IFSCC 2025 full paper (IFSCC2025-439)

"Sensitive Skin Redefined: Sustainable Solutions for Environmental Stress

-Discovery of Plant-Based TRPV1 Inhibitors-"

Masayuki Takaishi[1,2], Maori Kono[1,2], Kaori Motonami[1], Ayumi Yamaguchi[1], Megumi Kakito[1], Yuri Okano[3], Makoto Tominaga[4]

1. Mandom Corporation, 2. Osaka Univ., 3. SOLA SAS, 4. Nagoya Adv. Res. and Dev. Center, Nagoya City Univ.

1. Introduction

The skin serves as the primary interface between the body and the external environment and is constantly exposed to a variety of environmental stressors, such as ultraviolet (UV) radiation, air pollutants (e.g., PM2.5), dryness, and temperature fluctuations. These external factors disrupt skin homeostasis by impairing barrier function, inducing inflammatory responses, and promoting oxidative stress [1,2]. In recent years, in addition to these physical and chemical insults, increasing attention has been paid to the mechanisms underlying sensory discomfort of the skin, such as itching and stinging [3-8].

Among the various molecular players, Transient Receptor Potential Vanilloid 1 (TRPV1), a sensory receptor expressed in peripheral neurons, mediates nociceptive responses upon activation by stimuli such as heat, acidic pH, and capsaicin [9-11]. Activation of TRPV1 has been implicated not only in transient discomfort but also in the chronicity of neurogenic inflammation and hypersensitivity [10]. However, the impact of environmental factors on TRPV1 activity and the upstream molecular events involved in neural outgrowth remain unclear.

In the context of cosmetic science, there is a growing demand for skincare products that not only offer moisturizing and brightening effects but also provide a pleasant, irritation-free user experience, especially for individuals with sensitive skin. Therefore, targeting TRPV1 is a promising and innovative strategy for developing next-generation cosmetic ingredients.

In this study, we investigated the effects of various environmental stressors, including UVB, PM2.5, and oxidative stress, on the expression of neural outgrowth-related factors (NGF and semaphorin 3A) and TRPV1 activity. Furthermore, we evaluated the efficacy of Chamomilla recutita (chamomile) extract, identified through screening, as a potential TRPV1 antagonist, using both in vitro assays and in vivo human skin models. The findings of this study support the potential application of chamomile extract as a novel cosmetic ingredient aimed at alleviating sensory irritation and improving skin conditions for sensitive skin and under environmentally stressed conditions.

2. Materials and Methods

2.1. Cell Culture and Stable Cell Line Establishment

Full-length human TRPV1 (hTRPV1) cDNA was cloned into the pcDNA3.1 vector (Thermo Fisher Scientific). Human embryonic kidney 293 (HEK293) and HaCaT keratinocytes (HaCaT) were cultured in DMEM (Wako Pure Chemical Industries) supplemented with 10% fetal bovine serum (Biowest SAS, Caille, France), 100 U/mL penicillin (Life Technologies Corp., Carlsbad, CA, USA), and 100 µg/mL streptomycin (Life Technologies Corp.) at 37°C in a 5% CO2 incubator. The recombinant plasmids were transfected into HEK293 cells via electroporation, followed by antibiotic selection with 1 mg/mL geneticin. Single colonies were selected at 14 days post-transfection, amplified, and then passaged weekly. HEK293 cells stably expressing high levels of hTRPV1 and MOCK-transfected cells (MOCK) were established to investigate the pathways and secretory factors affected by hTRPV1 activation.

HEK293 cells were chosen because they do not endogenously express TRP channels or hTRPV1, but they exhibit crosstalk between various TRP channels, thus making them well-suited for detecting TRP channel-dependent responses with high sensitivity.

2.2. Ca^{2+} imaging

Ca^{2+} imaging experiments were performed using a Calcium Kit-Fluo 4 (DOJIN). The reagents were dissolved in a standard bath solution (140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose; pH 7.4 [adjusted with KOH]). Free cytosolic Ca^{2+} concentrations in HEK293 cells were measured by fura-4 microfluorometry with excitation at 490 nm and emission at 520 nm using an FDSS 7000 system (Hamamatsu Photonics, Japan). The calculated signal intensity ratio for 2 µM ionomycin was used as a control.

