VSL#3®

We get the gut

VSL#3® Clinical Summary

Summary of recent scientific publications for healthcare professionals

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Each study reference includes the abstract of the publication available and retrievable on PubMed.

Contents

Human

Effect of Short-Term Dietary Intervention and Probiotic Mix Supplementation on the Gut Microbiota of Elderly Obese Women

Cancello R, Turroni S, Rampelli S, Cattaldo S, Candela M, Cattani L, Mai S, Vietti R, Scacchi M, Brigidi P, Invitti C. *Nutrients, December 2019.*

Abstract

Accumulating literature is providing evidence that the gut microbiota is involved in metabolic disorders, but the question of how to effectively modulate it to restore homeostasis, especially in the elderly, is still under debate. In this study, we profiled the intestinal microbiota of 20 elderly obese women (EO) at the baseline (T0), after 15 days of hypocaloric Mediterranean diet administered as part of a nutritional-metabolic rehabilitation program for obesity (T1), and after a further 15 days of the same diet supplemented with a probiotic mix (T2). Fecal samples were characterized by Illumina MiSeq sequencing of the 16S rRNA gene. The EO microbiota showed the typical alterations found in obesity, namely, an increase in potential pro-inflammatory components (i.e., *Collinsella*) and a decrease in health-promoting, short-chain fatty acid producers (i.e., *Lachnospiraceae* and *Ruminococcaceae* members), with a tendency to reduced biodiversity. After 15 days of the rehabilitation program, weight decreased by (2.7 ± 1.5)% and the gut microbiota dysbiosis was partially reversed, with a decline of *Collinsella* and an increase in leanness-related taxa. During the next 15 days of diet and probiotics, weight dropped further by (1.2 ± 1.1)%, markers of oxidative stress improved, and *Akkermansia*, a mucin degrader with beneficial effects on host metabolism, increased significantly. These findings support the relevant role of a correct dietetic approach, even in the short term, to modulate the EO gut microbiota towards a metabolic health-related configuration, counteracting the increased risk of morbidity in these patients.

Evaluation of tolerability and major factors affecting the adherence to probiotics in irritable bowel syndrome

Laterza L, Napoli M, Petito V, Scaldaferri F, Gaetani E, Gasbarrini A. *Minerva Medica, June 2021.*

Abstract

Background: Probiotics have been evaluated in multiple clinical trials on irritable bowel syndrome (IBS). However, in real-life long-term compliance could be low. Our study is single-center, observational and prospective, aiming both to evaluate the adherence to prescription of probiotic therapy in real-life and to identify factors able to influence adherence to therapy.

Methods: Fifty patients diagnosed with IBS according to Rome IV and receiving a clinical prescription of a multistrain probiotic preparation (VSL#3® manufactured by Nutrilinea Srl and marketed in Italy by Ferring S.p.A., Milan, Italy) have been enrolled and 49 completed the follow-up. Two months after baseline a second visit was made to assess adherence and eventual reasons for discontinuation.

Results: Sixty percent of patients took all the prescribed probiotic therapy in real-life setting, with perceived benefits in more than 60% of cases. Among the 20 patients with reduced adherence, 5 took less than 50%, 12 took 50% and 2 took more than 50% but less than 80% of the prescribed doses. Principal reasons of not complete adherence among the 20 patients were: price of the product (8/20), mild adverse events (AEs) (6/20) and poor appreciation of flavour (3/20).

Conclusions: This study suggested that the adherence to probiotic therapy is affected by different factors in patients with IBS in a real-life setting. The main reason for lack of adherence was the price of the product. Other reasons are mild AEs (mainly bloating) and low palatability.

Human

A proof of concept pilot trial of probiotics in symptomatic oral lichen planus (CABRIO)

Marlina E, Goodman RN, Mercadante V, Shephard M, McMillan R, Hodgson T, Leeson R, Porter S, Barber JA, Fedele S, Smith AM. *Oral Diseases, September 2021.*

Abstract

Objective: To preliminary evaluate the clinical effects of probiotics in individuals with symptomatic oral lichen planus and the possible mechanisms of action.

Subjects and methods: A group of 30 individuals with symptomatic oral lichen planus were recruited in a randomised double-blind parallel group controlled (1:1) proof-of-concept pilot trial of probiotic VSL#3 vs placebo. Efficacy outcomes included changes in pain numeric rating scale, oral disease severity score and the chronic oral mucosal disease questionnaire. Adverse effects, home diary and withdrawals were assessed as feasibility outcomes. Mechanistic outcomes included changes in salivary and serum levels of CXCL10 and IFN-γ and in oral microbial composition.

Results: The probiotic VSL#3 was safe and well tolerated. We observed no statistically significant change in pain, disease activity, quality of life, serum/salivary CXCL10 or oral microbial composition with respect to placebo. Salivary IFN-γ levels demonstrate a trend for a reduced level in the active group ($p = 0.082$) after 30 days of probiotic consumption.

Conclusions: The present proof-of-concept study provides some weak not convincing indication of biological and clinical effects of probiotic VSL#3 in individuals with painful oral lichen planus. Further research in this field is needed, with the current study providing useful information to the design of future clinical trials.

Human

The effect of gastric acid suppression on probiotic colonization in a double blinded randomized clinical trial

Singh G, Haileselassie Y, Briscoe L, Bai L, Patel A, Sanjines E, Hendler S, Singh PK, Garud NR, Limketkai BN, Habtezion A. *Clinical Nutrition ESPEN, November 2021.*

Abstract

Background & aims: Probiotics contain living microorganisms consumed for their putative benefits on the intestinal microbiota and general health and a concept is emerging to use probiotic as a therapeutic intervention to reduce proton pump inhibitors (PPIs) negative effects, but data is lacking. The use of PPIs can result in disordered gut microbiota, leading to a risk of enteric infections. PPIs are frequently prescribed in the general practice setting for gastroesophageal reflux disease (GERD), peptic ulcer disease, and related conditions. Despite the availability and widespread use of probiotics and acid-suppressing medications, the effect of PPIs-induced gastric acid suppression on the survival and colonization of probiotics bacterial species is currently unclear. We hypothesized that gastric acid suppression may improve intestinal colonization of probiotics bacterial species and probiotic intervention may have a potential role in mitigating untoward effects of PPI.

Methods: In a randomized, double-blind, placebo-controlled study, healthy subjects were given either proton pump inhibitor (PPI, n = 15) or placebo (n = 15) over 6 weeks. All subjects then consumed multi-strain probiotics from weeks 2-6. Thirty participants (10 males, 20 females, age range: 18-56 years) were enrolled in the study. Shotgun metagenomic sequencing and untargeted metabolomics analyses were performed on stool samples collected at week 0, 2, and 6.

Results: Short term PPI treatment increased the microbial abundance of Streptococcaceae ($p = 0.004$), Leuconostacaceae ($p = 0.001$), and Pasteurellaceae ($p = 0.020$) at family level and corresponding genus levels. The metabolomic analysis of the stools revealed a change in 10 metabolites where Gly Arg Val and phenylacetic acid were consistently increased compared to the baseline. Probiotic intervention inhibited PPI-induced microbial changes such as a decrease in Leuconostacaceae family ($p = 0.01$) and led to an increase in metabolite 1H-Indole-4-carbaldehyde. Notably, PPI enhanced the colonization of certain probiotic bacterial species like Streptococcus thermophilus ($p < 0.05$) along with other species present in the multi-strain probiotic.

