International Journal of
Cosmetic Science

International Journal of Cosmetic Science, 2016, 1–7 doi: 10.1111/ics.12385

Grifolin derivatives from *Albatrellus ovinus* as TRPV1 receptor blockers for cosmetic applications

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Received 16 September 2016, Accepted 15 November 2016

Keywords: capsaicin, chemical analysis, claim substantiation *in vivo*/*in vitro*, sensitive skin/inflammation/allergy, TRPV1

Abstract

OBJECTIVE: Blocking the TRPV1 receptor is an interesting approach for the treatment of sensitive skin. Here we investigated the potential of grifolin derivatives from *Albatrellus ovinus* to act as TRPV1 receptor blockers and their potential to serve as cosmetic active ingredients.

METHODS: Binding characteristics of grifolin derivatives from *Albatrellus ovinus* were determined in competitive and functional *in vitro* assays to achieve IC_{50} values. The TRPV1 receptor was activated *in vivo* with capsaicin and noxious heat to investigate skin reddening, microcirculation, skin sensations and heat pain thresholds.

RESULTS: Grifolin derivatives extracted from *Albatrellus ovinus* proved to inhibit the TRPV1 receptor *in vitro* and *in vivo*. Besides suppression of the TRPV1 receptor activity upon chemical stimulation with capsaicin, thermal activation was shown to be inhibited as well by application of cosmetic formulations containing 3% *Albatrellus ovinus* extract. The reduction of stinging and burning sensations as well as reduction of reddening and microcirculation upon irritation with capsaicin or thermal stress proved efficacy *in vivo*.

CONCLUSION: Grifolin derivatives from *Albatrellus ovinus* are able to serve as fungal-derived TRPV1 receptor blockers with capability to serve as a cosmetic active ingredient on sensitive skin.

Résumé

OBJECTIF: Le blocage du récepteur TRPV1 est une approche intéressante pour le traitement des peaux sensibles. Ici, nous avons étudié le potentiel des dérivés de grifolin *d'Albatrellus ovinus* pour agir comme bloqueurs des récepteurs TRPV1 et leur potentiel pour servir d'ingrédients actifs cosmétiques.

METHODES: Les caractéristiques de liaison de dérivés de grifoline d'*Albatrellus ovinus* ont été déterminées dans des essais *in vitro* compétitifs et fonctionnels pour obtenir les valeurs de CI50. Le récepteur TRPV1 a été activé *in vivo* avec de la capsaïcine et de la chaleur nocive pour étudier la rougeur de la peau, la microcirculation, les sensations cutanées et les seuils de douleur thermique.

RESULTATS: Les dérivés de grifoline extraits *d'Albatrellus ovinus* ont montré une inhibition *in vitro* et *in vivo* du récepteur TRPV1. Outre la suppression de l'activité du récepteur TRPV1 par stimulation chimique avec la capsaïcine, on a montré que l'activation thermique était également inhibée par l'application de formulations cosmétiques contenant 3% d'extrait *d'Albatrellus ovinus*. La réduction des sensations de picotemente et de brûlure, ainsi que la réduction des rougeurs et de la microcirculation lors de l'irritation par la capsaïcine ou le stress thermique, ont prouvé leur efficacité *in vivo*.

CONCLUSION: Les dérivés de grifolin du champignon *Albatrellus ovinus* sont capables de servir de bloqueurs des récepteurs TRPV1, capables de servir d'ingrédient actif cosmétique sur les peaux sensibles.

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Introduction

Transient receptor potential channels play an important role in the sensing of changes in the proximity of cells. To date, six families of these transmembrane receptors are known with diverse modes of activation [1]. Among these, the TRPV1 (transient receptor potential vanilloid 1) receptor is a well-investigated member of the temperature-sensitive calcium channels. TRPV1 is predominantly located on nociceptive C or Aδ nerve fibres [2]. It is activated in case of noxious heat (>42°C), low pH and by chemical agonists rendering the receptor in an open state [3]. Certain metabolites and cytokines supporting inflammatory reactions like arachidonic acid and leukotrienes are also able to trigger the TRPV1 receptor [4, 5]. Opening the receptor leads to calcium influx which evokes an action potential transferred to the neuron and subsequently to the brain. This leads to the perception of pain, burning, stinging or tightness, which are typical epiphenomenons of sensitive skin. As such, chemical compounds like capsaicin which trigger the open state of the channel lead to burning sensations. Interestingly, a continuous or repeated stimulus with this compound causes a downregulation of the TRPV1 receptor and a desensitization, whereas stimulation with, for example, phenoxyethanol does not [6]. In contrast, acid application increases the expression of TRPV1 [7]. As such, the regulation of TRPV1 seems to be variable dependent on the external trigger. In hypersensitive skin, the number of TRPV1 receptors is elevated [8] making the skin more susceptible for activating impacts. Recent findings also suggest that distinctive single nucleotide polymorphisms (SNPs) may be responsible for sensitive skin [9]. Antagonists of the TRPV1 receptor prevent calcium influx leading to a soothing of the associated unpleasant sensations [10]. Interestingly, capsazepine, a potent TRPV1 receptor antagonist with an IC_{50} in the low micromolar range, can inhibit calcium influx induced by chemical agonists but not by other painful stimuli like heat or acids [11]. The reason might be the complex topology of

