology control of filamentous fungi is a key process variable and serves as an important parameter for implementation of reproducible cultivation processes. In order to develop solutions for reproducible bioprocess strategies of the non-sporulating basidiomycete *Schizophyllum commune* its morphology was investigated in detail.

We give an overview of the growth kinetics of morphology combined with modeling of dry cell weight (dcw) by morphological analysis. Thus, we investigated several macromorphological parameters for their potential to predict dcw concentrations. Additionally, further strategies were developed for the prediction of dcw concentrations depending on reactor scale (0. 1 - 30 L) and cultivation conditions. The simultaneous measurement of offline quantified (gravimetrically) dcw and at-line determination of morphology growth kinetics (microscopic) for the non-sporulating fungus allowed detailed evaluation of at-line dcw predictability. Our approach was compared with online methods using sensor techniques like optical density sensors. Summarized, our morphology approach may lead to a more reliable online analysis of dry cell weight using filamentous fungi which may provide new routes toward application in multivariate data analysis (e. g. identification of the "golden" batch) for fast process monitoring and optimization at different scales. Analysis of the major challenges of filamentous fungi cultivation in the future and potential solution approaches will also be outlined.

PO-51

Track: Other Areas

ANTI-OXIDATIVE AND ANTI-INFLAMMATORY EFFECTS OF EXERCISE AND DIETARY RESTRICTION IN LEAD-TREATED RATS

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Introduction: regular physical exercise up-regulates anti-oxidant and anti-inflammatory systems of the body. Caloric restriction has the same effects. On the other hand, main mechanism of lead toxicity is induction of oxidative stress and inflammation. The present study aimed to investigate the effects of exercise and dietary (caloric) restriction on oxidative/anti-oxidative and inflammatory markers in the blood of lead-administered rat.

Materials and Methods: male Sprague-Dawley rats were randomly divided into four groups: 1. Sedentary + normal calorie intake (Cont); 2. Sedentary + 65% dietary (caloric) restriction (DR); 3. Exercise by wheel + normal calorie intake (Ex); 4. Exercise by wheel + 65% dietary restriction (Ex+DR).

After six weeks lead acetate were administered to all animals. Blood samples were collected before and after lead injections. Oxidant/anti-oxidant factors (superoxide dismutase, glutathione peroxidase and malondialdehyde), inflammatory cytokine (TNF-Ä) and Lead concentration were assayed in the samples.

Results: lead administration increased peroxidation and inflammation markers and decreased anti-oxidant enzymes activities in the blood of the Cont group. Exercise (Ex group) or dietary restriction (DR group) prevented the lead-induced changes; While, simultaneous exercise and dietary restriction (Ex+DR group) aggravated lead-induced oxidative stress and inflammation.

Conclusion: exercise or caloric restriction prevented lead toxicity and simultaneous exercise and dietary restriction aggravated it.

Keywords: oxidative stress, inflammation, exercise, dietary restriction, lead toxicity, rat.

PO-16

Track: Plant and Environment

ANALYSIS OF EPL-1 GENE FUNCTION IN EXPRESSION OF GENES ASSOCIATED WITH *TRICHODERMA HARZIANUM* MYCOPARASITISM PROCESS AGAINST SCLEROTINIA SCLEROTIORUM

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Fungal species *Trichoderma harzianum*, are studied as plant pathogens biocontrol agents in soil, and its mechanism of biocontrol is a complex process, that can occur through different mechanisms or a combination thereof, including mycoparasitism. In this work, epl-1 gene were deleted in a *T. harzianum* strain and direct confrontation assays in plate between wild type and mutant *T. harzianum* strains and against the phytopathogenic fungus Sclerotinia sclerotiorum were performed. RNA (1 Ãg) from each sample was extracted, before and after hyphae contact than treated with DNase I (Fermentas) to remove genomic DNA. After this step, cDNAs strands were synthesized using the First Strand cDNA kit MaximaTM Synthesis, according to the manufacturer's instruction. After dilution of 1/50 times, the cDNA were used for Real-time PCR analysis using equipment CFX96TM using Bio-Rad® SsoFastTM EvaGreen Supermix (Bio-Rad) for signal detection, in accordance with manufacturer's instruction. Gene encoding actin was amplified as a reference to normalize the total amount of cDNA present in each reaction. The gene expression level was calculated according to the method 2- CT (Livak & Schmittgen, 2001), where the transcription rate of *T. harzianum* WT x *T. harzianum* WT before contact was used as a control sample. These calculated gene expression results were used for construction of graphs showing the differences in expression of these genes during the comparison. The results indicated that epl-1 gene deletion altered the expression modulation of all genes analyzed (exoglucanase, mannosidase, alpha-glucanase, acid phosphatase, phytase, chitinase [chit42] aspartyl protease, trypsin-like protease and serine protease [SprT]), indicating a potential role of Epl-1 in *T. harzianum* signaling process of genes involved in mycoparasitism interaction and recognition of own and host cell wall.

Keywords: *Trichoderma harzianum*; Phitopathogens Biocontrol, Mycoparasitism.

PO-49

Track: Industrial and Manufacturing

MASS TRANSFER ANALYSIS OF A DISTILLATION COLUMN SIEVE TRAY USING COMPUTATIONAL FLUID DYNAMICS

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Distillation is one of the most important separation techniques used in biotechnology industrial processes. Empirical studies on the flow dynamics in distillation columns are rare due to the large dimensions of these devices and the high investment needed, including measurement instrumentation. In this paper, a computational fluid dynamics simulation of a distillation column sieve trays is proposed. The main aim was to develop a model capable of predicting the column hydrodynamics and the mass transfer of the components transfer between the liquid and the vapor phases. A tridimensional, transient and multiphase model with the Euler-Euler approach was used. The model predicted liquid and gas velocity profiles, clear liquid height, component mass fraction and transfer efficiency, showing good agreement when compared with experimental reported values obtained from the literature. The predicted tray efficiency was 65%. The CFD tools proved to be capable to study the flow on columns internals and can be applied in the design and optimization of these devices.

Keywords: Distillation Column, Mass Transfer, Computational Fluid Dynamics.

PO-92

Track: Pharmaceutical Biotechnology

EFFECT OF SIMULATED HYPERGRAVITY ON THE GERMINATION OF *OCIMUM BASILICUM L*.

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Gravitational forces greater than 1G have been used to study the role of gravity on plant growth. *Ocimum basilicum* L. (sweet basil) is a plant whose essential oil has many therapeutic attributes. Ways of increasing basil production have been sought by researchers. Therefore, this project aimed to evaluate the effect of simulated hypergravity on the basil plant to determine the optimum cultivation protocol. Each sample consisted of 45 seeds grown on germination paper soaked with 80ml of water. They were submitted to hypergravity simulation (centrifuge), intermittently (8h at 7Gz & 16h at 1G), while the control group remained at rest. Germination was analyzed by two-way ANOVA after 1, 2, 3, 4, 8, 12 and 14 days of centrifugation. Significantly more germination was found on days 1, 2 and 3 for seeds under hypergravity in comparison to the controls. This difference was confirmed by the observed power reliability, which was 99%, 94% and 85% on days 1, 2 and 3, respectively. Values lower that 80% were found for the remaining experiment days. Hypergravity simulation increased basil germination after 1, 2 and 3 days centrifugation. The protocol chosen for use in sweet basil plant cultivation is 1 day of hypergravity simulation.

Keywords: Simulated hypergravity, germination, *Ocimum basilicum* L.

PO- 96

Track: Plant and Environment

CYTOSINE DNA METHYLATION IN PLANTS: A COMPARATIVE STUDY OF *IN SILICO* AND *IN VITRO*

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Epigenetics is defined as changes in gene expression and regulation without alteration in the DNA sequence. Enzyme mediated chemical modifications to the cytosine residues of DNA are referred as DNA cytosine methylation. The occurrence of cytosine methylation is one of the most frequent DNA chemical modifications at the CG, CHG and CHH (methylation islands), where H is a base other than G. The density of these islands present in a gene may relate its epigenetic regulation. Using the bisulfite sequencing method we studied a total of 154,339 cytosine methylation islands in several cotton (Gossypium spp.) lines and found that density of CG islands statistically differed ($p<0.0001$) from that of CHG and CHH. Further studies revealed that actually methylation island densities were also differed ($p<0.0001$) between CG and CHG/CHH islands revealing that there was a relationship between the densities of methylation islands and the actual cytosine methylation level determined using bisulfite sequencing. A total of 423 promoters ranging from 2000 to 3006 bp in length belonging to monocots and dicots were studied using in silico approaches. Results revealed that densities of CG and CHG islands differed ($p<0.0001$) between the promoters of the two classes. Further studies revealed that CG and CHG densities of promoters of Brassicaceae & Leguminosae, Poaceae & Solanaceae, and Poaceae & Brassicaceae significantly differed ($p<0.0001$) while these epigenetic mark densities were not differed between Solanaceae & Leguminosae. The following conclusions were drawn: (i) CG islands in plant genomes were also primary sites for the cytosine methylation as in mammalian genomes, (ii) cytosine methylation based epigenetic regulation of genes could be biased toward the density differences of CG, CHG and CHH and this phenomenon could be viewed as an effect of genetic on epigenetics.

Keywords: Bisulfite sequencing, dicots, epigenetics, monocots, promoters.

PO-48

Track: Pharmaceutical Biotechnology

DEVELOPMENT OF NOVEL NANOPARTICLE FORMULATIONS OF THE RETINOID St1926 IN COLORECTAL CANCER

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The promise of nanomedicine in drug delivery has recently gained widespread attention, especially in cancer targeted therapy. Combining nanotechnology with medicine enables more efficient drug delivery, increasing stability, bioavailability, and reducing drug toxicity which are common challenges in drug development. Hence, nanoparticles (NP) have been extensively studied in various cancer systems, and have made their way to the clinic. Their success can be attributed to their small size (typically 10-200 nm) in addition to their increased accumulation at tumor sites, *via* the enhanced permeability and retention effect and active targeting.

Cancer is one of the major causes of death worldwide; in particular, colorectal cancer is the third amongst men and women, highlighting the need for novel therapies. Retinoids constitute a promising class of anti-cancer compounds, where natural retinoids, such as all-trans-retinoic acid, have been used against various types of cancers. However, their clinical usage is hindered by undesirable side effects and acquired resistance, therefore, synthetic retinoids, which couple increased specificity and reduced toxicity, were developed. In particular, ST1926 is a synthetic retinoid that has shown promise in several solid and liquid tumors. Interestingly, it has reached Phase I clinical trials in ovarian cancer. However, ST1926 has recently been found to undergo glucuroconjugation in cancer patients, leading to its rapid excretion in urine, thus reducing its bioavailability and therapeutic efficiency.

Therefore, we developed novel ST1926 nanoparticles (ST-NP) and assessed their efficacy in an *in vitro* human colorectal cancer model: HCT-116 wild type and HCT-116 p53 null cell lines. ST-NP were developed using Flash NanoPrecipitation with the amphiphilic diblock copolymer polystyrene-b-ethylene oxide. This is a recently developed technique for the production of controlled size nanoparticles of drugs, providing high active loading efficiencies and drug loading contents. In one application, it was used to produce nanoparticles of the lead anti-cancer drug paclitaxel that improved its *in vivo* efficacy. In the current work, ST1926 was formulated into nanoparticles with a drug to polymer mass ratio of 1:5, which provided a stable formulation. The mean ST-NP diameter ranged between 174-230 nm, with a polydispersity index of 0. 345. Importantly, using the MTT cell viability assay, ST-NP exhibited potent anti-cancer activities as native ST1926 in tested colorectal cancer cells, at pharmacologically achievable concentrations. Future studies will assess the *in vivo* targeting and efficacy of ST-NP. Ultimately, our studies will support the use of ST-NP formulations in enhancing the stability and bioavailability of ST1926 in colorectal cancer.

Keywords: Nanoparticles, Drug Delivery, Colorectal Cancer, Retinoids.

PO-63

Track: Medical Biotechnology

MODULATION OF P-GLYCOPROTEIN, CYTOCHROME P450, AND GLUTATHIONE-*S*-TRANSFERASE BY EMODIN IN MULTIDRUG RESISTANCE HUMAN CANCER CELLS

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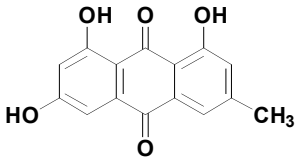
Resistance of cancer cells to chemotherapy is controlled by a decrease of intracellular drug accumulation, increase of detoxification, and diminished propensity of cancer cells to undergo apoptosis. ABC-membrane transporters together with intracellular metabolic enzymes contribute to the complex and unresolved phenomenon of multidrug resistance (MDR). Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is a naturally occurring anthraquinone present in many herb include *Fallopia japonica*, has diverse biological properties properties. However, it is also interesting in the field of cancer therapy. The mechanisms by which emodin might produce anticancer effects are not well understood. In this study, emodin was shown to increase Rho123 and calcein accumulation in a concentration dependent manner (1–500 ĀM) in Caco-2 cells by with IC_{50} 226. 64 ĀM and 51. 21 ĀM, recepectively. Morever, the treatment of CEM/ADR5000 with 10–100 ĀM emodin significantly inhibited the Rho123 and calcein efflux by 138–90%, and 107–87% as compared with verapamil (100%), respectively. The cytotoxicity of doxorubicin was enhanced by using 20 ĀM emodin; IC_{50} values were decreased from 4. 15 to 2. 27 ĀM, and from 33. 67 to 3. 57 ĀM, respectively. Furthermore, emodin significantly inhibited GST and cytochrome P450 enzyme activity in a dose dependent manner with IC_{50} values 0. 26 ĀM and 91. 13 ĀM, respectively. RT-PCR reveals a significantly down-regulation of ABC-transporters and of smetabolic enzymes mRNA levels in Caco-2 cell lines in response to emodin treatment. In conclusion, the inhibition of both ABC-transporters and of metabolic enzymes could explain the advantages of emodin in cancer therapy.



Emodin

PO- 97

Track: *Plant and Environment*

CYTOSINE METHYLATION DIFFERENCES BETWEEN LONG AND SHORT FIBER CELLS OF UPLAND COTTON

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Cotton (*Gossypium* spp.) plant is one of the important cash crops in many regions in the world. Cotton crop is not only used for the textile industry but also useful in feed and oil industries. Fiber is separated from the seeds using a process called ginning. Seeds of cotton plant consisted of two type of fiber; long fiber, also called as staple fiber and short fibers also called as fuzz fiber. Both of these fibers are produced from ovule epidermal cells. The mechanisms of differentiation of epidermal cells into staple and fuzz fiber cells are poorly understood. In this study the level of cytosine methylation levels between the two types of fiber cells were studied using bisulfite sequencing technique. Three types of cytosine methylations; CG, CHG and CHH were studied using 250-750 nucleotide regions of several different loci of Texas Marker -1 line (TM-1), a genetic standard in cotton research. Results clearly that there existed locus specific cytosine methylation levels between the short and long fiber cell. Some loci show high level of cytosine methylation in short fiber while the level of methylation decreased in the long fiber cells. In some other loci the situations reversed long fiber cell showed high level of methylations. In conclusion, our results revealed that there are cytosine level differences between long and short fiber cells of cotton some gene methylated while some other genes are unmethylated.

Keywords: Epigenetics, fiber development, bisulfite sequencing

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PO-38

Track: Medical biotechnology

NOVEL ROLES FOR PEROXIREDOXIN2 AS TARGET ANTIGENS FOR ANTI-ENDOTHELIAL CELL ANTIBODIES

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Anti-endothelial cell antibodies (AECA) were autoantibodies detected frequently in vasculitis.

We separated proteins extracted from HUVEC and HeLa cells respectively by 2-dimensional electrophoresis. By WB using vasculitis sera, we detected and identified antigens that were positive only in HUVEC using proteomics.

