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Investigation of a Melissa officinalis special extract on Cognition I
In vitro study on muscarinic properties

KEYWORDS: Melissa officinalis, lemon balm extract, in vitro study, muscarinic M1 receptor, cognition

Abstract *Melissa officinalis* (L.) leaves, Lemon balm, are used as food and in traditional medicine. The traditional application of Lemon balm focuses on calming and relaxing effects. Modern research has demonstrated new effects of Lemon balm on cognitive health. A *Melissa officinalis* special extract was used to investigate muscarinic receptor M1 binding properties in the following in vitro experiments. Competitive binding to muscarinic M1 receptor was determined by measuring muscarinic displacement using a human recombinant muscarinic M1 receptor expressed in CHO (Chinese Hamster Ovary cells). As a result, the special *Melissa officinalis* extract showed muscarinic receptor M1 binding underlining cognitive effects, which might impact the beneficial effects of the extract in different indications for cognition and mental health.

INTRODUCTION

Melissa officinalis, Lemon balm, is an annual eatable herb native to Europe. Its traditional medical applications focus on the essential oil components of the plant and on rosmarinic acid for relaxation properties. Currently there are several traditional herbal medicinal products on the market which promote Lemon balm for its calming and relaxing effects. More recent research demonstrated new effects of Lemon balm on cognitive performance (1, 2). Surprisingly human studies reported that not all Lemon balm extracts show these beneficial cognitive effects, even if the extracts have similar phytochemical specifications (standardization on rosmarinic acid) (1). In order to clarify this several in vitro studies were carried out to understand the mode of action behind the beneficial effects on cognitive performance of Lemon balm and how they differ from extracts with the traditional calming effects. Studies showed that the traditional relaxing effects may be linked to elevated levels of the neurotransmitter GABA (γ-Aminobutyric acid) by inhibition of GABA-transaminase (GABA T), an enzyme which degrades GABA. Rosmarinic acid is described to be responsible for this effect (3). The antioxidant protection provided by Lemon balm shows promises in reducing oxidative-related brain cell death which contributes to the development of degenerative diseases (4). Several studies showed that CNS cholinergic binding affinity to nicotinic and muscarinic receptors improves cognitive performance, e.g. attention, action selection, learning and memory improvement (5-7). Particularly the muscarinic receptors as part of the cholinergic system have been investigated for cognitive effects. Muscarinic receptors are members of G protein-coupled receptors (GPCRs). Up to now, five different subtypes (M1 – M5) have been identified. Activation of muscarinic receptors helps to improve cognitive processing by synchronization of oscillatory activity (rhythmic activity of neurons) and the consequent transition of the brain from a deactivated to an activated status. Disruption of oscillatory activity leads to cognitive deficits (6). The muscarinic 1 receptor is expressed with highest levels in the prefrontal cortex and the hippocampus, brain regions which are important for cognition (8). Muscarinic M1 receptor agonists are used as modulators for normal cognitive functions and reversal of cognitive deficits (9). To screen *Melissa officinalis* special extract on muscarin M1 receptor properties and to confirm the proposed in mode of action, in vitro experiments were selected, which determine the competitive binding to muscarinic M1 receptor by measuring muscarinic displacement. A human recombinant muscarinic M1 receptor expressed in CHO (Chinese Hamster Ovary cells) was used (10).

MATERIALS AND METHODS

Characterization of *Melissa officinalis* special extract

Melissa officinalis special extract is obtained by water extraction out of *Melissa officinalis (L.)* leaves, an annual eatable herbaceous plant native to Europe. *Melissa officinalis*, shown in figure 1 is a member of the family *Lamiaceae*. The extract is hydrophilic and can easily be dissolved. The extract has a raw material: extract ratio of 3.5:1 and is standardized on 6 percent rosmarinic acid. For HPLC analyses the European Pharmacopoeia 8 method 2.2.29 was applied.
Melissa officinalis extract (commercially available as Bluenesse®) was supplied by Vital Solutions GmbH, Germany (Dr. Sybille Buchwald-Werner). Three different *Melissa officinalis* extract batches were investigated. They were obtained out

of three different *Melissa officinalis* raw material batches. The extracts have been produced in the same way and have the same specifications.

Figure 1. *Melissa officinalis*

Sample preparation for *in vitro* study
A stock solution (300 mg/ml) was prepared in DMSO (Dimethylsulfoxid) and was then diluted with water to the applied test concentration.

Competitive binding to muscarinic M1 receptor
Muscarinic displacement was measured using a human recombinant muscarinic M1 receptor expressed in CHO (Chinese Hamster Ovary cells). The applied method is well established and was validated and carried out by Eurofins/ Cereb in France according to the method described by Dörje, F. et. al. (10).The radio ligand was [3H]pirenzepine. Incubation time was 60 min at room temperature. Non-specific binding was defined in the presence of 1 µM atropine and all assays were carried out in duplicates. Competition binding was performed with the extract at five different concentrations (0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml, 3 mg/ml), the radio ligand and the receptor. After incubation of the assay mix the receptor bound fraction was isolated by filtration on GF/C glass fiber. The amount of radio labeled ligand bound to the receptors was quantified in a liquid scintillation counter.
The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand. The data are expressed as specific binding of the radio ligand in the presence of the extract. The IC50 values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (nH, slope factor) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using Hill equation curve fitting. Two separated studies were carried out. Within the first study one sample and within the second study two *Melissa officinalis* special extract samples were investigated.

STATISTICS

The results are presented as mean. Results showing a receptor binding higher than 50 percent are considered to represent significant effects of the test compounds.

