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"Development and Application of an Eye Irritation Screening Method Based on TRPV1 Channels"

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1. Introduction

Sunscreen products are a primary way for consumers to mitigate the photoaging effects of ultraviolet (UV) on the skin[1,2]. However, many consumers report experiencing eye irritation, such as stinging and tearing, when using certain suncreen products, which negatively impacts their usage experience. This discomfort can lead to concerns about product safety, and some consumers may reduce the frequency or amount of sunscreen use due to these issues[3]. Therefore, it is crucial to understand which ingredients in sunscreen products cause eye irritation so that their amount can be reduced.

Currently, industry professionals speculate that UV filters or additives such as fragrances, preservatives, and alcohol may cause eye irritation. Chemical UV fiters, particularly Octocrylene (OCT) and oxybenzone, are often implicated as one of the causes for eye irritation. However, our research indicates that there is no clear scientific evidence to support these assumptions. Since eye stinging and tearing are manifestations of ocular irritation, identifying the specific components in sunscreen products that possess ocular irritancy is the focus of this study.

In this study, we evaluated the ocular irritation of two widely used UV filters, OCT and Ethylhexyl methoxycinnamate (OMC), through in vitro approaches. In addition to conventional toxicological assessments, including the Short Time Exposure (STE) assay and Bovine Corneal Opacity and Permeability (BCOP) assay, we further investigated their effects on the activation of Transient Receptor Potential Vanilloid (TRPV) ion channels. TRPV1 receptor is predominantly distributed in ocular sensory nerve fibers, can be activated by chemical substances, heat, and mechanical stimuli[4]. Activation of this receptor leads to an influx of extracellular Ca^{2+}, a rapid increase in intracellular Ca^{2+} concentration, the release of inflammatory mediators, and subsequent eye stinging[5,6].

Different from traditional toxicological experiments, we used TRPV1-overexpressing rabbit corneal epithelial cells (TRPV1-OE SIRC cells) as an experimental model. By conducting TRPV1 ion channel activation experiments, we attempted to explore the sensory-level irritation of UV filters on the eyes. This Method aims to provide a potential

explanation for the molecular mechanisms underlying eye stinging caused by sunscreens and to offer a scientific basis for optimizing the safety of sunscreen formulations.

2. Materials and Methods

2.1. Materials &instruments

Cells: Statens Serum institut RabbitCornea (SIRC) Cell line; SIRC transfected withTRPV1 virus.Reagents: 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT, Gibco, 2500251P); Isopropanol hydrochloride; PBS solution; DMEM (Sigma, 102540550); MEM; HBSS without phenol red; 0.9% NaCl; fluorescein sodium; 4% PFA; Probenecid (MCE, HY-B0545); Ionomycin (MCE, HY-13434); Manganous chloride (MCE, HY-109521A); Sodium hydroxide (Aladin, S580606); Calcium chloride (Aladin, C431202); glucose (Macklin, D769078); Fluo-3 AM, cell permeant (Yeasen, 40703ES72). Microplate reader: Molecular Devices, #SpectralMaxi3x, Molecular Devices, #SPECTRA MAX 190; BASF Opacitometer Kit3.0; Flow cytometer: Beckman, CytoFLEX.

2.2 STE Assay

The STE assay was conducted according to the OECD test guideline Nº 491[7]. Briefly, SIRC cells were seeded into 96-well plates and cultured until reaching to 80% confluency. Cells were treated with 5% and 0.5% (w/v) solutions of OCT or OMC prepared in complete medium for 5 minutes. Positive controls (0.01% sodium lauryl sulfate, SLS), negative controls (10% FBS-supplemented DMEM), and blank controls (Ca^{2+}/Mg^{2+}-free PBS) were included. After exposure, cells were incubated with 0.5 mg/mL MTT solution at 37°C under 5% CO_2 for 2h. The formazan crystals were dissolved in isopropanol-HCl (0.04 N HCl) for 1h in the dark. Absorbance was measured at 570nm using a microplate reader. Relative cell viability (%) was calculated as: Cell Viability (%)=[(OD_{570}sample−OD_{570}blank)/(OD_{570}solvent−OD_{570}blank)]×100.Eye irritation or serious eye damage of the exposure groups was classified in UN GHS categories according to cell viability values for concentrations at 5 and 0.05% of each test material as follows: “no category”, i.e. not classified for eye irritation or serious eye damage if cell viability was > 70% at 5% and 0.05% concentrations; “category 1”, i.e. serious eye damage when 5% and 0.05% concentrations triggered a cell viability of ≤ 70%; or “no prediction can be made” if cell viability for 5% and 0.05% concentrations were ≤ 70% and > 70%, respectively[8].