One of the identified proteins was found peroxiredoxin2 (Prx2) and anti-Prx2 antibodies were detected in 60% of Kawasaki disease (KD) patients. ELISA demonstrated the release of Prx2 from ECs stimulated by inflammatory cytokines. Various inflammatory cytokine secretion and expression of adhesion molecule on ECs were induced not only by anti-Prx2 antibodies but also by extracellular Prx2. These effects of Prx2 were enhanced by the appearance of anti-Prx2 antibodies and were inhibited by blocking toll-like receptor 4 (TLR4) signaling. Clinically, the duration of fever was longer in the anti-Prx2-positive group in KD.

Anti-Prx2 antibodies would be a useful marker for KD. Expression of endothelial adhesion molecules and inflammatory cytokine production were induced by both anti-Prx2 antibodies and extracellular Prx2, which would result in endothelial injury.

PO-62

Track: Industrial and Manufacturing

SCREENING AND ISOLATION OF *ASPERGILLUS* SPECIES FOR HYPER-PRODUCTION OF POLYGALACTURONASES

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Pectinase is an enzyme that breaks down pectin, a polysaccharide found in plant cell walls. Commonly referred to as pectic enzymes, they include pectolyase, pectozyme and polygalacturonase. Polygalacturonases (PG) are pectinolytic enzymes that have technological, functional and biological applications in food processing, fruit ripening and plant-fungus interactions. The aim of the study was to isolate the fungi from different vegetative/fruit wastes and screen them on the basis of enzyme activity. In the current study, 10 fungal strains were isolated from moldy vegetables and soil samples were collected from different vegetated fields. Among these isolates, 5 strains were selected and identified on the basis of morphological and enzymatic assays of *Aspergillus* species. One single strain was selected at 0. 5%, 1. 0%, 2. 0% and 2. 5% pectin enzyme activity. Strain "A" showed the maximum enzyme activity among the 5 selected strains, i. e. 1169. 85 U/ml/min.

PO-59

Track: Marine Biotechnology

INCIDENCE OF POSTOPERATIVE ENDOPHTHALMITIS AND THE POTENTIAL OF MARINE SPONGES AS NEW SOURCES OF ANTIBIOTICS IN EGYPT

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Purpose: To identify microbial pathogens in aqueous humor of patients with postoperative endophthalmitis using polymerase chain reaction (PCR) and conventional methods and studying antimicrobial activity of marine-derived bio products.

Methods: Fifty patients clinically diagnosed as postoperative endophthalmitis, mainly after cataract surgery, who attended the Research Institute of Ophthalmology were investigated in our study. A total of fifty persons were used as controls. Conventional methods, including direct microscopy and culture, and PCR were used. Antibacterial activity of crude extracts from sponges against microbial strains was determined by the agar-diffusion method.

Results: Among the fifty aqueous humor samples, 28% showed positive culture growth and 60% were PCR positive. When associated, culture and PCR allowed for a microbiological diagnosis in 76% of cases. Microorganisms cultured by conventional techniques matched those identified by PCR. The most common isolated microorganism was *Pseudomonas aeruginosa* (56%) followed by *Staphylococcus aureus* (46%). Aqueous and ethanolic extracts of sponge species showed inhibitory activity against *Staphylococcus aureus* more than *Pseudomonas aeruginosa*.

Conclusion: Detection of microbial DNA by PCR may prove a useful and rapid means of diagnosing postoperative endophthalmitis and facilitating management decisions when conventional culture is negative. Marine sponges are among the most promising sources of new antimicrobial substances.

Keywords: Postoperative endophthalmitis-polymerase chain reaction-Marine sponges-antimicrobial activity-Egypt.

PO-8

Track: Plant and Environment

DEVELOPMENT OF PVYO RESISTANCE TRANSGENIC POTATOES BY MHD MOTIF ENGINEERING IN SOLANUM TUBEROSUM

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In Solanaceae, potato virus YO (PVYO) is a widespread virus leading to severe damages such as necrosis, molting, and yield reduction. Previously, The resistance Y gene (Ry) of potato specifically confers resistance to PVY infection. Previously, potatoes resistant to PVYO infection were screened among the 32 Korean cultivars. We identified that Golden Valley has extremely resistance compare to Winter Valley highly susceptible to PVYO infection. The normal groups of Resistance (R) gene are composed of two classes, which are the CC-NB-LRR and the TIR-NB-LRR domain. The Resistance y (Ry) of Golden and Winter Valley include the TIR-NB-LRR class. Specifically, NB domain of Golden Ry gene (G-Ry) has Methionine-Histidine-Aspartic (MHD) motif, and the NB domain of Winter Ry gene (W-Ry) has Methionine-Histidine-Glycine (MHG) motif. MHD motif of R gene is very important region to virus resistance. To identify MHD motif of G-Ry and W-Ry, we performed that W-Ry converted MHG to MHD motif (mW-Ry) and G-Ry converted MHD to MHG (mG-Ry). We carried out Agrobacterium-mediated transformation in Winter valley. To identify PVYO resistance of transgenic potatoes, Ry and coat protein (CP) of virus were analyzed using RT-PCR. The results showed that the transgenic potatoes of mW-Ry did not show CP band, but transgenic potatoes of W-Ry constructs showed CP band. It suggests that MHD motif has PVYO resistance. These results can be also applied to the development of virus resistant potato.

Keywords: PVYO, Ry gene, Solanum tuberosum.

PO-21

Track: Pharmaceutical Biotechnology

ANTIOXIDANT ACTIVITY OF PROTOCATECHUIC ACID FROM PINUS DENSIFLORA AND DERIVATIVES

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Pinus densiflora contains several phenolic compounds that have various biological activities such as antimicrobial, antioxidant, antihypertension. However, the health beneficial effects of these compounds have rarely been reported. The methanolic extract of *P. densiflora* was tested for antioxidant capacity. To DPPH assay, antioxidant activity was determinded and fractionated each compounds with several solvents. Protocatechuic acid (PCA) isolated from ethyl acetate-soluble fraction exhibiting strong antioxidant activity and was purified by repeated silica gel column/HPLC. PCA is aromatic carboxylic acid containing 2-OH group bonded directly to unsaturation benzene ring. Then, PCA derivatives were examinded antioxidant acitivity. DPPH antioxidant of PCA derivatives results shows a clear pattern of effective antioxidant activity. The radical scavenging abilities of these acids depend greatly on the number and arrangement of phenolic hydroxyl groups. In the present study, we analyze the antioxidant activity of PCA and derivatives in murine endothelial cell line HepG2. Cytotoxicity screening was measured by the MTT method. We identify antioxidant activity using total ROS (as detected by DCF-DA), superoxide dismutase (SOD), glutathione (GSH) assay. This several assay results were same pattern like DPPH assay. So we confirm that antioxidant activity of PCA and derivatives were depends greatly on the number and arrangement of phenolic hydroxyl groups.

Keywords: Pinus densiflora, Antioxidant, Protocatechuic acid, HepG2.

PO-24

Track: Medical Biotechnology

ASSOCIATION BETWEEN DNA METHYLATION STATUS AND CLINICAL CANCER STAGE IN 3 TUMOR SUPPRESSOR GENES: IMPLICATION FOR RISK OF COLORECTAL CANCER RECURRENCE

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Aberrant DNA methylation has been shown to play an important role in carcinogenesis, but prospective evidence of an interaction between the molecular biomarker and clinical stage for prognosis of colorectal cancer is limited. The authors examined the DNA methylation status in tumor and matched normal tissues in patients after surgical resection of colorectal cancer (CRC) and to identify the association between clinical stage and DNA methylation for the risk of CRC recurrence. Pairs of specimens were obtained from the 215 CRC patients (430 samples) in Tri-Service General Hospital in Taiwan and used candidate gene approach to select 3 tumor suppressor genes involved in the pathways. Bisulfite-treated DNA was subjected to methylation-specific polymerase chain reaction (MS-PCR). The authors observed a joint effect with a significantly risk of recurrence by *CDKN2A* promoter methylation (HR=9. 56; 95% CI: 4. 24-21. 54), and the risk was 9. 56 (95% CI: 4. 48-20. 37) in *MLH1* and 11. 11 (95% CI: 5. 03-24. 51) in *MGMT*, respectively; 5-years accumulative recurrence rate was significantly higher in advanced stage with DNA methylation ($p<0.001$). There was an interaction between DNA methylation and clinical cancer stage that increased the recurrence risk of CRC, possibly because of molecular changes that could not be examined according to clinical pathology. The results can be a reference marker to evaluate the risk of CRC recurrence. The findings provide a new insight into the mechanism of DNA methylation of in different histological cancer stages and demonstrate the interaction that occurs in matched normal tissues.

Keywords: Colorectal cancer; recurrence; DNA methylation; Clinical stage; Interaction.

PO-39

Track: Other Areas

HIGH EFFICIENCY SORTING SYSTEM TO ASSESS ARSENIC TRIOXIDE (As_2O_3) INDUCED APOPTOSIS

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In these days, arsenic trioxide (As_2O_3) has been known as a drug for the treatment of leukemia in pathological and clinical fields. To investigate survival rate of leukemia treated with As_2O_3, various methods such as methyl thiazol tetrazolium assay, DNA fragmentation assay and florescent activated cell sorter have been utilized. Due to complexity of treating process, those methods require high cost. Therefore, we proposed negative dielectrophoresis (n-DEP) based sorting system which does not require immuno-labelling. The proposed system is employed for evaluation of As_2O_3 efficacy for leukemia by separating live/dead cells. To confirm the feasibility of separation principle, we theoretically analyze the correlation between n-DEP force and the hydrodynamic force acting on cells since those forces are the main factors that determine separation efficiency. Based on theoretical results, we selected the input conditions for voltage and flow rates for each micro channel. After we observe apoptotic ratio according to treated time of 24hours, finally, separation test is performed with mixture of live/dead (80%/20%) leukemia. Conclusively, we demonstrated high separation efficiency of over 90% which is competitive performance compared to conventionally stained method.

PO-23

Track: Medical Biotechnology

NOVEL STRUCTURED BIODEGRADABLE OSTEOCHONDRAL SCAFFOLD FOR ARTICULAR CARTILAGE REPAIR

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There are currently around 2. 4 million people affected with focal cartilage defects in the United States with only about 800,000 getting treatment due to a lack of a better solution. Articular cartilage itself is a fairly complex structure that has multiple alignments, where in the top superficial layer, the alignment of the collagen fibrils are parallel to the movement of the joint to assist in resistance to shear stress, while in the bottom radial zone, the fibrils are perpendicularly aligned to help resist compressive stresses. Current solutions cannot reproduce the morphological alignments of the normal articular cartilage. Additionally treatments like microfracture and autologous chondrocyte implantation cannot usually reproduce hyaline cartilage, only inferior fibrocartilage leading to degenerative tissue and the need for repeated procedures.

To overcome these limitations, we have developed a novel structured biodegradable osteochondral scaffolds for repairing articular cartilage injuries. The osteochondral scaffold consists of an outer microspheric shell made of a composite biodegradable polycaprolactone (PCL) and poly (lactic – glycolic) acid (PLGA) for protection of the growing tissue from mechanical stresses. The inside parts are two porous PCL spiral scaffolds with one laden with aligned nanofibers to induce alignment of the cellular extracellular matrix molecules (ECM) to serve as the cartilage scaffolds, and the other porous PCL spiral scaffolds to serve as bone scaffolds separately. To induce differentiation of the stem cells, hyaluronic acid (HYA) (20% w/w) and chondroitin sulfate (CS) (0. 2% w/w) were crosslinked onto cartilage portion of the scaffold using 48 mM EDC/ 6 mM NHS chemistry. To test the efficacy of the system, rat bone marrow stem cells (BMSCs) were seeded onto the cartilage portion of the scaffolds. The western blot, MTS assay, and confocal imaging were performed at days 1, 7, 14, and 28. The DMEM supplemented with 0. 025% HYA with 16% FBS was used for the cartilage scaffold culture. Western blot probing for collagen type II and aggrecan was performed using Ā-actin as a standard. Confocal imaging using anti-collagen type II and anti-aggrecan with secondary antibody conjugated with FITC with DAPI for nucleic staining was performed. For the bone portion of the scaffold, instead of HYA and CS being electrospun with the fibers, hydroxyapatite (HA) was mixed with the PCL solution at a 20% w/w ratio. The rat BMSCs were seeded onto the bone portion of the scaffolds. The DMEM with 16% FBS with 100 nM dexamethasone, 10 mM Ā-glycerophosphate, and 0. 05 mM L-ascorbic acid were used for the bone scaffold culture. To determine differentiation of BMSCs on the bone scaffold portion, RT-PCR was used to determine expression of alkaline phosphatase (ALP), osteonectin (ON), osteopontin (OPN), bone morphogenetic protein 2 (BMP-2), and osteocalcin (OCN).

Laser confocal microscopy showed that there was aggrecan and collagen type II secretion according to the alignment of the nanofibers. There was significantly more collagen type II secretion in the scaffolds with CS and HYA. Western blotting showed that there was a significant increase in aggrecan and collagen type II secretion in the aligned nanofibers combined with CS and HYA when compared to the plate control or nanofibers with no HYA and CS. For the bone scaffold part, the PCR characterization showed that there was a significant increase in all ALP, ON, OPN, BMP-2, and OCN in the scaffolds combined with HA when compared to the control without HA. For both the cartilage and bone scaffold, there was an increase in cellular proliferation throughout the 28 days of culture. Cyclic compressive testing of the complete scaffold at 1. 6 MPa loading in a confined vessel with PBS for 10,000 cycles showed that the entire scaffold was able to sustain physiological loads.

From these results, it can be determined that the scaffold can cause the secretion of collagen type II and aggrecan, the main characteristics of hyaline cartilage. This shows that when combined with the BMSCs, the scaffold should be able to take advantage of one's own cells to regenerate the osteochondral tissue. By combining an osteochondral scaffold model with aligned nanofibers to take advantage of one's own bone marrow stem cells, more functional cartilage can be regenerated. Additionally by regenerating cartilage and bone simultaneously, delamination can be minimized. Overall, with a scaffold that can regenerate cartilage that is physiological similar to that of natural cartilage for better functionality, quality of life can be greatly improved.

PO-52

Track: Other Areas

RELATIONSHIP BETWEEN PULSE WAVE VELOCITY, METABOLIC SYNDROME AND FRAMINGHAM RISK SCORE IN KOREAN

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Various measures of pulse wave velocity (PWV) are increasingly recognized as important in predicting cardiovascular disease. However, evidence that PWV can be used to provide a reliable assessment for the treatment of heart disease is still lacking. There need to be more evidence supporting the use of the PWV in different populations. To determine the factors influencing relationship between PWV, Metabolic Syndrome (MetS) and Framingham Risk Score (FRS), we studied in habitants of Seoul, Korea.

We analyzed 3,350 Korean subjects (1,926 male, 1,443 female) aged between 20 and 78 years (43. 3±8. 9). Unpaired t-test was used to compare between mean values of the components of MetS, FRS and PWV (arotic PWV: the common carotid to femoral artery, arm PWV: the brachial to radial artery, leg PWV: the femoral to dorsalis pedis artery). Ten-year risk for coronary heart disease (CHD) was estimated by the FRS. Receiver-operating characteristic (ROC) curves and their respective areas under the curve (AUCs) were used to compare the ability of the PWV to predict FRS categories and subjects with or without Metabolic Syndrome. The present study shows that FRS increased significantly with increasing number of components of MetS. Leg PWV (AUC, 0. 79) was a better predictor of CHD than arotic PWV (AUC, 0. 78) and arm PWV (AUC, 0. 76) in intermediate and high levels of 10-year risk. Leg PWV (AUC, 0. 81) was also a significantly better predictor of MetS than arotic PWV (AUC, 0. 80) and arm PWV (AUC, 0. 79) in the number of metabolic abnormalities. This study provided evidence that PWV has the potential to predict of cardiovascular disease (CVD) in Korean.

