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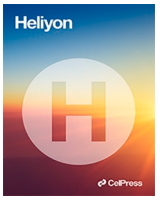

Research article

Regulatory effects of *Ishige okamurae* extract and Diphlorethohydroxycarmalol on skin barrier function

Seon Gyeong Bak [a], Hyung Jin Lim [b], Yeong-Seon Won [c], Sang-Ik Park [d], Sun Hee Cheong [e,**], Seung Jae Lee [a,f,*]

[a] *Functional Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Jeongeup, 56212, Republic of Korea*
[b] *Scripps Korea Antibody Institute, Chuncheon, Republic of Korea*
[c] *Division of Research Management, Department of Bioresource Industrialization, Honam National Institute of Biological Resource, Mokpo, Republic of Korea*
[d] *Department of Veterinary Pathology, College of Veterinary Medicine and BK21 FOUR Program, Chonnam National University, Gwangju 61186, Republic of Korea*
[e] *Department of Marine Bio Food Science, Chonnam National University, Yeosu, 59626, Republic of Korea*
[f] *Applied Biological Engineering, KRIBB School of Biotechnology, University of Science and Technology, Daejeon, 34113, Republic of Korea*

A R T I C L E I N F O

Keywords:
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2,4-Dinitrochlorobenzene
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Ceramide

A B S T R A C T

Ethnopharmacological relevance: The pharmacological potential of marine organisms remains largely unexplored. *Ishige Okamurae,* commonly known as Pae, is extensively distributed over Asia. Its antioxidant, antibacterial, antiobesity, and anti-inflammatory properties are also being investigated.

Aim of the study: In most cases of atopic dermatitis, the stratum corneum, the outermost layer of the epidermis, is damaged, causing symptoms such as dryness and hyperproliferation of the epidermis. In particular, the disruption of cell junctions leads to damage of the skin barrier, exacerbating the disease and becoming a target for therapeutic development. Our study aims to investigate of Ishige okamurae extract (IOE) and a major compound derived from it, called Diphlorethohydroxycarmalol (DPHC), can help strengthen the skin barrier in animals with atopic dermatitis induced by 2,4-dinitrochlorobenzene (DNCB).

Materials and methods: In keratinocyte cell lines, HaCaT cells, the cytotoxicity of IOE and DPHC was assessed by MTT analysis. The gene expression of skin barrier factors and tight junctions were determined by real-time PCR in tumor necrosis factor-α/interferon-γ-stimulated HaCaT cells. In addition, JAK/STAT signaling pathway was performed to evaluating the mechanism of drugs by Western blot. Next, we studied the effects of IOE and DPHC on the skin of animals with DNCB-induced atopic dermatitis. We measured the expression of genes related of the skin barrier and tight junctions in their ear tissue.

Results: As a result, IOE and DPHC confirmed that the expression of skin barrier proteins (thymic stromal lymphopoietin, filaggrin, loricrin, and involucrin) was improved in the DNCB-induced atopic dermatitis model and HaCaT cells. In addition, the expression of tight junction-related proteins (claudin, occludin, and tight junction protein-1) were improved.

* Corresponding author. Functional Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Jeongeup, 56212, Republic of Korea.
** Corresponding author. Department of Marine Bio Food Science, Chonnam National University, Yeosu, 59626, Republic of Korea.
E-mail addresses: sunny3843@jnu.ac.kr (S.H. Cheong), seung99@kribb.re.kr (S.J. Lee).

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Conclusion: IOE and DPHC ameliorated the atopic dermatitis lesions through alleviating the pro-inflammatory responses and tight junction protein destruction. Our results suggest that IOE and DPHC could be promising candidates for enhancing skin barrier function.