2.3. Whole-cell patch-clamp

Whole-cell patch-clamp recordings were performed one day after transfection. The standard bath solution was the same as that used in the Ca^{2+} imaging experiments. The pipette solution contained 140 mM KCl, 5 mM EGTA, and 10 mM HEPES (pH 7.4, adjusted with KOH). Data from the whole-cell voltage-clamp recordings were sampled at 10 kHz and filtered at 5 kHz for analysis (Axon 200 B amplifier with pCLAMP software; Axon Instruments, Sunnyvale, CA, USA). The membrane potential was clamped at –60 mV, and voltage ramp-pulses from –100 to +100 mV (500 ms) were applied every 5 s for all conditions studied. All experiments were performed at room temperature (24–26°C).

2.4. mRNA Expression Analysis

Real-time PCR was performed to assess mRNA expression levels. Total RNA was extracted using a Maxwell RSC simplyRNA Tissue Kit (Promega). Single-stranded cDNA was synthesized from 1 µg of total RNA using the Superscript IV VILO Master Mix (Thermo Fisher Scientific). Real-time PCR was performed using LightCycler 96 (Roche). Target mRNA levels were normalized to those of the housekeeping gene, β-actin, and the relative mRNA expression levels were calculated by dividing the values of treated samples by those of untreated controls.

2.5.High-Throughput Screening

A high-throughput calcium imaging assay was conducted to screen a library of 2,700 compounds s (TargetMol Chemicals) as potential hTRPV1 modulators. Assay robustness was evaluated by calculating the Z'-factor using the following formula:

$$Z' = 1 - \frac{3 \times (SD_{\text{control}} + SD_{\text{blank}})}{|\text{Mean}_{\text{control}} - \text{Mean}_{\text{blank}}|}$$

A plate was considered valid if the Z′-factor was ≥ 0.5 and the percentage of cells responsive to 60 nM capsaicin exceeded 70%. From this compound library, hit candidates were selected based on the changes in intracellular calcium concentration following compound treatment. The EC_{50} values of the selected compounds were determined by nonlinear regression using a four-parameter logistic model in GraphPad Prism 7. The compounds which showed hTRPV1 activity in the calcium imaging assay were validated using the whole-cell patch-clamp technique to confirm their activity and identify them as hit compounds.

2.6. Ethical approval

Human skin sections were obtained from BioPredic International (Rennes, France). Skin samples were obtained from healthy donors after obtaining written informed consent, and the procurement and handling of tissues complied with the ethical principles outlined in the Declaration of Helsinki (2013). The in vivo experiments were conducted with the approval of the Ethics Committee of Mandom Corporation. All procedures were performed in accordance with applicable laws and institutional guidelines, and written informed consent was obtained from all participants.

2.7. Human Sensory Irritation Study

The sensory irritation tests were conducted under controlled conditions at a temperature of 21–23°C and a relative humidity of 45%–55%. Areas of neck skin were wiped with a wet towel and acclimatized for 10 min prior to testing. Blind randomized half-region (left vs. right) trials were performed by applying two types of samples to the neck region, one containing the hTRPV1 antagonist and the other without. The amount of base formulation applied was 250 µL. The subjects evaluated pricking, stinging, burning, and itching sensations at 1, 3, 5, 7, and 10 min after compound application, following the criteria summarized in Table I. The total sensory irritation score was calculated at each time point.

In this continuous-use study, male participants in their 30s to 50s were enrolled. To assess the sensory reactivity following repeated use, 2% lactic acid was used as the test stimulant, and the cumulative irritation score over a 3-minute period was calculated. The base formulation volume applied was 750 µL. Sensory parameters, such as pain and discomfort, were assessed using the same evaluation criteria described in Table I.

Table.1 Criteria for sensitivity scoring

Sensory perception	Score	Scoring criteria
Itching	5	Unbearable intense sensation
Slightly unusual	4	
Stinging pain	3	
Painful prickly sensation	2	Distinct sensation
Burning sensation	1	
	0	Obscure sensation

2.8. Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using one- or two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test or Dunnett's test, as appropriate. Student's t-test was used for comparisons between two groups. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using the GraphPad Prism software (version 9.0; GraphPad Software, San Diego, CA, USA).