Acknowledgement

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Keywords: Pulse Wave Velocity, Metabolic Syndrome, Framingham Risk Score.

PO-34

Track: Plant and Environment

VISUALIZING AND DEVELOPING CONTEMPORARY BIOREMEDIATION TECHNOLOGIES

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The population explosion in the world has resulted in an increase in area of polluted soil and water. The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment. This paper aimed to identify the major research and development (R&D) trends of bioremediation technologies as well as to build the core technology category framework through collecting United States Patent and Trademark Office's (USPTO) patent information. The traditional approach for patent inquiry into technologies is too general to meet the needs of specific industries. Moreover, some patents are placed in incorrect categories, making it difficult for decision makers to carry out R&D planning, patent portfolio planning, technology positioning, and technology forecasting. To address these issues, the Patent Co-citation Analysis (PCA) method and the factor analysis technique were applied to categorize the patents of bioremediation and to identify the main technology trends of it. The result revealed that the major R&D trends cover the fields of anaerobic degradation, aerobic degradation, anaerobic reductive dechlorination, bioaugmentation, and bioventing.

Next, the paper further applied the fuzzy Delphi method following the former study results chiefly derived from the mentioned PCA, in order to investigate and decide the most appropriate bioremediation technologies for a case country Taiwan, who had had emergent needs on the aspect of developing novel bioremediation technologies. At this point, the result showed that both "anaerobic degradation" and "anaerobic reductive dechlorination" are two better bioremediation technology candidates for Taiwan, mainly due to the fact that the country had had pollution problems with varying industries as well as agricultural lands all along.

It was subsequently suggested for future researches to consider how different environmental, economic, technological and social scenarios should lead to corresponding bioremediation technology exploitation.

Keywords: Bioremediation, Technology Category, USPTO, Patent Co-citation Analysis, Fuzzy Delphi Method.

PO-46

Track: Industrial and Manufacturing

CO-CULTURE IN IMMOBILIZED-CELL HOLLOW FIBER MEMBRANE BIOREACTOR FOR EFFECTIVE BIOETHANOL PRODUCTION ON GLUCOSE AND XYLOSE

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Lignocellulose is the most abundant renewable source of biomass available for conversion into bioethanol. In processing lignocellulosic biomass into bioethanol, major concerns are ethanol yield and fermentable sugars in the lignocellulosic hydrolysate. The major sugars in the hydrolysate are glucose and xylose. There is currently no wild-type microorganism that could efficiently accomplish the fermentation of this sugar mixture, so co-culture is a promising approach. However, the use of xylose-fermenting microorganism for co-culture in bioethanol production is limited due to their sensitivity to inhibitors, low ethanol tolerance and carefully regulated oxygen requirement. In this presentation, we developed a Submerged Hollow Fiber Membrane Bioreactor (SHFMB) with immobilized *Zymomonas mobilis* and *Pichia stipitis* for simultaneous co-fermentation of glucose and xylose in lignocellulosic hydrolysate. This SHFMB has been shown to facilitate efficient bioethanol production by shielding the cells from inhibitors present in lignocellulosic hydrolysate and from bioethanol inhibition in the fermentation broth. Operating parameters including the number of hollow fibers used, the concentrations of sugars and inhibitors, as well as the effects of aeration on the complete conversion of both sugars to ethanol have also been investigated.

PO-37

Track: Regenerative Medicine

APPLICATION OF BIOPOLYMERS IN BIOMEDICAL DEVICES: ADVANCES IN BIOMATERIALS AND FABRICATION METHODS

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Tissue engineering has emerged as a promising alternative approach in the treatment of malfunctioning or lost organs. In this approach, a temporary scaffold is needed to serve as an adhesive substrate for the implanted cells and a physical support to guide the formation of the new organs. In addition to facilitating cell adhesion, promoting cell growth, and allowing the retention of differentiated cell functions, the scaffold should be biocompatible, biodegradable, highly porous with a large surface/volume ratio, mechanically strong, and malleable. The scaffold degrades while a new organ or tissue is formed. A number of three-dimensional porous scaffolds fabricated from various kinds of biodegradable materials have been developed. The poly(! -hydroxy acids) are the principal biodegradable and bioresorbable polymers used in tissue engineering. In developing and selecting bioresorbable scaffolds, the degradation

time is fundamental for successful biocompatibility and biofuncionality. This work intends to be a contribution through a critical review of advances in biomaterials and fabrication methods for application in bone tissue engineering.

Keywords: Tissue engineering, biomaterial, scaffold.

PO-33

Track: Industrial and Manufacturing

NOVEL SYNTHESIS OF PYRIDINE AND 3-PICOLINE FROM GLYCEROL AND AMMONIA OVER ALKALINE-ACID SEQUENT-TREATED ZEOLITE CATALYSTS IN SERIES-CONNECTED TWO-STAGE FIXED-FED REACTOR

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Pyridine and 3-picoline were synthesized, for the first time, by employing a 20 wt % glycerol as raw material and in a series-connected two-stage fixed-fed reactor. Two kinds of catalysts, the alkaline-acid sequent-treated HZSM-22, namely AaT-HZSM-22, and ZnO loaded on the alkaline-acid sequent-treated HZSM-5, namely ZnO/AaT-HZSM-5, were respectively employed for the dehydration of glycerol to acrolein in the first stage of reactor and the condensation of acrolein and ammonia in the second stage of reactor. The catalysts showed an appreciably high stability and good regeneration performance, while maintaining an above 60 % total yield of pyridine and 3-picoline. Contrastively, the employment of either the AaT-HZSM-22 and ZnO/AaT-HZSM-5 catalysts in a single-stage reactor or the HZSM-22 and ZnO/HZSM-5 ones in the two-sage reactor provided only very low total yield of pyridine and 3-picoline. The catalysts were characterized by XRD, N2-physisorption, SEM, FT-IR and NH3-TPD, and the results indicated that mesoporosity was generated and acid strength decreased in the AaT-HZSM-22 and ZnO/AaT-HZSM-5 catalysts, relative to the HZSM-22 and ZnO/HZSM-5 ones. Therefore, both the employment of two-stage reactor and catalysts with mesopore-structure and suitable acidity were favorable to the generation of pyridine and 3-pyridine from the reaction of glycerol and ammonia.

Keywords: *Glycerol; Pyridine; 3-picoline; alkaline; HZSM-5.*

PO-69

Track: Other Areas

POSSIBLE ROLE OF CARFILZOMIB IN THE PREVENTION AND TREATMENT OF CHEMICALLY INDUCED LIVER CANCER IN RATS

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The possible therapeutic effect of irreversible proteasome inhibitor, carfilzomib against hepatocellular carcinoma induced chemically by chronic administration of diethylnitrosoamines (DENA) was investigated.

Hepatocellular carcinoma induced by DENA in male Wistar rats was manifested biochemically by significant elevation of serum Ā-feto protein (AFP) and carcinoembryonic antigen (CEA). In addition, hepatic cancer was further confirmed by a significant increase in hepatic tissue growth factors; vascular endothelial growth factor (VEGF), transforming growth factor Äl (TGF-Äl) and basic fibroblast growth factor (FGF). Moreover a marked increase in matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-1 (TIMP-1) content were also observed, along with a profound decrease in hepatic endostatin and metallothonein level.

Treatment of rats with the selected doses of carfilzomib produced a significant protection against hepatic cancer. The present results claimed that chosen doses of carfilzomib succeeded in suppressing serum tumor markers level AFP and CEA. Furthermore, the drug reduced the elevated level of hepatic growth factors, MMP-2 and TIMP-1 induced by the carcinogen. The antitumor effect of carfilzomib was also accompanied by augmentation of hepatic content of endostatin and metallothionein. Histopathological examination of liver tissues also correlated with the biochemical observations. It could be concluded that treatment with carfilzomib confers a possible antitumor effect against hepatocellular carcinoma induced by DENA model in rats.

PO-30

Track: Other Areas

THE ANTIBACTERIAL EFFECTS OF FATTY ACID SALTS ON ORAL BACTERIA

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Introduction

Streptococcus mutans is normally within the oral cavity and the leading cause tooth decay. Fatty acid salts are one of the surfactant and known to have potent antibacterial activities. However, few studies have addressed the effect of fatty acid salts on oral bacterial. In this study, we evaluated the antibacterial activity of fatty acid salts to Streptococcus mutans.

Methods

The antibacterial activity of 9 fatty acid salts tested on spores of Streptococcus mutans NBRC 13955. Fatty acid salts were prepared by mixing the fatty acid with the appropriate amount of KOH. The antibacterial method, the bacterial suspension was mixed with sample of fatty acid potassium.

Result and Conclusions

C10K, C12K, C14K, C18:1K, C18:2K and C18:3K were effective to decrease survival rate of *S. mutans* at 175mM. The minimum inhibitory concentration of C18:2K and C18:3K was 5. 5 mM. This result indicates that C18:2K and C18:3K have high antibacterial activity against *S. mutans*. Moreover, we investigated the effects of mixtures of fatty acid salts. Mixture of C12K and short-chain fatty acids potassium showed high antibacterial activity.

These results indicate that C12K, C18:2K and C18:3K has high antibacterial activity against *S. mutans* and has great potential for antimicrobial agents.

Keywords: Antibacterial effects, fatty acid salts, oral bacteria.

PO-44

Track: Other Areas

DETERMINATION OF Ca AND Fe IN BLOOD OF GRMD DOG SUBMITED TO A SYSTEMIC TRANSPLANTATION OF STROMAL CELLS (hASCs) USING NAA AND FRX TECHNIQUES

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The use of alternative analytical techniques to investigate specific electrolytes in body fluids (mainly blood, serum and urine) has increased in past few years, presenting significant progress in clinical practices. Since 2004 Neutron Activation Analysis (NAA) and more recently X-ray Fluorescence (XRF) techniques have been applied to this clinical finality at IPEN/CNEN-SP, in collaboration with other research centers from Brazil. Several investigations in veterinary medicine, immunology and genetic fields were performed and also applications for medical diagnosis studies; particularly for Duchenne Muscular Dystrophy (DMD). The DMD is an illness of a hereditary character that affects approximately 1 in every 3,600 to 6,000 live male births in the world. Nowadays, many promising therapeutic strategies have been developed in animal models with DMD. An animal model which has a phenotype, which is similar to human patients with DMD, has been bred in Brazil: Golden Retriever Muscular Dystrophy dogs (GRMD). In these dogs, muscle degeneration and fibrosis are predominating, leading to a progressive loss of structure and muscle function, as in humans (once they have a comparable muscle mass to that of a human being).

Recently, the Human Genome Research Center (Biosciences Institute in Brazil) has shown that human adipose derived from stromal cells (hASCs) when injected systemically into GRMD dog cephalic vein paw are able to reach, engraft, and express human dystrophin in the host GRMD dystrophic muscle (up to 6 months after transplantation). This shows an improvement in the functional performance of injected animals without any immunosuppressant. In this study, Ca and Fe were investigated in whole blood during this period (before starting the transplantation process and after six months); due to functions they play in muscle to keep them healthy. Nonetheless, this disease is caused by a mutation of the dystrophin gene. The absence of dystrophin (a protein present in muscles) permits the excess of calcium to penetrate the sarcolemma (cell membrane). This protein is altered causing a critical muscular dysfunction in several body functions, such as: calcium homeostasis and dysfunction in the mechanisms of membrane permeability causing a degeneration of the membrane that involves the muscular cell, leading to its death. Another clinical point to be considered is related to Fe concentrations in blood. The hemochromathosis (overload of iron) can cause joint pain, abdominal pain, weakness, fatigue or significant organ damage, and that can eventually trigger another health problem for the DMD patients.

The present results have showed that this therapeutic procedure does not affect the Ca and Fe levels. Furthermore, some improvements in their physiological structure and mobility have been observed, suggesting that there is a need to continue this therapeutic strategy for DMD patients.

Keywords: Steam cells, DMD, whole blood, Ca, Fe, NAA, FRX.

PO-32

Track: Other Areas

THE OPTIMUM CONDITION FOR ENZYME PRODUCTION IN LIQUID CULTURE OF *ASPERGILLUS KAWACHII*

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Aspergillus kawachii of the shochu koji mold produces two kinds of Ā-amylase, one is an acid-unstable Ā-amylase and the other is an acid-stable Ā-amylase. In liquid culture, acid-unstable Ā-amylase was produced abundantly, but, acid-stable Ā-amylase was not produced. Therefore, in this study we investigated optimum condition of production of acid-

stable Ä-amylase in liquid culture. Aspergillus kawachii NBRC 4308 was used. The conidia of *A. kawachii* were inoculated into 100 ml of altered SLS medium in baffled flasks and cultivated with shaking at 30 ^{o}C and 200 rpm for 72 h. First we experimented with co-culture of A. kawachii and lactobacillus in order to get control of pH in altered SLS medium. But high production of acid-stable Ä-amylase was not obtained. Next we experimented with yoghurt or milk made an addition to liquid culture. As a result, high production of acid-stable Ä-amylase (964 U/g-substrate) was obtained when milk made an addition to liquid culture. We investigated details of high production of acid-stable Ä-amylase. The result is that it was indicated possibility of development of shochu making technology with the use of this liquid culture method.

Keywords: Acid-stable Ä-amylase, liquid culture, *Aspergillus kawachii*.

PO-13

Track: Industrial and Manufacturing

THE HYDRODYNAMIC PERFORMANCE AND EFFICIENCY OF THE IMMOBILIZED BIOCATALYST FOR THE AIR PURIFICATION IN THE BIOTRICKLING FILTER

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Introduction

The biological air purification and odour control of volatile organic compounds becomes cheap, efficient and produces no secondary pollutants. In this process in addition to microorganisms active element is the packing, on which microorganisms are immobilized. In this regard, from the hydrodynamic performance of the packing depends the energy intensity and overall process efficiency.

The aim of the study was to find high packing with minimum pressure drop by means of hydrodynamic experiments and experiments in a pilot plant for purification ventilation emissions of tobacco factory.

Materials and Methods

The experiments were carried out on a column. Diameter of the column is 200 mm, height of the column is 1000 mm, height of packing is 800 mm. Air flow amounted to 200 mÄh, which corresponds to a velocity flow W0 = 0-1 m/s in the calculation of the total cross section empty apparatus. Air temperature was 20 °C, barometric pressure is 101,3 kPa.

Results

The experiments were subjected to random and regular packings such as active carbon, balls of silica gel and wood chips, zeolite, foam (mixture), pine-cone, ceramic Raschig rings, HPCM packing and promising Combined packing. The Combined packing from lavsan grids which are gas-liquid distributor of the flow and from polymeric fibers, which are filamentary carrier as shown efficiency for purification tobacco ventilation emissions up to 99%, and pressure drop is 200 Pa/m. These values allow the high efficiency and prospects using the packing of the biological air purification.

Conclusions

The efficiency of biological air-purification depends on the conditions created in the apparatus for the microorganisms, the key element is the packing. From the properties of the packing (geometric, natural, hydrodynamic) will depend the intensity of the air-purification process as a whole. As evidenced by the work done.

Keywords: Air-purification, biofiltration, packing, biotrickling filter, pressure drop.

PO-36

Track: Medical Biotechnology

FOOTBALL RELATED CONCUSSIONS & NEUROBEHAVIORAL IMPAIRMENT: WHAT IS THE RELATIONSHIP?

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Gap in Knowledge

Although recent research suggests concussions may have long-term consequences, no clear dose-response relationship (The number of concussions and the level of impairment) has ever been established in football [1]. There is also insufficient evidence to determine whether the long-term effects reported in professional retired athletes also occur following retirement at a younger age/level of play [2]. With pver 200 million Americans involved in organized sports, the long-term effects of concussions represents a significant public health concern [3]. At present, there are no practical methods of predicting which athletes are likely to develop impairments and which are not [5,6].

Objective

Our primary objective was to establish if there is a relationship between concussions and later-life function, and if so to quantify the magnitude of the dose-response relationship for each level of play (youth, high school, collegiate, professional). We also sought to establish a practical method of assessing exposure.

