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abstract

A novel neurocosmetic soothing active, a biomimetic peptide inspired by the analgesic component of sea anemone venom, immediately comforts sensitive skin after the first application. An *in vitro* assay showed that the peptide reduced the activation of the TRPV1 pain receptor. To ensure improved skin uptake, the peptide was incorporated into a soft sphere carrier system based on shea butter. In clinical studies, the soothing active increased the electrical current perception threshold of the skin, which is an indicator for reduced skin reactivity and decreased skin sensitivity by reducing the response to skin irritation.

Introduction

Environmental Factors Lead to Uncomfortable Skin

Approximately half of the population considers their skin to be sensitive skin [1]. There are three main types of sensitive skin that are caused by I) reduced barrier function, II) inflammation with an intact barrier function and III) – the most common type in otherwise healthy individuals – skin that overreacts to environmental factors with irritation without barrier impairment and inflammation [2]. The reaction of type III sensitive skin can range from a feeling of slight discomfort to frequent visible signs of skin irritation, such as redness. This type of sensitive skin is characterized by an enhanced reactivity to common stimuli [3] – for example, wind, heat, clothes, sunlight and pollution. The result is skin that feels tight, itchy or even has a burning sensation. The main reason for this reaction is a sensory response that is too strong.

The Molecular Cause of Sensitive Skin

The Transient Receptor Potential (TRP) channels play an important role in the transduction of pain from a variety of environmental stimuli. The most important member of the TRP channel family is the TRPV1 vanilloid receptor. This receptor responds to different irritants such as heat, acids and certain chemical compounds, e.g. capsaicin, by opening up the transmembrane channel to enable the influx of Ca^{2+} ions into the cell [4]. This in turn leads to the activation of signaling pathways in the cell that ultimately results in an itching or painful sensation. TRPV1 receptors are most common in sensory nerve cells. However, they are also ex-

pressed in keratinocytes in the epidermis as these cell types are one of the first to encounter external stimuli and can therefore quickly communicate the pain signal to underlying nerve cells (Fig. 1). An increase of the nerve response to harmless environmental influences in sensitive skin is often due to an overreaction of the TRPV1 receptor. This can be the result of a lower activation threshold of TRPV1 as well as higher expression levels of TRPV1 in sensitive skin. In addition to being responsible for skin discomfort, TRPV1 plays a role in skin aging. In photo-aged human skin, TRPV1 is often overexpressed [5]. Furthermore, the constant activation of TRPV1, for example through heat and infrared radiation, leads to inflammation as well as to the upregulation of enzymes that destroy collagen in the skin and this could result in premature skin aging. The solution for sensitive skin is to strengthen the tolerance level by reducing the reactivity of TRPV1.

