7+8/17 | Volume 143 | Thannhausen, July 28, 2017

sofw journal

Home & Personal Care Ingredients & Formulations

7/8
2017
english

sun care

Innovative Formulation Technique for a New Broad Spectrum, Water Resistant, SPF 25+ Daily Facial Moisturizer

Current Formulation Aspects for Sunscreens

Pickering Emulsions: a Sustainable Strategy to Boost the Water Resistance Performance of Sunscreens

Renewable Green Emollient to Solubilize Filters in Sunscreen Lotions

Maximizing the Benefits and Minimizing the Dangers of Sun Exposure

hair care

The Multi-functional Silk Polypeptide as New and Promising Strategy against Pollution

regulation

Future Regulatory Changes Affecting Cosmetic Ingredients

skin care

The Role of the TRPV1 Receptor in Thermal Ageing

The Role of the TRPV1 Receptor in Thermal Ageing

E. Besic Gyenge*, S. Breitenbach*, B. Suter*, S. Hettwer*, B. Obermayer*

abstract

The TRPV1 receptor is a key sensor for thermal and chemical stresses in the skin. Continuous stimulation of this receptor leads to sensitive skin and premature skin ageing. *Albatrellus ovinus* extract contains grifolin derivatives which block the receptor. Binding experiments revealed IC_{50} values in the low micromolecular range and blocking of neuronal CGRP release was determined by ELISA. *In vivo* studies with a TSAII neurosensory analyser and IR-A irradiation demonstrated a benefit for the skin by using a cosmetic formulation containing *Albatrellus ovinus* extract in heat stress situations. *Albatrellus ovinus* extract is suitable to sooth sensitive skin and protects against thermal and chemical stressors. The contribution of blocking the TRPV1 receptor to prevent thermal ageing is discussed.

Introduction

Skin ageing, as a progressive and very complex development, is not only a result of intrinsic, genetically determined processes. Skin ageing is mainly accelerated by extrinsic factors, coming from the environment. The skin loses its youthful appearance by developing wrinkles and discolorations, especially on the hands, face and décolleté. As those body parts are exposed to sunlight the most, it is obvious that chronic exposure to solar radiation is a major trigger factor for skin ageing. The solar radiation is composed of 7 % UV, 39 % visible and 54 % infrared radiation (IR) [1]. Not only UV but also infrared radiation can harm the skin. As such, at least 60 % of solar radiation can cause skin damage or lead to irritated or sensitive skin and subsequently to photoageing (dermatoheliosis). Photoageing is a cumulative process that progresses over the years.

UVB rays are a primary mutagen that can penetrate through the epidermis and reach the stratum papillare of the dermis. Exposure to UVB will mainly result in direct DNA and protein damage and inflammation. In the short term, it will create sunburn and hyperalgesic skin. In the long run, it will lead to severe photoaging and cancer. UVA rays are able to penetrate deeper into the dermis and are the main cause for dermal decomposition. A prolonged UVA radiation creates reactive oxygen species (ROS) which destroy elastin and collagen, leading to a reduced thickness of the dermis. These processes also trigger inflammation which enhances the dermal degradation due to collagenase release. Recently, it was discovered that infrared (IR-A) radiation also contributes significantly to photoageing processes [2]. Infrared also produces ROS and plays an important role in inflammation. It impairs the dermal-epidermal junction, upregulates several matrix metalloproteinases and reduces the expression of key genes of the extracellular matrix like collagen and elastin. As such, chronic exposure to IR-A plays a more important role in premature skin aging than previously expected. Even a relatively small increase in temperature will lead to collagen degradation [3] and free radical formation will be substantially increased [4]. It is quite clear that IR or heat exposure trigger the same processes leading to skin ageing as is observed for exposure to UV radiation. As infrared radiation resembles heat, we can use the term "thermal ageing" for heat and IR induced photoageing.