1. Introduction

Maintaining a robust skin barrier function is crucial to balancing water levels and shielding against foreign antigens and allergens from the environment. Itching, a fundamental symptom of atopic dermatitis, causes skin scratching, exacerbating epidermal damage and skin inflammation resulting in skin barrier destruction. The human epidermis is composed of a middle layer that is distinct from the basal layer of the basement membrane and is formed toward the supraspinal layer and the granular layer, and finally the outermost stratum corneum. The primary cells of the epidermis, keratinocytes, has a crucial role in preserving the skin barrier by binding specific components such as involucrin (IVL), filaggrin (FLG), and loricrin (LOR) [1]. FLG is crucial for maintaining the skin barrier, as it contributes to the formation of the outermost layer by aggregating the keratinocyte matrix [2]. FLGs then participate in epidermal differentiation, which is an important mechanism that constitutes the skin barrier. Usually, FLG exists in the form of pro-FLG and undergoes conversion into FLG via phosphorylation and proteolysis catalyzed by serine proteases [3]. However, due to skin xerosis caused by damage and lack of moisture in the skin barrier, FLG undergoes degradation into components that constitute natural moisturizing factors, such as free amino acids like sodium pyrolidone carboxylic acid and urocanic acid [4]. IVL and LOR contribute to the outer structure of keratinocytes and assist in the maturation process of the epidermis [2]. IVL forms a cross-linked scaffold with other proteins [5], and LOR is a protein insoluble within the keratinized cell envelope, Constituting 80 % from the total protein [6]. Therefore, these components play important roles in maintaining skin barrier functions and homeostasis. In atopic dermatitis, thymic stromal lymphopoietin (TSLP) is generated by epithelial cells, including keratinocytes, and it functions as an activator of inflammation. The cytokine TSLP activates myeloid dendritic cells, which in turn triggers an inflammatory Type 2 T helper (Th2) response [7,8]. Skin barrier component proteins are dissolved by Th2 cytokines generated by TSLP, such as interleukin (IL)-4, IL-13, and IL-31, which results in abnormalities in skin function [9]. Furthermore, a recent study shown that the function of the epidermal barrier is also harmed by Th17 cells and Th22-mediated cytokines, such as IL-17 and IL-22 [10]. Th2-mediated cytokines may stimulate Th17 and Th22 cells even more, leading to dysfunctions in the epidermal barrier [11]. Therefore, Th2-mediated skin disease mice are an appropriate model for research on developing therapeutic agents for skin barrier destruction and dysfunctions. Seaweeds have historically been extensively utilized in Asian nations for their nutritional and therapeutic properties, as they are rich in various natural bioactive substances including proteins, sterols, polysaccharides, and polyphenols [12,13,13]. *Ishige okamurae* is a consumable brown seaweed found along the entire southern coast, Jeju Island, Japan, and China, predominantly in the intertidal region of the southern coast and Jeju Island. *Ishige okamurae* exhibit a range of bioactive characteristics, including anti-inflammatory and anti-obesity properties [14], antioxidant [15], and anti-aging effects [16]. Diphlorethohydroxycarmalol (DPHC) represents a prevalent bioactive constituent found in *Ishige okamurae*. According to earlier research, DPHC possesses strong anti-inflammatory [16], anti-diabetic [17], antioxidant [18] and anti-melanogenesis properties [19], atopic dermatitis effect [20]. Recent studies have demonstrated that Ishige okamurae Celluclast extract offers potent protection against ultraviolet-B (UVB)-induced skin damage and photoaging [21]. Another study revealed that a novel glyceroglycolipid isolated from *Ishige okamurae* improved photoaging and counteracted inflammation in UVB-induced HaCaT cells [22]. Additionally, DPHC has been shown to protect human dermal fibroblasts from UVB-induced damage in vitro [23]. However, there are not enough studies on the skin barrier, despite the fact that research on plaque and DPHC is being done on a large scale. In this study, animal models with DNCB-induced atopic dermatitis were employed to investigate the therapeutic potential of IOE and its key bioactive compound, DPHC, in restoring skin barrier integrity. Specifically, we aimed to assess the effect of IOE and DPHC on the breakdown of the epidermal barrier by examining the expression of key skin barrier proteins, including FLG, IVL, and LOR, in keratinocytes. Additionally, the study sought to elucidate the underlying molecular mechanisms by which these compounds modulate keratinocyte differentiation and inflammatory pathways involving TSLP, Th2 cytokines, and other mediators such as IL-17 and IL-22, which contribute to epidermal barrier dysfunction. Through this approach, we aimed to clarify how IOE and DPHC function as agents that enhance skin barrier integrity and counteract the effects of AD.

2. Materials and methods

2.1. Cell culture

The HaCaT human keratinocyte cell line was maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10 % heat-inactivated fetal bovine serum and 1 % penicillin-streptomycin. The cells were cultured at 37 °C under conditions of 90–95 % humidity and 5 % CO_2.

2.2. Real-time quantitative polymerase chain reaction (Real-time PCR)

Using TRIzol reagent, total RNA was extracted from HaCaT cells and ear tissues from each group. First-strand complementary DNA (cDNA) was synthesized using a PrimeScript 1st Strand cDNA Synthesis Kit from Takara Bio Inc., (Shiga, Japan). Quantitative PCR was conducted using a Bio-Rad T100 thermal cycler (Bio-Rad, Hercules, CA, USA) following the manufacturer's protocol. Real-time PCR

was performed using a Step One Plus Real-time PCR System with TaqMan probes and TaqMan Real-time PCR Master Mix (Applied Biosystems, Foster City, CA, USA. 18s rRNA or GAPDH was used as an endogenous control for normalization. The TaqMan primers and probes were purchased from Applied Biosystems, Thermo Fisher Scientific (Table S1).

2.3. Western blotting

Following the extraction of total protein from the cells, 100 μL of cell lysis buffer (Cell Signaling Technology, Danvers, MA, USA) was used to lyse the samples. he lysed samples were then vortexed, incubated for 30 min on ice, then centrifuged for 10 min at 4 °C at 13,000 rpm. The supernatants were collected and quantified using a DC protein assay kit (Bio-Rad, Hercules, CA, USA). Subsequently, an 8–12 % SDS-PAGE gel was employed to separate equal amounts of protein lysate, which were later transferred onto a polyvinylidene fluoride membrane. Following blocking with 5 % bovine serum albumin in tris-buffered saline, the membrane was exposed to a primary antibody specific to the target protein, washed, and then treated with a horseradish peroxidase-conjugated IgG secondary antibody. The bands were visualized using a West-Queen RTS Western Blot Detection Kit (iNtRON Bio, Seongnam, Korea). The antibodies utilized were procured from Cell Signaling Technology (Table S2).

2.4. Animals

Female BALB/c mice aged 6 weeks were procured from Samtako in Osan, Korea. The mice were provided with unrestricted access to standard rodent chow and filtered water throughout the duration of the study. They were housed in a controlled laminar airflow setting with a 12-h light/dark cycle, maintained at a temperature of 22 ± 2 °C, and a relative humidity of 55 ± 5 %. Each cage accommodated five mice. The animal care and experimental procedures adhered to the guidelines outlined by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Korea Research Institute of Bioscience and Biotechnology (KRIBB-AEC-20265, KRIBB-AEC-21103).