Conclusion: Acid suppression enhanced certain probiotic associated bacterial colonization and probiotics in turn suppressed PPI-mediated intestinal microbial alterations. Thus, probiotics in combination with PPI might be a beneficial strategy that allows probiotic colonization and suppress PPI-induced microbial perturbations. CLINICAL TRIALS.

Human

Long-Term Effects of a Web-Based Low-FODMAP Diet Versus Probiotic Treatment for Irritable Bowel Syndrome, Including Shotgun Analyses of Microbiota: Randomized, Double-Crossover Clinical Trial

Ankersen DV, Weimers P, Bennedsen M, Haaber AB, Fjordside EL, Beber ME, Lieven C, Saboori S, Vad N, Rannem T, Marker D, Paridaens K, Frahm S, Jensen L, Rosager Hansen M, Burisch J, Munkholm P.
Journal Of Medical Internet Research, December 2021.

Abstract

Background: The long-term management of irritable bowel syndrome (IBS) poses many challenges. In short-term studies, eHealth interventions have been demonstrated to be safe and practical for at-home monitoring of the effects of probiotic treatments and a diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs). IBS has been linked to alterations in the microbiota.

Objective: The aim of this study was to determine whether a web-based low-FODMAP diet (LFD) intervention and probiotic treatment were equally good at reducing IBS symptoms, and whether the response to treatments could be explained by patients' microbiota.

Methods: Adult IBS patients were enrolled in an open-label, randomized crossover trial (for nonresponders) with 1 year of follow-up using the web application IBS Constant Care (IBS CC). Patients were recruited from the outpatient clinic at the Department of Gastroenterology, North Zealand University Hospital, Denmark. Patients received either VSL#3 for 4 weeks (2 × 450 billion colony-forming units per day) or were placed on an LFD for 4 weeks. Patients responding to the LFD were reintroduced to foods high in FODMAPs, and probiotic responders received treatments whenever they experienced a flare-up of symptoms. Treatment response and symptom flare-ups were defined as a reduction or increase, respectively, of at least 50 points on the IBS Severity Scoring System (IBS-SSS). Web-based ward rounds were performed daily by the study investigator. Fecal microbiota were analyzed by shotgun metagenomic sequencing (at least 10 million 2 × 100 bp paired-end sequencing reads per sample).

Results: A total of 34 IBS patients without comorbidities and 6 healthy controls were enrolled in the study. Taken from participating subjects, 180 fecal samples were analyzed for their microbiota composition. Out of 21 IBS patients, 12 (57%) responded to the LFD and 8 (38%) completed the reintroduction of FODMAPs. Out of 21 patients, 13 (62%) responded to their first treatment of VSL#3 and 7 (33%) responded to multiple VSL#3 treatments. A median of 3 (IQR 2.25-3.75) probiotic treatments were needed for sustained symptom control. LFD responders were reintroduced to a median of 14.50 (IQR 7.25-21.75) high-FODMAP items. No significant difference in the median reduction of IBS-SSS for LFD versus probiotic responders was observed, where for LFD it was -126.50 (IQR -196.75 to -76.75) and for VSL#3 it was -130.00 (IQR -211.00 to -70.50; P>.99). Responses to either of the two treatments were not able to be predicted using patients' microbiota.

Conclusions: The web-based LFD intervention and probiotic treatment were equally efficacious in managing IBS symptoms. The response to treatments could not be explained by the composition of the microbiota. The IBS CC web application was shown to be practical, safe, and useful for clinical decision making in the long-term management of IBS. Although this study was underpowered, findings from this study warrant further research in a larger sample of patients with IBS to confirm these long-term outcomes.

Human

Probiotic Therapy With VSL#3® in Patients With NAFLD: A Randomized Clinical Trial

Derosa G, Guasti L, D'Angelo A, Martinotti C, Valentino M C, Di Matteo S, Bruno GM, Maresca AM, Gaudio GV, Maffioli P. *Frontiers in Nutrition, May 2022.*

Abstract

Aim: To evaluate if VSL#3® [a high-concentration multi-strain probiotic mix containing one strain of *Streptococcus thermophilus* BT01, three strains of *Bifidobacteria* (*B. breve* BB02; *B. animalis* subspecies [subsp.] *lactis* BL03, previously identified as *B. longum* BL03; and *B. animalis* subsp. *lactis* BI04, previously identified as *B. infantis* BI04), and four strains of *Lactobacilli* (*L. acidophilus* BA05, *L. plantarum* BP06, *L. paracasei* BP07, and *L. helveticus* BD08, previously identified as *L. delbrueckii* subsp. *bulgaricus* BD08)] therapy could improve hepatic parameters.

Methods: We enrolled 60 Caucasian patients aged ≥ 18 years of either sex with the diagnosis of non-alcoholic fatty liver disease (NAFLD), according to practice guidance, in a double-blind, placebo-controlled study. Patients were randomized to take placebo or VSL#3®, 2 sachets/day in the morning for 3 months. VSL#3® and placebo were self-administered.

Results: We did not observe any change in body mass index (BMI), circumferences, fasting plasma glucose (FPG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and adiponectin (ADN) with neither treatment. A statistically significant triglycerides (Tg) decrease ($p < 0.05$ vs. baseline, and $p < 0.05$ vs. placebo, respectively) and high-sensitivity C-reactive protein (Hs-CRP) decrease ($p < 0.05$ vs. baseline) was observed in the group of patients being treated with VSL#3® compared with placebo. Transaminases and gamma-glutamyltransferase (γ-GT) were significantly reduced in VSL#3® group ($p < 0.05$ vs. baseline and placebo, respectively) compared with the placebo group. Aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio and hepatic steatosis index (HSI) were significantly lower than the VSL#3® group ($p < 0.05$ vs. baseline and placebo, respectively) compared with the placebo group. All patients reported an improvement or the disappearance of hepatic steatosis.

Conclusion: Probiotic therapy with VSL#3® ameliorates hepatic parameters and echography grading, while reducing Tg and the inflammatory status, without any difference between men and women.

Human

Study on Effects of Probiotics on Gut Microbiome and Clinical Course in Patients With Critical Care Illnesses

Saikrishna K, Talukdar D, Das S, Bakshi S, Chakravarti P, Jana P, Karmakar S, Wig N, Das B, Ray A.
Microbial Ecology, May 2023.

Abstract

Ventilator-associated pneumonia (VAP) is a nosocomial infection contracted by ventilator patients in which bacteria colonize the upper digestive tract and contaminated secretions are released into the lower airway. This nosocomial infection increases the morbidity and mortality of the patients as well as the cost of treatment. Probiotic formulations have recently been proposed to prevent the colonization of these pathogenic bacteria. In this prospective observational study, we aimed to investigate the effects of probiotics on gut microbiota and their relation to clinical outcomes in mechanically ventilated patients.

For this study, 35 patients were recruited (22 probiotic-treated and 13 without probiotic treatment) from a cohort of 169 patients. Patients in the probiotic group were given a dose of 6 capsules of a commercially available probiotic (VSL#3®:112.5 billion CFU/cap) in three divided doses for 10 days. Sampling was carried out after each dose to monitor the temporal change in the gut microbiota composition. To profile the microbiota, we used a 16S rRNA metagenomic approach, and differences among the groups were computed using multivariate statistical analyses. Differences in gut microbial diversity (Bray Curtis and Jaccard distance, p-value > 0.05) between the probiotic-treated group and the control group were not observed. Furthermore, treatment with probiotics resulted in the enrichment of Lactobacillus and Streptococcus in the gut microbiota of the probiotic-treated groups.