The TRPV1 (transient receptor potential vanilloid 1) receptor is a well investigated member of the temperature sensitive calcium channels. Cosmetic use of TRPV1 receptor blockers for soothing sensitive skin was already reported elsewhere [5]. TRPV1 is not only involved in pain perception. Instead, it also plays an important role in premature skin ageing. Concepts on the influence of heat and thereby induced skin inflammation and ageing effects by activation of the TRPV1 receptor are still not available. It is known that TRPV1 levels are higher in the skin of elderly people, suggesting that this receptor is involved in the intrinsic ageing process [6]. Moreover, UV radiation increases TRPV1 levels, which implies TRPV1 is involved in photoageing. Here, we are proposing a new concept of skin ageing called thermal skin ageing (**Fig. 1**), which is caused by continuous heat stress on the skin. Besides neurons, keratinocytes also display the TRPV1 receptor on their surface [7, 8]. TRPV1 is triggered by temperatures higher than 40 °C and evokes an inflammatory response which leads to elevated MMP1 release and subsequently increased collagen degradation [8, 9]. As such, inhibition of TRPV1 would be a good approach for preventing thermal skin ageing, besides providing a soothing effect as a neurocosmetic [8, 9]. As an inhibiting agent we used a formulation containing 3 % *Albatrellus ovinus* extract (DEFENSIL®-SOFT; INCI: Propanediol, Albatrellus Ovinus Extract, Citric Acid). This extract from an

edible mushroom contains bioactive phenolic compounds such as grifolin, neogrifolin, and scutigeral.

Materials and Methods

IC_{50} -Measurements

Measurements were performed as described in [10]. In brief, a mixture of 0.2 nM [3H]- radiolabeled resiniferatoxin (RTX) and 100 nM non- labelled RTX were incubated with TRPV1 receptor. The release of radioligand upon binding of a competitive compound was detected by scintigraphy. To calculate the IC_{50} values, the Hill equation was fitted to the acquired data points.

CGRP-Release

To test the ability of grifolin to inhibit neuronal calcitonin gene-related peptide (CGRP) release by upstream inhibition of the TRPV1 receptor, sensory neurons co-cultivated with normal human epidermal keratinocytes (NHEK) were stimulated 30 minutes with 30 µM capsaicin to activate the TRPV1 receptor. The CGRP release was investigated by quantitative ELISA technique in the presence of different grifolin concentrations.

In vivo Studies

All studies were performed in accordance with the principles of good laboratory practice (GLP), good clinical practice (GCP), and in compliance with the quality assurance system requirements. The studies were in accordance with the World Medical Association's Declaration of Helsinki. All study participants signed a written informed consent at the beginning of the study. Details of the study protocols are depicted in the results section.

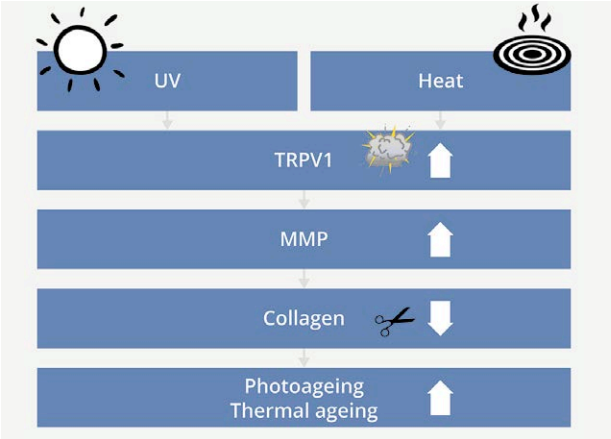

Fig. 1 TRPV1 plays a substantial role in photoageing and thermal ageing of the skin. A model of the role of TRPV1 in UV- and heat-induced MMP1 expression: Heat and UV light trigger TRPV1 and increase its expression. Activation of TRPV1 then increases MMP1 expression and stimulates collagen and matrix protein degradation, eventually leading to skin ageing.