3. Results

3-1. Assessment of environmental factor-induced modulation of neurite outgrowth-related gene expression in HaCaT cells

To investigate the effects of external environmental factors on neurite outgrowth, HaCaT cells were treated with 0.5 mM hydrogen peroxide, 1 mJ/cm² UVB, and 100 µg/mL PM2.5. No cytotoxicity was observed at these concentrations (data not shown). The expression of nerve growth factor (NGF) and semaphorin 3A (sema3A), two key regulators of neurite outgrowth, was assessed as an indicator of neurogenic modulation (Fig. 1).

Analysis of NGF gene expression revealed a significant upregulation at 24 h post-exposure under all conditions studied. In contrast, *SEMA3A* expression was significantly downregulated in both hydrogen peroxide- and PM2.5-treated groups. Upon UVB exposure, *SEMA3A* expression showed a transient decrease at 6 h post-treatment, followed by a significant increase at 24 h.

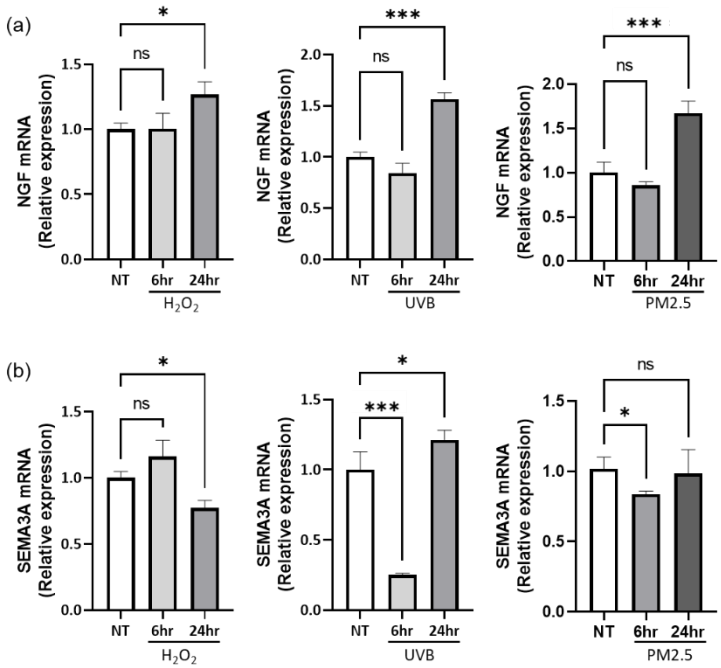

Fig. 1. Effects of external factors on *NGF* (a) and *SEMA3A* (b) expression in HaCaT cells.
(mean ± SEM, n = 3, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, One-way ANOVA test for each time point of the NT sample vs. the treatment sample)

3-2. Evaluation of hTRPV1 activation in human biopsy skin ex vivo following environmental stimuli

We focused on the activity of hTRPV1, a sensory receptor expressed in peripheral nerves and a member of the TRP channel family, which is known to mediate the perception of both acute and chronic stimuli. In this study, we used human skin biopsy samples that retained multiple components such as nerve fibers, inflammatory cells, sebaceous glands, and sweat glands. This ex vivo model offers an experimental environment that is more representative of the actual external exposure than conventional keratinocyte monocultures.

Preliminary tests were performed to determine the conditions required to prevent significant tissue damage. Based on these aforementioned findings, UVB irradiation doses were set at 50 mJ/cm² and 100 mJ/cm², and the exposure concentrations for PM2.5 were each set at 300 µg/mL. Following exposure, culture supernatants were collected from the treated biopsy samples and applied to cell lines stably expressing hTRPV1. The hTRPV1 activity was assessed using Ca^{2+} imaging. The results revealed that compared to supernatants from untreated skin biopsies, those derived from skin exposed to any of the environmental factors significantly enhanced hTRPV1 activation in response to capsaicin, a known hTRPV1 agonist (Fig. 2).