Our results demonstrated that probiotics might lead to favorable alterations in gut microbiome characteristics. Future studies should focus on the appropriate dosages and frequency of probiotics, which can lead to improved clinical outcomes.

Ecology and Machine Learning-Based Classification Models of Gut Microbiota and Inflammatory Markers May Evaluate the Effects of Probiotic Supplementation in Patients Recently Recovered from COVID-19

Laterza L, Putignani L, Settanni CR, Petito V, Varca S, De Maio F, Macari G, Guarrasi V, Gremese E, Tolusso B, Wlderk G, Pirro MA, Fanali C, Scaldaferri F, Turchini L, Amatucci V, Sanguinetti M, Gasbarrini A.
International Journal of Molecular Sciences, April 2023.

Abstract

Gut microbiota (GM) modulation can be investigated as possible solution to enhance recovery after COVID-19. An open-label, single-center, single-arm, pilot, interventional study was performed by enrolling twenty patients recently recovered from COVID-19 to investigate the role of a mixed probiotic, containing Lactobacilli, Bifidobacteria and *Streptococcus thermophilus*, on gastrointestinal symptoms, local and systemic inflammation, intestinal barrier integrity and GM profile. Gastrointestinal Symptom Rating Scale, cytokines, inflammatory, gut permeability, and integrity markers were evaluated before (T_0) and after 8 weeks (T_1) of probiotic supplementation. GM profiling was based on 16S-rRNA targeted-metagenomics and QIIME 2.0, LEfSe and PICRUSt computational algorithms. Multiple machine learning (ML) models were trained to classify GM at T_0 and T_1. A statistically significant reduction of IL-6 ($p < 0.001$), TNF-α ($p < 0.001$) and IL-12RA ($p < 0.02$), citrulline (p value < 0.001) was reported at T_1. GM global distribution and microbial biomarkers strictly reflected probiotic composition, with a general increase in Bifidobacteria at T_1. Twelve unique KEGG orthologs were associated only to T_0, including tetracycline resistance cassettes. ML classified the GM at T_1 with 100% score at phylum level. Bifidobacteriaceae and *Bifidobacterium* spp. inversely correlated to reduction of citrulline and inflammatory cytokines. Probiotic supplementation during post-COVID-19 may trigger anti-inflammatory effects though Bifidobacteria and related-metabolism enhancement.

In vitro

Compositional Quality and Potential Gastrointestinal Behavior of Probiotic Products Commercialized in Italy

Vecchione A, Celandroni F, Mazzantini D, Senesi S, Lupetti A, Ghelardi E. *Frontiers in Medicine, February 2018.*

Abstract

Recent guidelines indicate that oral probiotics, living microorganisms able to confer a health benefit on the host, should be safe for human consumption, when administered in a sufficient amount, and resist acid and bile to exert their beneficial effects (e.g., metabolic, immunomodulatory, anti-inflammatory, competitive).

This study evaluated quantitative and qualitative aspects and the viability in simulated gastric and intestinal juices of commercial probiotic formulations available in Italy. Plate counting and MALDI-TOF mass spectrometry were used to enumerate and identify the contained organisms. *In vitro* studies with two artificial gastric juices and pancreatin-bile salt solution were performed to gain information on the gastric tolerance and bile resistance of the probiotic formulations. Most preparations satisfied the requirements for probiotics and no contaminants were found. Acid resistance and viability in bile were extremely variable depending on the composition of the formulations in terms of contained species and strains.

In conclusion, this study indicates good microbiological quality but striking differences in the behavior in the presence of acids and bile for probiotic formulations marketed in Italy.

Impact of the Trophic Effects of the Secretome From a Multistrain Probiotic Preparation on the Intestinal Epithelia

Petito V, Greco V, Laterza L, Graziani C, Fanali C, Lucchetti D, Barbaro MR, Bugli F, Pieroni L, Lopetuso LR, Sgambato A, Sanguinetti M, Scaldaferri F, Urbani A, Gasbarrini A. *Inflammatory Bowel Diseases, December 2020.*

Abstract

Background: Probiotics are defined as live, nonpathogenic bacteria that confer health benefits beyond their nutritional value. In particular, VSL#3 exhibits demonstrated efficacy in the management of diseases characterized by an increased intestinal permeability. Our study aimed to understand how VSL#3 promotes gut health by secreting bioactive factors and identify which human pathways are modulated by secretome derived from the VSL#3 formula.

Methods: Two different lots of VSL#3 were used, and Caco-2 cell line was treated with conditioned media (CM) prepared using 1 g of the probiotic formula. We evaluated the effects of the probiotics on cellular proliferation and apoptosis by cytometry and the expression of tight junction proteins by western blotting. A proteomics analysis of both culture media and the whole proteome of Caco-2 cells treated with VSL#3-CM was performed by nano-ultra performance liquid chromatography - tandem mass (nUPLC MS/MS) spectrometry.

Results: The probiotic formula increased cell proliferation, decreased cellular apoptosis cells, and increased re-epithelialization in the scratch assay. Several peptides specifically synthetized by all the species within the probiotic preparation were recognized in the proteomics analysis. Human proteins synthesized by CaCo-2 cells were also identified.

Conclusions: To our knowledge, this manuscript describes the first evaluation of the probiotic secretome, and the results showed that the improvement in intestinal barrier functions induced by probiotics seems to be accompanied by the modulation of some human cellular pathways.

In vivo

Use of the synbiotic VSL#3 and yacon-based concentrate attenuates intestinal damage and reduces the abundance of Candidatus Saccharimonas in a colitis-associated carcinogenesis model

Dos Santos Cruz BC, Conceição LLD, Mendes TAO, Ferreira CLLF, Gonçalves RV, Peluzio MDCG.
Food Research International, September 2020.

Abstract

Individuals with inflammatory bowel disease are at high risk of developing colitis-associated cancer; thus, strategies to inhibit disease progression should be investigated. The study aimed to explore the role of the synbiotic (probiotic VSL#3® and yacon-based concentrate) in a colitis-associated carcinogenesis model. IL-10$^{-/-}$ mice were induced to carcinogenesis with 1,2-dimethylhydrazine and divided into two experimental groups: control and synbiotic.

Manifestations of colitis, colon histology, expression of antioxidant enzymes, production of organic acids and intestinal microbiota were evaluated. The use of the synbiotic showed benefits, such as the preservation of intestinal architecture, increased expression of antioxidant enzymes and the concentration of organic acids, especially butyrate. It was also observed different microbial community profiles between the groups during the study. Together, these factors contributed to mitigate the manifestations of colitis and improve intestinal integrity, suggesting the potential benefit of the synbiotic in intestinal diseases.