Methods

We studied 200 former football players, including: 53 former professional (NFL); 16 semi-professional; 67 college; 57 high school; and 7 youth level players. They had mean age of 47.5 years. Participants approximated their concussion exposure using four different definitions derived from the literature: a) a spontaneous estimate of the number of concussions sustained throughout their lives; b) another estimate after being provided with an evidenced-based modern definition of concussion; c) an estimate of the number of concussions they experienced with a loss of consciousness (LOC); and d) a total of their "*significant or major*" concussions for which participants could provide more detail (e. g. age, activity, length of LOC). They also completed the **Behavior Rating Inventory of Executive Function (BRIEF-A)** [7], a well-validated standardized self-report measure of neurobehavioral function and the **Center for Epidemiologic Studies Depression Scale (CES-D)** [8]. BRIEF-A scores were converted into age-appropriate T-scores that yield an overall composite score (Global Executive Composite [GEC]), two index scores, (Behavioral Regulation Index [BRI] and Metacognition Index [MI]), and nine clinical scales. Each self-reported concussion history was evaluated and **corrected for recall bias heteroscedasticity** [8].

Next, we assessed the relationship between each concussion definition using independent **linear regression** models adjusted for age and education. For all linear regression analyses, alpha was set to 0. 0125 after applying Bonferroni's correction. Bootstrap analysis was performed on each regression's beta-coefficient to ensure **internal validity** and **predictive accuracy**. Finally, we determined whether the level-of-play effected the dose-response relationship by taking the difference (t-test) in the concussion dose-effect in high.

Results

Mood and depression did not have a significant dose-response relationship to any of the four concussion definitions. The estimate of concussions based on a definition significantly regressed with executive dysfunction (GEC $\ddot{A}$ = 2. 55, SE = 0. 52, p <. 0125), cognitive impairment (MI $\ddot{A}$ = 2. 20, SE = 0. 62, p <. 0125), and behavioral impairment (BRI $\ddot{A}$ = 2. 62, SE = 0. 58, p <. 0125). In a separate regression, the *major* concussions also regressed with GEC (Ä = 3. 72, SE = 1. 33, p <. 0125) and MI (Ä = 4. 01, SE = 1. 38, p <. 0125). The beta-coefficients (dose-response) *appear* to be larger for "*major concussions*" as qualitatively compared to other definitions, though no additional analysis was performed. Interestingly, there was no significant difference in the dose-effects between levels, suggesting concussions lead to the same long-term decline in NFL players as they do for high school players. Our preliminary findings have important implications for public health but require validation.

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PO-28

Track: Other Areas

THE EFFECT OF FATTY ACID SALT FOR BAKING PROPERTY OF WHEAT FLOUR DOUGH

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Introduction

In recent years, surface acting agent has used as bread improver in bread making, which effects of increasing specific loaf volume. Therefore, we focused attention to fatty acid salt which is one of the surface acting agents as new food additive. In this study, we tried to additive fatty acid salt as bread improver in bread making and investigated the dough expansion force.

Material and Methods

Dough Expansion Ability Test

Bread dough was consisted of 100 g of flour, other materials and fatty acid salt (potassium myristate: C14K ,350 mM and pH 10. 5). The concentration of C14K in the dough was 5 %, 10 %, 15 % and 20 % relative to flour weight.

150 g of dough was filled from bottom of the cylinder and fermented at 30 ℃, 85 % humidity for 120 min. The height of the expansion in the dough was measured and determined its expansion ability.

Result

Dough expansion ability of control bread was 390 ml after 60 min and 460 ml after 120 min. However, 15 % of C14K adding to the dough were the highest abilities, each value was 510 ml after 60 min and 570 ml after 120 min.

Keywords: Fatty acid salts, bread-making, food additive.

PO-25

Track: Other areas

BOUND TO NEURONS EXTRACELLULAR EXO- AND ENDO-METALLOPEPTIDASES; ENGINEERING OF PROTECTED THERAPEUTIC PEPTIDES

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Therapeutic peptides are increasingly used as analgetic and antiinflammatory agents, as drugs for the treatment of cancer, neurodegenerative and other diseases. However in the body, the peptides are attacked by proteolytic enzymes and rapidly lose their therapeutic efficiency. Therefore, it is of pivotal importance to produce protected therapeutic peptides that much longer would remain stable. A prerequisite for the successful design of the protected therapeutic peptides is the identification and characterization of peptidases that may come into contact with the peptides. We have found that isolated from mammalian brain axonal endings of neurons (synaptosomes) possess pronounced peptidase activity. Therefore, in brain, interneuron contacts (synapses) possess extracellular peptidase activity (or activities) associated with the neurons. Mild treatment of highly purified synaptosomes with nonionic detergent Triton X-100 (0. 1%, 0"! , 30 min) detaches from the synaptosomes a group of ecto (external) metallopeptidases (1,10-phenantrolin inhibits their activities). They were named NEMPs (Neuronal Ecto MetalloPeptidases). The peptidases were partially separated by means of gel electrophoresis under non-denaturizing conditions and characterized according to the type of activity.

Altogether four NEMPs were found:

1. Carboxypeptidase (NEMP1)

2. Aminopeptidase (NEMP2)

3. Endopeptidase A (NEMP3)

4. Endopeptidase B (NEMP4)

The exopeptidases (NEMP1 and NEMP2) can split dipeptides, but not dipeptide ( )ala-his (carnosine). Therefore carnosine present at the C-end, at the N-end or at the both ends of a peptide protect it from NEMP1, from NEMP2 or from the both, correspondingly. Instead of carnosine, present in its composition ( )alanine can be used for the peptide ends protection. Some specific properties of certain NEMPs were detected. NEMP1 is composed of rather short polypeptides, which tend to form multimers. A specific feature of NEMP3 is its activation by carnosine, ( )alanine and some other substances. Being activated, NEMP3 splits peptides predominantly near proline, alanine, phenylalanine, lysine and arginine residues. However some internal bonds containing these residues proved to be stable. The data obtained in this ongoing research will be useful for engineering of therapeutic peptides reliably protected from degradation in brain intercellular medium, in particular, in the area of synapses.

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PO-5

Track: Plant and Environment

UREA FERTILIZATION: EFFECTS ON GROWTH, NUTRIENT UPTAKE AND ROOT DEVELOPMENT OF THE BIODIESEL PLANT, CASTOR BEAN *(RICINUS COMMUNIS L)*

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An indoor pot culture experiment was conducted in the growth chamber during the period of vegetative growth to evaluate the influence of inorganic nitrogen fertilizer in the form of urea on nutrient uptake, growth and root development of castor bean plant. The Nitrogen Fertilizer treatments imposed in the experiment were: Control (N_0), no nitrogen and others at the rate of 60lb N/acre (N_1), 90lb N/acre (N_2) and 120lb N/acre (N_3) respectively. Effect of higher nitrogen concentration indicated considerable increases in castor growth including vegetative growth and the plant components biomass. Elevated nitrogen fertilizer increased height and other morphological and physiological parameters (Leaf and petiole length, intermodal distance, root numbers) including the root, shoot dry wt, root/ shoot ratio, nitrogen and crude protein content in plants. Among the plant components, shoot, root dry weight and root shoot ratio had the greatest decrease under N deficiency, while root/shoot carbon ratio increased under N deficiency.

No statistical different was observed with treatments in shoot and root N% and shoot C% in plants although root carbon content was significantly higher with lowest nitrogen level compared to elevated levels. Significant increases of carbon content in plants at N_0 showed some tendency of this crop to adjust with lower nitrogen levels. Also no statistical difference was observed in root and shoot N ratio, while the root and shoot carbon ratio was found significant at N_0 compared to other treatments. However the concentration of carbon and nitrogen were found higher in shoot than root in all applied treatments. After harvesting the residual nitrogen effect in soil was also found significant with elevated nitrogen level compared with other treatments and control.

Keywords: Castor bean, Growth, Urea, Nitrogen uptake, Root/Shoot Ratio.

PO-1

Track: Pharmaceutical Biotechnology

THE CHROMATIN REMODELING AGENT BmL-210 SUPPRESSES PROLIFERATION AND INDUCES APOPTOSIS IN HUMAN MYELOID LEUKEMIA CELLS

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In our studies we focused ourselves on a novel chromatin remodeling agent, histone deacetylase inhibitor (HDACI) - Bml-210. We found that Bml-210 at concentrations 10 and 20 ĀM inhibits the growth of human promyelocytic leukemia HL-60 and NB4 cell lines and promotes apoptosis in a dose and time dependent manner. Bml-210 alone induces HL-60 cell differentiation to granulocyte-like cells. This correlated with decreased HDAC protein levels and HDAC activity loss. HDAC inhibitor Bml-210 at concentration 20 ĀM causes apoptosis in both cell lines and this correlates with increased expression level of FasL protein and further activation of caspase-8. In NB4 cells HDAC inhibitor Bml-210 induced apoptosis both *via* caspases 8/3 and caspase 9 activation.

The comprehensive proteomic analysis of control and Bml-210 treated NB4 cells was performed. Total proteins were isolated, fractionated in two-dimensional gel electrophoresis (2DE) and visualized by staining with Colloidal Coomassie G-250. Proteins of interest were cut-out and prepared for mass spectrometry analysis. After in-gel tryptic-digestion the peptides were chromatographically separated using Agilent 1100 HPLC system with the flow splitter and analyzed by electrospray ionization MS in positive ionization mode using the ion trap "HCTultra PTM Discovery System" (Bruker Daltonics). In total over 35 proteins were identified and only few of them showed expression differences in comparison

of untreated and treated leukemic cells. We showed that PCNA protein was downregulated after leukemic cell treatment with Bml-210 and some others, like LGUL protein (involved in regulation of NF-kappa-B activity), RANG protein (involved in cell cycle regulation), CLIC1 and EFHD proteins were upregulated in Bml-210 treated cells. These proteins could be important for apoptotic processes in leukemic cells treated with HDACI Bml-210.

We suggest that Bml-210 as a chromatin remodeling agent, can be promising anticancer agent, especially in leukemia treatment, by inducing apoptosis and regulating proliferation through the modulation of histone acetylation, gene and protein expression.

Keywords: acute myeloid leukemia, histone deacetylase inhibitor, Bml-210, proteomics, apoptosis.

Acknowledgement

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PO-31

Track: Other Areas

THE EFFECTS OF FATTY ACID POTASSIUM ON *CLADOSPORIUM CLADOSPRIOIDES*

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Cladosporium cladosporioides is one of the most often detected fungi in the indoor environment, and causes pollution and deterioration. Therefore, the creation of antifungal agents with high safety and antifungal effect is required. Fatty acid salts are known that have antibacterial activities. This report describes the antifungal activity of fatty acid salts against *Cladosporium cladosporioides* NBRC 30314. Potassium salts of 9 fatty acids (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C18:1, C18:2, C18:3) were prepared by mixing the fatty acid with the appropriate amount of KOH solution to a concentration of 175 mM and pH 10. 5. The antifungal method, the spore suspention (3. 0Ã104 spores/mL) was mixed with sample of fatty acid potassium (final concentration of 175 mM). C8K, C10K, C12K, C14K was effective to decrease 4 log unit of the fatty acids potassium incubated time for 10 min. Especially, C12K was the most high antifungal activity and the MIC of C12K was 0. 7 mM. C12K mixed with C10K or C14K. C12K was effective to decrease survival rate when mixing C10K or C14K the higher than the MIC. These results indicate that C12K has high antifungal activity against *C. cladosporioides* and suggest the fatty acid potassium have great potential for indoor application.

Keywords: Fatty acid salts, antifungal effect, *Cladosporium cladosporioides.*

PO-15

Track: Pharmaceutical Biotechnology

SYRIAN HONEYBEE VENOM - ISOLATION OF ITS MAJOR COMPONENTS AND ITS TOXICO-PHARMACOLOGICAL-POTENTIAL

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Bee venom (BV) is widely used as a medicament in various diseases; it is known that bee venom *Apis mellifera* is a complex mixture of proteins and peptides exhibiting the most diverse biological activities. Venom was collected from Syrian beehives during using electric shock method and its LD50 was determined by Spearman-Karber method equaled 2. 78 mg/ kg. Melittin, the major component of BV, Apamin, Mast cell degranulating peptide (MCD) and Phospholipase (PLA2) was isolated using RP-HPLC C18 column, and identified using MALDI-TOF-MS, and HLPC-

triple Quadruple mass Spectrometer analysis. Venom which collected locally and the obtained Melittin and PLA2 have significant antibacterial effects against Gram-positive and negative-bacteria. Local bee venom in relatively lower concentrations showed nearly the same effect as purified venom purchased from Sigma. In this work, antibacterial activity of local bee venom proved to be concentration-dependent. It has an inhibitory bacteriostatic effect at low concentrations and exhibits a bactericidal activity at high concentrations. Our results revealed that gram positive bacteria, e.g., ***Listeria monocytogenes,*** and ***Staphylococcus aureus,*** were more sensitive to venom and its isolated peptides than the gram negative e. g. ***Escherichia coli, Salmonella enterica, Yersinia kristensenii***, ***Klepsiella pneumoniae*** and ***Klepsiella oxytoca***. This antibacterial property of bee venom may be used for medical purposes and drug preparations, or by some modifications for food preservation.

Additionally, whole bee venom (1mg/g) and melittin (1 and 3 mg/g) treatment was found to significantly accelerate wound contraction and re-epithelialisation as wound sizes decreased dramatically and healed within 5 days in both venom and Melittin treated rats compared with 8 days in the controls in a rat full-thickness excision wound model. But at venom concentration 3mg/g no good results were found. These findings suggested that topical venom in -low concentration- and Melittin treatment for skin defects should be very effective in preventing and reducing the wound and scar sizes. However, further studies are needed to evaluate the precise mechanism of epithelial cell proliferation induced by Melittin treatment.

PO-3

Track: Plant & Environment

SIMULATION OF *NEOCHLORIS OLEOABUNDANS* CELL GROWTH AT DIFFERENT SOURCE LIGHT INTENSITIES AND PHOTOBIOREACTOR DIAMETERS

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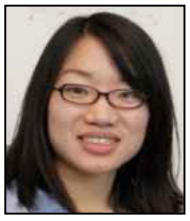

A model for microalgal growth kinetics was developed by integrating equations derived from the Beer-Lambert law and that derived from a modified Monad equation. The Beer-Lambert law relates the local light intensity to the location inside a photobioreactor (PBR), microalgal biomass concentration, and source light intensity. The local light intensity is integrated to obtain the average light intensity through the entire volume of the PBR. And finally, the modified Monad equation was used to relate the apparent specific growth rate of microalgae to average light intensity. The model was verified with experimental data obtained from microalga *Neochloris oleoabundans* cultures in 3L PBR converted from standard stirred-tank Bioflo 110 bioreactors. Model parameters including the maximum specific growth rate (μ_{max}), the interrelation of microalgal cells to light intensity (I_k) and exponent constant (n) were determined by means of non-linear least squares method with Microsoft Excel, employing experimental data obtained at a source light intensity of 90 $W \cdot m^{-2}$. The values of these equation constants were μ_{max}= 0. 0620 h^{-1}; I_k= 6. 98 $W \cdot m^{-2}$ and n=1. 16 with a regression coefficient of r^2=0. 9983. The model was then verified using the experimental results obtained at 180 $W \cdot m^{-2}$ source light intensity and used to simulate microalgal cultivation at different PBR diameters and source light intensities to provide guidelines for PBR design and process optimization.

PO-102

Track: Medical Biotechnology

EFFECT OF MATERNAL ORAL INGESTION OF ASPIRIN ON BIRTH WEIGHT OF WISTAR RATS

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Background and Objective: Aspirin, acetylsalicylic acid, is the most frequently consumed medication in pregnancy, mostly taken without prescription because of headache or a minor ailment. This experimental study was carried out to examine the influence of maternal oral ingestion of aspirin on the newborn birth weight of wistar rats.