2.3. BCOP Assay

The BCOP assay was performed according to the OECD test guideline Nº 437[9]. Fresh bovine corneas without epithelial damage were excised with a 2–3 mm scleral rim and mounted on corneal holders. Both anterior and posterior chambers were filled with phenol red-free MEM medium pre-equilibrated at 32 °C. After 1–2 h of equilibration, baseline corneal opacity was measured using a BASF Opacitometer 3.0. Test compounds, negative controls (MEM medium), and positive controls (1% Triton X-100) were applied to the epithelial surface (n = 3 replicates per group) and incubated for 10 minutes at 32 °C. Post-exposure, corneas were rinsed, re-equilibrated for 2h, and final opacity values were recorded. Fluorescein permeability was assessed by incubating corneas with 1.0 mL of 4 mg/mL fluorescein sodium for 90 minutes. The posterior chamber medium was collected, and absorbance at 490 nm was measured. The In Vitro Irritancy Score (IVIS) was calculated as: IVIS=Mean corrected opacity+15×Mean corrected fluorescein OD.The corneal irritancy levels

of exposure groups were determined based on the obtained IVIS values as follows: “No Category” (IVIS ≤ 3); “No Prediction Can Be Made” (3 < IVIS ≤ 55); or “Category 1” (IVIS > 55)[8].

2.4 TRPV1-Mediated Ca^{2+} Influx Assay

2.4.1 Cell Viability Assessment

TRPV1-OE SIRC cells were seeded into 96-well plates and cultured to 80% confluency. Cells were treated with serial dilutions of OCT or OMC (0.05–0.2 mg/mL) for 24h. 5 mg/mL MTT solution was added to each well and incubated for 3h at 37°C. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm. Relative cell viability (%) was calculated as: Cell Viability (%)=[(OD_{570}sample−OD_{570}blank)/(OD_{570}solvent−OD_{570}blank)]×100.

2.4.2 Ca^{2+} Influx Assay

TRPV1-OE SIRC cells were seeded into 96-well plates and cultured for 24h, and then were loaded with 5μM Fluo-3 AM in PHF buffer (pH 7.4) for 30 minutes at 37°C. Following the initiation of the assay, the baseline fluorescence was monitored for 50 seconds by Flow cytometer in the absence of the sample. The measurement was then paused, and The test sample was subsequently added , after which the measurement was resumed for an additional 204 seconds. For Ca^{2+} influx analysis, appropriate time intervals were selected. The Ca^{2+} signals within these intervals were normalized to the average baseline fluorescence value (i.e., the fluorescence signal recorded prior to sample addition), which was designated as 100%. The relative fluorescence intensity for each group was calculated based on this normalization.

2.5 Statistical analysis

Three independent assays were performed, and the data analyzed using GraphPad Prism version 5.01 software. Statistical significance was considered as $p<0.05$.

3. Results

3.1 STE Assay Results

As shown in Tables 1 and 2, under the experimental conditions conducted in accordance with OECD Test Guideline 491(2023), the cell viability of both OCT and OMC was greater than 70%, indicating that neither of them not classified for eye irritation or serious eye damage. According to the Globally Harmonized System (GHS) classification criteria, neither compound required classification for ocular irritation.