2.5. Induction of atopic dermatitis-like lesions using DNCB

A cohort of 25 mice was segregated into five distinct groups (n = 5): phosphate-buffered saline (PBS) as the vehicle control, DNCB vehicle (PBS), DNCB in combination with IOE at doses of 50 and 100 mg/kg, and DNCB combined with dexamethasone (DX) at a dose of 1 mg/kg. The initial phase of sensitization involved the application of DNCB (2 %, 20 μL/ear) once to each ear during the first week. Subsequently, both ears of each BALB/c mouse were subjected to DNCB challenge (1 %, 20 μL/ear) twice per week for a duration of 3 weeks. IOE (at doses of 50 and 100 mg/kg) or DX (1 mg/kg) was orally administered via gavage for five consecutive days per week, coinciding with the DNCB challenge. In the case of the atopic dermatitis model, DPHC (25 mg/kg) was used as a substitute for IOE for analysis purposes.

2.6. Histological assay

Mouse-ear tissues were preserved by fixation in a 4 % paraformaldehyde solution in PBS with a pH of 7.4. Subsequently, 4 μm-thick sections of the paraffin-embedded tissues were subjected to staining using the hematoxylin and eosin (H&E) technique.

2.7. Enzyme-linked immunosorbent assay (ELISA)

Serum IgE levels were assessed through the utilization of ELISA kits manufactured by Thermo Fisher Scientific, located in Waltham, MA, USA, in accordance with the provided guidelines. The optical density was quantified at 450 nm employing a microplate reader.

2.8. Statistical analysis

Statistical analysis was conducted utilizing Prism 5 software developed by GraphPad Software in San Diego, CA, USA. The results are expressed as the mean ± standard deviation derived from nine separate experiments. Statistical significance was assessed through a one-way analysis of variance and subsequent Tukey's multiple comparisons test.

3. Results

3.1. IOE maintained the skin barrier function and tight junction gene expression in HaCaT cells

We investigated whether the expression of key skin barrier proteins is impacted by IOE in keratinocytes. Pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ induce the generation of TSLP in keratinocytes [24]. It was examined if IOE has an impact on TSLP expressed by TNF-α/IFN-γ. Fig. 1 shows how TNF-α/IFN-γ activation enhanced TSLP expression, which was subsequently concentration-dependently reduced by IOE. Furthermore, TNF-α/IFN-γ-treated cells exhibited a substantial reduction in FLG, LOR, and IVL gene expression when compared with control cells. However, IOE treatment restored expression in a dose-dependent manner (Fig. 1). This suggests that IOE restores the skin barrier protein by reducing the expression of TSLP. Tight junctions have been reported to perform complementary functions with the stratum corneum as a skin barrier [25,26]. We

then examined whether IOE had an impact on the expression of the tight junction-forming proteins such as tight junction-1 (TJP-1), occludin (Ocln), and claudin (Cldn). When compared to the control, the TNF-α/IFN-γ treatment dramatically reduced the gene expression of Cldn, Ocln, and TJP-1. However, the expression was dose-dependently restored by IOE treatment (Fig. 2A). In addition, gene expression of serine palmitoyltransferase (SPT) 1 and 2, as the enzyme that regulates the synthesis of ceramide through *de nove*, was significantly decreased in TNF-α/IFN-γ-treatment compared to control, and IOE treatment restored expression in a dose-dependent manner (Fig. 2B). Moreover, TNF-α/IFN-γ treatment led to a substantial increase in ceramidase gene expression as compared to control, while IOE therapy resulted in a dose-dependent decrease in expression (Fig. 2B). Ceramide is a representative water-retaining substance that encircles keratinocytes, and ceramide is reduced in atopic dermatitis. Thus, a single objective of atopic dermatitis treatment may be ceramide production and maintenance. These findings suggested that IOE's protection of the epidermal barrier had an anti-atopic impact.

3.2. IOE suppressed the JAK/STAT signaling pathway in HaCaT cells

Previous studies have reported that TSLP is involved in activation of STAT3 signaling pathway [27]. To investigate the effect of IOE on the mechanism of action of improving the skin barrier, the intracellular signaling pathway stimulated by TNF-α/IFN-γ was evaluated. When HaCaT cells were activated TNF-α/IFN-γ, they displayed higher signaling molecule phosphorylation. However, IOE pretreatment inhibited TNF-α/IFN-γ-induced phosphorylation of JAK2 and STAT3 (Fig. 3, Fig. S4). Furthermore, the phosphorylation of JAK2 and STAT3 was markedly reduced by the greatest quantity of IOE. Our findings show that IOE significantly influences atopic skin lesions by influencing inflammatory response of keratinocytes.