Methods: The experimental animals were 10 albino rats consisting of 8 females and 2 males with weights ranging between 200g and 250grams. The 2 males were separated from the 8 females which were then divided into 2 groups of 4 each (A and B). Three female wistar rats were then removed from each group to form classes A and B, while the remaining 2 females formed the control class C. Each was then placed in an enclosure for 2 days with a male after which they were examined for signs of copulation. Water containing aspirin in varying doses was then administered to the rats in classes A and B on the various gestational days. Off-springs from the wistar rats were later weighed and their weights recorded.

Results: The birth weights of the off springs decreased significantly in group B and slightly in group A. The birth weights of the off springs in the control group were observed to be within the normal range of 5 and 6 grams for wistar rats.

Conclusion: Maternal ingestion of aspirin led to the production of off-springs with low birth weight, the severity depending on the dosage of aspirin administered.

Keywords: Aspirin, wistar rats, gestational days, newborn and birth weight.

PO-101

Track: Medical Biotechnology

USE OF ELASTIC SCATTERING SPECTROSCOPY FOR *EX VIVO* ANALYSIS OF NEOPLASTIC COLORECTAL TISSUE: A PILOT STUDY

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Background: Spectrometry has been increasingly used to examine biological tissues to achieve non-invasive techniques, able to differentiate healthy and neoplastic tissues, by observing electromagnetic spectra. This study focused on the development of a method for transmittance measurement of healthy and neoplastic human colonic mucosa samples by Elastic Scattering Spectroscopy.

Materials and Methods: 21 spectra from healthy and 21 from neoplastic tissue of variable thickness were investigated using a white light Xenon Lamp and an optical fiber in order to measure transmittance. Spectra were acquired using a CCD spectrometer (Edmond) connected to a computer with a specific software (BWTeck®). The average transmittance of the healthy samples was used to set *the cut off* value and to compare it with pathological findings. Hereafter, setup was improved dissecting samples at 150 µm thickness (Thermo Scientific, Microm HM 550) and analyzing them by a Perkin Elmer spectrometer (Lambda 900).

Results: Mean transmittance in the two groups was significantly different (99%). By performing Mann-Whitney test was found a P=0.0011. Sensitivity and specificity resulted respectively 87.5% and 71.4%. Improved setup shows that neoplastic specimens have a lower scattering index at every wavelength.

Conclusions: Healthy and neoplastic colonic mucosa show different spectra when analyzed by ESS.

PO-50

Track: Industrial and Manufacturing

DEVELOPMENT OF BIOCATALYTIC FILM CARRYING RECOMBINANT *PSEUDOMONAS FLUORESCENS* AS A WHOLE CELL CATALYST

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Tyrosinase is an enzyme that oxidizes L-tyrosine into L-DOPA and cross-links multiple L-DOPA into covalently bonded macromolecules. By using this pathway, we can covalently link any molecule that contains phenol moieties and manufacture a gel-like platform for multiple applications including biocatalysts. In this research, we tried to manufacture a self-assembling oil degrading biocatalyst using tyrosinase- and lipase-secreting *Pseudomonas fluorescens*. We cloned *Streptomyces* antibioticus *tyrosinase* melC2 and secretion mediator melC1 into expression vector and expressed them in *Pseudomonas fluorescens*. We also cloned a thermostable lipase with an ABC-transporter for its secretory productio. In order to determine the amount of secreted tyrosinase, we measured the specific activity of tyrosinase by purifying them with histidine affinity chromatography. Furthermore, we measured the specific activity of lipase secreted by *P. fluorescens*. Finally, we incubated tyrosinase- and lipase-secreting cells in phenol conjugated chitosan and observed the formation of a catalytic film. From the experimental results, we successfully showed that tyrosinase-secreting *P. fluorescens* have the ability to self-assemble into gels and films *via* conjugation of chitosan containing phenol moieties, and that the secreted lipase have activities enough to be applicable in making lipid-degrading films. In the future, we expect to improve film-manufacturing technology into oil-breaking catalysts applicable in large scale such as oil spillage and also broaden the application involving different catalytic activities.

Keywords: Tyrosinase, *Pseudomonas fluorescens*, biocatalyst, catechol chemistry, lipase.

PO-29

Track: Other areas

BIOLOGICAL CONTROL OF MOLD SPORE BY FATTY ACID SALTS

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Introduction Penicillium fungi and Aspergillus fungi are one of the most common contaminants introduced by accident during the production of the food. Therefore, the fungicides which have high antifungal activity and safety for human are required. In this study, antifungal activity of fatty acid salts which is main component of soap against *P. pinophilum* and *A. niger* were investigated. Material and methods Potassium butyrate(C4K), caproate(C6K), caprylate(C8K), caprate(C10K), laurate(C12K), myristate(C14K), oleate(C18:1K), linoleate(C18:2K) and linolenate(C18:3K) were used as nine fatty acid salts. All samples are 350 mM and pH 10. 5. The antifungal method, the spore suspension (3. 0Ā104 spores/mL) was mixed with sample of fatty acid potassium (final concentration of 175 mM). Results and discussion C10K was most effective, and C8K and C12K also were effective to decrease survival of *P. pinophilum*. (4 log unit). C10K completely inhibited its growth at 7 days. However, no obvious change was observed in tested fatty acid salts against *A. niger*. These results suggest C10K has great potential in the field of biological control agents. Since there is no antifungal effect on C10K against *Aspergillus niger*, it is necessary to evaluate the antifungal activity of other fatty acids and the derivatives, in future.

Keywords: Fatty acid salts, *Penicillium pinophilum*, antifungal activity.

PO-60

Track: Plant and Environment

ENVIRONMENTAL FRIENDLY PROCESSES FOR HEAVY OIL UPGRADING IN 21ST CENTURY

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Some heavy metals such as vanadium and nickel in crude oil have an adverse effect on the refinery processes and act as a poison on catalysts used in catalytic cracking, hydrogenation and hydro-desulphurization processes. Among strategies for removing metals from crude oil, chemical processes such as solvent extraction, and catalytic hydro-processing have been commonly used because of their effectiveness. These are often expensive and produce secondary pollution in the environment. Biological upgrading can be an environmental friendly alternative approach for improving of heavy oil. Microorganisms and enzymes can degrade complex molecule structure of heavy crude oils to small fragments and under specific conditions. Heavy oil bio-upgrading can be attracted much attention because those are safe, simple, environmental friendly, low cost, low waste and also need to fewer process steps to upgrade heavy oil.

This work reviews the recent trends about demetallizing of the metallic compounds from crude oil using microorganisms and biological processes.

PO-66

Track: Plant and Environment

ENHANCED PHOSPHORUS USE EFFICIENCY IN AVP-OX ROMAINE LETTUCE

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Crops produced on calcareous soils in arid regions receive large annual applications of phosphorus (P) fertilizer for optimal yield and quality. However, declining P mineral reserves, erratic fertilizer costs, and concerns about water pollution, have created incentives for improved efficiency. While we have developed management practices, such as soil and plant tissue testing and improved fertilizer placement, the possibility of genetic modifications to crops for improved fertilizer use efficiency has received little attention. Recently, it has been shown that over-expression of type I H+-pyrophosphatase AVP1 (AVP, *Arabidopsis* vacuolar pyrophosphatase) contribute positively to many plant energetic processes including general growth, nutrient acquisition, and stress response. This genetic modification enhances nutrient uptake by affecting the abundance and activity of the plasma membrane H+-ATPase in a manner that correlates with apoplastic pH alterations and rhizosphere acidification. The objective of this project was to evaluate the potential for using AVP1 overexpression (AVP1-OX) modified romaine lettuce (*Lactuca sativa*) for improved P use efficiency under arid-land cropping systems. AVP1-OX romaine lettuce displayed enhanced rhizosphere acidification and larger shoot and root growth compared to controls under P limitations in the growth chamber. Greenhouse and field production data show that AVP1-OX romaine lettuce outperformed conventional romaine lettuce at all levels of P.

PO-11

Track: Plant and Environment

SITE SPECIFIC WIRING OF REDOX ENZYMES FOR THEIR USE IN BIOFUEL CELLS

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Enzyme based biofuel cells suffer from poor electron transfer rates between the catalyst's active site and the electrode surface. Our approach solves this problem by 'wiring' the enzyme site-specifically to the electrode by using unnatural amino acids (UAA) incorporation technology. This way we want to achieve direct electron transfer (DET) from the electrode surface to the enzyme's active site, rendering a soluble mediator, redundant. The redox enzyme chosen, CueO from *E. coli*, was cloned into a pET vector and overexpressed in *E. coli* BL21. Experiments were done to optimize the expression of the enzyme to achieve high yields. We successfully incorporated the UAA p-azido-l-phenylalanine and propargyl-l-lysine into the protein in several positions close to the enzyme active site. The optimization of the UAA position and the subsequent attachment of a linker will allow good communication between the electrode and the active site leading to efficient DET. The oriented site-specific wiring onto gold coated electrodes was done using azide-alkyne Cu(I) catalyzed Huisgen cycloaddition and a short 'wire' molecule. The generation of the Cu(I) ions was done using several methods, including a novel approach, to electrochemically reduce Cu(II) to Cu(I). In the future we will further investigate and characterize this system in order to optimize the electron transfer pathway. The improved electron transfer rates from the electrode to the enzyme may improve the power output and efficiency of the system, and create a new generation of biofuel cells as well as other bioelectronic devices.

Keywords: Biofuel cells, click reaction, site-specific wiring, unnatural amino acids, UAAs.

PO-83

Track: Plant and Environment

MORPHO-BIOPHYSIOCHEMICAL ASSESSMENT OF SALINITY TOLERANCE IN SUGARCANE

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Sugarcane is a typical glycophyte exhibiting stunted growth or no growth under salinity and reducing yield. Salinity which affect crop productivity in one or more of the ways by adversely affecting yield per unit area and time, by eliminating some of the outstanding varieties from commercial cultivation. Salinity is a limiting factor for harnessing the yield potential of varieties. Besides soil reclamation measures, it is important to develop varieties that has inherent capacity to tolerate saline soil and minimize the yield penalty. Ten varieties of sugarcane (*Saccharum officinarum* L.) were screened for their salinity stress tolerance in the experiment. The experimental soil is sandy loam with initial pH 6. 8 and ECe (Electrical Conductivity of the extract of a saturated soil paste) is 1. 39 dSm-1. We create level of salinity, 8 dSm- by adding desired amount of NaCl, mixed thoroughly into the soil before filling in pot. Each germplasm was potted in normal (without salt application) and ECe 8 dSm-1 (pre-maintained salt) in three replication. The experiment was performed in complete randomized (factorial) design (CRD) consisting of 3 replications of each variety in both normal (1. 39 dSm-1) and saline (8 dSm-1). During formative phase (60-150 days of crop age), biochemical parameter like chlorophyll content, proline content, memebrane stabitllity index and Relative water content were determined for selected genotypes. In the salt stressed leaves of all genotypes decreased significantly, but the extent of decrease was variable among different genotypes. Proline content in salt stressed plantlets of all sugarcane genotypes increased markedly, except in genotypes Co 419, Co 85036, Co 7704 and Co 775. Analysis of variance showed significant differences for five yield related traits among the nine varieties under control and salinity treatments. The results indicated that varieties Co 99004, Co 87002 and Co 94010 were the most tolerant while varieties Co 419, Co 85036 and Co 7704 were the most sensitive ones.

Keywords: Salinity, sugarcane, abiotic stress, saline treatment.

PO-82

Track: Plant and Environment

EFFECT OF DIFFERENT PHYTOHORMONE REGIME AND pH ON MICRO PROPAGATION OF THREE SUGARCANE VERIETIES

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Micro propagation is currently the only realistic means of achieving rapid, large- scale production of disease-free quality planting material as seed canes of newly developed varieties in order to speed up the breeding and commercialization process in sugarcane Sugarcane (*Saccharum* spp. Complex) is the most valuable commercial crop in the world. It holds not only sugarcane and distillery industries but also a key position in the international economy by earning foreign exchanges. The contemporary sugar industry plays many significant roles in relation to food, energy and economic security. Present investigation deals with the effect of different phytohormone combinations on *in vitro* growth responses of shoot tip explants of sugarcane varieties CoS 99259, CoS 98259 and CoS 96258. Shoot tip explants are suitable explants for shoot regeneration via axilary bud for *in vitro* micropropagation of sugarcane varieties. In the present study high frequency in induction of shoot & shoot regeneration from axilary bud formation was recorded in explants achieved on MS medium supplemented with BAP, Kinetin or NAA in different concentration. The micropropagated shoots of sugarcane were successfully rooted on half strength MS liquid medium containing NAA (5. 0 mg/l) and sucrose (50 mg/l). Study for perfect chemical conditions to develop efficient protocol was also done best results were obtained at pH 6. 0 in both the varieties.

Keywords: Callus culture, sugarcane, shoot induction, root induction, *in vitro* micropropagation.

PO-26

Track: Plant and Environment

METHYLMERCURY INDUCED REGION-SPECIFIC PROTEIN CHANGES IN THE MARMOSET MONKEY BRAIN

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Methylmercury (MeHg) is a well-known neurotoxin; however, the molecular mechanisms of its target to the nervous system are unclear. The aim of this study is to identify and analyze the proteome changes in the four regions (cerebellum(C), frontal lobe(F), occipital lobe(O) and thalamus(T)) of the marmoset brain that are induced by MeHg using proteomic techniques. 102 analytes in cerebellum, 62, 89 and 67 in Frontal lobe, Occipital lobe and Thalamus were found with significant abundance changes. Functional analysis of these proteins revealed the important categories in the four regions of brain which respond to MeHg toxicity. The categorized proteins were then integrated into the network of protein and protein interactions to obtain a systematic view of biological process of the changed proteome induced by MeHg in each of the brain region. The four regions of the brain displayed the differential functional categories exposed to MeHg. Compared to the other 3 regions, the most variance in affected cellular functions and toxicity pathways were found in the cerebellum. This study presents a novel view of MeHg toxicity in functional classifications by comparison of the different regions of marmoset brain, and provides the insights into the molecular changes and targets in functional studies of MeHg neurotoxicity.

Keywords: Methylmercury; Shotgun proteomics; Neurotoxicity; Marmoset Monkey.

PO-68

Track: Industrial and Manufacturing

PRODUCTION AND APPLICATION OF THERMOSTABLE LACCASE AND XYLANASE IN PULP BIOBLEACHING

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Enzymatic delignification of pulp provides an environment-friendly bleaching strategy. However, the costs of the enzymes to be used for biobleaching need to be reduced, and the enzyme activities need to be higher to make the process feasible. Thermostable laccase and xylanase have advantages in their higher reaction rates and longer life time at the elevated temperatures occurring in pulp-bleaching process. Thermostable laccase gene of *Thermus thermophilus* and xylanase B gene of *Thermotoga maritime* were cloned and overexpressed in *Escherichia coli*. In gene expression system pHsh, the expression of target gene is under control of an alternative Sigma factor, Sigma 32 of *E. coli*, which not only eliminates the cost for a chemical inducer such as IPTG, but also activates chaperon expression and allows host cells to grow to high densities. By using pHsh vectors, the expression levels of thermostable laccase and xylanase reached 52% and 32% of total soluble protein of *E. coli*. The recombinant laccase and xylanase were easily purified by a step of heat treatment and applied to biobleaching tests over wheat straw pulp. The optimal biobleaching process includes treating 10% wheat straw pulp at 85°C, pH 4. 8 for 120 min with 10 U xylanase or 5 U laccase per gram of dry pulp. Compared with conventional straw pulp bleaching process, the treatment of thermostable laccase or xylanase can reduce the use of sodium hydroxide and the produce of alkaline waste water, save over 25% of hydrogen peroxide or chlorine dioxide, and increase the strength of paper as well. The combination of the laccase and xylanase gave higher brightness of wheat straw pulp. Therefore, the biobleaching of pulp by using thermostable laccase and xylanase can be economically beneficial to paper industry.