Table 1. The experimental results of eye irritation of OCT detected by STE *Assay*

Group	OD	Relative cell vi alibity （%）	Irritancy Level	UN GHS Classifi cation
BC	0.0327±0.0005	--	Non-irritating or slightly irritating	No Category
NC	0.9640±0.0647	93.71±6.51	Non-irritating or slightly irritating	No Category
SC	1.0265±0.0561	100.00±5.65	Non-irritating or slightly irritating	No Category
5.00%	0.7519±0.0326	73.25±3.17	Non-irritating or slightly irritating	No Category
0.05%	0.9276±0.0217	90.36±2.11	Non-irritating or slightly irritating	No Category
PC	0.2416±0.0030	21.02±0.30	Corrosiveness (irreversible eye d amage)	Category 1

Table 2. The experimental results of eye irritation of OMC detected by STE *Assay*

Group	OD	Relative cell vial ibity （%）	Irritancy Level	UN GHS Classifi cation
BC	0.0327±0.0005	--	Non-irritating or slightly irritating	No Category
NC	0.9640±0.0647	93.71±6.51	Non-irritating or slightly irritating	No Category
SC	1.0265±0.0561	100.00±5.65	Non-irritating or slightly irritating	No Category
5.00%	0.8756±0.0194	85.30±1.89	Non-irritating or slightly irritating	No Category
0.05%	0.9450±0.0298	92.06±2.90	Non-irritating or slightly irritating	No Category
PC	0.2416±0.0030	21.02±0.30	Corrosiveness (irreversible eye damage)	Category 1

3.2. BCOP Assay Results

The BCOP assay, conducted in accordance with OECD Test Guideline 437 (2023), confirmed the non-irritant classification of OCT and OMC (Tables 3 and 4). The IVIS values of both compounds were less than 3, which is below the threshold defined for irritation classification and is consistent with the GHS “No Category” designation.

Table 3. The experimental results of eye irritation of OCT detected by BCOP *Assay*

Group	Mean corrected opacity	Mean corrected f luorescein OD	IVIS	UN GHS Clas sification	Irritancy Level
NC	0.4057±0.5287	0.0463±0.0017	1.1007±0.5055	No Category	Non-irritating
PC	30.5718±5.5956	0.9555±0.2121	44.9038±8.7666	Unpredictable	Unpredictable
OCT	-0.6831±0.2201	0.0487±0.0031	0.0469±0.2608	No Category	Non-irritating

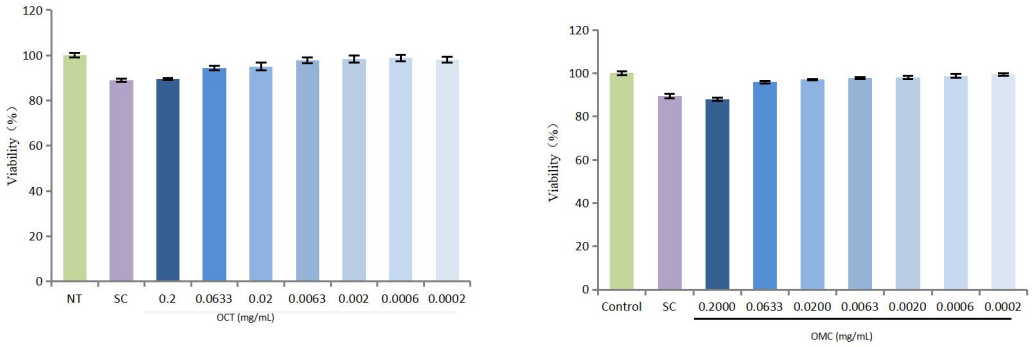
Table 4. The experimental results of eye irritation of OMC detected by BCOP *Assay*

Group	Mean corrected opacity	Mean corrected f luorescein OD	IVIS	UN GHS Clas sification	Irritancy Level
NC	0.4057±0.5287	0.0463±0.0017	1.1007±0.5055	No Category	Non-irritating
PC	30.5718±5.5956	0.9555±0.2121	44.9038±8.7666	Unpredictable	Unpredictable
OMC	-0.2771±1.2763	0.0523±0.0090	0.5079±1.1708	No Category	Non-irritating