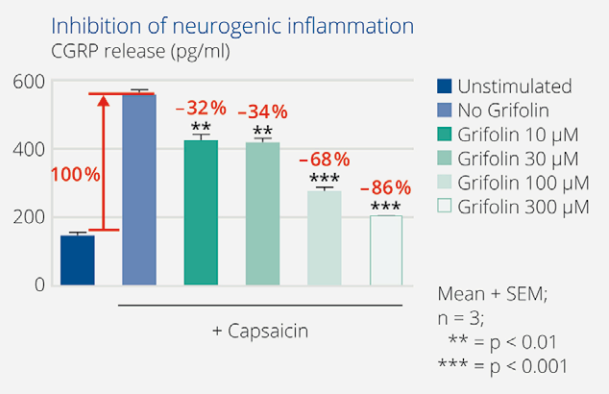

Fig. 2 Grifolin reduces the irritation of nerve endings. Nerve cell-keratinocyte co-cultures were pre-incubated with solutions containing grifolin or not, and were then stimulated with 300 nM capsaicin. The release of the pro-inflammatory neuropeptide CGRP and percentage of Inhibition are depicted. CGRP release was inhibited by grifolin in a dose-dependent manner. Mean + SEM are given. Students' t-test.

Results

Grifolin, Neogrifolin and Scutigeral Show Excellent Affinity to TRPV1

Grifolin derivatives from *Albatrellus ovinus* showed high binding potency (IC_{50}) in the low micromolar range (15-30 µM). Scutigeral showed the highest potency with 14.7 µM. Grifolin and neogrifolin also proved to be very effective (18.8 µM and 30.8 µM).

Grifolin Counteracts Neuron Driven Inflammation

Grifolin showed a concentration-dependent inhibitory effect on the capsaicin-induced CGRP release in sensory neuron-NHEK co-cultures (**Fig. 2**). As little as 10 µM Grifolin (~0.0003 %) already inhibited 1/3 of the CGRP release and the inhibitory effect reached 86 % at 300 µM, without affecting cellular viability.

Albatrellus Ovinus Extract Protects from Heat Stress and Soothes Hyperalgesic Skin

In a double-blind, placebo controlled, randomized study, 17 subjects with Caucasian skin in the range of 26-63 years were analysed for their heat pain threshold with a TSA II neurosensory analyser (Medoc, Israel). A 9 cm^2 thermode was placed medial anterior on the forearm and a temperature ramp with a slope of 0.5 °C/s between 32 °C and 50 °C was applied. At the heat pain threshold, the study participant hit a button to stop the heating process. After twice daily application of a formulation containing 3 % *Albatrellus ovinus* extract or a placebo on the one arm or the other over a period of 14 days, the measurement was repeated. Subsequently, a cream containing 240 µM capsaicin (ABC-Cream, Hansaplast, Germany) was applied on both arms and the heat pain thresholds were recorded after 60 minutes to investigate hyperalgesic skin.

No differences in the heat-pain thresholds were observed between the left and right arms of the study participants at baseline. After 14 days of applying the test formulations, no significant increase in the heat-pain threshold was observed for the placebo, while a significant 1.5 °C increase was detected for *Albatrellus ovinus* extract (**Fig. 3**, left). Induction of hyperalgesic skin with capsaicin revealed a significant decrease of 1.4 °C in the heat-pain threshold for the placebo, but not for verum compared to the baseline value (**Fig. 3**, right).

Albatrellus Ovinus Extract Ameliorates IR-induced Barrier Damage and Skin Reddening

In an anecdotal study on two male subjects (34 and 44 years) with Caucasian skin, two areas on the inner side of the forearm were treated with a formulation containing 0 % (placebo) or 3 % *Albatrellus ovinus* extract (verum). 30 minutes after application, the skin was exposed for 10 minutes to a standard broad spectrum IR radiator (Philips Infrared PAR38 150W) at 200 mW/cm^2 in distinct circular areas. This IR dose is equivalent to 80 minutes of European midday sun. The skin reddening upon IR exposure was monitored by colorimetric analysis using a skin colorimeter CL400 (a*-value; Courage & Kazhaka Electronic, Germany). The effect on the skin barrier was monitored by tewametry (TEWL, Tewameter TM300, Courage & Kazhaka Electronic, Germany).