Synbiotic VSL#3 and yacon-based product modulate the intestinal microbiota and prevent the development of pre-neoplastic lesions in a colorectal carcinogenesis model

Dos Santos Cruz BC, da Silva Duarte V, Giacomini A, Corich V, de Paula SO, da Silva Fialho L, Guimarães VM, de Luces Fortes Ferreira CL, Gouveia Peluzio MDC. *Applied Microbiology and Biotechnology, September 2020.*

Abstract

Colorectal cancer is a public health problem, with dysbiosis being one of the risk factors due to its role in intestinal inflammation. Probiotics and synbiotics have been used in order to restore the microbiota balance and to prevent colorectal carcinogenesis. We aimed to investigate the effects of the probiotic VSL#3® alone or in combination with a yacon-based prebiotic concentrate on the microbiota modulation and its influence on colorectal carcinogenesis in an animal model. C57BL/6J mice were divided into three groups: control (control diet), probiotic (control diet + VSL#3®), and synbiotic (yacon diet + VSL#3®). The diets were provided for 13 weeks and, from the third one, all animals were subjected to induction of colorectal cancer precursor lesions. Stool samples were collected to evaluate organic acids, feces pH, βglucuronidase activity, and microbiota composition. The colon was used to count pre-neoplastic lesions and to determine the cytokines. The microbiota composition was influenced by the use of probiotic and synbiotic. Modifications were also observed in the abundance of bacterial genera with respect to the control group, which confirms the interference of carcinogenesis in the microbiota. Pre-neoplastic lesions were reduced by the use of the synbiotic, but not with the probiotic. The protection provided by the synbiotic can be attributed to the modulation of the intestinal inflammatory response, to the inhibition of a pro-carcinogenic enzyme, and to the production of organic acids. The modulation of the composition and activity of the microbiota contributed to beneficial changes in the intestinal microenvironment, which led to a reduction in carcinogenesis.

KEY POINTS:

- Synbiotic reduces the incidence of colorectal cancer precursor lesions.
- Synbiotic modulates the composition and activity of intestinal microbiota.
- Synbiotic increases the abundance of butyrate-producing bacteria.

In vivo

The Role of Intestinal Microbiota and Mast Cell in a Rat Model of Visceral Hypersensitivity

Li YJ, Li J, Dai C. *Journal of Neurogastroenterology and Motility, September 2020.*

Abstract

Background/aims: To explore the role of intestinal flora and mast cells in visceral hypersensitivity (VH).

Methods: The experimental animals were divided into 4 groups: control group, VH group, VH + VSL#3 group, and VH + ketotifen group. Stool samples were collected from each group (n = 3) for a further analysis using 16S ribosomal DNA gene sequence. Visceral sensitivity was evaluated by abdominal withdrawal reflex (AWR) score. Colon tissues of rats were obtained from each group. Mast cells were detected by toluidine blue staining. The degranulation of mast cells was assessed by transmission electron microscopy.

Results: VH rat model could successfully be induced by acetic acid enema combined with partial limb restraint method. Compared with rats in the control group, AWR score, number of mast cells, and degranulation of mast cells were increased in the VH rats, which could be reduced by administration of ketotifen or probiotic VSL#3. *Clostridium sensu stricto* 1 abundance was higher in the VH group compared to the control group, which could be restored by application of probiotic VSL#3.

Conclusions: Probiotic VSL#3 decreases visceral sensitivity in VH rats. The mechanism may be related to mast cell and intestinal flora. Change of *Clostridium sensu stricto* 1 abundance may be a basis for VH observed in irritable bowel syndrome and may be prevented by specific probiotic administration.

Evaluation of the efficacy of probiotic VSL#3 and synbiotic VSL#3 and yacon-based product in reducing oxidative stress and intestinal permeability in mice induced to colorectal carcinogenesis

Cruz BCDS, de Sousa Moraes LF, De Nadai Marcon L, Dias KA, Murad LB, Sarandy MM, Conceição LLD, Gonçalves RV, Ferreira CLLF, Peluzio MDCG. *Journal of Food Science, March 2021.*

Abstract

The objective of the present study was to evaluate the effect of probiotic VSL#3 isolated or associated with a yacon-based product (synbiotic) on oxidative stress modulation and intestinal permeability in an experimental model of colorectal carcinogenesis. Forty-five C57BL/6J mice were divided into three groups: control (standard diet AIN-93 M); probiotic (standard diet AIN-93 M and multispecies probiotic VSL#3, 2.25 × 10^9 CFU), and synbiotic (standard diet AIN-93 M with yacon-based product, 6% fructooligosaccharides and inulin, and probiotic VSL#3, 2.25 × 10^9 CFU). The experimental diets were provided for 13 weeks. The probiotic and the yacon-based product showed antioxidant activity, with the percentage of DPPH radical scavenging equal to 69.7 ± 0.4% and 74.3 ± 0.1%, respectively. These findings contributed to reduce hepatic oxidative stress: the control group showed higher concentration of malondialdehyde (1.8-fold, p = 0.007 and 1.5-fold, p = 0.035) and carbonylated protein (2-fold, p = 0.008 and 5.6-fold, p = 0.000) compared to the probiotic and synbiotic groups, respectively. Catalase enzyme activity increased 1.43-fold (p = 0.014) in synbiotic group. The crypt depth increased 1.2-fold and 1.4-fold with the use of probiotic and synbiotic, respectively, compared to the control diet (p = 0.000). These findings corroborate the reduction in intestinal permeability in the probiotic and synbiotic groups, as measured by the percentage of urinary lactulose excretion (CON: 0.93 ± 0.62% × PRO: 0.44 ± 0.05%, p = 0.048; and CON: 0.93 ± 0.62% × SYN: 0.41 ± 0.12%, p = 0.043). In conclusion, the probiotic and synbiotic showed antioxidant activity, which contributed to the reduction of oxidative stress markers. In addition, they protected the mucosa from damage caused by chemical carcinogen and reduced intestinal permeability.

PRACTICAL APPLICATION: The relationship between intestinal health and the occurrence of various organic disorders has been demonstrated in many studies. The use of probiotics and prebiotics is currently one of the main targets for modulation of intestinal health. We demonstrated that the use of a commercial mix of probiotic bacteria (VSL#3) isolated or associated with a yacon-based prebiotic, rich in fructooligosaccharides and inulin, is able to reduce the oxidative stress and intestinal permeability in a colorectal carcinogenesis model. These compounds have great potential to be used as a food supplement, or as ingredients in the development of food products.

In vivo

Synergistic Effect of Omega-3 and Probiotic Supplementation on Preventing Ligature-Induced Periodontitis

Doğan B, Kemer Doğan ES, Özmen Ö, Fentoğlu Ö, Kırzıoğlu FY, Calapoğlu M.
Probiotics and Antimicrobial Proteins, May 2021.

Abstract

Omega-3 and probiotics were shown to improve periodontal health by modulating the host immune response. Recently, the combination of omega-3 and probiotics has been shown to have a potential synergistic effect on host modulation. The aim of this study was to evaluate the prophylactic role of an omega-3 and probiotic combination on alveolar bone loss (ABL) via inflammatory response in an experimental periodontitis model. Forty-three rats were divided into 5 groups as control (C, n = 8), periodontitis (P, n = 8), omega-3 + periodontitis (O, n = 8), probiotic + periodontitis (Pro, n = 10), and omega-3 + probiotic + periodontitis (OPro, n = 9). Additionally to a standardized diet, omega-3 and/or probiotics were supplemented with oral gavage to the O, Pro, and OPro groups for 44 days. Periodontitis was induced by ligature to the P, O, Pro, and OPro groups on the 30th day for 2 weeks. ABL levels were measured histopathologically, and serum interleukin (IL) 1β, IL6, and IL10 levels were analysed by enzyme-linked immunosorbent assay. ABL increased in all periodontitis groups (P, O, Pro, and OPro), compared to C group. Compared to P group, all oral gavage groups (O, Pro, and OPro) revealed decreased ABL, which was lowest in OPro group. IL1β and IL6 decreased and IL10 increased in OPro group, compared to P group. In conclusion, prophylactic administration of omega-3 and probiotic combination reduced ABL and improved serum IL1β, IL6, and IL10 levels more than their single use.