Keywords: Over-expression; pulp biobleaching; thermostable enzymes.

PO-78

Track: Medical Biotechnology

EXPRESSION OF CagP FROM *HELICOBACTER PYLORI* AND PRELIMINARY STUDY OF ITS BIOLOGICAL FUNCTION

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Helicoabacter pylori (*H. pylori*), a kind of bacteria colonization in the stomach, causes gastric diseases and mucosal injury through secretion of virulence factors (CagA) and special structrue. Previous studies have indicated that virulence factors of *H. pylori* (CagA and so on) are delivered into host cells via the type IV secretion system (T4SS). T4SS is a multiple tunnel-like structure that is consisted of 14 structural proteins, various chaperonins and some unknown proteins. The T4SS, located on the bacteria membrane (including inner and outer membrane), has the major function of translocation of virulence factors, signal transduction and induction of IL-8. At present, although the characterization of the structural proteins and chaperonins has been identified, the characterization of some proteins remains unclear. This study is aimed at the T4SS protein CagP and focused on the subcellular localization, function, interaction, mechanism in the process of reconizing and delivering virulence factors and involvement in the *H. pylori* pathogenicity and biofilm formation. All of these results will shed light on the biological properties, the mechanism of recognizing and delivering virulence factors of T4SS. Meanwhile, this study might explore mechanism of pathogenicity in *H. pylori*.

PO-74

Track: Plant and Environment

MICROBIAL DIVERSITIES AND POTENTIAL APPLICATION OF ENHANCED OIL RECOVERY IN A LOW TEMPERATURE HEAVY OIL RESERVOIR

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Indigenous microbes in the injection well of T6186 and the production well of T6073 from heavy crude oil reservoir in Xinjiang were analyzed. Dominant microbes in the samples from the production well of T6073 and the injection well of T6186 was uncultured Desulfobacterales (GQ354918, 66. 4%) and uncultured bacteria (AY327241, 47%). Particularly, potential microbes for microbial enhanced oil recovery (MEOR) were *Pseudomonas stutzeri*, spirochete, Bacteroides, Azoarcus, Desulfobium, Desulfuromonas, Desulfobacterium, among which *Pseudomonas stutzeri* and Desulfobium were introduced into the oil reservoir and identified as exogenous. Microbial communities in the enrichments with and without molasses were also analyzed. Microbes in the enrichment with molasses (1. 4%) showed abilities of profile control and oil flooding. While microbes in the enrichment without molasses showed abilities of biosurfactant producing and desulfuration. Strains that produced peptide biosurfactant were isolated. The strains degraded and emulsified the crude oil from the well of T6191 with a decrease of oil viscosity of 30%. The strains were identified as *Bacillus brevis*, *Bacillus cereus* and *Bacillus licheniformis*. When the isolated strains were cocultured together, the surface tension of the enrichment was reduced to 25mN/m. Crude oil before and after microbial treatment were analyzed through Chromatography and Mass Spectroscopy. Obtained results indicated that the relative abundances of saturated hydrocarbons and asphaltene increased, while the relative abundances of aromatics and resin decreased. Biosurfactant-producing bacteria stimulated the growth of microbial groups to reduce the viscosity of crude oil and make crude oil move smoothly. All results showed that activating indigenous microbial enhanced oil recovery will have great potential application in the heavy oil reservoir.

Keywords: Indigenous microbial communities, microbial enhanced oil recovery, *Pseudomonas stutzeri*, heavy oil reservoir, viscosity reducing.

PO-43

Track: Other Areas

GRAPHENE OXIDE-BASED BIOPOLYMER COMPOSITES SYNTHESIZED IN SUPER-CRITICAL CO_2 AND THEIR ADSORPTION BEHAVIOR

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Graphene oxide (GO) was functionalized covalently with a biopolymer, poly(2-hydroxyethyl) methacrylate (HEMA), by dispersion polymerization in supercritical carbon dioxide system. The structure of GO-PHEMA composite was characterized by Fourier transform infrared spectroscopy, X-ray diffraction, scanning electron microscopy, energy-dispersive x-ray spectrometer and thermogravimetric analyses. The adsorption behavior of the composite to methylene blue (MB) organic dye was observed through UV-visible spectrum, where the effect of adsorbent dosage, pH, contact time, and dye concentration were investigated. Adsorption parameters and kinetics showed that the adsorption data were fit well into the Freundlich adsorption isotherm and followed a pseudo-second order reaction, with the correlation coefficient (R^2) of 0. 991. The maximum adsorption capacity was predicted to 31. 2 mg of dye per gram of adsorbent. Experimental results indicate that the prepared composite can remove 99. 8% of dye under the optimum operation condition. Further biomedical applications are also being investigated.

Keywords: Graphene oxide-Biopolymer composite, PHEMA, Bioadsorption, Supercritical CO_2.

PO-89

Track: Other Areas

NEW ORIGINAL ANTICONVULSANT ALTERS THE PROPERTIES OF BENZODIAZEPINE RECEPTORS "CENTRAL" AND "PERIPHERAL" TYPES IN BRAIN CORTEX OF "HEAVY DRINK" RATS

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Purpose: Alcohol abuse induces neuroadaptive alterations of benzodiazepine receptors (BDR), that modulate GABAAR, and GABA mediation in brain regions, associated with reward function in the brain, that serve alcohol addictions. Studying the effects of drugs that have modulatory effects on neuronal receptors, in particular the GABAA-benzodiazepine receptors, can be the basis for understanding the formation of alcohol motivation and addiction, to develop new approaches to the treatment of this disease.

Materials and methods: Experimental animals - Wistar rats (n = 250) used in the experimental model of alcoholism. Properties of BDR "synaptosomal" and "mitochondrial" types were examined in respective membrane fractions obtained from brain cortex of rats with experimental alcoholism and treating of new anticonvulsant meta-chloro-benzhydrylurea (m-hBGU) by radioreceptor assay (RRA) with [^{3}H]flunitrazepam and [^{3}H]Ro5-4864.

Results: As a result of screening in terms of consumption of 15% alcohol and water in the rat were divided into 3 major groups of animals. 1st group consisted of rats preferred to ethanol in testing - "heavy drink" rats under the terms of the experiment were chronic alcoholism (15% alcohol as the sole source of drinking for 10 months); 2nd group consisted of rats preferred to ethanol - "non-heavy drink" male contained no access to the entire period of ethanol; third group - the rats, "non-prefer" alcohol rats – contained in the water. Parts of animals in each group were administered m-hBGU, 100 mg / kg for 14 days. Introduction of m-hBGU rats caused a significant decrease in alcohol consumption in animals preferring alcohol (1st and 2nd groups) by 82. 7% from baseline and 75% from baseline, respectively ($p < 0.05$). On alcohol consumption by rats prefer water, m-hBGM no effect.

Reducing alcohol consumption by rats occurred on 2-3rd day receiving m-hBGU and persisted as a backdrop on the drug, and after its cancellation within 2 weeks of observation. Thus, the introduction of m-hBGU resulted in a significant reduction of ethanol intake animals long who preferred the water ethanol. Comparative study of kinetic parameters (Kd and Bmax) of [^{3}H]flunitrazepam and [^{3}H]Ro5-4864 binding with synaptosomal ("central" type) and mitochondrial ("peripheral" type) membranes showed that properties of BDR in membranes from brain cortex of male rats with different preference to alcohol and showed that affinity of [^{3}H]flunitrazepam and [^{3}H]Ro5-4864 binding with membranes was decreased, but capacity of receptors was increased in brain cortex of "heavy drink" and "non-heavy drink" male rats compared with "non-prefer" alcohol rats. Administration of anticonvulsant m-hBGU increased affinity of BDR in brain cortex of "heavy drink" rats, that induced mediation of GABA in brain of these rats and reduced alcohol consumptions.

Conclusions: our study revealed that new anticonvulsant m-chBHU has a normalizing effect on GABAA - receptor function in "heavy drink" rats, and reduced alcohol consumptions in these animals.

PO-18

Track: Regenerative Medicine

NATIVE TENDON SLICES USED AS THE TISSUE ENGINEERED SCAFFOLD FOR LIGAMENT REPAIR

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Functional restoration of the injured ligaments is still a great challenge clinically. Tissue engineered approaches provide the possibility to enhance ligament healing and regenerate ligament tissue. Ideal scaffold for tissue engineered ligament would possess the mechanical properties similar to the normal ligament, the environment for good cell viability throughout the matrix, improvement of collagen synthesis for ligament regeneration, and compatibility with surrounding tissue. Various biological and synthetic materials have been used for the scaffolds of tissue engineered ligament. Successful application of these scaffolds is still limited. In this presentation, canine infraspinatus tendons were sectioned in longitudinal slices with a thickness of 50 micrometer. The mechanical properties of the slices were measured. The gene expression of bone marrow stromal cells (BMSC), which had been seeded on the decellularized slices, was investigated. The engineered composite of multilayer acellular tendon slices seeded with BMSC was used to repair a rabbit patellar tendon defect. It was found tendon slice had stronger mechanical properties than normal collagen constructs, and stimulated BMSC to synthesize tenomodulin, a biomarker of tendon and ligament. The cells in the engineered composite of multilayer tendon slices could survive *in vivo* and express a ligament phonotype. We conclude that native tendon slices can be used as the tissue engineered scaffold for ligament repair.

Keywords: Tissue Engineered Scaffold, Ligament Repair, Native Tendon Slices.

PO-12

Track: Plant and Environment

ENGINEERING OF MICROORGANISMS FOR FULL FUEL OXIDATION IN HYBRID BIOFUEL CELLS

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Biofuel cells are electrochemical devices which convert chemical energy to electrical energy using redox enzymes as catalysts. Microbial fuel cells (MFCs) are biofuel cells which use microorganisms that can catalyze the release of electrons from organic matter and transfer them to various electron carriers that are electrochemically active. In enzymatic biofuel cells, purified enzymes are used as catalysts.

Yeast surface display (YSD) is a powerful tool for engineering the affinity, specificity, and stability of proteins. Recently, we were able to show that redox enzymes displayed on the surface of yeast in an anode compartment of a MFC improved its performance, since membrane transport limitations are overcome. In this type of hybrid microbial-enzymatic biofuel cell, further oxidation of the fuel performed by the microorganisms generated power output as well, but with significantly lower efficiency, due to lower electron density and non-efficient electron transport pathway. Complete oxidation of the fuel can be achieved by expression of a series of enzymes, creating a cascade that mimics the full oxidation of the fuel to carbon dioxide in a biochemical pathway.

In order to display a cascade on the surface, a proteinogenic scaffold will be expressed on the cell"s surface coupled to separately expressed redox enzymes, each expressed scaffold containing a unique linker fitting a specific site on one redox enzyme, dictating its correct location in the enzymatic cascade.

Keywords: Hybrid biofuel cells, Yeast surface dispaly.

PO-56

Track: Other Area: Food

THE DEGRADATION OF MILK PROTEIN AND FAT WITH THE USE OF YARROWIA LIPOLYTICA HYDROLASES

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The aim of the study was the application of noncommercial hydrolases isolated from yeast *Yarrowia lipolytica,* originating from mould cheese, for degradation of milk protein and fat. The enzymes were isolated from submerged culture of yeast, conducted in bioreactor in medium enriched with casein and fat industry waste products at pH 7. 5.

After 48-hours the culture was centrifuged at 4^{o}C. The resulted supernatant, containing extracellular enzymes, was concentrated with the use of 18 kDa membrane, while the obtained biomass after washing with Sorensen buffer pH 7. 0, was sonificated at 4^{o}C for 15 minutes for isolation of intracellular hydrolases. The obtained yeast enzyme preparations were as follows: extracellular (1) and intracellular (2). Also third Y. lipolytica enzyme preparation was prepared as an enzymatic cocktail (3) – by mixing preparation (1) and (2) at the ratio 1:1 (v:v).

The proteolytic activity of enzymes was determined against casein, the lipolytc activity was measured against butyrate-pNP. The intracellular proteolitic activities were analyzed as the aminopeptidase activity against Leu-pNA, carboxypeptidase activity against Z-Glu-Tyr and di- and tri-peptidase activities against peptide substrates: Ala-Pro and Ala-Gly-Gly, respectively.

All enzyme preparations were used for degradation of calcium paracaseinate, containing 7,0% of protein and 17,5 % of fat. The degradation was conducted for 48h at 35^{o}C. The proteolysis was monitored by the determinations of water soluble nitrogen, free amino groups content (FAG; fractions soluble in PTA and in water) and by the RP-HPLC and electrophoretically. The degradation of milk fat was analyzed by the determination of fee fatty acids release on GC. Also analysis of volatile compounds in hydrolysates was performed on GC/MS after their extraction with the use of SPME.

The higher proteolytic changes of paracaseinate were observed in samples degraded with the extracellular enzyme preparation (1) than with intracellular enzymes (2). After 48h of reaction the concentration of FAG in analyzed samples were 9718 ĀM Gly/100g and 3425 ĀM Gly/100g, respectively. Combining of both enzyme preparations extra- and intracellular (3) significantly influenced the level of protein degradation expressed by the concentration of peptides and free amino acids.

The use of enzymatic cocktail (3) brought up more intensive lipolysis of milk fat, causing release of 9 463mg FFA/100g. Comparing to activity of intracellular preparation (2) it was *ca.* two times higher, while to extracellular (1) only 20%. The use of yeast enzymatic cocktail (3) to degradation of milk components caused also the increase of volatile compounds content, among which short-and middle-chain fatty acids predominated.

Acknowledgment

This work was supported by reasearch grant POIG. 01. 03. 01-02-080/12, co-financed by the European Union from the European Regional Development Found.

Keywords: Yeast, Yarrowia lioplytica, hydrolytic enzymes, milk and fat degradation.

PO-55

Track: Pharmaceutical Biotechnology

THE EFFECT OF STORAGE CONDITIONS ON THE STABILITY OF BIOLOGICAL ACTIVITY OF MILK PROTEIN HYDROLYSATES

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The aim of the study was to evaluate stability of biological activities of milk proteins hydrolyzates during storage. The hydrolyzates of whey protein concentrate (WPC 80) and sodium caseinate were produced with noncommercial proteolytic enzymes: serine protease isolated from pumpkin *Cucurbita ficifolia* and serine extracellular protease isolated from submerged culture of yeast *Yarrowia lipolytica*. The hydrolysis of substrates was carried out at temp. 37^{o} C and pH 8. 0 for 24 h. The extent of their proteolysis level was monitored by the determination of the hydrolysis degree (DH), free amino groups release and by the RP-HPLC chromatography. In obtained protein hydrolysates following biological activities were determined: antioxidant activity as a free radicals DPPH scavenging activity, power reduction of Fe (III) and chelating Fe (II) activities, antidiabetic activity expressed as inhibitory activity against dipeptidyl peptidase (DPP-IV) and Ä-glucosidase. Also angiotensin converting enzyme (ACE) inhibitory activity in hydrolyzates was investigated.

The effect of cooling storage at temp. 4°C (of sterilized hydrolysates by tyndallization), freezing storage at temp -20°C and freeze drying on biological activity of milk protein hydrolysates was evaluated. The stability of all these biological activities were controlled after 0, 2, 4, 6 months.

It was shown that hydrolyzates obtained after sodium caseinate and whey proteins degradation with serine proteases of plant and yeast origin displayed significant biological activities. The WPC 80 hydrolysate degraded with *Yarrowia lipolytica* serine protease exhibited especially high antioxidant properties. Its free radicals DPPH scavenging activity was 0. 76 ÃM Trolox/mg, while its reduction power of Fe (III) activity reached the level of 43. 7 Ãg Fe^{3+}/mg. Sodium caseinate hydrolized with *Cucurbita ficifolia* serine protease appeared to reveal high inhibitory activities against ACE (IC_{50}=8. 41 mg/ml), DPP-IV (IC_{50}=10. 67 mg/ml) and Ä-glucosidase (IC_{50}=16. 34 mg/ml).