3.3. IOE ameliorated atopic lesions on DNCB-induced atopic dermatitis mice

We assessed the anti-atopic dermatitis effects of IOE using an animal model of atopic dermatitis produced by DNCB. DNCB is a powerful agent for the induction of atopic lesions. As shown in Fig. 4A, mice treated with DNCB had considerably worsened atopic skin lesions. Histopathological analysis by H&E staining showed the increases in ear edema and immune cell infiltration. Additionally, after receiving DNCB treatment, ear thickness increased (Fig. 4B). On the other hand, oral IOE treatment reduced DNCB-induced atopic skin lesions (Fig. 4A and B). Serum IgE levels were measured since they are a prominent feature of lesions from atopic dermatitis. In addition, IOE decreased serum total IgE levels in comparison to the DNCB-induced group (Fig. 4C). Therefore, our findings suggest that IOE prevents the release of inflammatory mediators associated with atopic dermatitis in mice with DNCB-induced atopic dermatitis.

3.4. IOE regulated the skin barrier function on DNCB-induced atopic dermatitis mice

Next, we investigated at the manner in which IOE affected the expression of TSLP in the ear tissues of atopic dermatitis model mice that was induced by DNCB. As shown in Fig. 5, the RNA levels of TSLP were increased by DNCB and decreased by IOE. In addition, the

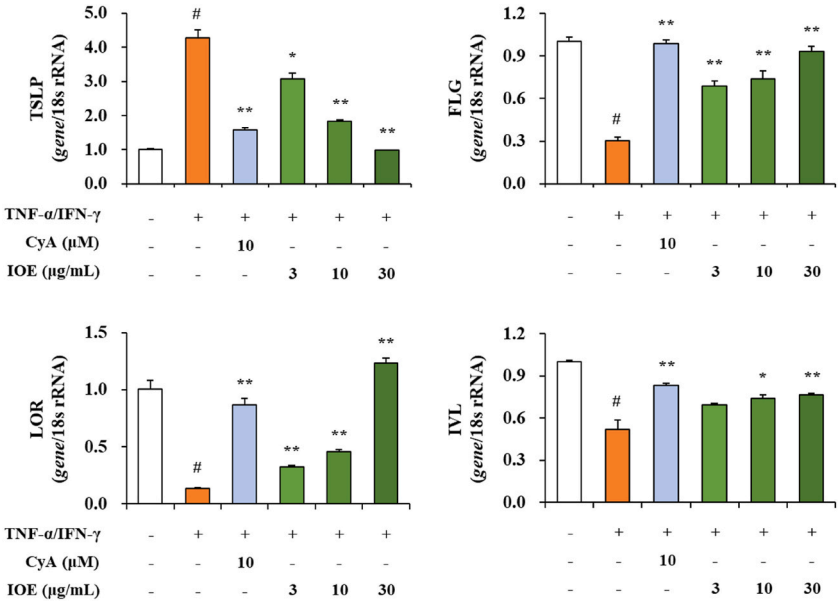

Fig. 1. Illustrates the impact of IOE on the expression levels of skin barrier genes in HaCaT cells stimulated with TNF-α/IFN-γ. Skin barrier protein levels were assessed through real-time quantitative PCR. Statistical analysis indicated a significant difference (*$p < 0.05$, **$p < 0.01$) when compared to the TNF-α/IFN-γ-stimulated group.

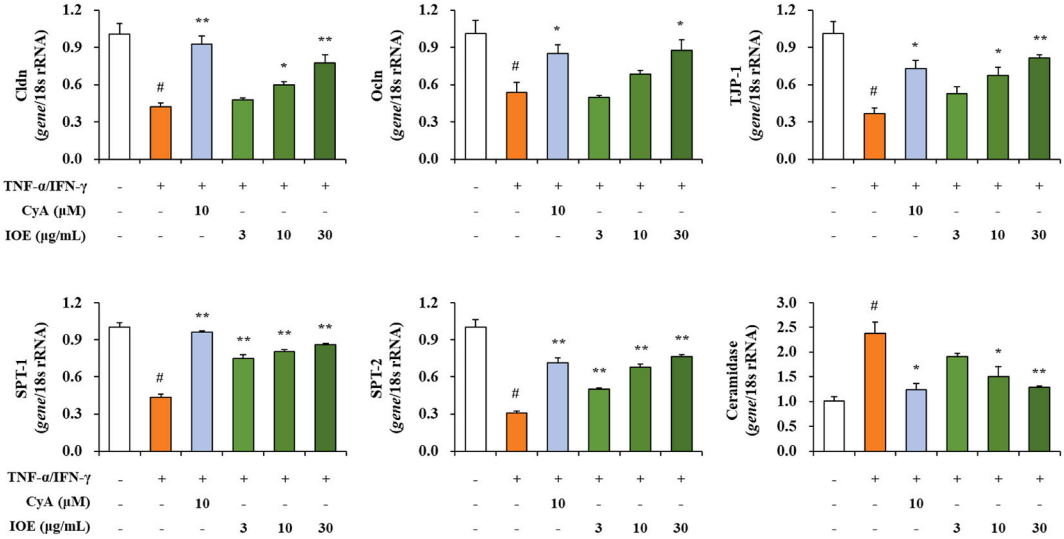

Fig. 2. Displays the influence of IOE on the gene expression levels of tight junction and stratum corneum proteins in TNF-α/IFN-γ stimulated HaCaT cells. The quantification of skin barrier proteins was conducted using real-time quantitative PCR. Statistical analysis indicated a significant difference (*$p < 0.05$, **$p < 0.01$) when compared to the TNF-α/IFN-γ-stimulated group.