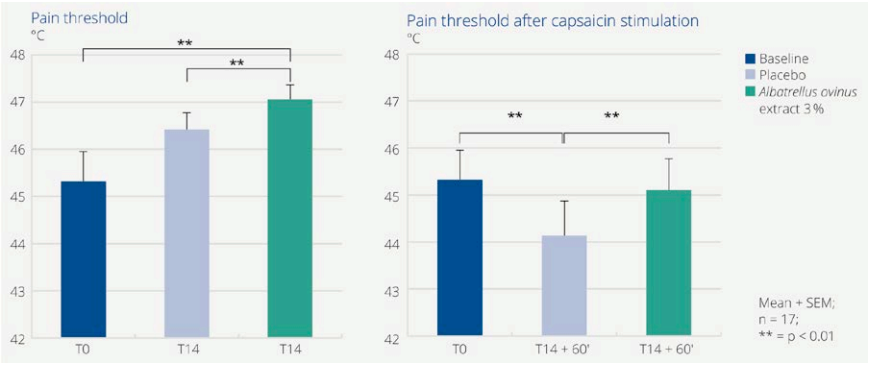

Fig. 3 *Albatrellus ovinus* extract increases the heat pain threshold. Left: 3 % *Albatrellus ovinus* extract applied for 14 days medial anterior on the forearm increased the heat pain threshold significantly ($p < 0.01$) by 1.5 °C. The placebo formulation did not show a significant increase. Right: Activation of the TRPV1 receptor with 240 µM capsaicin decreased the heat pain threshold significantly ($p < 0.01$) on the placebo treated arm while for the formulation containing 3 % *Albatrellus ovinus* extract no significant change was observed compared to the baseline. N = 17. Mean + SEM are given. Students' t-test.

Directly after IR-A irradiation, both areas showed pronounced reddening (**Fig. 4**). While the inflammatory reaction in the *Albatrellus ovinus* treated region stayed within the IR exposed area, the reddening in the placebo treated region scattered outside the exposed area. The pronounced reddening remained strongly visible for 30 minutes and vanished after about 1 - 2 hours on the verum side. This was also reflected in the a-values recorded in parallel. On the placebo side, a quite persistent erythema was induced vanishing only after 5 hours. By investigation of the TEWL during the recovery phase, a pronounced increase of transepidermal water loss could be recorded, which was significantly enhanced in the placebo treated area compared to the verum treated area (**Fig. 4** bottom left). Similar results were recorded for another subject.

Discussion

CGRP is a subsequent mediator of TRPV1 receptor activation [11]. Release of CGRP leads to enhanced blood circulation and inflammatory reactions resulting in skin ageing. By suppressing the activation of the TRPV1 receptor, an attenuated inflammatory reaction can be expected. *In vitro* measurement of TRPV1 receptor induced CGRP release revealed an inhibitory effect of grifolin (**Fig. 2**). This result, as well as the improvement of capsaicin induced heat pain threshold, attenuated skin reddening and TEWL by application of *Albatrellus ovinus* extract, points to an important shutdown of downstream reactions upon TRPV1 receptor activation. CGRP plays an important role in the maintenance of psoriasiform dermatitis [12]. While denervation of the skin led to an improvement of the condition, an application of CGRP had the opposite effect. Additionally, in atopic dermatitis, CGRP harbouring nerve fibres are elevated as well as circulating levels of CGRP. A reduction of this inflammatory mediator may thus contribute to maintain a normal skin function.

Importantly, as the TRPV1 receptor is mainly a thermal receptor, infrared (or heat stress) is a natural trigger of TRPV1 receptor activation. Irradiation of the skin with 200 mW/cm² for 10 minutes, corresponding to 80 minutes exposure to European midday sun, caused a mild erythema on the skin of a test person (**Fig. 4**). A pronounced reddening of verum and placebo treated areas was observed and confirmed by measuring the a-value. While the erythema disappeared after 1 - 2 hours on