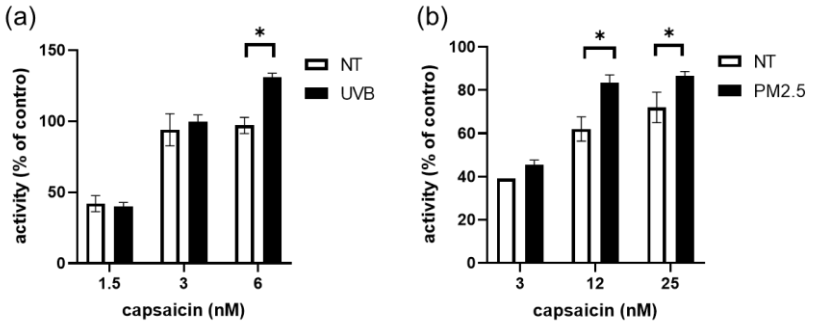

Fig. 2. Modulation of hTRPV1 activation by media conditioned with UVB (a) and PM2.5 (b).
(mean ± SEM, n = 3, * $p < 0.05$; One-way ANOVA for each concentration of NT sample vs. treatment sample).

3-3. Screening of hTRPV1 antagonists to alleviate sensation of skin irritation

To reduce the sensation of skin irritation, we hypothesized that the inhibition of hTRPV1 activity would be effective and screened for hTRPV1 antagonists. As a first step toward establishing a reliable evaluation system, we utilized a cell line stably expressing hTRPV1 and performed calcium imaging to monitor the cellular responses to capsaicin. Based on these experiments, we determined that a final capsaicin concentration of 30 nM yielded a Z′-factor exceeding 0.5, indicating sufficient assay quality. Under these conditions, the signal-to-noise (S/N) ratio was found to be 1.5.

Subsequently, a compound library comprising 2,700 compounds was screened, and 254 compounds with potential hTRPV1 antagonistic activities were then identified. Of these, 19 exhibited reproducible antagonistic effects without apparent cytotoxicity. For further validation,

both Ca^{2+} imaging and patch-clamp electrophysiology, which are capable of directly assessing ion channel activity, were employed. This dual-method approach confirmed the hTRPV1 inhibitory activity of 8 of the 19 compounds (Fig.3a,b).

Among the eight candidates studied, the chamomile-derived extract was identified as a feasible ingredient for cosmetic applications. The extract demonstrated consistent hTRPV1 inhibitory activity in both calcium imaging and patch-clamp assays, suggesting its potential use in alleviating skin irritation (Fig. 3c).

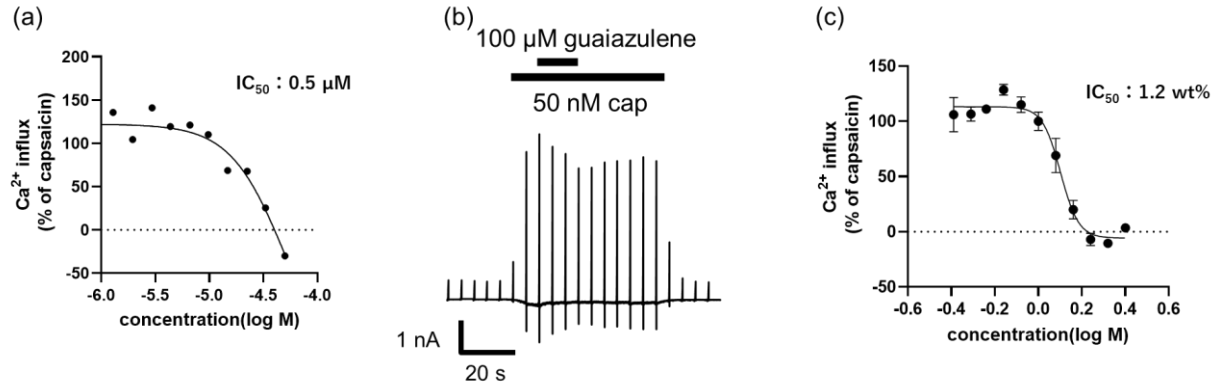

Fig. 3. Inhibitory effects of a novel hTRPV1 antagonist and cosmetic material on hTRPV1 activity. (a) Inhibitory effects of guaiazulene, one of the active compounds identified in our screening as an hTRPV1 antagonist. (mean ± SEM, n = 3–5). (b) A representative whole-cell capsaicin (50 nM)-evoked hTRPV1 current that was inhibited by guaiazulene (100 μM). (c) Inhibitory effect of a chamomile-derived extract subsequently found to contain guaiazulene. (mean ± SEM, n = 4-6).