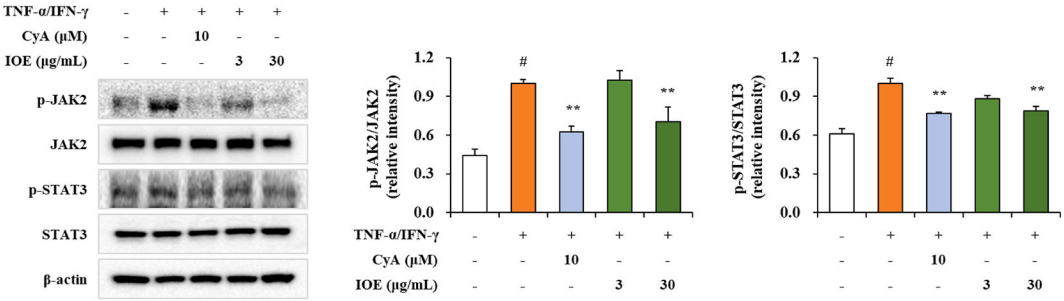

Fig. 3. Demonstrates the impact of IOE on intracellular signal transduction in TNF-α/IFN-γ stimulated HaCaT cells. The cells were pre-treated with 3 or 30 mg/mL IOE for 1 h before being stimulated with TNF-α/IFN-γ for 30 min. Total protein was extracted using Thermo Fisher total protein extraction reagent.

effect of IOE on the expression of FLG, LOR, and IVL, which are major constituent proteins of the skin barrier, was investigated at the RNA level. When comparing the DNCB group to the normal group, there was a substantial drop in the gene expression of FLG, LOR, and IVL. However, the IOE group was able to restore expression in a way that was dose-dependent (Fig. 5).

3.5. DPHC maintained the skin barrier function and tight junction gene expressions in HaCaT cells

In previous findings, IOE demonstrated the anti-atopic impact. Thus, it was determined that DPHC was effective in finding an active ingredient that would demonstrate the effectiveness of IOE. We examined whether important skin barrier proteins and TSLP expression are impacted by DPHC in keratinocytes. The gene expression of TSLP was significantly increased in the TNF-α/IFN-γ treatment group compared to the control group. There was a dose-dependent decrease in the expression of DPHC. In addition, the gene expression of the skin barrier proteins FLG, LOR, and IVL was significantly decreased in the TNF-α/IFN-γ treatment group compared to the control group. However, DPHC treatment restored expression in a dose-dependent manner (Fig. 6). Then, the impact of DPHC on the expression of tight junction proteins was examined in this study. The gene expression levels of Cldn, Ocln, and TJP-1 were significantly reduced in response to TNF-α/IFN-γ treatment compared to the control group. However, treatment with DPHC led to a dose-dependent restoration of their expression levels, as illustrated in Fig. 7A. Furthermore, the gene expression of SPT-1 and SPT-2 was significantly decreased following TNF-α/IFN-γ treatment compared to the control group. Yet, DPHC treatment resulted in a dose-dependent recovery of their expression levels, as depicted in Fig. 7B. Additionally, the gene expression of ceramidase was markedly elevated in response to TNF-α/IFN-γ treatment compared to the control group. Nevertheless, DPHC treatment resulted in a dose-dependent reduction in its expression levels, as illustrated in Fig. 7B. These findings suggest that DPHC exhibits anti-atopic properties by safeguarding the skin barrier.

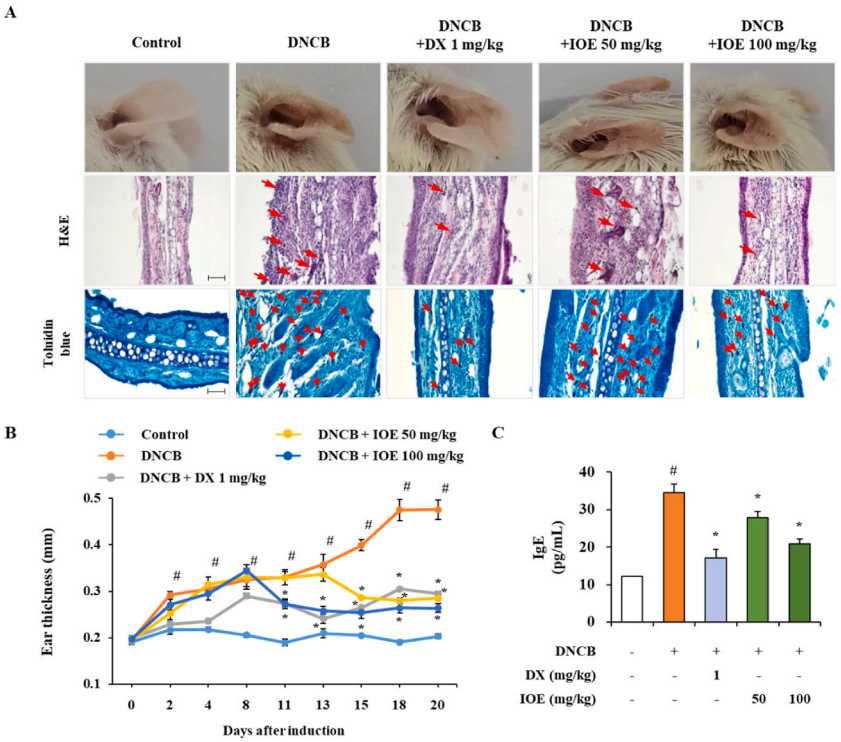

Fig. 4. Showcases the effect of IOE on skin lesions and IgE levels in a DNCB-induced atopic dermatitis mouse model. (A) The image depicts skin lesions on the ears of mice with DNCB-induced atopic dermatitis 24 h after the final DNCB treatment. Ear thickness was measured using a dial thickness gauge 24 h post-DNCB induction (B), while serum IgE levels were quantified using ELISA (C). Statistical analysis indicated a significant difference (*p < 0.05, **p < 0.01) when compared to the DNCB-treated group.