RESULTS

The experiments were designed to determine muscarinic M1 receptor binding properties of *Melissa officinalis* special extract. Results are shown in figure 2. The three samples showed dose-dependent effects with receptor binding higher than 50 percent. Receptor binding higher than 50 percent is considered to represent significant effects. All three samples showed significant muscarinic M1 receptor binding properties.

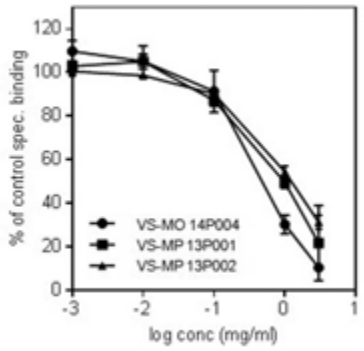

Figure 2. Competitive binding at the muscarinic M1 receptor of [3H]pirenzepine under the influence of *Melissa officinalis* special extracts

Assay 1	IC_{50}-value (mg/ml)
VS-MO 13P001	1.26
VS-MO 13P001	0.78
Assay control (pirenzepine)	0.0094 (= 27nM)

Assay 2	IC_{50}-value (mg/ml)
VS-MO 14P004	0.45
Assay control (pirenzepine)	0.0059 (= 17nM)

Figure 3. Binding affinities of three *Melissa officinalis* special extracts to the muscarinic M1 receptor (IC50 values)

DISCUSSION

The experiments clearly demonstrated significant muscarinic M1 receptor binding properties of *Melissa officinalis* special extract. Muscarinic receptors as part of the cholinergic system have been reported to be important for cognitive effects, like alertness and memory.
The binding property of *Melissa officinalis* extract might be highly dependent on the *Melissa officinalis* leaf raw material used to obtain the extract. Other *in vitro* studies investigating the potential CNS cholinergic activity of different Lemon balm extracts confirmed that not all extracts exhibit the expected different CNS cholinergic receptor binding properties (11). An extract with negligible cholinergic receptor binding showed in humans well known effects consistent with its long traditional use as a mild sedative/ anxiolytic, but did not enhance memory. *Melissa officinalis* extract screened for muscarinic binding in human brain tissue had the same calming effects, but furthermore also improved alertness and memory performance. This property could be confirmed in several human studies, which demonstrated that a Melissa officinalis extract screened for muscarinic M1 receptor binding properties in human brain tissue provides the well-known calming effects, but furthermore also improves alertness and memory performance (1, 2, 12) .Due to the origin of the raw material, being plants with different genetic backgrounds and grown under different climatic conditions, certain variability in the raw material composition is expected.

Consequently the selection and control of a suitable *Melissa officinalis* leaf raw material with cholinergic receptor binding properties is of high importance if the resulting Lemon balm extract is intended to be used to support mental health. Referring to the presented results a reproducible muscarinic M1 receptor binding assay would be suitable to confirm the targeted activity for the selection of suitable *Melissa officinalis* leaf raw materials to produce a Lemon balm extract for mental health.
For many plant extracts their physiological effects are known, but these effects cannot be linked to dedicated molecules. A combination of traditional knowledge plus innovative technologies has to be applied to develop state of the art extracts. New and innovative extracts, complying with regulatory requirements need to demonstrate a stable quality with regards to safety and efficacy aspects. Phytochemical and in vitro studies as combined quality control methods allow standardization on activity of extracts and natural products. These methods deliver reliable results and are able to detect variations in activity which might be related to different plant varieties, cultivation techniques and extraction conditions.

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The workshop will provide in its first part an introduction on the skin structure and the permeability properties of the stratum corneum, on the effect cosmetic formulations can have on local gene expression patterns and on the sphingolipid metabolism. Subsequently, specialists from university and industrial research will provide an overview on different delivery systems today available to cosmetic formulators.

Target audience: industry researchers, specialists in formulation, consultants, buyers, developers, decision makers, product specialists, marketing people, etc.

Crucial role of the tissue structure for stratum corneum permeability
Marek HAFTEK, MD, PhD Directeur de Recherches CNRS-Laboratoire de Recherche Dermatologique, MatriX Faculté de Médecine et de Pharmacie UCBL1, Lyon, France

Gene modulation in skin cells : a tool to assess the effect of formulations on skin
Dr. Thierry ODDOS Pharmacology Department, Johnson and Johnson Santé Beauté France, Campus de Maigremont, Val de Reuil, France

Environmental biological influences on sphingolipids metabolism in epidermis.
Dr. Iuliana POPA University of Paris XI, School of Pharmacy, Châtenay-Malabry, France

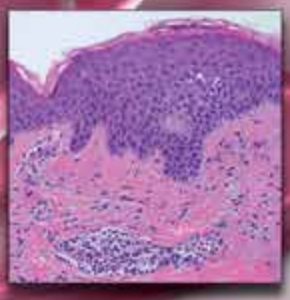
Last innovations in the field of carrier systems for cosmetic actives delivery
Dr. Gabriele BLUME Sopharcos, Research & Development, Consulting Pharmacy & Cosmetics, Steinau an der Strasse Germany

Developing suitable formulations for skin application containing lipid nanoparticles
Dr. Marilene Sofia RODRIGUES ESTANQUEIRO Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

Innovative system for actives delivery in cosmetic formulation: balancing the innovation with the regulatory requirements
Serena TONGIANI, Ph.D. R&D Director, ANGELINI, S. PALOMBA, Acraf S.p.A., Pomezia (RM) Italy

Moderator: **Florian T. E. Weighardt, PhD**, Associate Editor of **H&PC Today, TKS Publisher**





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