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the TRPV1 receptor which is a homotetramer consisting of four binding sites for small molecules. In dependence on the interaction of these molecules with the TRPV1 receptor, the conformation will be in an open or closed state [12]. Typically, two molecules of a binder are sufficient to induce the conformational change [13]. Potent TRPV1 receptor blockers with convincing *in vitro* and *in vivo* data exist [6, 10, 14–16]. However, an inhibitory effect on temperature dependent activation *in vivo* has not yet been demonstrated for TRPV1 receptor blockers in cosmetic use.

By far, more plant-derived compounds are known to activate TRPV1 receptors than inhibiting them [17]. A promising group of compounds for the use as TRPV1 receptor antagonists in cosmetics are prenylated phenols which can be found in the basidiocarps of the edible fungus *Albatrellus confluens*. Among these, grifolin, neogrifolin, confluentin and scutigeral were already investigated for their potential to bind and modulate the TRPV1 receptor [18]. Scutigeral and confluentin do not act as antagonists for the TRPV1 receptor but desensitize it. Grifolin and neogrifolin showed antagonistic activity in the low micromolar range. As a promising candidate to isolate TRPV1 antagonists, *Albatrellus ovinus*, a commercially available source of *Albatrellaceae*, was investigated for the content of grifolin derivatives and their capability to inhibit the TRPV1 receptor *in vitro* and *in vivo*.

Material and methods

Extraction of *Albatrellus ovinus* was done by incubation of the dried mushroom fruit bodies in 1,3 propanediol and subsequent filtration of the liquid phase.

Identification of grifolin derivatives

Albatrellus ovinus extract was subjected to HPLC-MS on an Agilent Series 1100 HPLC including autosampler and DAD detector coupled with a Micromass LCT mass spectrometer with ESI ion source and a Sedere Sedex-75 evaporative light scattering detector (ELSD). The used column was a Waters Symmetry C18 at 0.4 mL min^{-1} at 40°C. Compounds were separated in a standard gradient of water and acetonitrile both containing 0.1% (v/v) formic acid and visualized by detecting at 275 nm. Compounds were identified due to their mass and UV spectra and confirmed with ^{1}H-NMR and ^{13}C-NMR spectroscopy. Pure compounds were isolated by preparative HPLC.

IC_{50} determination for binding of prenylated phenols

Measurements were performed as described in Ref. [19]. In brief, a mixture of 0.2 nM [^{3}H]-radiolabelled resiniferatoxin (RTX) and 100 nM non-labelled RTX was incubated with TRPV1 receptor. The release of radioligand upon binding of a competitive compound was detected by scintillation counting. To calculate the IC_{50} values, the Hill equation was fitted to the acquired data points.

Functional inhibition of the TRPV1 receptor

Functional inhibition of the TRPV1 receptor was investigated by measuring calcium ion influx with patch clamp experiments in CHO cells stably transfected with cDNA for the TRPV1 receptor. Cells were passaged at a confluence of 50-80% in Ham's medium and nutrition mixture F-12 supplemented with 9% FCS and 0.9% Pen/Strep solution. For the measurements, cells were transferred into 35-mm dishes containing 2 mL culture complete medium. Cells were cultivated at 37°C at 5% CO_2. Grifolin was dissolved in DMSO to achieve a 10 mM solution. The required working concentrations were made by dilution in bath solution (137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM D-glucose, pH 7.4). Measurements were done in triplicate. 300 nM capsaicin was applied together with different concentrations of grifolin. Cells were continuously perfused at 1 mL min^{-1} in a dish under the microscope and attached to the electrode in whole-cell patch configuration. After a stable seal was established, cells were clamped to a potential of 80 mV. TRPV1 receptor currents were activated with 300 nM capsaicin until the currents were similar for at least two subsequent capsaicin applications. Afterwards, 300 nM capsaicin together with grifolin was added and the current was recorded. For control of sensitivity, 5 μM capsazepine was used.

Inhibition of CGRP release

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

Calculation of Hill coefficients from publication data

The corresponding IC_{50} plots were digitalized with WebPlotDigitizer [18, 20-22] and the Hill equation was fitted to the extracted data points with GraphPad Prism 6.05 (Graph Pad Software, U.S.A.).

In vivo studies

All studies were performed in accordance with the principles of good laboratory practice (GLP) and good clinical practice (GCP) and in compliance with the quality assurance system requirements. Studies were conducted with respect to World Medical Association in the Declaration of Helsinki. All study participants signed a written informed consent at the beginning of the study.