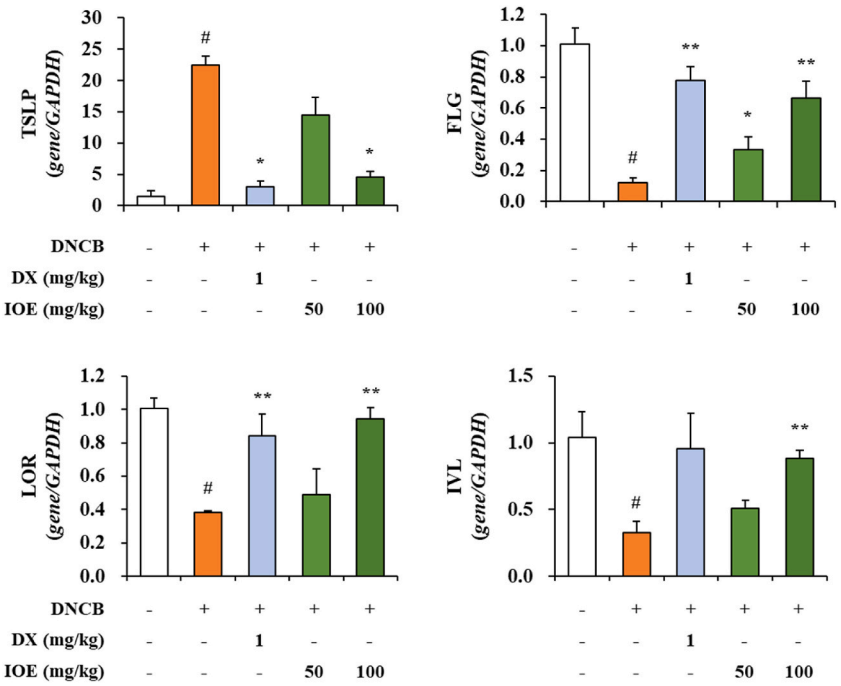

Fig. 5. Examines the impact of IOE on skin barrier gene expression and production in a DNCB-induced atopic dermatitis mouse model. Total RNA was extracted from ear tissues, and the levels of skin barrier proteins such as TSLP, FLG, LOR, and IVL were assessed via real-time PCR. Statistical analysis indicated a significant difference (*p < 0.05, **p < 0.01) when compared to the TNF-α/IFN-γ-stimulated group.

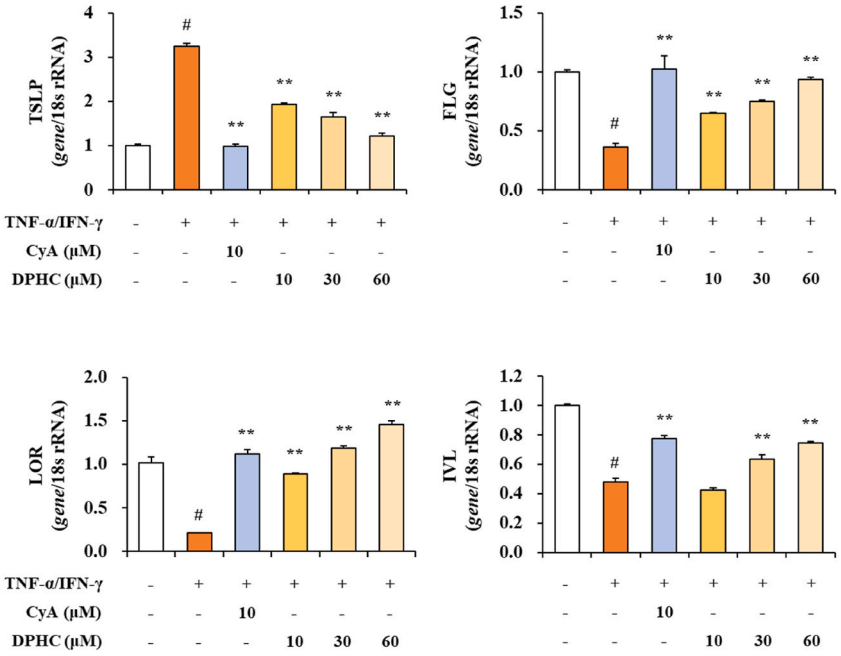

Fig. 6. Illustrates the impact of DPHC on the expression levels of skin barrier genes in HaCaT cells stimulated with TNF-α/IFN-γ. The quantification of skin barrier proteins was conducted through real-time PCR. Statistical analysis indicated a significant difference (*p < 0.05, **p < 0.01) when compared to the TNF-α/IFN-γ-stimulated group.