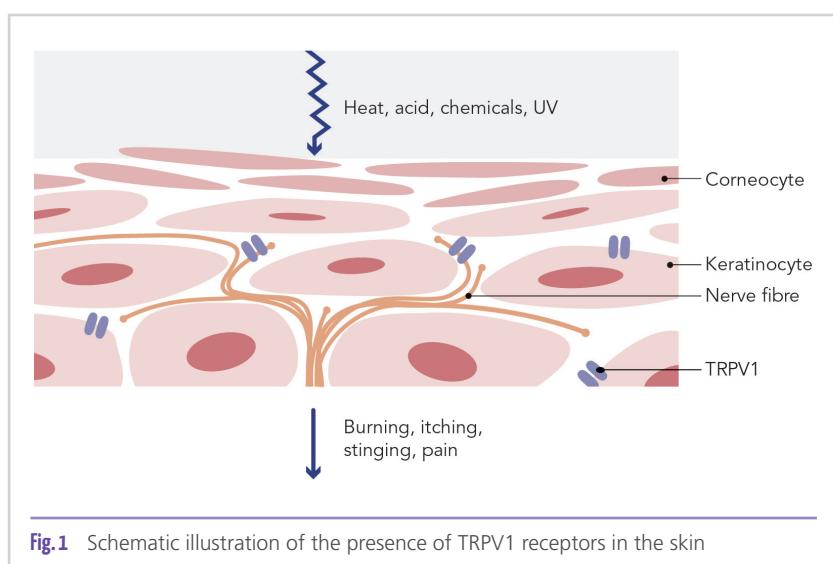


Fig. 1 Schematic illustration of the presence of TRPV1 receptors in the skin

A Solution for Sensitive Skin Derived from Sea Anemone Venom

Heteractis crispa, the leathery sea anemone, inhabits tropical and subtropical waters (Fig. 2). It is home to many anemone-fish species that use the anemone for shelter. A lesser known fact is, as the sea anemone is soft and vulnerable, it produces a venom to protect itself from predators and also to immobilize prey. The venom of the leathery sea anemone consists of proteins and peptides that interact with a variety of cellular targets. Scientists have recently discovered an interesting small protein called APHC1 in the venom of the leathery sea anemone that represented the first polypeptide inhibitor of the TRPV1 receptor [6]. It was further shown that treatment with APHC1 had ameliorating effects on the pain response in acute and chronic pain models [7]. This protein is therefore a very interesting molecule for targeting TRPV1 in sensitive skin to reduce irritation.

In order to render the soothing effect of the sea anemone protein available for cosmetic formulations, a pentapeptide (RRRFV) was designed to model the TRPV1 binding sequence. This was realized in collaboration with scientists at Venomtech (UK) who are experts in venom-based drug discovery. For sustainability and purity reasons, the peptide is produced synthetically. Furthermore, the pentapeptide was incorporated into a soft sphere carrier system based on shea butter in order to increase its penetration into the skin and to protect the peptide molecules in the formulation against degradation. The resulting soothing active [SensAmone P5; INCI: Pentapeptide-59 (and) Hydrogenated Lecithin (and) Butyrospermum Parkii (Shea) Butter (and) Phenethyl Alcohol (and) Ethylhexylglycerin (and) Maltodextrin (and) Aqua/



Fig. 2 *Heteractis crispa* sea anemone specimen

Water] was tested *in vitro* and *in vivo* for its skin comforting abilities.

Materials and Methods

Whole-cell Patch Clamp Technique

Patch clamp is a standard technique to assess the properties of ion channels. This involves attaching a glass electrode to a cell and recording the current or voltage change in response to stimuli.

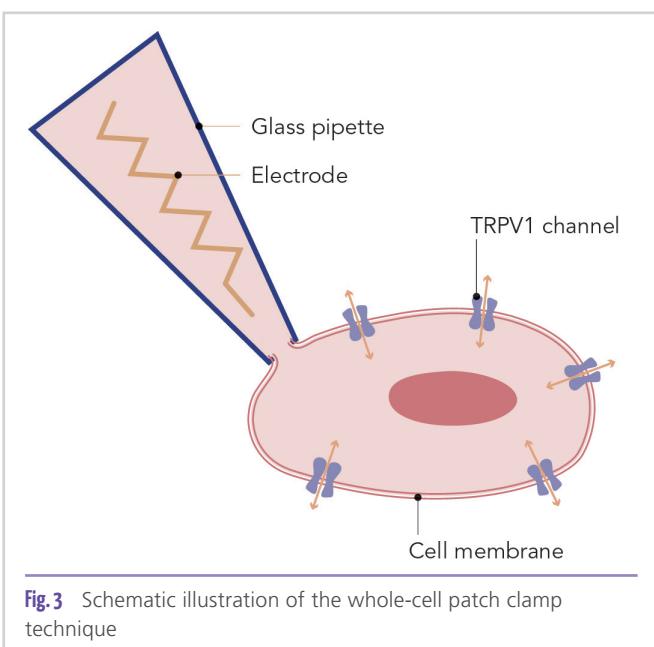
CHO cells that stably express TRPV1 receptors were clamped with a pipette tip on the cell surface and the membrane across the pipette tip was ruptured to assure electrical access to the cell interior and the cell was clamped to a potential of -80 mV. TRPV1 receptors were activated by 300 nM capsaicin followed by an application containing the test items (APHC1 protein or pentapeptide-59) and 300 nM capsaicin. A final application of capsaicin was applied to prove any signal reduction was due to direct action on TRPV1 and not deterioration of the seal or the cell. In order to confirm maximal inhibition a control treatment was performed with 10 µM capsazepine (TRPV1 antagonist).