In vivo study with grifolin

Double-blind, placebo-controlled, randomized, crossover study. Thirty female subjects with Caucasian skin with a positive skin response to topical application of 100 μM capsaicin (i.e. 'stingers') in the age range of 18–55 years (average 35.2) were divided into two groups. Both groups applied the same base formulation (water, glycerine, disodium EDTA, *Limnanthes Alba*, *Butyrospermum Parkii*, capsaicin (100 μM), butylhydroxyanisole, steareth-2, steareth-21, cetearyl alcohol, propylene glycol, diazolidinyl urea, methylparaben, propylparaben) with 5 mM grifolin (verum) or without (placebo). In a split face approach, half of the subjects applied the grifolin containing formulation on day 1 and placebo on day 2 on the nasolabial fold area, whereas the other half applied the placebo on day 1 and the grifolin containing formulation on day 2 to one side of the nasolabial fold area. As a reference, a saline solution was applied on the non-treated side. Scores of unpleasant perceptions were recorded 3 min after product application. Scoring was 0 = no perception, 1 = doubtful, barely perception, 2 = slight perception, 3 = moderate perception, 4 = strong perception.

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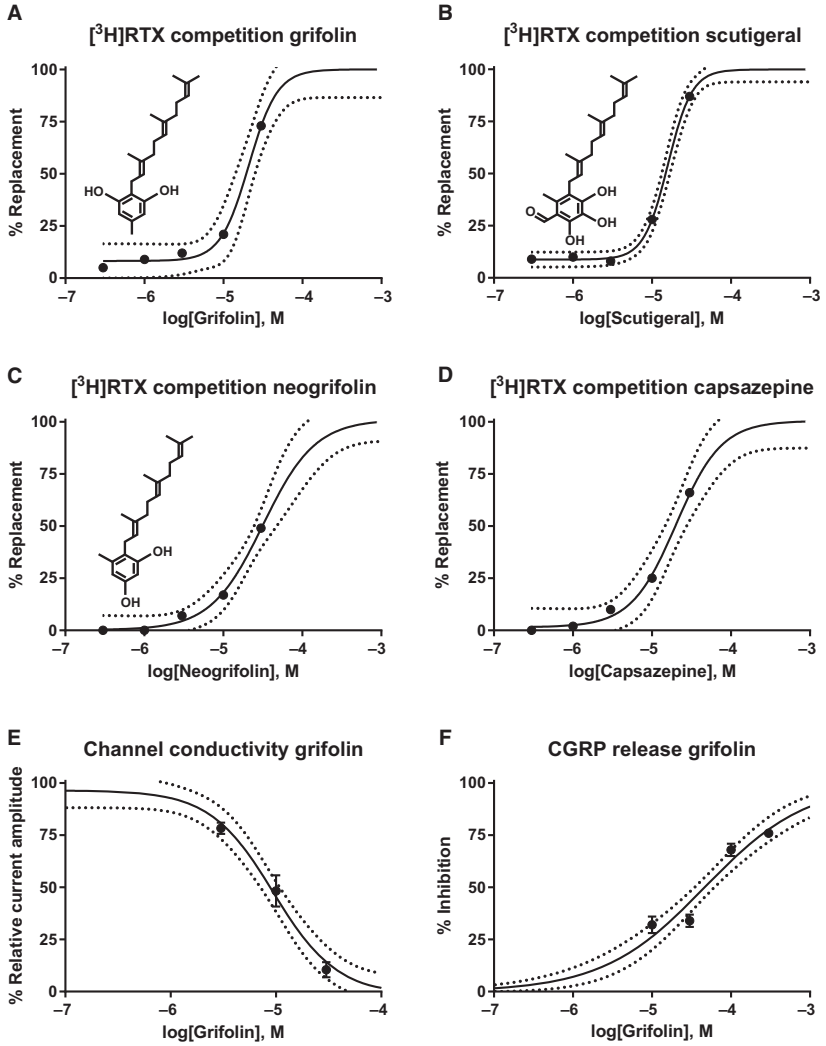

Figure 1 Grifolin derivatives are potent TRPV1 receptor modulating compounds. A–D: IC_{50} determinations for the binding of grifolin derivatives to the TRPV1 receptor were carried out with a competitive [^{3}H] resiniferatoxin (RTX) competition assay. For all derivatives, the IC_{50} values were in the low micromolar range (Table I). Functional inhibition of TRPV1 receptor stably expressed in CHO cells confirmed this result (E). Subsequent triggering of the signalling cascade leading to CGRP release can be blocked by grifolin with an IC_{50} of 46.4 μM (F). The dashed line represents the 95% confidence interval of the fits.