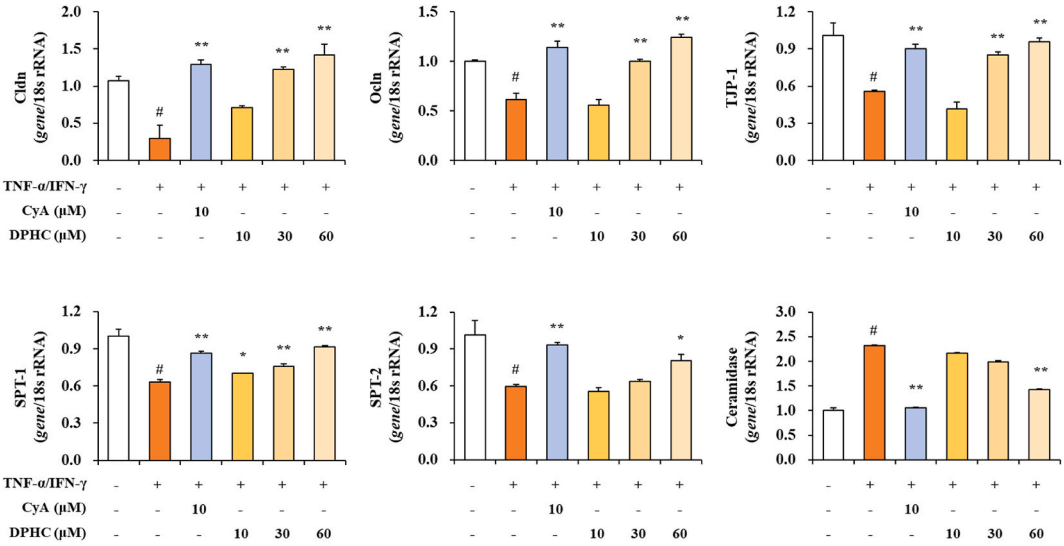

Fig. 7. Illustrates the impact of DPHC on the tight junction and SPT gene expression levels in TNF-α/IFN-γ-stimulated HaCaT cells. The quantification of skin barrier proteins was conducted through real-time PCR. Statistical analysis indicated a significant difference (*p < 0.05, **p < 0.01) when compared to the TNF-α/IFN-γ-stimulated group.

3.6. DPHC suppressed the JAK/STAT signaling pathway in HaCaT cells

To explore the mechanism of action of DPHC, the intracellular signaling pathway stimulated by TNF-α/IFN-γ was evaluated. TNF-α/IFN-γ-stimulated HaCaT cells showed an increase in the phosphorylation of signaling molecules. However, pretreatment with DPHC suppressed the TNF-α/IFN-γ-induced phosphorylation of JAK2, and STAT3 (Fig. 8, Fig. S5). Moreover, JAK2 and STAT3 phosphorylation was markedly inhibited by the greatest concentration of DPHC. Our findings showed that via regulating keratinocyte inflammatory responses, DPHC significantly modulates atopic skin lesions.

3.7. DPHC ameliorated atopic lesions on DNCB-induced atopic dermatitis mice

Using an animal model of atopic dermatitis produced by DNCB, we assessed the anti-atopic dermatitis effects of DPHC. The DNCB-treated mice's atopic skin lesions were noticeably even worse, as Fig. 9A shows. H&E staining histopathological examination revealed increased immune cell infiltration and ear edema. Serum IgE levels were measured since they are a prominent feature of lesions from atopic dermatitis. In comparison to the DNCB-induced group, DPHC also reduced the blood levels of total IgE (Fig. 9C). Therefore, our findings suggest that in mice with DNCB-induced atopic dermatitis, DPHC has an anti-atopic dermatitis effect.

3.8. DPHC regulated the skin barrier function on DNCB-induced atopic dermatitis mice

Next, we investigated to see how DPHC affected the mRNA expression of TSLP in the ear tissue of atopic dermatitis model mice that was induced by DNCB. DNCB raised TSLP whereas DPHC lowered it. Furthermore, the impact on the mRNA level of FLG, LOR, and IVL expression-three important skin barrier component proteins was examined. When comparing the DNCB group to the normal group, there was a substantial drop in the gene expression of FLG, LOR, and IVL. But as shown in Fig. 10, the DPHC group was able to restore expression in a dose-dependent manner.

4. Discussion

Atopic dermatitis is accompanied by pathophysiological changes that impair skin barrier function. In this study, using an in vivo animal model, we examined the effectiveness of IOE and DPHC on atopic dermatitis. Specifically, we examined whether IOE or DPHC generated from *Ishige okamurae* might enhance the recovery of skin barrier function in atopic dermatitis. Atopic dermatitis is characterized by dry skin and disruption of the skin barrier. Epithelial cells in the skin stimulated by antigen exposure release factors such as TSLP to trigger Th2 immune responses and inflammatory mediators such as cytokines and chemokines [8,28]. IgE is a crucial component that is generated by B cells that have been stimulated by Th2 cytokines and is involved in Th2 immune responses [29]. Thus, TSLP and serum IgE levels can be the target for the treatment of atopic dermatitis. In this study, both TNF-α/IFN-γ-treated HaCaT cells and DNCB-induced atopic dermatitis animal models exhibited significant increases in the expression of TSLP and serum IgE, which are key indicators of inflammatory and allergic responses. These elevated levels suggest a heightened inflammatory state and a disruption of skin barrier integrity in both models. However, treatment with IOE or DPHC resulted in a notable, dose-dependent reduction in TSLP expression and serum IgE levels compared to the TNF-α/IFN-γ-treated or DNCB-induced groups (Figs. 4 and 9). Specifically, higher concentrations of IOE and DPHC corresponded to more pronounced decreases in these markers, suggesting an inhibition of the inflammatory pathways associated with atopic dermatitis. These findings indicate that both IOE and DPHC may exert therapeutic effects by modulating immune responses, reducing inflammatory cytokines, and restoring the disrupted skin barrier in atopic conditions. Therefore, IOE and DPHC could be promising candidates for the development of treatments targeting atopic dermatitis and other inflammatory skin disorders.