The obtained results showed that storage of hydrolyzates of both milk proteins degraded with noncommercial proteases, indepent on method of preservation and conditions, did not cause significant changes in their bioactivities ($p<0.05$), even during 6 months.

Acknowledgment

This work was financially supported by the National Science Center. Project no 2011/01/B/NZ9/04297

Keywords: Noncommercial enzymes, milk protein hydrolyzates, bioactive peptides, storage stability.

PO-40

Track: Pharmaceutical Biotechnology

APPLICATION STUDIES ON SYNTHESIS OF ENANTIOPURE ALCOHOLS BY ETHYLBENZENE DEHYDROGENASE

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Nowadays chiral alcohols are one of the most important and valuable synthons for the production of various biologically active compounds i. e. pharmaceuticals, agrochemicals or flavor compounds. In our studies we focus on possibility of their biotechnological production via enantioselectivity oxidation of hydrocarbons by ethylbenzene dehydrogenase.

Ethylbenzene dehydrogenase (EBDH) is a bacterial enzyme coming from denitrifying bacterium

Aromatoleum aromaticum (Azoarcus sp.) where it is involved in the anaerobic mineralization of ethylbenzene. This molybdenum/iron-sulfur/heme protein catalyzes hydroxylation of ethylbenzene to (S)-1-phenylethanol with 100% enantioselectivity.

Our previous studies have shown that EBDH catalyzes stereospecific synthesis of alkylaromatic and alkylheterocyclic secondary chiral alcohols [1, 2]. More than 30 hydrocarbon substrates are oxidized to the alcohols with 100% enantiomeric selectivity. For this reason, EBDH seems to be an effective biocatalyst for the synthesis of the optically pure compounds valuable for fine-chemical pharmaceutical industry.

To meet requirements of industrial application, it is necessary to improve enzyme economic feasibility in technological processes. Immobilization of the enzymes on solid supports is a very effective way to increase enzyme stability and operational lifetime. Moreover, it facilitates the separation of enzymes from reaction media, hence the recovery and purification of the final products from the reaction mixture becomes simpler and more efficient.

In the presented study EBDH was covalently immobilized onto functionalized cellulose support (Granocel type). The immobilization procedure and reaction conditions were optimized. Additionally, the impact of enzyme's purity and aerobic/anaerobic conditions on storage stability of the homogenous EBDH was determined. As a result it was possible to extend the effective enzyme activity in reaction conditions from 4 hours to more than 10 days.

Keywords: Ethylbenzene dehydrogenase, chiral alcohols, immobilization

Acknowledgements

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Mateusz Tataruch acknowledges the financial support from the project Interdisciplinary PhD Studies "Molecular sciences for medicine" (co-financed by the European Social Fund within the Human Capital Operational Program).

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PO- 99

Track: *Plant and Environment*

A COMPARATIVE ANALYSIS OF PAH BIOREMEDIATION BY LIGNINOLYTIC WHITE ROT FUNGI

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White rot fungi responsible for extensive degradation of the complex natural recalcitrant polymer, lignin, are amongst the most studied microorganisms for the degradation of polyaromatic hydrocarbons (PAH). Ligninolytic and PAH degradation activities of selected white rot fungi indigenous to a Zimbabwean forest were evaluated against the most studied model white rot fungus *Phaneroachaete chrysosporium (BKM-F-1767 ATCC 24725)*. Evaluation of the ligninolytic activities showed that the isolates *T. versicolor, T. cingulata, T. pocas* and *DSPM95* produced laccases and manganese peroxidase and no lignin peroxidase activity while *P. Chrysosporium* produced all the three ligninolytic enzymes. From the biodegradation studies of representative PAHs: fluorene, phenantherene, anthracene, pyrene and Benzo(a)anthracene in static batch cultures, *T. versicolor, T. pocas* and *DSPM95* could degrade more fluorene and phenanthrene than *P. chrysosporium*. Anthracene degradation was in the order *DSPM95>P. chrysosporium>T. pocas> T.versicolor >T. cingulata*. Exclusion of fluorene and phenanthrene from the PAH mixture resulted in increased degradation for higher molecular weight pyrene and benzo(a)anthracene. GC-MS evaluation of the degradation showed that most metabolites formed were progressively degraded by day 31 except for *P. chrysosporium* where the main metabolite remained undegraded. No metabolites were accumulated in *T. cingulata* cultures as compared to the other fungi.

PO-94

Track: Plant & Environment

EVALUATION OF VICTORIAN MARINE ISOLATE *SCHIZOCHYTRIUM SP.* DT9 FOR BIODIESEL PRODUCTION

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Microalgae are of interest as a renewable source of biofuels. Algal biofuels offer advantages over first and second generation fuels, since they overcome the food *versus* fuel concerns. Thraustochytrids, which are members of the phytoplankton group, are potentially useful for the production of omega-3 fatty acids, carotenoid and biofuel. We explored local Victorian (Barwon water, Australia) marine environments and isolated thraustochytrids with high lipid contents. In this study the new *Schizochytrium* sp. DT9 was explored for its suitability for biodiesel production. High yield of biomass and lipid were achieved with the use of glycerol as a carbon source in the fermentation medium. Direct trans-esterification using a single step process, followed by extraction of lipid was compared with two-step trans-esterification. The single step process requires less solvent than the two-step process and so is the preferable method. We investigated the impact of different solvents and catalysts during trans-esterification for direct trans-esterification method. After optimisation the single step process gave a high oil yield that was comparable with that obtained for the two-step process.

Keywords: Thraustochytrids, Biofuel, microalgae, trans-esterification

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PO-2

Track: Plant and Environment

PROTEOMIC PROFILE OF THE DANDELION (TARAXACUM OFFICINALE) POLLEN

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Pollen of various plant species has different content and amount of proteins. Unlike some other flowering plants dandelion's pollen is an inferior food source because they are missing some essential amino acids. In principal the proteome of dandelion pollen is not identified and here we present a comprehensive analysis of the proteins from hand- and bees-collected dandelion Taraxacum officinale pollen. Total proteins from pollen grains were isolated using the method described by Sheoran (Sheoran *et al*., Sex Plant Reprod, 2006) with some modification. Isolated proteins were fractionated by two-dimensional electrophoresis (2DE) and visualized by staining with Colloidal Coomassie G-250. Computer assisted analysis was performed for comparison of protein profile maps typical for hand- and bee-collected dandelion pollen.

Proteins from hand-collected pollen after tryptic-digestion in solution were supplied for mass spectrometry analysis by 4000 QTRAP (AB Sciex, Framingham, USA). It was identified over one thousand proteins. Among the identified proteins we could distinguish several groups agreeably their possible cellular function: i) enzymes important for metabolic reactions and other catalytic cellular processes (glucose 6 phosphate isomerase, NADPH dehydrogenase, malate dehydrogenase, fructokinase etc.), ii) proteins involved in cellular processes of translation (eukaryotic translation initiation factor 5A, elongation factor Tu chloroplastic, etc.); iii) chromatin structure (histones H4, H2B fragments); iv) structural proteins (actin, tubulin etc.); v) energy related proteins (glyceraldehyde 3-P dehydrogenase, pyrophosphate energized vacuolar membrane proton pump, putative calcium transporting ATPase 7 plasma membrane, etc.); vi) signaling proteins (calmodulin, Ras related protein Rab 2, Serine threonine protein phosphatase 2A, mitogen activated protein kinase, etc.). Computer assisting methods were adjusted for combining results obtained with mass spectrometry and fractionated proteins in 2DE system. To our knowledge this proteomic study is the first comprehensive analysis by comparison the protein profiles characteristic to hand- and bee-collected dandelion pollen.

Keywords: Dandelion pollen, protein analysis

Acknowledgement

This research was funded by a grant (No. SVE-01/2012) from the Research Council of Lithuania.

PO-91

Track: Other areas

SCREENING OF NOVEL FUNGUS ISOLATED THE SOIL CONTAMINATED AUTOMOTIVE LUBRICANTS FOR HYDROCARBON DEGRADATION POTENTIAL

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In the present study it has been found potential abilities for bioremediation and biosurfactant production the fungus isolated from the soil in Ribeirão Preto County, São Paulo State (Brazil), at 21°06"42. 80"S 47°49"0. 34"W. After sequencing of 18S ribosomal RNA gene partial, we submitted the sequence in NCBI / BLAST showed similarity with uncultured fungus clone not described in the literature. The biomass equivalent to 0. 0064 g dry weight was inoculated in the following treatments in medium minimal. The ability for using as carbon sources: 40; 50; 60; 70; 75; 80 and 85% of diesel S10, 5% of kerosene, 4% of thinner solvent and 4% acetone measured by dry weight showed: 0. 7918; 0. 07508; 0. 8518; 0. 7638; 0. 8238; 0. 8326; 1. 113 g for diesel S10 respectively, 0. 14 g for kerosene, 0. 023 g for thinner solvent and 0. 32 g for acetone. Fungal Biomass was inoculated on sheep blood agar plate and positive strain caused lysis of the blood cells showing a colorless transparent ring around the colony. Hemolysis can also be show with purified biosurfactant. These results suggest that the novel fungus can be for used for the bioremediation of soils polluted by such compounds, treatment of oily residues of petroleum refineries and production biosurfactant potential. Financial Support: FAPESP.

Keywords: Alkane hydroxylase, alkB, Soils polluted.

PO-77

Track: Medical Biotechnology

CHARACTERIZATION OF CagQ IN CAG PATHOGENICITY ISLAND OF *HELICOBACTER PYLORI*

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Helicobacter pylori (*H. pylori*), one of the most pathogens in gastric disease, is estimated to inhabit at least half of the world's human population. Cytotoxin-associated (CagA) is one of the most of the important virulent factors of *H. pylori* and delivered from the bacterium into the cytoplasm of the bacterium-attached gastric epithelial cell via the type IV secretion system (T4SS) that encoded by the Cag pathogenicity island (Cag-PAI). T4SS of *H. pylori* is composed of structural proteins and chaperones. There are some chaperones, which is common in the type III secretion system. The role of these proteins in T4SS is unclear. Therefore, this study is aimed at CagQ from T4SS chaperonins. We will focus on their subcellular localization, interaction and interaction with T4SS, CagA by molecular biology and immunology. We will also explore *H. pylori* pathogenicity *in vivo* and *in vitro*. All of these results will shed light on the role of the chaperonins in secretion of the effector protein in T4SS, the foundation to perfect T4SS structure and explore mechanism of pathogenicity in *H. pylori*.

PO-81

Track: Business Development

GLOBAL COMPARISON OF SEASONAL INFLUENZA VACCINE COST-EFFECTIVENESS IN LOW AND HIGH INCOME COUNTRIES

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Background:

Influenza-like illness accounts for a large portion of morbidity, mortality, and economic burden worldwide, and the use of influenza vaccine is considered expanding in both developed and developing countries. However, strategies on whom to vaccinate and what the proportions to be applied to different age-groups in different countries is poorly understood.

Objective:

In this chapter I aim to determine if seasonal influenza vaccination is cost-effective in different low and high income countries and how different it is between different age and high risk groups.

Methods:

I employed a compartmental epidemic model with the compartments susceptible, infectious, asymptomatic, recovered, hospitalized and dead. The epidemic model integrates the effects of vaccination every year. The models were age-structured with six age groups and high and low risk individuals. I embedded the epidemic model in an epidemic-economic model to calculate the total costs. The cost-effectiveness analysis look not only at the direct medical cost savings and averted illness, but also at the effects on broader economic impacts including labor productivity.

Preliminary results:

The results show that low and high income countries benefit the most from strategies that vaccinate children at high risk. These strategies appear cost-saving for seasonal influenza in low and high income countries.

Keywords: flu vaccine, cost-effectiveness, global comparison.

PO-42

Track: Medical Biotechnology

CONNECTION BETWEEN ZINC, HEDGEHOG SIGNALING AND AUTISM

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Recent research estimates autism spectrum disorders (ASD) costs ~137 billion on US economy annually. However, the etiology in ASD is poorly understood, with no direct diagnostic test or cure so far. Low zinc status in autism is well established. However, the biological function of zinc in ASD remains largely unknown. In humans, Hedgehog (Hh) pathway has the pleiotropic influences in many neurodevelopmental and brain diseases. Recently, serum levels of Hh protein were found to be significantly increased in children with ASD and the levels of Hh were positively correlated with the severity of autism. However, how Hh signaling is mechanistically connected to ASD is completely unclear.

In this study, the effect of zinc on the auto-processing of Hh precursor was examined and we found that Zn2+ inhibits Hh autoprocessing by NMR spectroscopy and thermodynamic study. Related in cell studies are ongoing as well. Our study suggests Hh could be a new pathway in ASD pathogenesis. Zinc-Hh interaction may provide a useful starting point for understanding how genetic and environmental risk factors in ASD converge to affect the same signaling systems at critical times of development, which could not only help develop a novel therapeutic intervention for neuronal protection in ASD, but may also serve as an early biomarker to detect ASD.

Keywords: Autism, Hedgehog signaling, Zinc, Biomarker.

PO-79

Track: Plant & Environment

ISOLATION AND CHARACTERIZATION OF A *BACILLUS CEREUS* COLD-ACTIVE BATERIOPHAGE

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Cold-active bateriophage is one of the most important biological factors to maintain the balance of the glacier ecosystem. A culturable Bacillus cereus cold-active bateriophage MYBP22 was isolated from Mingyong glacier. The MYBP22 constituted like "bullet" type head and can not bend the short straight tail，and there are 10 to 12 sub-rod-like object in the neck is mainly characterized. The head showed a polyhedral stereoscopic symmetrical structure with the size of 59nm Ā 32nm. The tail is divided into two sections formed a fine thick “spike-like” structure, length of about 43nm. The MYBP22 belongs to Podoviridae. MYBP22 with infection activity at 4^{o}C, among 4~37^{o}C can form plaques, and with good infection activity under 4^{o}C ； The optimum temperature of infection is 20^{o}C ， the optimal infection pH is 8, the optimum multiplicity of infection of 0. 1，and is sensitive to the chloroform. The capsid protein analysis showed that MYBP22 with high content of the main strips at 52 KDa, speculated that it might is the main structural protein. In addition, complete genome sequencing showed that the MYBP22 genome is double-stranded DNA, the size is 18,609 kb with total of 15 restriction sites and the G+C content of 36. 36%. The predicted ORF number is 22.

PO-58

Track: Industrial and manufacturing: Bioprocess engineering and optimization

THE PRODUCTION OF *DEBARYOMYCES HANSENII* KILLER TOXINS ON INDUSTRIAL SCALE

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The yeasts *D. hansenii* produce in an acidic medium (pH 4,0-4. 6) killer toxins active against other species of yeasts and filamentous fungi. In laboratory tests, a medium containing glucose and organic nitrogen was used for their synthesis.

The study investigated the possibility of the production scaleincrease using bioreactors of following working capacity: 2L, 50L, 100L, 1000L. The growth of yeast biomass, killer activity and utilization of glucose were monitored during cultivation.

In all bioreactors similar growth profiles were observed. Active growth started immediately after inoculation with a maximum specific growth rate at a level 0,25h^{-1}. The stationary growth phase was reached at 22h-26h of the process with biomass level of approx. 20 g/L. In the bioreactor with a working volume of 2L yeast killer toxin synthesis began at the end of the logarithmic phase, whereas in cultures with a higher working capacity toxin biosynthesis began with the start of the logarithmic growth phase. In each case, the highest level of toxin activitywas observed after reaching by yeasts a stationary growth phase. The scaling up of the process caused the increase of killer activity levels from 230 aU/ml to 1540 aU/mL.

Acknowledgement

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Keywords: *Debaryomyces hansenii*, killer toxins, bioreactor culture.