In vivo study with *Albatrellus ovinus* extract

Double-blind, placebo-controlled, randomized, crossover study. Twenty-one female subjects with Caucasian skin with a positive response to topical application of 114 μM capsaicin (i.e. 'stingers') in the age range 21–56 years (average 36.2) were divided into two groups. Both groups applied the same base formulation (water, caprylic/capric triglyceride, glycerine, propanediol, phenoxyethanol, carbomer, xanthan gum, caprylyl glycol, sodium hydroxide, citric acid) with 3% *Albatrellus ovinus* extract (verum) or without (placebo). Half of the subjects applied the verum containing formulation on day 1 on both nasolabial fold areas, and the other half used placebo. The application was done *vice versa* on day 2. Five minutes after application, in a split face approach, one side of the nasolabial fold was irritated by application of 114 μM capsaicin solution whereas on the other side saline solution was applied. Scores of unpleasant perceptions were recorded 3 min after product application. Scoring was 0 = absent perception, 1 = light perception, 2 = moderate perception, 3 = significant perception. The values were corrected by the baseline perceptions with saline. The reddening was evaluated by recording a-values with a skin colorimeter CL400 (Courage & Khazaka Electronic, Germany) after 10 min. Microcirculation was recorded with a Periflux PF5000 device (Perimed, Sweden). Blood perfusion values were detected as the mean of 3-min continuous measurements.

Determination of heat pain thresholds

Double-blind, placebo-controlled, randomized study. Seventeen subjects with Caucasian skin (eight male, nine female) in the age range of 26–63 years (average 43.7) were analysed for their heat

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Table I IC_{50} values of grifolin derivatives and capsazepine as control molecule. Columns 1 and 2 refer to the [^{3}H] resiniferatoxin (RTX) competition assay. Reference values for the Hill coefficients were calculated upon data given in the corresponding references ('Hill coefficient ref.') and originate from functional assays. 'IC50 functional assay' refers to current amplitude measurements on patch-clamped CHO cells stably expressing the TRPV1 receptor. Corresponding values from the literature are depicted in the last column

	IC50 binding (μM)	Hill coefficient	Hill coefficient ref.	IC50 functional assay (μM)	IC50 functional assay ref. (μM)
Grifolin	18.8	1.84	1.40*	8.6	26.0*
Neogrifolin	30.8	1.50	2.86*	n.d.	7.1*
Scutigeral	14.7	2.41	1.75†	n.d.	19.2†
Capsazepine	18.2	1.69	1.95‡	n.d.	6.9‡

*Hellwig *et al.* [18].
†Szallassi *et al.* [21].
‡El Kouhen *et al.* (2006); n.d. not determined.

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 from 32°C to 50°C with 0.5°C s^{-1} was applied. The study subjects interrupted the heating ramp by pressing a button when the individual heat pain threshold was reached. The mean of three subsequent measurements was taken into account for learning and adaptation processes. By this, a more accurate result was achieved although a slight desensitization of the skin was observed from measurement to measurement. After 14-day application of formulations (see study above) containing 3% *Albatrellus ovinus* extract or placebo twice daily on the one arm or the other, the measurement was repeated. On day 14, a cream containing 240 μM capsaicin (ABC-Creme, Hansaplast, Germany) was applied on both arms. Heat pain thresholds were recorded after 60 min.

Results

In vitro studies

Inhibition of the TRPV1 receptor by grifolin derivatives from Albatrellus ovinus

Dried *Albatrellus ovinus* was extracted with 1,3 propanediol and investigated for the presence of TRPV1 receptor antagonists. HPLC analysis revealed three main compounds which could be assigned to grifolin, neogrifolin and scutigeral in concordance with Hellwig *et al.* [18] shown for *Albatrellus confluens*. The amount of these grifolin derivatives varied between 2.3 and 4.6 mg mL^{-1} which corresponds to up to 12.8 mM grifolin derivatives.

The IC_{50} values for the binding of isolated grifolin derivatives from *Albatrellus ovinus* to the TRPV1 receptor turned out to be in the low micromolar range in agreement with previous investigations (Fig. 1A–D, Table I). For all compounds, a Hill coefficient between 1.5 and 2.4 was determined. The Hill coefficient reflects how many small molecules bind to a receptor. In this case, about two molecules are necessary to fully block the TRPV1 receptor activity. In addition to the pure binding properties, the functional inhibition of the TRPV1 receptor was investigated for grifolin. Addition of 10 μM grifolin to the non-stimulated system did not provoke calcium ion influx which confirmed the inhibitory action of grifolin rather than a desensitization action known from scutigeral (not shown). The resulting IC_{50} value of 8.6 μM for grifolin (Fig. 1E) is even lower than the reported value of 26 μM [18]. Inhibition of TRPV1 receptor-dependent CGRP release on cultured neurons by grifolin revealed an IC_{50} of 46.4 μM (Fig. 1F).