Current Perception Threshold Measurements

The current perception threshold (CPT) was measured using a Neurometer (Neurotron, Inc., Baltimore, USA). For Neurometer measurements, a sinusoidal electrical stimulus is applied to the skin via surface electrodes at different frequencies to target different nerve populations. 250 Hz stimulates small myelinated nerve fibers that transmit fast pain, temperature and pressure sensation and 5 Hz stimulates small unmyelinated nerve fibers that transmit dull pain and temperature and are responsible for itching. The CPT is determined by the amount of electrical stimulus needed for it to be felt by the volunteer. In a placebo-controlled double-blind clinical study, 31 female volunteers (mean age: 47 years) with sensitive skin applied once a cream containing 2 % soothing active on one half of the face and the corresponding placebo cream on the other half. The CPT was measured before and two hours after the single application.

Lactic Acid Stinging Test

In a placebo-controlled double-blind clinical study, 31 female volunteers (mean age: 47 years) with sensitive skin applied a cream containing 2 % soothing active on one half of the face and the corresponding placebo cream on the other half twice daily for 28 days. Before treatment and 4 weeks after the start of treatment, the skin sensitivity was assessed by the lactic acid facial stinging test using an aqueous 5 % lactic acid solution applied on the nasolabial fold. The assessment for stinging, burning, itching was rated for each criterion on a 4 point scale (0; no stinging, 1; slight sting; 2; moderate sting; 3; severe sting) at 1 minute intervals for 9 minutes.



Results and Discussion

Peptide Derived from Sea Anemone Inhibits TRPV1

To assess the ability of the pentapeptide to inhibit the activation of the TRPV1 receptor and to compare it to the sea anemone venom protein APHC1, the whole-cell patch-clamp technique was used. With this method, the electric current change that occurs upon receptor activation and subsequent ion channel opening can be measured with an electrode (**Fig. 3**). The TRPV1 receptors were activated with capsaicin in the presence or absence (control) of either the full-length protein APHC1 or the pentapeptide. Both APHC1 and the pentapeptide reduced TRPV1 activation upon capsaicin irritation. The pentapeptide however, re-

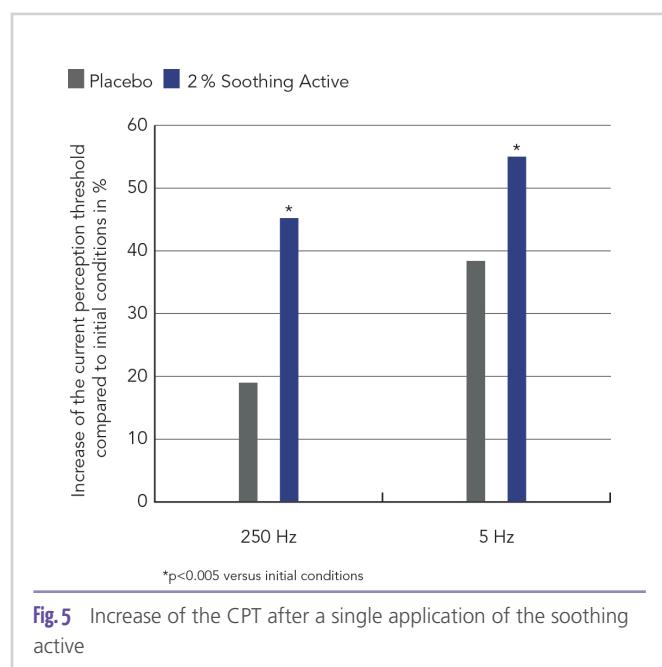
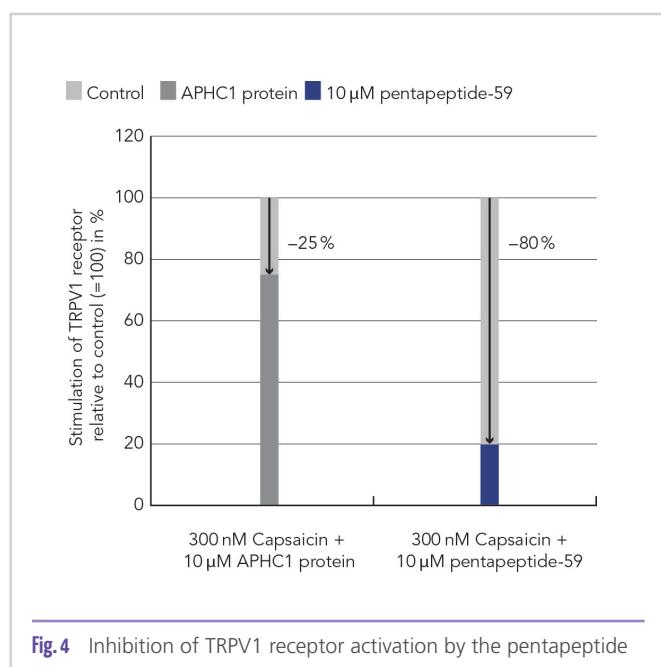
duced receptor activation by 80 %, a considerably stronger TRPV1 inhibition than with the full-length protein, which reduced receptor activation by 25 % (**Fig. 4**). This shows that the pentapeptide is an efficient inhibitor of TRPV1 receptor activation.