Propionate and Butyrate Produced by Gut Microbiota after Probiotic Supplementation Attenuate Lung Metastasis of Melanoma Cells in Mice

Chen L, Zhou X, Wang Y, Wang D, Ke Y, Zeng X. *Molecular Nutrition & Food Research, June 2021.*

Abstract

Scope: The beneficial effects of probiotics in reducing gastrointestinal inflammation and in preventing colorectal cancer have been reported, but the mechanism underlying the immunomodulatory effect of probiotics in inhibiting extra-intestinal tumor progression remains unclear.

Methods and results: This study shows that probiotic supplementation attenuate lung metastasis of melanoma cells in mice. Feeding mice with VSL#3 probiotics change the composition and proportion of gut microbiota. The changes in gut bacteria composition, such as in the abundance of Lachnospiraceae, Streptococcus, and Lachnoclostridium, are associated with the production of short-chain fatty acids in the gut. The concentrations of propionate and butyrate are upregulated in gut and blood after feeding VSL#3, and the increase in propionate and butyrate levels promotes the expression of chemokine (C-C motif) ligand 20 (CCL20) in lung endothelial cells and the recruitment of T helper 17 (Th17) cells to the lungs via the CCL20/chemokine receptor 6 axis. The recruitment of Th17 cells decreases the number of tumor foci in lungs and attenuates the lung metastasis of melanoma cells in mice.

Conclusions: The results provide new information on the role and mechanisms of action of probiotics in attenuating extra-intestinal tumor metastasis.

In vivo

Sex-Dependent Effects of Intestinal Microbiome Manipulation in a Mouse Model of Alzheimer's Disease

Kaur H, Nookala S, Singh S, Mukundan S, Nagamoto-Combs K, Combs CK. *Cells, September 2021.*

Abstract

Mechanisms linking intestinal bacteria and neurodegenerative diseases such as Alzheimer's disease (AD) are still unclear. We hypothesized that intestinal dysbiosis might potentiate AD, and manipulating the microbiome to promote intestinal eubiosis and immune homeostasis may improve AD-related brain changes. This study assessed sex differences in the effects of oral probiotic, antibiotics, and synbiotic treatments in the *App*$^{NL-G-F}$ mouse model of AD. The fecal microbiome demonstrated significant correlations between bacterial genera in *App*$^{NL-G-F}$ mice and Aβ plaque load, gliosis, and memory performance. Female and not male *App*$^{NL-G-F}$ mice fed probiotic but not synbiotic exhibited a decrease in Aβ plaques, microgliosis, brain TNF-α, and memory improvement compared to no treatment controls. Although antibiotics treatment did not produce these multiple changes in brain cytokines, memory, or gliosis, it did decrease Aβ plaque load and colon cytokines in *App*$^{NL-G-F}$ males. The intestinal cytokine milieu and splenocyte phenotype of female but not male *App*$^{NL-G-F}$ mice indicated a modest proinflammatory innate response following probiotic treatment compared to controls, with an adaptive response following antibiotics treatment in male *App*$^{NL-G-F}$ mice. Overall, these results demonstrate the beneficial effects of probiotic only in *App*$^{NL-G-F}$ females, with minimal benefits of antibiotics or synbiotic feeding in male or female mice.

Effect of probiotics on olanzapine-induced metabolic syndrome in Wistar albino rats

Syed M, Nayak V. *Biomedicine, 2022.*

Abstract

Introduction and Aim: Olanzapine is the most efficacious second-generation antipsychotic (SGA) used in the treatment of schizophrenia and at the same time, it is known to cause metabolic syndrome (MS). The aim was to assess the adequacy of probiotics in fighting the unfriendly impacts of olanzapine treatment such as weight gain, hyperlipidaemia, and hyperglycaemia in the olanzapine-induced MS model in rats.

Materials and Methods: Thirty-six Wistar rats were divided into six groups (n=6), and were treated for 28 days as follows: Group-I: normal saline 1 ml/kg/day orally, Group-II: olanzapine 2 mg/kg/day i.p., Group-III: probiotic: 0.6 g/kg/day orally, Group-IV: probiotic: 1.2 g/kg/day orally, Group-V: olanzapine 2 mg/kg/day i.p., + probiotic: 0.6 g/kg/day orally, Group-VI: olanzapine 2 mg/kg/day i.p. + probiotic: 1.2 g/kg/day orally. Bodyweight, fasting blood glucose (FBG), and lipid profile were assessed at baseline and the end of each week. Data were analysed by applying repeated measures ANOVA, followed by a post-hoc Bonferroni test. P-value <0.05 was considered statistically significant.

Results: There was a significant increment in the body weight, FBG, total cholesterol, and triglycerides level after olanzapine treatment ($p<0.001$), and similarly a decline in the body weight, FBG, total cholesterol, and triglycerides level in the probiotic-treated groups ($p<0.001$). There was a decrease in weight gain and FBG levels caused by olanzapine in the probiotic-treated groups.

Conclusion: Probiotics forestalled the advancement of hyperlipidaemia, hyperglycaemia, decreased weight, and an increase in FBG levels induced by olanzapine. A long-term evaluation should also be directed to assess probiotics' impact on olanzapine-induced MS and their plausible mechanism.

In vivo

The Effects of Probiotics and Omega-3 Fatty Acids in Liver Steatosis Induced in Rats by High-Fructose Corn Syrup

Kizilaslan N, Erdem NZ, Katar M, Gevrek F. *International Journal of Clinical Practice, January 2022.*

Abstract

Aims: This study was designed to reveal the effect of probiotics and omega-3 fatty acids in a fatty liver model in rats induced by high-fructose corn syrup (HFCS).

Methods: In the study, 40 male Wistar Albino rats were used, and these rats were divided into five groups. HFCS was added to the drinking water (30% solution) of four groups (Groups 2, 3, 4, and 5) for three weeks, and the animals were fed ad libitum. At the end of three weeks, the rats in Groups 3, 4, and 5 were administered omega-3 fatty acids (400 mg/kg) and probiotics (1.5×10^9 cfu/mL/day) with the gavage method for four weeks. The body weights of rats were weighed and recorded before starting the experiment, at the end of the third week, and before the animals were sacrificed at the last week, all at the same hour. By subtracting the remaining amount of food and water from the daily food and water amount, the amount of food and water consumed was calculated. These values were recorded for seven weeks. At the end of the seven weeks, the rats were sacrificed after blood specimens and tissues were taken.

Results: Analyzing the changes in the food intake of each group within itself throughout the experiment, it was observed that there was an increase in the food intake in the control group; from the starting week to the last week, the food intake amount of the HFCS group began to decrease particularly after the second week; and it began to decrease after the third week in the groups that were administered probiotics and omega-3 fatty acids. The changes in the sacrifice weights in the HFCS + omega-3 fatty acid, HFCS + probiotic, and HFCS + probiotic + omega-3 fatty acid groups were found to be lower than that in the HFCS group. The maximum levels of glucose, ALT, ALP, serum cholesterol, triglyceride and AST were found to be in the HFCS group. It was determined that the minimum mean steatosis level was in the control group, while the maximum steatosis level was in the HFCS group.

Conclusions: As a result, there was a protective effect of probiotic and omega-3 fatty acid.