In vivo studies

Grifolin reduces capsaicin-induced TRPV1 receptor activity in vivo

As a proof of concept that blocking of the TRPV1 receptor with grifolin derivatives has an *in vivo* effect on sensitive skin, we tested a formulation with 5 mM grifolin isolated from *Albatrellus ovinus* on 30 subjects with sensitive skin. All of the study participants reacted positively on the application of capsaicin on the nasolabial fold. In this study, 100 μM capsaicin was included in the test formulation to induce misperceptions by activation of the TRPV1 receptor. A 5 mM grifolin containing formulation leads to a pronounced and significant reduction of misperceptions to a score of 3.1 compared to placebo (score 5.8; Fig. 2A). The burning and stinging perceptions on the nasolabial fold were significantly reduced ($P < 0.05$/ $P < 0.001$; Fig. 2B).

Albatrellus ovinus extract reduces capsaicin-induced TRPV1 receptor activity in vivo

The proof of concept study with isolated grifolin was reproduced using 3% *Albatrellus ovinus* extract on 21 subjects with sensitive skin. Basically, the same beneficial effects were observed as reported for the study using pure grifolin (Fig. 2C, D). A significant difference in the score of misperception between placebo (3.1) and verum (1.6) was observed. Besides these subjective scorings, the reddening reaction as well as the microcirculation was investigated instrumentally. The *a*-value was substantially lower by treatment with verum (5.1 units for placebo compared to 0.5 units for verum) and an increase of the microcirculation was prevented (Fig. 2E, F).

Heat pain threshold is elevated by application of Albatrellus ovinus extract

Capsaicin is a widely used compound to activate the TRPV1 receptor but is usually not a natural trigger of TRPV1 receptor activation in the skin. The main function of the TRPV1 receptor is to serve as a temperature sensor to avoid overheating of the tissue leading to cell death. To test for the ability of *Albatrellus ovinus* extract to interfere with this trigger, we investigated the thermal activation of TRPV1 receptor with a TSAII NeuroSensory Analyzer on 17 subjects. This

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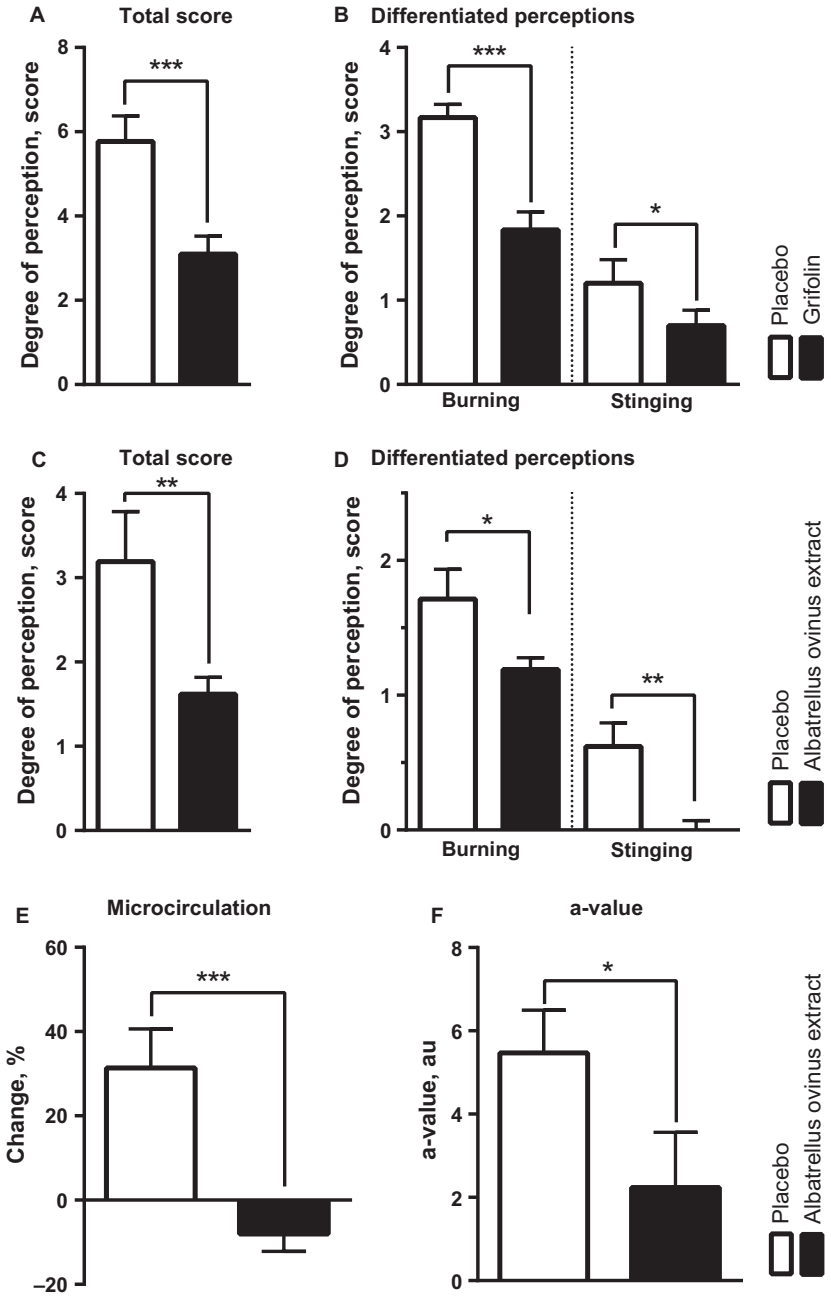