3.3.The results of TRPV1-Mediated Ca^{2+} Influx Assay

3.3.1 Cytotoxicity assessment of OCT and OMC

As shown in Figures 1, treatment with OCT and OMC at concentrations ranging from 0.0002 to 0.2 mg/mL resulted in cell viability values consistently above 85% ($P > 0.05$ compared to the untreated control group), indicating no significant cytotoxicity under these conditions. Based on these findings, three non-cytotoxic concentrations—0.2, 0.1, and 0.05 mg/mL—were selected for subsequent experiments to evaluate the sensory irritation potential of the compounds.



(**a**) (**b**)

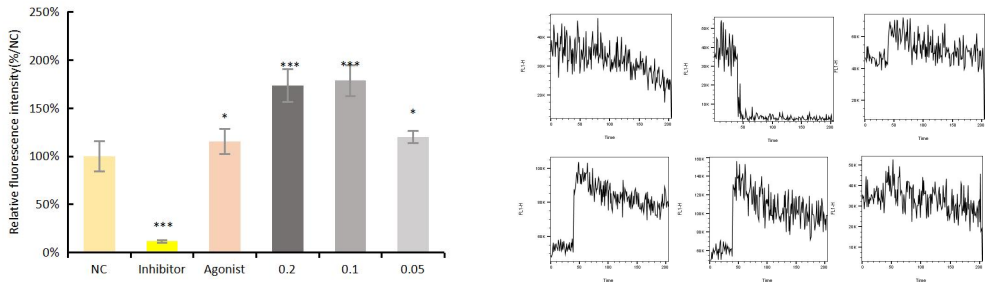
Figure 1. The results of the cytotoxicity test: (a) Cytotoxicity *assessment* results of TRPV1-OE SIRC cells by OCT; (b) Cytotoxicity *assessment* results of TRPV1-OE SIRC cells by OMC.

3.3.2 The results of the influence of OCT and OMC on Ca^{2+} Influx

As illustrated in Figure 2 and 3, the TRPV1-OE SIRC cells demonstrated robust functional validation of the TRPV1 overexpression model. Compared to the negative control group (NC), the inhibitor-treated group (I) exhibited a significant reduction in relative Ca^{2+} fluorescence intensity ($P < 0.05$), whereas the agonist-treated group (A) showed a marked increase in Ca^{2+} fluorescence intensity ($P < 0.05$). These results confirm that the TRPV1-OE SIRC cells retain physiological responsiveness to pharmacological modulators.

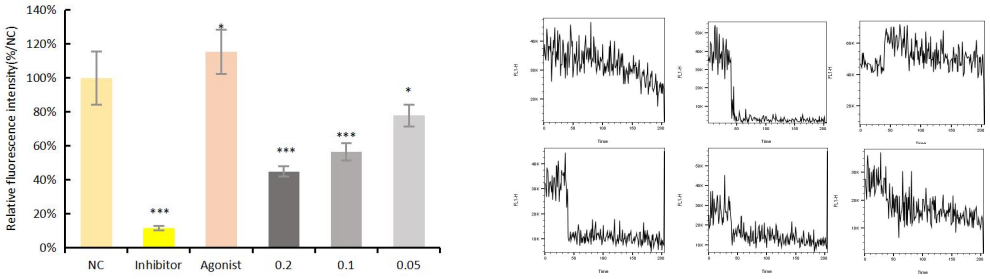
In the OCT treatment groups (Figure 2), Ca^{2+} fluorescence intensity increased significantly. At concentrations of 0.2, 0.1, and 0.05 mg/mL, the relative fluorescence intensity rose by 73.37% ($P < 0.01$), 78.59% ($P < 0.01$), and 20.07% ($P < 0.05$), respectively, compared to the NC group. This indicates that OCT strongly activates TRPV1 channels, triggering extracellular Ca^{2+} influx.