The Beneficial Effect of Probiotics Supplementation on Penicillin Induced Focal Seizure in Rats

Kızılaslan N, Sumbul O, Aygun H. *Neurochemical Research, January 2022.*

Abstract

The focal epilepsy is a chronic neurological brain disorder which affects millions of people in the world. There is emerging evidence that changes in the gut microbiota may have effects on epileptic seizures. In the present study, we examined the effect of probiotics on penicillin-induced focal seizure model in rats. Male Wistar Albino rats (n: 21) were randomly divided into three groups: control (no medication), penicillin and penicillin + probiotic. Probiotic VSL#3 (12.86 bn living bacteria/kg/day) was given by gavage for 30 days. The seizures were induced by intracortical injection of penicillin G (500 IU) into the cortex. An ECoG recordings were made for 180 min after penicillin G application. The spike frequency and the amplitude were used to assess the severity of seizures. Tumor necrosis factor (TNF-α), nitric oxide (NO) and interleukin (IL-6) levels in the brain were studied biochemically. Our results indicated that probiotic supplementation improved focal seizures through increasing the latency ($p < 0.001$) and decreasing the spike frequency ($p < 0.01$) compared to the penicillin group. Penicillin-induced seizure in rats significantly enhanced TNF-α ($p < 0.01$), NO ($p < 0.01$) and IL-6 ($p < 0.05$) compared to the control. Probiotic supplementation significantly decreased IL-6 ($p < 0.05$), TNF-α ($p < 0.01$) and NO ($p < 0.001$) compared to the penicillin group. When the body weights were compared before and after the experiment, there was no difference between the control and penicillin groups, but it was observed that the body weight decreased after probiotic supplementation in the penicillin + probiotic group. Probiotic supplementation may have anti-seizure effect by reducing proinflammatory cytokine and NO levels in epileptic rat brain.

In vivo

Probiotic supplementation alleviates absence seizures and anxiety- and depression-like behavior in WAG/Rij rat by increasing neurotrophic factors and decreasing proinflammatory cytokines

Aygun H, Akin AT, Kızılaslan N, Sumbul O, Karabulut D. *Epilepsy & Behavior, February 2022.*

Abstract

Aim: Epilepsy is one of the most common chronic brain disorders that affect millions of people worldwide. In the present study, we investigated the effects of probiotic supplementation on absence epilepsy and anxiety-and depression-like behavior in WAG/Rij rats.

Material and method: Fourteen male WAG/Rij rats (absence-epileptic) and seven male Wistar rats (nonepileptic) were used. The effects of probiotic VSL#3 (12.86 bn living bacteria/kg/day for 30 day/gavage) on absence seizures, and related psychiatric comorbidities were evaluated in WAG/Rij rats. Anxiety-like behavior was evaluated by the open-field test and depression-like behavior by the forced swimming test. In addition, the brain tissues of rats were evaluated histopathologically for nerve growth factor [NGF], brain-derived neurotrophic factor [BDNF], SRY sex-determining region Y-box 2 [SOX2] and biochemically for nitric oxide [NO], tumor necrosis factor-alpha [TNF-α] ,and Interleukin-6 [IL-6].

Results: Compared to Wistar rats, WAG/Rij rats exhibited anxiety- and depression-like behavior, and had lower BDNF, NGF and SOX2 immunoreactivity, and higher TNF-α, IL-6 levels in brain tissue. VSL#3 supplementation reduced the duration and number of spike-wave discharges (SWDs) and exhibited anxiolytic or anti-depressive effect. VSL#3 supplement also increased the NGF immunoreactivity while decreasing IL-6, TNF-α and NO levels in WAG/Rij rat brain.

Conclusion: The findings of the present study showed that neurotrophins, SOX2 deficiency, and pro-inflammatory cytokines may play a role in the pathogenesis of absence epilepsy. Our data support the hypothesis that the probiotics have anti-inflammatory effect. The present study is the first to show the positive effects of probiotic bacteria on absence seizures and anxiety- and depression-like behavior.

Altered gut microbiota composition with antibiotic treatment impairs functional recovery after traumatic peripheral nerve crush injury in mice: effects of probiotics with butyrate producing bacteria

Rodenhouse A, Talukder MAH, Lee JI, Govindappa PK, O'Brien M, Manto KM, Lloyd K, Wandling GD, Wright JR, Chen See JR, Anderson SL, Lamendella R, Hegarty JP, Elfar JC. *BMC Research Notes, February 2022.*

Abstract

Objective: Antibiotics (ABX) are widely used for life-threatening infections and also for routine surgical operations. Compelling evidence suggests that ABX-induced alterations of gut microbiota composition, termed dysbiosis, are linked with diverse disease states including neurological and neurodegenerative conditions. To combat the consequences of dysbiosis, probiotics (PBX) are widely used. ABX-induced dysbiosis is reported to impair neurological function after spinal cord injury. Traumatic peripheral nerve injury (TPNI) results in profound neurologic impairment and permanent disability. It is unknown whether ABX treatment-induced dysbiosis has any impact on TPNI-induced functional recovery, and if so, what role medical-grade PBX could have on TPNI recovery.

Results: In this study, ABX-induced dysbiosis and PBX-induced microbiota enrichment models were used to explore the potential role of gut microbiome in TPNI. Stool analysis with 16S ribosomal RNA (rRNA) gene sequencing confirmed ABX-induced dysbiosis and revealed that ABX-induced changes could be partially restored by PBX administration with an abundance of butyrate producing bacteria. Pre-injury ABX significantly impaired, but pre-injury PBX significantly improved post-TPNI functional recovery. Importantly, post-injury PBX protected against pre-injury ABX-induced functional impairment. These findings demonstrate that reestablishment of gut microbiota composition with butyrate producing PBX during ABX-induced dysbiosis could be a useful adjuvant therapy for TPNI.

In vivo

Synbiotic modulates intestinal microbiota metabolic pathways and inhibits DMH-induced colon tumorigenesis through c-myc and PCNA suppression

Dos Santos Cruz BC, da Silva Duarte V, Sousa Dias R, Ladeira Bernardes A, de Paula SO, de Luces Fortes Ferreira CL, do Carmo Gouveia Peluzio M. *Food Research International, June 2022.*

Abstract

The use of probiotic and synbiotic is a promising strategy to modulate the intestinal microbiota, and thereby modify the risk of diseases. In this study, the effect of probiotic VSL#3, isolated or associated with a yacon-based product (PBY), on the functional metabolic pathways of the microbiota, in a colorectal carcinogenesis model, was evaluated. For this, mice induced to carcinogenesis were fed with standard diet AIN-93 M (CON), diet AIN-93 M and VSL#3 (PRO) or diet AIN-93 M with yacon and VSL#3 (SYN). The SYN group showed a highly differentiated intestinal community based on the MetaCyc pathways. Of the 351 predicted functional pathways, 222 differed between groups. Most of them were enriched in the SYN group, namely: amino acid biosynthesis pathways, small molecule biosynthesis pathways (cofactors, prosthetic groups, electron carriers and vitamins) carbohydrate degradation pathways and fermentation pathways. In addition, the synbiotic was able to stimulate the anti-inflammatory immune response and reduce the gene expression of PCNA and c-myc.

Thus, we conclude that the synbiotic impacted more significantly the metabolic functions of the microbiota compared to the isolated use of probiotic. We believe that the enrichment of these pathways can exert antiproliferative action, reducing colorectal carcinogenesis. The prediction of the functional activity of the microbiota is a promising tool for understanding the influence of the microbiome on tumor development.