3-4. Evaluation of chamomile extract for reducing skin sensory irritation and improving sensitive skin condition

To validate the irritation-reducing effects of the chamomile extract identified through screening, a stinging test was conducted on the neck. The results demonstrated that a formulation containing 0.3% chamomile extract significantly suppressed skin irritation induced by vanillyl butyl ether (VBE), a hTRPV1 agonist (Fig. 4).

Furthermore, to evaluate the impact of long-term use of chamomile extract on skin sensation and condition, a continuous-use study was conducted over eight weeks involving participants with heightened sensitivity to skin irritation. The participants were divided into two groups: one using a model lotion containing chamomile extract and the other using a placebo formulation. In the chamomile extract group, stinging test scores were significantly reduced after repeated applications compared to baseline values. In contrast, the placebo group showed a tendency toward decreased skin hydration, whereas no such decrease was observed in the chamomile extract group, suggesting the maintenance of skin condition (Fig. 5).

These findings indicate that chamomile extract may help improve hypersensitive skin conditions by modulating the activity of overactive hTRPV1 channels and contribute to maintaining healthy skin. Thus, chamomile extract is a promising and suitable ingredient for skin care formulations designed for sensitive skin.

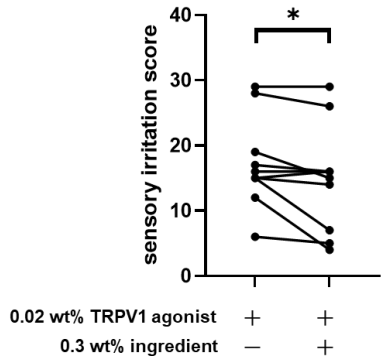

Fig. 4. Sensory irritation responses evaluated by aqueous solution application to the neck area. (mean ± SEM, n = 10; paired t-test, * $p < 0.05$)

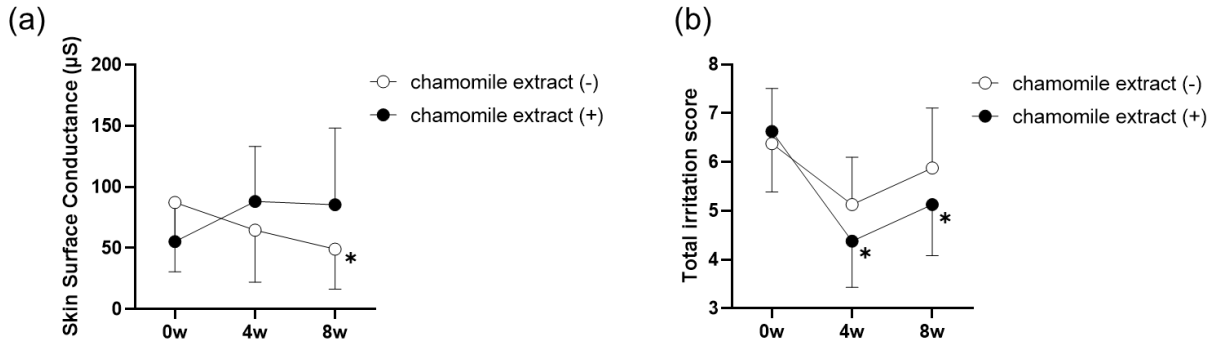

Fig. 5. Effects of long-term application of the model lotions on skin hydration (a) and sensory irritation (b). (mean ± SEM, n = 10, unpaired two-tailed t-test; * $p < 0.05$).
Statistical analysis was performed by comparing each treatment group (4w or 8w) with baseline (0w). No significant differences were observed between the groups at baseline (a: $p = 0.18$, b: $p = 0.88$, unpaired t-test).

4. Discussion

Numerous studies have reported the impact of external environmental factors such as UV radiation and atmospheric pollutants on the skin. However, the present study specifically focused on the effects of environmental stimuli on neurite outgrowth and the sensory receptor hTRPV1, which is expressed at the distal ends of peripheral nerves. Although the disruption of skin barrier function by UVB and the deleterious effects of particulate matter (PM2.5) on the skin have been documented [12,13], their potential involvement in the induction of skin sensory irritation and the underlying mechanisms remain unknown.