PO-57

Track: Other area: Systems biology

THE HIGH STRUCTURE CONSERVATION OF AUTONOMOUS LINEAR PLASMIDS ISOLATED FROM *DEBARYOMYCES HANSENII* YEAST STRAINS

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Yeasts may possess linear dsDNA plasmids, similarly like bacteria, algae, filamentous fungi and plants. These plasmids usually occur in systems of two or three plasmids. The largest one in the system is called an autonomous, due to the fact that it encodes the genes responsible for its replication and transcription.

This is the first time that the autonomous plasmids from *Debaryomyces hansenii* yeast species were sequenced. In the study six plasmid sequences in the range of ca. 15. 0 - 21 kb were obtained by NGS. Each plasmid contained the fragment consisted of ORFs possibly encoding: specific DNA polymerase with terminal protein (TP), the capping enzyme, helicase, SSB protein, two subunits of RNA polymerase, TRF1 and three proteins of unknown function. All ORFs were arranged in the specific order precisely consistent with the observed in previously known autonomous plasmids from other yeas species: *Kluyveromyces lactis* (pGKL2), *Schwanniomyces etchellsii* (pPE1B), *Millerozyma acaciae* (pPac1-1), *Lachancea kluyveri* (pSKL). However, *D. hansenii* plasmids contain additional DNA fragments of length from 1. 5 to 7 kb.

Acknowledgements

This work was financially supported by Polish Ministry of Science and Higher Education. Project N N 312 258138.

Keywords: *Debaryomyces hansenii*, linear plasmids, DNA sequence.

PO-76

Track: Medical Biotechnology

PROCESSING OF A NOVEL ELECTROPOLYMERIZED SILK FIBROIN HYDROGEL MEMBRANE AND ITS CHARACTERIZATION

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Silk fibroin can be made into various forms of biocompatible materials in which silk hydrogel has been investigated extensively as a 3D medical tissue engineering materials due to its excellent properties. Here we report a novel method for the preparation of electropolymerized silk fibroin hydrogel membrane (ESFHM), which is formed on a nanoporous film as a barrier on the principle of electropolymerization by our home-made device. When the regenerated silk fibroin solution in Tris buffer was added into the reservoir with negative charge and the silk molecules immigrated toward with positive charge at a higher DC voltage, the ESFHM formed on the barrier film. The barrier film with a MWCO of 10kDa is favorable to the formation of the ESFHM. The effects of environmental conditions on the formation and characterizations of the ESFHM were studied to gain additional insight into the process and control of the material properties. The semi-transparent ESFHM with optimal properties could be obtained on the barrier film in Tris buffer (pH 6. 55-7. 55) at 80VDC. The more intact molecules of the regenerated silk fibroin can be made into the more superior ESFHM in mechanical properties. The ESFHM was predominantly a mixture of Ä-sheets and Ā-helix crystalline structures. Methanol immersion could slightly improve the crystallinity of the ESFHM. SEM observation showed that the ESFHM characterizes 3D mesh structure woven by a chain of silk fibroin nanoparticles with size of about 30 nanometers as a pearl necklace. The swelling ratio could be up to 1056. 4%. *In vitro* biological tests indicated that ESFHM was degradable and could satisfy the cell adhesion and growth requirements. Therefore, the ESFHM is a promising candidate for loading bioactive protein and appropriate cells, artificial skin or using for transplantation. The method reported here could be used to prepare for another novel electropolymerized macromolecular hydrogel membranes such as chitosan.

Keywords: ESFHM, nanoparticles, electropolymerized method, functional materials.

PO-65

Track: Regenerative Medicine

EFFECTS OF ZHICHAN POWDER ON SIGNAL TRANSDUCTION AND APOPTOSIS-ASSOCIATED GENE EXPRESSION IN THE SUBSTANTIANIGRA OF PARKINSON'S DISEASE RATS

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Previous studies have shown that Zhichan powder elevated immunity and suppressed oxidation in mice. Rat models of Parkinson's disease were induced by stereotaxically injecting 6-hydroxydopamine into the substantia nigra. The rat models were intragastrically treated with Zhichan powder, which is composed of milkvetch root, ginseng, bunge swallowwort root, himalayan teasel root, Magnolia officinalis, Ligustrum lucidum Ait. and szechwan lovage rhizome. Immunohistochemistry and reverse transcription-PCR results demonstrated that mRNA and protein expression of tumor necrosis factor receptor 1, Fas, caspase-8, cytochrome C, Bax, caspase-3, and p53 significantly increased, but Bcl-2 expression significantly decreased in the substantia nigra of rats with Parkinson's disease. Following Zhichan powder administration, mRNA and protein expression of tumor necrosis factor receptor 1, Fas, caspase-8, cytochrome C, Bax, caspase-3, and p53 diminished, but Bcl-2 expression increased in the rat substantia nigra. These results indicate that Zhichan powder regulates signal transduction protein expression, inhibits apoptosis, and exerts therapeutic effects on Parkinson's disease

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PO-64

Track: Regenerative Medicine

GENE THERAPY IN A MOUSE TUMOR MODEL OF BREAST CANCER BY si-RNA-MEDIATED DOWN-REGULATION OF STAT3

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Breast carcinoma is one of the most common forms of cancer, with a high prevalence and mortality rate worldwide. Signal transducer and activator of transcription 3 (STAT3) plays a key role in tumor cell survival and proliferation, angiogenesis, apoptosis. It is aberrantly activated in several types of cancers, including breast cancer. We assessed the therapeutic effects using a DNA vector-based STAT3-specific small interfering RNA (pSi-STAT3) on a murine breast cancer model. We observed the tumor growth in evry groups and further discussed the mechanism underlying. STAT3 was significantly downregulated at both the mRNA and protein levels in the pSi-STAT3 group. The growth of the tumors was significantly reduced in the pSi-STAT3-treated mice. Flow cytometry revealed that the number of early apoptotic cells was significantly elevated in the pSi-STAT3 group. Moreover, in the pSi-STAT3 group, the mRNA expression of the STAT3 downstream genes Bcl2 and cMyc was also significantly inhibited, and immunohistochemistry revealed that the expression of STAT3, HIF1 and PCNA protein were reduced in the tumor tissues. Our results suggested that STAT3-specific siRNA significantly suppressed tumor growth in breast cancer-bearing mice. It might be a useful therapeutic strategy in malignancies.

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ADDITIONAL ABSTRACTS

PO-103

Track: Others

THE TYROSINASE INHIBITION ACTIVITY OF MULBERROSIDE A FROM THE BRANCH BARK OF MULBERRY

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A bioactive ingredient in an ethanol extract from the branch bark of cultivated mulberry Husang-32 (*Morus multicaulis* Perr.) was isolated using a macroporous resin column. The primary component, which was purified by semi-preparative HPLC-DAD, was identified as mulberroside A (MA) by LC-MS, ^{1}H and ^{13}C NMR spectra. In total, 4.12 g MA was efficiently extracted from one kilogram of mulberry bark. The enzymatic analysis showed that MA inhibited the generation of dopachrome by affecting the activities of monophenolase and diphenolase of tyrosinase *in vitro*. This analysis indicated that MA and oxyresveratrol (OR) exhibited strong inhibition of the monophenolase activity with IC_{50} values of 1.29 μmol/L and 0.12 μmol/L, respectively. However, the former showed weaker inhibitory activity than the latter for diphenolase. For the monophenolase activity, the inhibitory activity of MA and OR was reversible and showed mixed type 1 inhibition. Additionally, the inhibition constant K_I values were 0.385 μmol/L and 0.926 μmol/L, respectively, and the K_{IS} values were 0.177 μmol/L and 0.662 μmol/L, respectively. However, MA showed competitive inhibition of diphenolase activity, and K_I was 4.36 μmol/L. In contrast, OR showed noncompetitive inhibition and $K_I = K_{IS}$=2.95 μmol/L. Taken together, these results provide important information concerning the inhibitory mechanism of MA on melanin synthesis, which is widely used in whitening cosmetics.

Keywords: mulberry; branch bark, mulberroside A; tyrosinase inhibition; monophenolase; diphenolase.

PO-104

Track: *Medical Biotechnology*

PROCESSING OF A NOVEL ELECTROPOLYMERIZED SILK FIBROIN HYDROGEL MEMBRANE AND ITS CHARACTERIZATION

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Silk fibroin can be made into various forms of biocompatible materials in which silk hydrogel has been investigated extensively as a 3D medical tissue engineering materials due to its excellent properties. Here we report a novel method for the preparation of electropolymerized silk fibroin hydrogel membrane (ESFHM), which is formed on a nanoporous film as a barrier on the principle of electropolymerization by our home-made device. When the regenerated silk fibroin solution in Tris buffer was added into the reservoir with negative charge and the silk molecules immigrated toward with positive charge at a higher DC voltage, the ESFHM formed on the barrier film. The barrier film with a MWCO of 10kDa is favorable to the formation of the ESFHM. The effects of environmental conditions on the formation and characterizations of the ESFHM were studied to gain additional insight into the process and control of the material properties. The semi-transparent ESFHM with optimal properties could be obtained on the barrier film in Tris buffer (pH 6.55-7.55) at 80VDC. The more intact molecules of the regenerated silk fibroin can be made into the more superior ESFHM in mechanical properties. The ESFHM was predominantly a mixture of Ä-sheets and Ā-helix crystalline structures. Methanol immersion could slightly improve the crystallinity of the ESFHM. SEM observation showed that the ESFHM characterizes 3D mesh structure woven by a chain of silk fibroin nanoparticles with size of about 30 nanometers as a pearl necklace. The swelling ratio could be up to 1056.4%. In vitro biological tests indicated that ESFHM was degradable and could satisfy the cell adhesion and growth requirements. Therefore, the ESFHM is a promising candidate for loading bioactive protein and appropriate cells, artificial skin or using for transplantation. The method reported here could be used to prepare for another novel electropolymerized macromolecular hydrogel membranes such as chitosan.

Keywords: ESFHM, nanoparticles, electropolymerized method, functional materials.

SL-184(b)

Track: *Medical Biotechnology*

SOUTH AFRICAN MEDICINAL PLANTS SHOW ANTI-CANCER SPLICING ACTIVITY

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Disruption of exon recognition and misregulation of alternative splicing are a common cause of human diseases including cancer progression. Currently the analysis of cancer-specific alternative splicing is a promising step forward in basic and translational molecular biology. Traditional medicine has a long history and is still the major source of medicine in developing countries. Approximately 70% of the South African population consults traditional healers, perpetuating the need for scientific appraisal of traditional medicine as a means to establish its efficiency and safety. Also, pharmacological and phytochemical insights into several plants have led to the discovery of novel chemicals and therefore novel drugs. Alternatively, such novel chemical structures can serve as lead compounds/templates for the design of new drugs. The aim was to ascertain if the South African medicinal plants have anticancer splicing activity. 10c cells were treated with *Tulbhaghia violacea* and *Cotyledon orbiculata*, followed by mRNA extraction and RT-PCR. The results showed that *Tulbhaghia violacea* and *Cotyledon orbiculata* extracts have anti-cancer splicing activity on the BCLX and the AXL apoptosis genes. Additionally *Cotyledon orbiculata* extract has an anticancer splicing activity of the angiogenesis gene VEGF165. VEGF Elisa also confirmed the VEGF165 VEGF165b splicing switch. We have shown that South African medicinal plants have anti-cancer splicing activity. We are continuing to screen more medicinal plants and will select those extracts with anti-cancer splicing activity for further studies. These further studies should identify numerous splicing pathways and completely elucidate the splicing target compounds that may serve as novel anti-cancer drugs or lead compounds.

PO-103

Track: Plant and Environment

EXPERIMENTAL AND THEORETICAL STUDIES ON STEROID C25 DEHYDROGENASE FROM *STEROLIBACTERIUM DENITRIFICANS*

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Steroid C-25 dehydrogenase (S25DH), a new molybdenum enzyme isolated from denitrifying bacterium *Sterolibacterium denitrificans* Chol-1ST [1], catalyzes regioselective hydroxylation at the C-25 tertiary carbon atom of the aliphatic side chain in cholesterol and its derivatives. The enzyme is express only under anaerobic and catalyzes transfer of the oxygen atom from a water molecule to the substrate [2].

Reaction catalyzed by S25DH is proposed as an alternative method for synthesis of 25-hydroxycholesterol (25-HC) in place of the currently used multistep chemical synthetic procedure. 25-HC is an important regulatory compound that is involved in a complex regulation of cells of the human immunological system [3]. However, up to date lack of a cheap commercial source of 25-HC limits studies of its physiological and immunological role and its potential medical application.

S25DH was purified under anaerobic conditions and aerobic with ferrocenium (III) tetrafluoroborate as an oxygen protectant [1, 4]. Then the enzyme was tested as a catalyst for production of 25-hydroxylated cholesterol.

In this presentation we show an optimized reaction conditions for synthesis of 25-HC in aqueous medium. The main obstacle to high conversion, i.e. poor solubility of cholesterol, was circumvented by substitution of cholesterol with its more soluble derivatives such as cholesteryl hemisuccinate or addition of solubilizers such as a mixture of !-cyclodextrin and short chain alcohols or glycols.

Moreover, we present the homology model of S25DH based on the template of ethylbenzene dehydrogenase (EBDH) from *Aromaticum aromatoleum* EbN1 [4] (identity 40 %, similarity 96 %). Molecular dynamic simulations and docking

experiments for known S25DH substrates we used to study the substrates binding mode and crucial interactions between amino acid residues and the docked ligands.

Acknowledgement

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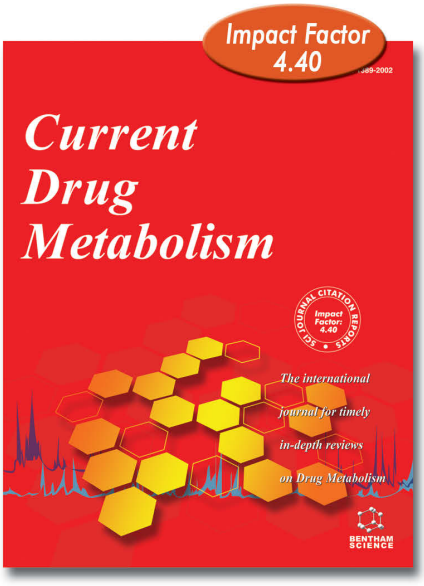

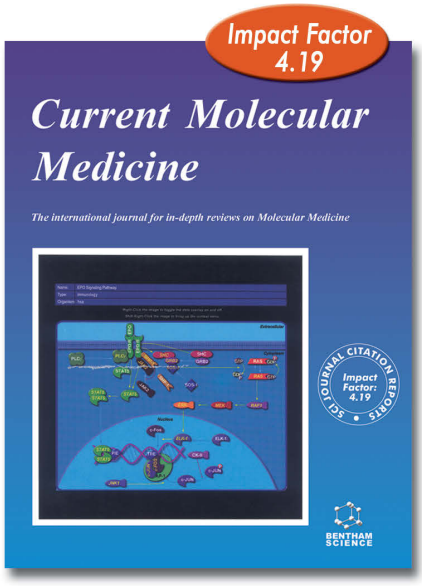

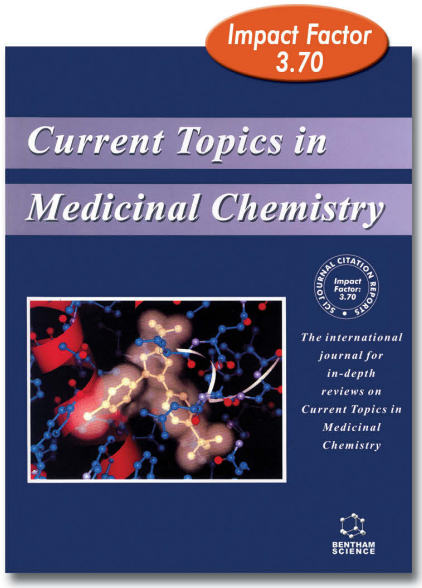

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