Effects of probiotic supplementation on very low dose AFB1-induced neurotoxicity in adult male rats

Sahin GA, Karabulut D, Unal G, Sayan M, Sahin H. *Life Sciences, July 2022.*

Abstract

Aims: Aflatoxin B1 (AFB1) is the most toxic and common form of AF found in food and feed. Although AFB1 exposure has toxic effects on many organs, studies on the brain are limited. Moreover, to the best of our knowledge, there is no study on the effect of probiotics on AFB1-induced neurotoxicity. Therefore, we aimed to evaluate the possible effects of probiotics on AFB1-induced neurotoxicity in the brain.

Main methods: Thirty-two adult male Wistar rats were divided into four groups: Vehicle (VEH), Probiotic (PRO) (2.5×10^{10} CFU/day VSL#3, orally), Aflatoxin B1 (AFB1) (25 µg/kg/week AFB1, orally), and Aflatoxin B1 + Probiotic (AFB1 + PRO) (2.5×10^{10} CFU/day VSL#3 + 25 µg/kg/week AFB1, orally). At the end of eight weeks, rats were behaviorally evaluated by the open field test, novel object recognition test, and forced swim test. Then, oxidative stress and inflammatory markers in brain tissues were analyzed. Next, brain sections were processed for Hematoxylin&Eosin staining and NeuN and GFAP immunostaining.

Key findings: Probiotic supplementation tended to decrease oxidative stress and inflammatory markers compared to the AFB1 group. Besides, brain tissues had more normal histological structures in VEH, PRO, and AFB1 + PRO groups than in the AFB1 group. Moreover, in probiotic groups, GFAP immunoreactivity intensity was decreased, while NeuN-positive cell number increased in brain tissues compared to the AFB1 group.

Significance: Probiotics seem to be effective at reducing the neurotoxic effects of AFB1. Thus, our study suggested that especially Bifidobacterium and Lactobacillus species can improve AFB1-induced neurotoxicity with their antioxidant and anti-inflammatory effects.

In vivo

Probiotic colonization dynamics after oral consumption of VSL#3® by antibiotic-treated mice

Theriot C, Thanissery R, O'Flaherty S, Barrangou R. *Microbiome Research Reports, July 2022.*

Abstract

Background: The ability of probiotic strains to provide health benefits to the host partially hinges on the survival of gastrointestinal passage and temporary colonization of the digestive tract. This study aims to investigate the colonization profile of individual probiotic strains comprising the commercial product VSL#3® and determine their impact on the host intestinal microbiota.

Methods: Using a cefoperazone-treated mouse model of antibiotic treatment, we investigated the impact of oral gavage with ~10^8 CFU commercial VSL#3® product on the intestinal microbiota using 16S-based amplicon sequencing over 7 days.

Results: Results showed that probiotic strains in the formulation were detected in treated murine fecal samples, with early colonization by *Streptococcus thermophilus* and *Lactiplantibacillus plantarum* subsp. *plantarum,* and late colonization by *Lacticaseibacillus paracasei subsp. paracasei, Bifidobacterium breve and Bifidobacterium animalis subsp. lactis.* Overall, VSL#3® consumption is associated with increased alpha diversity in the cecal microbial community, which is important in the context of antibiotic consumption. Probiotic supplementation resulted in an expansion of Proteobacteria, Bacteroidetes, and Actinobacteria, especially Bifidobacteriaceae and Lachnospiraceae, which are associated with *Clostridioides difficile* resistance in the murine gut.

Conclusion: This study illustrates the need for determining the ability of probiotics to colonize the host and impact the gut microbiota, and suggests that multiple doses may be warranted for extended transient colonization. In addition, follow-up studies should determine whether VSL#3® can provide resistance against *C. difficile* colonization and disease in a mouse model.

In vivo

Probiotics and prebiotics alleviate behavioral deficits, inflammatory response, and gut dysbiosis in prenatal VPA-induced rodent model of autism

Adıgüzel E, Çiçek B, Ünal G, Aydın MF, Barlak-Keti D. *Physiology & Behavior, September 2022.*

Abstract

Autism spectrum disorders are neuropsychiatric conditions characterized by social interaction and communication disorders and repetitive stereotypical behaviors. These disorders are also accompanied by an inflammatory status. Bidirectional communication between microbiome, gut, and brain has been discovered as a major mechanism influencing core symptoms and biomarkers of autism. Therefore, the modulation of the gut microbiota in autism has recently attracted interest. In this study, probiotic- and prebiotic-mediated modulation of the gut microbiota was compared in terms of different symptoms and findings in an experimental autism model. Valproic acid (VPA) (500 mg/kg) was administered to Wistar rats (on prenatal day 12.5) to induce autistic-like behaviors. Based on the supply of probiotics and prebiotics, animals were grouped as control (saline), autistic-like (prenatal VPA), probiotic (prenatal VPA + 22.5 × 10^9 cfu/day probiotic), prebiotic (prenatal VPA + 100 mg/day prebiotic), and combined treatment (prenatal VPA + 22.5 × 10^9 cfu/day probiotic + 100 mg/day prebiotic). After the treatment process, behavioral tests (social behaviors, anxiety, stereotypical behavior, sensorimotor gating, and behavioral despair) and biochemical analyses (serum and brain tissue) were conducted, and the quantities of some phyla and genera were determined in stool samples. Significant positive effects of probiotic and combined treatments were observed on the sociability, social interaction, and anxiety parameters. In addition, all three treatments had positive effects on stereotypical behavior. However, the treatments did not affect sensorimotor gating deficits and behavioral despair. Further, probiotic treatment reversed the VPA-induced increase and decrease in serum IL-6 and IL-10 levels, respectively. Combined treatment also significantly increased the IL-10 levels. Prenatal VPA exposure decreased 5-hydroxytryptamine (5-HT) levels in the prefrontal cortex of the brain; however, combined treatment reversed this decrease. Prenatal VPA exposure also caused a decrease in Bacteroidetes/Firmicutes ratio in the gut microbiota, while the probiotic treatment significantly increased this ratio. These findings indicate that probiotic- and prebiotic-mediated microbial modulation may represent a new therapeutic approach to alleviate autistic-like symptoms.

Nerve injury-induced gut dysbiosis contributes to spinal cord TNF-α expression and nociceptive sensitization

Lee J, Lee G, Ko G, Joong Lee S. *Brain, Behavior, and Immunity, March 2023.*

Abstract

The impact of the gut microbiota on glial cell growth and maturation via the gut-brain axis is highlighted herein. Considering that glial activation is crucial for onset and maintenance of neuropathic pain, we assessed the putative involvement of gut microbiota in the pathogenesis of neuropathic pain. Depletion of mouse gut microbiota with chronic antibiotics cocktail treatment prevented nerve injury-induced mechanical allodynia and thermal hyperalgesia both in male and female mice. Furthermore, post-injury treatment with antibiotics cocktail relieved ongoing pain in neuropathic pain-established mice. Upon recolonization of the gut microbiota after cessation of antibiotics, nerve injury-induced mechanical allodynia relapsed. Depletion of gut microbiota accompanied a decrease in nerve injury-induced TNF-α expression in the spinal cord. Notably, nerve injury changed the diversity and composition of the gut microbiome, which was measured by 16 s rRNA sequencing. We then tested if probiotic administration ameliorating dysbiosis affected the development of neuropathic pain after nerve injury. Probiotic treatment for three weeks prior to nerve injury inhibited nerve injury-induced TNF-α expression in the spinal cord and pain sensitization. Our data reveal an unexpected link between the gut microbiota and development and maintenance of nerve injury-induced neuropathic pain, and we propose a novel strategy to relieve neuropathic pain through the gut-brain axis.