Our findings demonstrated that exposure to external environmental factors induces changes in the expression of genes associated with neurite outgrowth, including *NGF* and *SEMA3A*. Notably, the balance between *NGF* and *SEMA3A* was altered following exposure. Previous reports have suggested that environmental stressors such as low humidity disrupt this bal-

ance [3, 4], thereby influencing axonal extension in sensory neurons. Thus, the present data suggest that chronic exposure to environmental irritants may promote the abnormal elongation of nerve fibers toward the epidermis, potentially heightening skin sensitivity [14].

Moreover, we observed that exposure of the skin to external environmental factors led to an increase in hTRPV1 channel activity, potentially mediated by soluble factors secreted from keratinocytes and other epidermal cells. These results indicate that certain cosmetic ingredients capable of activating hTRPV1 may cause sensory irritation. Conversely, the concomitant use of hTRPV1 antagonists may serve as a practical approach for mitigating these effects.

In our 8-week repeated-use study, a formulation containing chamomile extract demonstrated not only a significant reduction in sensory irritation, as assessed by stinging test scores, but also showed marked preservation of skin hydration during the dry winter season. While the placebo group exhibited a decline in skin moisture, the chamomile-treated group maintained hydration levels, suggesting that the chamomile extract may help maintain skin barrier function and moisture retention. These findings imply that the inhibitory effect of the extract on hTRPV1 activity contributed not only to the reduction in perceived irritation but also to the maintenance of healthy skin conditions. It is conceivable that suppression of skin dryness prevented excessive neurite outgrowth, thereby lowering the observed stinging scores.

hTRPV1 is involved in the perception of noxious stimuli and the initiation of inflammatory responses in the skin. Therefore, the suppression of hTRPV1 activity observed in this study may have contributed to the attenuation of subclinical inflammatory processes, leading to improved skin conditions and reduced irritation. Collectively, these findings support the potential of chamomile extract as a beneficial cosmetic ingredient for individuals with sensitive skin, owing to its ability to modulate hTRPV1 activity and simultaneously enhance skin comfort and integrity.

5. Conclusion

This study demonstrated that external environmental factors, including UVB, PM2.5, and oxidative stress, influence the expression of neural outgrowth-related factors and hTRPV1 channel activity, suggesting their involvement in sensory skin discomfort. Furthermore, the extract of the hTRPV1 antagonist chamomile significantly reduced skin irritation and prevented the deterioration of skin conditions associated with dryness and impaired barrier function. These results highlight the potential of chamomile extract as an effective ingredient for improving sensitive skin conditions with promising applications in cosmetic formulations.

6. References

[1]. Ferrara F, et al., J Cosmet Dermatol, 23(8), 2644–2656 (2024).
[2]. Biniek K, et al., Proc Natl Acad Sci U S A, 109(42), 17111–17116 (2012).
[3]. Moniaga CS, et al., Acta Derm Venereol, 100(2), 5609 (2020).
[4]. Jiao Q, et al., J Cosmet Dermatol, 23(5), 1518–1526 (2024).
[5]. Misery L, et al., J Eur Acad Dermatol Venereol, 30(Suppl.1), 2–8 (2016).

[6]. Li Z, et al., J Ginseng Res, 46, 115–125 (2022).
[7]. Li L, et al., Antioxidants (Basel), 13(8), 1002 (2024).
[8]. Tominaga M, et al., J Dermatol Sci, 55, 40–46 (2009).
[9]. Caterina MJ, et al., Nature, 389, 816–824 (1997).
[10]. Tominaga M, et al., J Neurobiol, 61, 3–12 (2004).
[11]. Fujita F, et al., Proceedings of the 26th IFSCC Congress, Buenos Aires, 20–23 September (2010).
[12]. Molagoda IMN, et al., Antioxidants (Basel), 10(9), (2021).
[13]. Kono M, et al., Toxicol Res, 39(1), 1–13 (2023).
[14]. Tominaga M, et al., Br J Dermatol, 158(4), 842–844 (2008).