Figure 2 Grifolin derivatives sooth reactions provoked by TRPV1 receptor activation on the skin. A: Scoring of unpleasant perceptions of an emulsion containing 5 mM grifolin in comparison with placebo. Both formulations contained 100 μM capsaicin to induce the corresponding skin reactions at the nasolabial fold. Perception was scored 3 min after application. B: Separation of burning and stinging perceptions. $N = 30$. Mean ± SEM values are given. C–F: Similar study with 3% *Albatrellus ovinus* extract. C and D analogous to A and B. E: Capsaicin-induced microcirculation is pronounced with placebo but reduced with verum. F: Skin reddening is less pronounced with verum. $N = 21$. Mean + SEM values are given. Statistics: Student's *t*-test: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ for all graphs.

diagnostic device is able to detect the individual heat pain threshold by heating up a thermode placed on the subject's skin. The heat pain threshold was determined on the forearm medial anterior to 45.5 ± 0.5°C (Fig. 3A). There was no difference between the left and right arms of the study participants (not shown). After 14 days of application of a formulation containing 3% *Albatrellus ovinus* extract, the heat pain threshold increased significantly by 1.5°C. At the same time, the heat pain threshold increased by 0.7°C on the placebo treated arm, which was not significant over the initial value. *Albatrellus ovinus* extract was significantly superior over placebo. To

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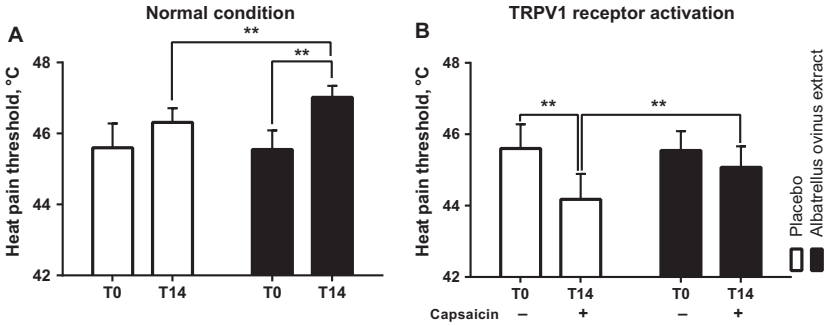

Figure 3 *Albatrellus ovinus* extract increases the heat pain threshold. A: 14-day application of a formulation containing 3% *Albatrellus ovinus* extract medial anterior of the forearm increased the heat pain significantly ($P < 0.01$) by 1.5°C. The placebo formulation did not show a significant increase. B: Upon activation of the TRPV1 receptor with 240 μM capsaicin, the heat pain threshold decreased significantly ($P < 0.01$) on the placebo treated arm, whereas for the formulation containing 3% *Albatrellus ovinus* extract, no significant change was observed compared to the initial values. $N = 17$. Mean + SEM values are given. Student's *t*-test: ** = $P < 0.01$.

evaluate the synergy of different triggers activating the TRPV1 receptor, a cream containing 240 μM capsaicin was applied at the end of the study. After 60 min, the heat pain threshold was recorded, which dropped significantly by 1.4°C for placebo and 0.48°C for *Albatrellus ovinus* extract (not significant) compared to the baseline condition (Fig. 3B).

Discussion

Albatrellus ovinus proved to be a suitable source for TRPV1 modulating compounds. Up to 4.6 mg mL^{-1} grifolin derivatives were selectively extracted with 1,3-propanediol which corresponded to 20% of the dry residue. The IC_{50} values of these derivatives for the binding to the TRPV1 receptor were in the expected low micromolar range with Hill coefficients between 1.5 and 2.4 (Fig. 1A–C, Table I). Similar Hill coefficients were calculated in previous measurements [18, 20, 21] and reflect the multiple binding site characteristic of the tetrameric TRPV1 receptor (Table I). As no cooperative binding characteristic was observed, Hill coefficients between 1.5 and 2.4 mean that 1-2 binding sites are occupied and induce allosteric changes in the protein which impede further binding. For grifolin, a Hill coefficient of 1.4 in a functional patch clamp assay was determined which matches the literature value [18].