Single Treatment with the Soothing Active Reduces Skin Reactivity

To test the reduction of skin reactivity *in vivo*, a placebo-controlled double-blind clinical study with 31 female volunteers with sensitive skin was conducted. The volunteers applied a cream containing 2 % soothing active on one half of their face and the corresponding placebo cream on the other half. After just a single application, an electrical current was applied to the volunteer's skin and the current perception threshold (CPT) was measured, which is the amount of electrical stimulus needed for it to be felt by the volunteer. The higher the CPT, the less reactive is the skin. At two different frequencies which target different sensory nerve cell types in the skin, a single application of 2 % soothing active led to a significant increase of the CPT (**Fig. 5**), indicating that the reactivity of the skin to external stimuli is reduced.

Reduction of Skin Sensitivity by the Soothing Active

In a second placebo-controlled double-blind clinical study, the reduction of skin reactivity was measured via lactic acid stinging test. For this, 31 female volunteers with sensitive skin applied a cream containing 2 % soothing active on one half of the face and the corresponding placebo cream on the other half twice daily for 28 days. A significant reduction in skin sensitivity was observed after treatment with



2 % soothing active (**Fig. 6**). Therefore, the soothing active can help protect sensitive skin from overreacting to environmental stimuli.

Conclusion

A novel biomimetic peptide was designed to inhibit pain receptor activation similar to a sea anemone venom protein. The resulting soothing active reduces skin sensitivity and reactivity by inhibiting the activation of the TRPV1 pain receptor present in the skin. The result is a comforted and calm skin that is more resistant to environmental stresses.

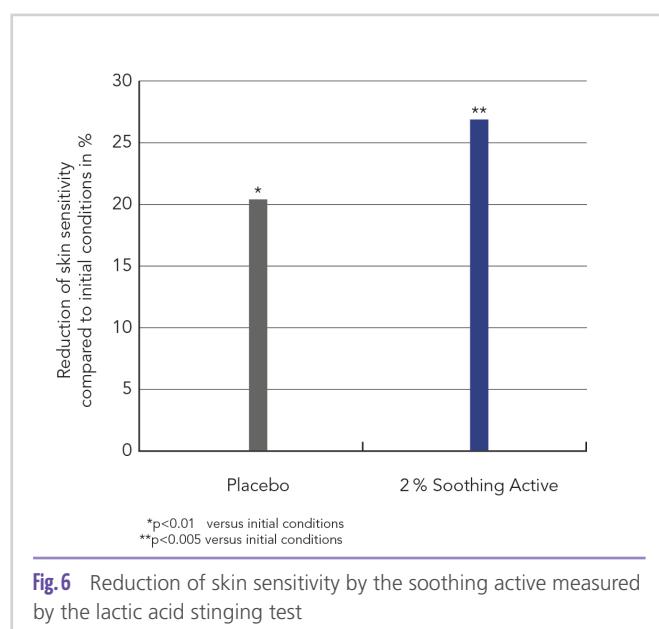


Fig. 6 Reduction of skin sensitivity by the soothing active measured by the lactic acid stinging test

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