In vivo

Probiotic Formulation VSL#3 Interacts with Mesenchymal Stromal Cells To Protect Dopaminergic Neurons via Centrally and Peripherally Suppressing NOD-Like Receptor Protein 3 Inflammasome-Mediated Inflammation in Parkinson's Disease Mice

Zhou L, Han D, Wang X, Chen Z. *Microbiology Spectrum, January 2023.*

Abstract

Systemic immunomodulation is increasingly recognized among the beneficial effects of mesenchymal stromal cells (MSCs) in treatment of Parkinson's disease (PD), while the underlying mechanism is not fully understood. With the growing popularity of using probiotics as an adjuvant approach in PD treatment, concerns about the added effects of probiotics have been raised. In addition to the molecular mechanism mediating the neuroprotective effects of MSCs, the combined effects of a probiotic formulation, VSL#3, and MSC infusion were also evaluated in PD mice. The animals were weekly treated with human MSCs (hMSCs) via the tail vein, VSL#3 via the gastrointestinal tract, or their combination six times. hMSCs, VSL#3 alone, and their combination markedly ameliorated the decreased striatal dopamine content, loss of dopaminergic neurons in the substantia nigra, increased levels of proinflammatory cytokines in serum, as well as tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) mRNAs in striatum and peripheral tissues induced by MPTP. Furthermore, hMSCs, VSL#3, and their combination notably downregulated mRNA expression of NOD-like receptor protein 3 (NLRP3) and caspase-1 in brain and peripheral tissues of PD mice. These results suggest that hMSCs, VSL#3, and their combination prevent neurodegenerative changes in PD mice via anti-inflammatory activities in both the central and peripheral systems, possibly through suppressing the NLRP3 inflammasome. Moreover, two-way analysis of variance (ANOVA) indicated that VSL#3 interacts with hMSCs to attenuate neurodegeneration and inhibit NLRP3 inflammasome-mediated inflammation without altering the effects of hMSCs. Major findings of our study support the usage of probiotic formulation VSL#3 as an adjuvant therapy to hMSC infusion in PD treatment.

IMPORTANCE This study provides evidence for the neuroprotective activities of human umbilical cord MSCs from the aspect of anti-inflammation actions. hMSCs inhibit the NLRP3 inflammasome and MPTP-induced inflammation in both brain and periphery to relieve the degenerative changes in dopaminergic neurons in PD mice. Furthermore, as an additional therapeutic agent, probiotic formulation VSL#3 interacts with hMSCs in suppressing the NLRP3 inflammasome as well as the central and peripheral anti-inflammatory effects to exert neuroprotective actions in PD mice without altering the actions of hMSCs, suggesting the potential of VSL#3 as an adjuvant therapy in PD treatment. The findings of the present study give a further understanding of the anti-inflammatory activity and the molecular mechanism for the beneficial effects of MSCs as well as the potential application of probiotic formulation as an adjuvant approach to MSC therapy in PD treatment.

In vivo

A probiotic formulation protects the dopaminergic neurons via attenuating the intestinal inflammation in mice of Parkinson's disease

Zhou L, Han D, Zheng T, Wang X, Xie H. *Research Square, May 2023.*

Abstract

Objective

Targeting the intestinal inflammation becomes a strategy for Parkinson's disease (PD) treatment. This study investigated the neuroprotective effects of a probiotic formulation, VSL#3® formulation, and the involvement of the anti-inflammation, in particular the intestinal inflammation.

Materials and Methods

The probiotics was orally administrated to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD for six weeks.

Results

The striatal content of dopamine and its metabolites, the survival of dopaminergic neurons in the substantia nigra were substantially increased in probiotics treatment mice compared to PD mice. The pro-inflammatory cytokines in the striatum were significantly suppressed while the anti-inflammation mediators were dramatically up-regulated by probiotics. The probiotics attenuated the intestinal inflammation via regulating the gut microbial composition. The mRNA expression of Tumor Necrosis Factor-α *(TNF-α)* and *Interleukin-1β (IL-1β)* mRNA significantly decreased in probiotic treatment mice compared to PD mice. Besides, the circulating levels of pro-inflammatory cytokines were notably decreased, indicating the blocked transfer of inflammatory cytokine from gut via blood.

Conclusion

Probiotics protect dopaminergic neurons in PD mice by attenuating the neuroinflammation via inhibiting the intestinal inflammation, which is acquired by restoring the imbalanced gut microbial composition, providing evidence for the idea of targeting the intestinal inflammation as well as using probiotics for PD treatment.

Microbiological

Comparative genomic analysis of the multispecies probiotic-marketed product VSL#3

Douillard FP, Mora D, Eijlander RT, Wels M, de Vos WM. *Plos One, February 2018.*

Abstract

Several probiotic-marketed formulations available for the consumers contain live lactic acid bacteria and/or bifidobacteria. The multispecies product commercialized as VSL#3 has been used for treating various gastro-intestinal disorders. However, like many other products, the bacterial strains present in VSL#3 have only been characterized to a limited extent and their efficacy as well as their predicted mode of action remain unclear, preventing further applications or comparative studies. In this work, the genomes of all eight bacterial strains present in VSL#3 were sequenced and characterized, to advance insights into the possible mode of action of this product and also to serve as a basis for future work and trials. Phylogenetic and genomic data analysis allowed us to identify the 7 species present in the VSL#3 product as specified by the manufacturer. The 8 strains present belong to the species Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus helveticus, Bifidobacterium breve and B. animalis subsp. lactis (two distinct strains). Comparative genomics revealed that the draft genomes of the S. thermophilus and L. helveticus strains were predicted to encode most of the defence systems such as restriction modification and CRISPR-Cas systems. Genes associated with a variety of potential probiotic functions were also identified. Thus, in the three Bifidobacterium spp., gene clusters were predicted to encode tight adherence pili, known to promote bacteria-host interaction and intestinal barrier integrity, and to impact host cell development. Various repertoires of putative signalling proteins were predicted to be encoded by the genomes of the Lactobacillus spp., i.e. surface layer proteins, LPXTG-containing proteins, or sortase-dependent pili that may interact with the intestinal mucosa and dendritic cells. Taken altogether, the individual genomic characterization of the strains present in the VSL#3 product confirmed the product specifications, determined its coding capacity as well as identified potential probiotic functions.

Development of omics-based protocols for the microbiological characterization of multi-strain formulations marketed as probiotics: the case of VSL#3

Mora D, Filardi R, Arioli S, Boeren S, Aalvink S, de Vos WM. *Microbial Biotechnology, August 2019.*

Abstract

The growing commercial interest in multi-strain formulations marketed as probiotics has not been accompanied by an equal increase in the evaluation of quality levels of these biotechnological products. The multi-strain product VSL#3 was used as a model to setup a microbiological characterization that could be extended to other formulations with high complexity. Shotgun metagenomics by deep Illumina sequencing was applied to DNA isolated from the commercial VSL#3 product to confirm strains identity safety and composition. Single-cell analysis was used to evaluate the cell viability, and β-galactosidase and urease activity have been used as marker to monitor the reproducibility of the production process. Similarly, these lots were characterized in detail by a metaproteomics approach for which a robust protein extraction protocol was combined with advanced mass spectrometry. The results identified over 1600 protein groups belonging to all strains present in the VSL#3 formulation. Of interest, only 3.2 % proteins showed significant differences mainly related to small variations in strain abundance. The protocols developed in this study addressed several quality criteria that are relevant for marketed multi-strain products and these represent the first efforts to define the quality of complex probiotic formulations such as VSL#3.

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