The *in vivo* tests revealed a significant ($P < 0.001$) calming effect of 5 mM grifolin in an O/W formulation which was subclassified in significant reductions in burning and stinging perceptions (Fig. 2A, B). In all cases, verum was superior over placebo ($P < 0.05$–0.001). This experiment served as a proof of concept study to investigate whether grifolin, one of the TRPV1 receptor antagonists found in *Albatrellus ovinus* extract, was able to show *in vivo* effects. The subsequent study with 3% *Albatrellus ovinus* extract confirmed the positive results of pure grifolin for the fungal-derived extract (Fig. 2C,D). In 3% *Albatrellus ovinus* extract, 0.4 mM grifolin derivatives are present. This is approximately 50-fold higher than the IC_{50} for grifolin (8.6 μM) allowing full inhibition of the receptor significantly reducing skin reddening and microcirculation (Fig. 2E, F) upon capsaicin treatment. The *in vitro* reduction of CGRP release (Fig. 1F) goes in line with this observation. Induction of hyperalgesic skin by the activation of the TRPV1 receptor with the chemical model substance capsaicin may seem to be quite an artificial system with poor practical relevance in the cosmetic field as no one would apply this compound to the skin without medical need. However, it has been shown that some chemical compounds relevant in cosmetics like phenoxyethanol or allergens can activate the TRPV1 receptor after a short lag time [6] and a TRPV1 receptor blocker to prevent a burning and stinging perception induced by chemical compounds is therefore of an advantage.

On the other hand, as the TRPV1 receptor is mainly a thermal receptor, infrared (or heat stress) is a natural trigger of TRPV1 receptor activation. To substantiate beneficial effects of TRPV1 receptor antagonists on thermally activated TRPV1 receptors in the skin, a controlled study with 17 participants was conducted. A 14-day treatment with 3% *Albatrellus ovinus* extract lead to a significant increase of the heat pain threshold by 1.5°C compared to the initial condition and placebo (Fig. 3A). That means the participants became less sensitive to thermal stress. This is the first study showing that thermal activation of the TRPV1 receptor can be delayed *in vivo* with a cosmetic ingredient. Additional activation of the TRPV1 receptor with capsaicin confirmed the protective effect of *Albatrellus ovinus* extract. Unlike with placebo, the verum-treated skin kept its heat tolerance on initial level when irritated with capsaicin (Fig. 3B). A beneficial effect of blocking the TRPV1 receptor on hyperalgesic skin can thus be proposed. This study envisages that *Albatrellus ovinus* extract has the potential to protect sensitive skin from the synergistic effects of multiple trigger factors activating the TRPV1 receptor leading to unpleasant sensations.

Sensitive skin might be associated with a decrease in thermal pain thresholds. Our own studies did not reveal a correlation between the heat pain threshold and a self-evaluated rating of the sensitivity of the skin (not shown). We consider that the term 'sensitive skin' must not reflect subjective parameters but is a result of intrinsic and/or extrinsic trigger factors with multiple effects. There was also no correlation between age and heat pain threshold (not shown), in concordance with previous findings [23]. Studies on people with different hair colour revealed significant differences in the heat pain threshold of black haired and red haired by 1.4°C [24] and recent studies strengthened a potential genetic influence of SNPs in the TRPV1 receptor gene [9]. Skin inflammation was shown to be associated with the release of inflammatory and nociceptive mediators and resulted in significant tissue hyperalgesia in case of UV-B-induced sunburn [25] with a reduction of the heat pain threshold by 6°C. Thus, the heat-sensitive TRPV1 receptor may be an interesting target for the relief of heat pain on hyperalgesic skin and suppression of inflammatory reactions.

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Acknowledgement

Research and development was done by RAHN AG.

Conflict of interest

All authors are employees of RAHN AG, Switzerland.