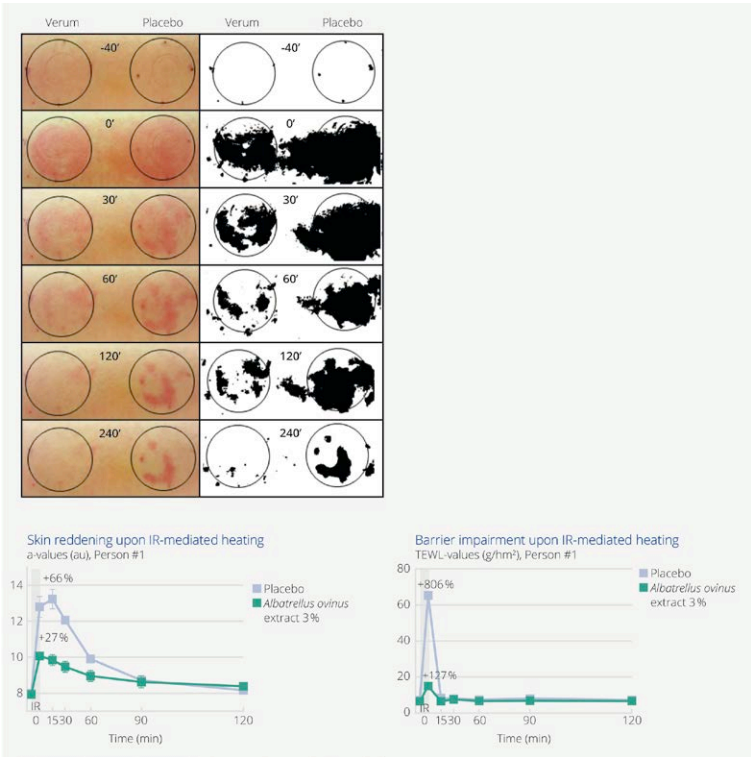

Fig. 4 Infrared induced erythema is less severe upon blocking of the TRPV1 receptor. Top: The left circular area on the forearm medial anterior was treated with 3 % *Albatrellus ovinus* extract, the right side with a placebo. 10 minutes irradiation with an IR-A lamp induced a mild erythema on both sides, which vanished significantly faster on the verum treated side. Left panels: original photographs; right panels: black and white conversion. Bottom left: a-values plotted against recovery time. The IR-A lamp was switched off at t = 0 min. Faster recovery was achieved with verum. Bottom right: Development of TEWL plotted against recovery time. IR-A irradiation caused a higher TEWL in placebo compared to verum.

the verum side, it took 5 hours for the placebo side. It can be concluded that TRPV1 receptor inhibition by the grifolin derivatives lead to a quicker decrease in CGRP level and eventually to a quicker reduction of microcirculation. Consequently, we assume less inflammatory reactivity on the verum side and as such, less skin ageing (**Fig. 5**). The enormous increase of the TEWL can be mainly considered as an increased evaporation due to the heating of the tissue and the increased activity of sweat glands. Since the presence of 3 % *Albatrellus ovinus* extract significantly reduced this effect ($p < 0.001$), the presence of TRPV1 receptor blockers presumably had a beneficial effect on the skin barrier. Indeed, it was found that sensitive skin shows a trend to higher TEWL values which can hence be reduced by blocking the TRPV1 receptor [13]. These findings and our studies suggest that the topical use of natural TRPV1 receptor antagonists, particularly grifolin derivatives extracted from Albatrellus ovinus, has the capacity to attenuate skin ageing and reduces uncomfortable skin sensations caused by chemicals, heat and irradiation with UV and IR on normal skin as well as on sensitive skin.