Conversely, OMC treatment (Figure 3) induced a concentration-dependent suppression of Ca^{2+} fluorescence intensity. At 0.2, 0.1, and 0.05 mg/mL, the relative fluorescence intensity decreased by 55.04% ($P < 0.01$), 43.43% ($P < 0.01$), and 21.97% ($P < 0.05$), respectively, compared to NC. This suggests that OMC does not lead to the activation of the TRPV1 channel and thus does not cause Ca^{2+} influx.



(**a**) (**b**)

Figure 2. The influence of OCT on Ca^{2+} flow: (a) Fluorescence intensity analysis; (b) Calcium flow change diagram.



(**a**) (**b**)

Figure 3. The influence of OMC on Ca^{2+} flow: (a) Fluorescence intensity analysis; (b) Ca^{2+} flow change diagram.

4. Discussion

Traditional ocular irritation assessment methods, such as the BCOP and the STE, classified both UV filters as non-irritating or minimally irritating, with no classification under the GHS. The inability of these two methods to distinguish the differences in ocular irritation between the two UV filters may arise from the fact that their testing principles are based on simulating corneal damage, which represents a relatively severe form of structural tissue damage. Specifically, the BCOP method quantifies corneal opacity and changes in sodium fluorescein permeability to reflect tissue damage, such as protein denaturation or stromal swelling, while the STE method assesses acute toxicity by measuring cell viability[10,11].

We hypothesize that the eye irritation experienced by consumers after applying sunscreen may not induce corneal damage but rather represents a sensory stimulation, distinct from tissue irritation. To investigate this hypothesis, we examined the effects of two UV filters on the TRPV1 ion channel activity, aiming to elucidate the potential mechanism underlying UV filter-induced sensory irritation.

TRPV1, a core receptor for pain transmission, is widely distributed in the sensory nerve fibers of the cornea and can be activated by chemical stimuli (such as capsaicin), heat, or mechanical pressure, thereby mediating the influx of extracellular Ca^{2+}. Experimental data showed that OCT significantly activated $Ca2^{+}$ signaling in TRPV1-OE SIRC cells within the concentration range of 0.05–0.2mg/mL (with fluorescence intensity increasing by 20.07%–73.37%, $P<0.01$), indicating that it triggers Ca^{2+} influx by directly or indirectly activating the TRPV1 channel. This process can further induce the release of inflammatory mediators (such as substance P and bradykinin), amplify pain signal transmission, and ultimately lead to neurogenic inflammatory responses, such as ocular stinging and tearing. In stark contrast, OMC failed to activate TRPV1 at the same concentrations.

These results highlight the fundamental differences in assessment strategies between traditional toxicological methods and novel detection models based on pain receptor mechanisms. BCOP and STE are more suitable for identifying substances with direct

tissue-destructive properties, but their “blind spot” for neurogenic irritation may lead to incomplete assessments of the safety of certain ingredients. For example, in this study, although OCT was classified as “non-irritating” by traditional methods, the sensory discomfort it causes through TRPV1 activation may reduce consumer compliance, thereby affecting product market performance. Therefore, the development of in vitro models based on pain receptors such as TRPV1 not only fills the sensitivity gap of traditional methods but also provides a target for the optimization of cosmetic formulations. For example, screening for TRPV1 antagonists or replacing UV filters with potential agonistic activity (such as OCT) could reduce the risk of ocular irritation.

5. Conclusion

This study successfully developed a novel ocular irritation screening method based on the activation mechanism of the TRPV1 channel, which exhibits significantly higher sensitivity compared to traditional toxicological models (BCOP and STE). By quantitatively analyzing the dynamic changes in intracellular Ca^{2+} signaling, this method is capable of identifying cosmetic ingredients that may induce ocular pain. The breakthrough of this study lies in the first-ever elucidation of the TRPV1-dependent mechanism underlying UV filter-induced ocular discomfort at the molecular level, thereby providing a possible scientific evidence for traditional empirical assumptions. The developed method not only offers a highly sensitive tool for the safety assessment of cosmetic ingredients but also guides formulation optimization to reduce the use of sensory irritants, thereby enhancing product comfort and market acceptance.

6. Reference

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