References

1. Owsianik, G., Talavera, K., Voets, T. and Nilius, B. Permeation and selectivity of TRP channels. *Annu. Rev. Physiol.* **68**, 685–717 (2006).
2. Stander, S., Moormann, C., Schumacher *et al.* Expression of vanilloid receptor subtype 1 in cutaneous sensory nerve fibers, mast cells, and epithelial cells of appendage structures. *Exp. Dermatol.* **13**, 129–139 (2004).
3. Szallasi, A., Cortright, D.N., Blum, C.A. and Eid, S.R. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. *Nat. Rev. Drug. Discov.* **6**, 357–372 (2007).
4. Huang, S.M., Bisogno, T., Trevisani, M., Al-Hayani, A., De Petrocellis, L., Fezza, F. *et al.* An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 8400–8405 (2002).
5. Shin, J., Cho, H., Hwang, S.W., Jung, J., Shin, C.Y., Lee, S.Y. *et al.* Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 10150–10155 (2002).
6. Li, D.G., Du, H.Y., Gerhard, S., Imke, M. and Liu, W. Inhibition of TRPV1 prevented skin irritancy induced by phenoxyethanol. A preliminary *in vitro* and *in vivo* study. *Int. J. Cosmet. Sci.* doi:10.1111/ics.12340 (2016).
7. Schicho, R., Florian, W., Liebmann, I., Holzer, P. and Lippe, I.T. Increased expression of TRPV1 receptor in dorsal root ganglia by acid insult of the rat gastric mucosa. *Eur. J. Neurosci.* **19**, 1811–1818 (2004).
8. Facer, P., Casula, M.A., Smith, G.D., Benham, C.D., Chessell, I.P., Bountra, C. *et al.* Differential expression of the capsaicin receptor TRPV1 and related novel receptors TRPV3, TRPV4 and TRPM8 in normal human tissues and changes in traumatic and diabetic neuropathy. *BMC Neurol.* **7**, 11 (2007).
9. Sun, L., Wang, X., Zhang, Y., Wang, T., Li, X. and Ma, Y. The evaluation of neural and vascular hyper-reactivity for sensitive skin. *Skin Res. Technol.* **22**, 381–387 (2016).
10. Kueper, T., Krohn, M., Haustedt, L.O., Hatt, H., Schmaus, G. and Vielhaber, G. Inhibition of TRPV1 for the treatment of sensitive skin. *Exp. Dermatol.* **19**, 980–986 (2010).
11. Walpole, C.S., Bevan, S., Bovermann, G., Boelsterli, J.J., Breckenridge, R., Davies, J.W. *et al.* The discovery of capsazepine, the first competitive antagonist of the sensory neuron excitants capsaicin and resiniferatoxin. *J. Med. Chem.* **37**, 1942–1954 (1994).
12. Jordt, S.E. and Julius, D. Molecular basis for species-specific sensitivity to "hot" chili peppers. *Cell* **108**, 421–430 (2002).
13. Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D. and Julius, D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **389**, 816–824 (1997).
14. Sulzberger, M., Worthmann, A.C., Holtzmann, U., Buck, B., Jung, K.A., Schoelermann, A.M. *et al.* Effective treatment for sensitive skin: 4-t-butylcyclohexanol and licochalcone A. *J. Eur. Acad. Dermatol. Venereol.* **30**(Suppl 1), 9–17 (2016).
15. Schoelermann, A.M., Weber, T.M., Arrowitz, C., Rizer, R.L., Qian, K. and Babcock, M. Skin compatibility and efficacy of a cosmetic skin care regimen with licochalcone A and 4-t-butylcyclohexanol in patients with rosacea subtype I. *J. Eur. Acad. Dermatol. Venereol.* **30**(Suppl 1), 21–27 (2016).
16. Schoelermann, A.M., Jung, K.A., Buck, B., Gronniger, E. and Conzelmann, S. Comparison of skin calming effects of cosmetic products containing 4-t-butylcyclohexanol or acetyl dipeptide-1 cetyl ester on capsaicin-induced facial stinging in volunteers with sensitive skin. *J. Eur. Acad. Dermatol. Venereol.* **30**(Suppl 1), 18–20 (2016).
17. Vriens, J., Nilius, B. and Vennekens, R. Herbal compounds and toxins modulating TRP channels. *Curr. Neuropharmacol.* **6**, 79–96 (2008).
18. Hellwig, V., Nopper, R., Mauler, F., Freitag, J., Ji-Kai, L., Zhi-Hui, D. *et al.* Activities of prenylphenol derivatives from fruitbodies of Albatrellus spp. on the human and rat vanilloid receptor 1 (VR1) and characterisation of the novel natural product, confluentin. *Arch. Pharm. (Weinheim)* **336**, 119–126 (2003).
19. Szallasi, A., Goso, C., Blumberg, P.M. and Manzini, S. 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**, 728–733 (1993).
20. El Kouhen, R., Surowy, C.S., Bianchi, B.R., Neelands, T.R., McDonald, H.A., Niforatos, W. *et al.* A-425619 [1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea], a novel and selective transient receptor potential type V1 receptor antagonist, blocks channel activation by vanilloids, heat, and acid. *J. Pharmacol. Exp. Ther.* **314**, 400–409 (2005).
21. Szallasi, A., Biro, T., Szabo, T., Modarres, S., Petersen, M., Klusch, A. *et al.* A non-pungent triprenyl phenol of fungal origin, scutigeral, stimulates rat dorsal root ganglion neurons via interaction at vanilloid receptors. *Br. J. Pharmacol.* **126**, 1351–1358 (1999).
22. Ankit Rohatgi, WebPlotDigitizer 3.10, http://arohatgi.info/WebPlotDigitizer, May, (2016)
23. Lautenbacher, S., Kunz, M., Strate, P., Nielsen, J. and Arendt-Nielsen, L. Age effects on pain thresholds, temporal summation and spatial summation of heat and pressure pain. *Pain* **115**, 410–418 (2005).
24. Liem, E.B., Joiner, T.V., Tsueda, K. and Sessler, D.I. Increased sensitivity to thermal pain and reduced subcutaneous lidocaine efficacy in redheads. *Anesthesiology* **102**, 509–514 (2005).
25. Moore, C., Cevikbas, F., Pasolli, H.A., Chen, Y., Kong, W., Kempkes, C. *et al.* UVB radiation generates sunburn pain and affects skin by activating epidermal TRPV4 ion channels and triggering endothelin-1 signaling. *Proc. Natl. Acad. Sci. U.S.A.* **110**, E3225–E3234 (2013).

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