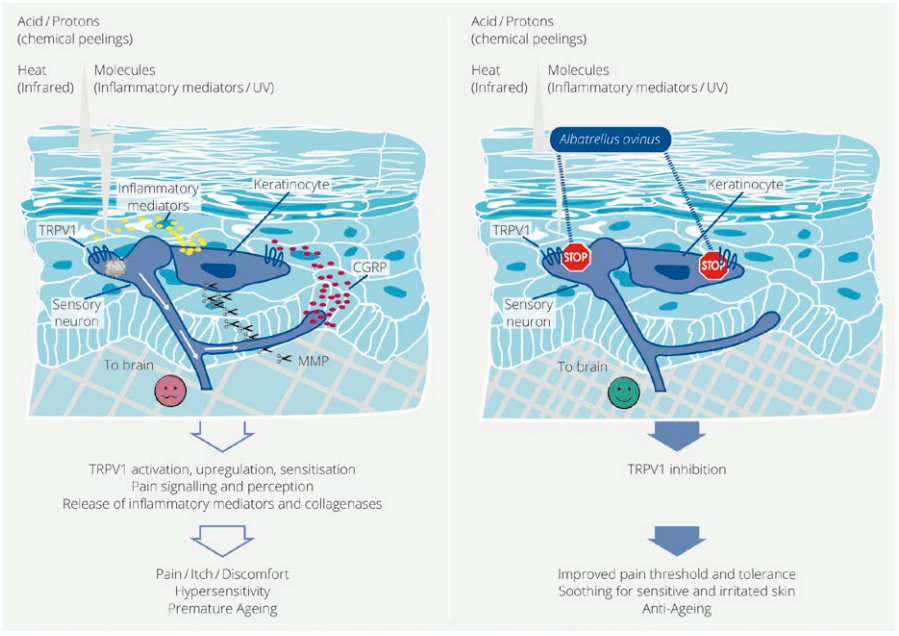

Fig. 5 Next-generation neuro-cosmetics. The pain receptor TRPV1 acts as a neuro-sensor and responds to potentially harmful stimuli such as heat, acids, and internal/external inflammatory molecules. Activation of TRPV1 leads to pain, itching and discomfort, as well as inflammatory stress and matrix degradation. Selective inhibition and calming of this receptor not only provides immediate pain relieve for sensitive or irritated skin but also prevents TRPV1-mediated premature ageing.

Bibliography

[1] *Kochevar, M.A.* and *A.P. Pathak*, Dermatology in General medicine, ed. Fitzpatrick. New York: McGraw-Hill.

[2] *Robert, C., et al.*, 2015. Low to moderate doses of infrared A irradiation impair extracellular matrix homeostasis of the skin and contribute to skin photodamage. Skin Pharmacol Physiol, 28(4): 196-204.

[3] *Shin, M.H., et al.*, 2012. Chronic heat treatment causes skin wrinkle formation and oxidative damage in hairless mice. Mech Ageing Dev, 133(2-3): 92-8.

[4] *Jung, T., et al.*, 2010. Effects of water-filtered infrared A irradiation on human fibroblasts. Free Radic Biol Med, 48(1): 153-60.

[5] *Hettwer, S., et al.*, 2016. Grifolin derivatives from *Albatrellus ovinus* as TRPV1 receptor blockers for cosmetic applications. Int J Cosmet Sci.

[6] *Lee, Y.M., Y.K. Kim,* and *J.H. Chung,* 2009. Increased expression of TRPV1 channel in intrinsically aged and photoaged human skin *in vivo.* Exp Dermatol, 18(5): 431-6.

[7] *Lee, Y.M., et al.*, 2009. A novel role for the TRPV1 channel in UV-induced matrix metalloproteinase (MMP)-1 expression in HaCaT cells. J Cell Physiol, 219(3): 766-75.

[8] *Lee, Y.M., S.M. Kang,* and *J.H. Chung,* 2012. The role of TRPV1 channel in aged human skin. J Dermatol Sci, 65(2): 81-5.

[9] *Li, W.H., et al.*, 2007. Transient receptor potential vanilloid-1 mediates heat-shock-induced matrix metalloproteinase-1 expression in human epidermal keratinocytes. J Invest Dermatol, 127(10): 2328-35.

[10] *Szallasi, A., et al.*, 1993. Competitive inhibition by capsazepine of [3H]resiniferatoxin binding to central (spinal cord and dorsal root ganglia) and peripheral (urinary bladder and airways) vanilloid (capsaicin) receptors in the rat. J Pharmacol Exp Ther, 267(2): 728-33.

[11] *Nakanishi, M., et al.*, 2010. Acid activation of Trpv1 leads to an up-regulation of calcitonin gene-related peptide expression in dorsal root ganglion neurons via the CaMK-CREB cascade: a potential mechanism of inflammatory pain. Mol Biol Cell, 21(15): 2568-77.

[12] *Granstein, R.D., et al.*, 2015. Calcitonin gene-related peptide: key regulator of cutaneous immunity. Acta Physiol (Oxf), 213(3): 586-94.

[13] *Seidenari, S., M. Francomano,* and *L. Mantovani,* 1998. Baseline biophysical parameters in subjects with sensitive skin. Contact Dermatitis, 38(6): 311-5.

*contact

Dr. Emina Besic Gyenge
Sandra Breitenbach
Brigit Suter
Dr. Stefan Hettwer
Barbara Obermayer | barbara.obermayer@rahn-group.com

RAHN AG
Dörflistrasse 120
8050 Zürich | Switzerland