The overexpression of inflammatory cytokines combined with atopic dermatitis causes impairment of skin function by disrupting tight junction proteins (Cldn, Ocln, and TJP-1) and cell-to-cell binding proteins (FLG, LOR, and IVL) [26]. In the present study, the DNCB-induced atopic dermatitis group exhibited significantly reduced levels of key skin barrier proteins, including FLG, LOR, and IVL, compared to the normal group. These proteins play a crucial role in maintaining skin barrier integrity, and their reduced expression correlates with compromised skin function and the manifestation of atopic dermatitis symptoms, such as itching. However, treatment with IOE or DPHC led to a marked restoration of FLG, LOR, and IVL levels in the DNCB group, indicating a recovery of skin barrier function and an improvement in atopic dermatitis symptoms (Figs. 5 and 10). Similarly, in HaCaT cells, which serve as an in vitro model for studying skin barrier-related proteins, TNF-α/IFN-γ treatment resulted in a significant decrease in the expression of FLG, LOR, and IVL compared to the untreated control. This reduction mirrors the inflammatory response and barrier dysfunction observed in atopic dermatitis. However, IOE and DPHC treatment restored the levels of these proteins in a dose-dependent manner, further reinforcing their potential role in enhancing skin barrier integrity and mitigating inflammatory damage (Figs. 1 and 6). These findings suggest that IOE and DPHC promote the recovery of skin barrier proteins, contributing to the alleviation of atopic dermatitis symptoms and restoration of epidermal homeostasis. To close the gap between cells and attach them to one another, tight junctions are found underneath the stratum corneum [30]. It has been documented that atopic dermatitis-induced damage to tight junctions can trigger a Th2-mediated immune response [31]. The expression levels of tight junction proteins, including Cldn, Ocln, and TJP-1, were assessed in HaCaT cells to evaluate their role in maintaining skin barrier integrity. Upon treatment with TNF-α/IFN-γ, there was a significant reduction in the expression of these tight junction proteins compared to the control group, indicating impaired tight junction function and compromised skin barrier integrity. However, treatment with IOE or DPHC led to a dose-dependent restoration of Cldn, Ocln, and TJP-1 expression (Figs. 2A and 7A). These results suggest that IOE and DPHC help repair tight junctions, contributing to improved skin barrier function and potentially alleviating inflammatory responses in conditions such as atopic dermatitis. Ceramides play an important role in maintaining the lamellar integrity of the epidermal barrier [32]. Ceramides depletion has been proposed as a causative element for breakdown of the epidermal barrier, which in turn causes clinical manifestations of dullness and dryness such epidermal hyperplasia in the atopic dermatitis epidermis [33,34]. The expression of serine SPTs, key enzymes involved in ceramide biosynthesis, was also evaluated in HaCaT cells. Ceramides are essential lipids that play a crucial role in maintaining skin barrier integrity and hydration. The results showed that TNF-α/IFN-γ treatment significantly reduced SPT expression, which likely contributed to a decrease in ceramide production and subsequent impairment of the skin barrier. However, treatment with IOE or DPHC led to a dose-dependent increase in SPT expression (Figs. 2B and 7B). This suggests that IOE and DPHC can restore ceramide synthesis by

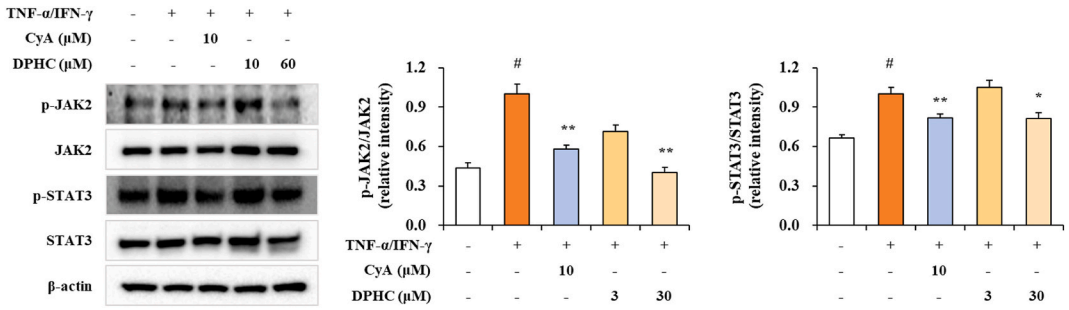

Fig. 8. Shows the effect of DPHC on intracellular signal transduction in TNF-α/IFN-γ-stimulated HaCaT cells. Prior to being stimulated with TNF-α/IFN-γ for 30 min, HaCaT cells were pre-exposed to either 10 or 60 mg/mL of DPHC for a duration of 1 h. Subsequently, total protein was isolated using the Thermo Fisher total protein extraction reagent. Statistical analysis indicated a significant difference (*p < 0.05, **p < 0.01) when compared to the TNF-α/IFN-γ-stimulated group.

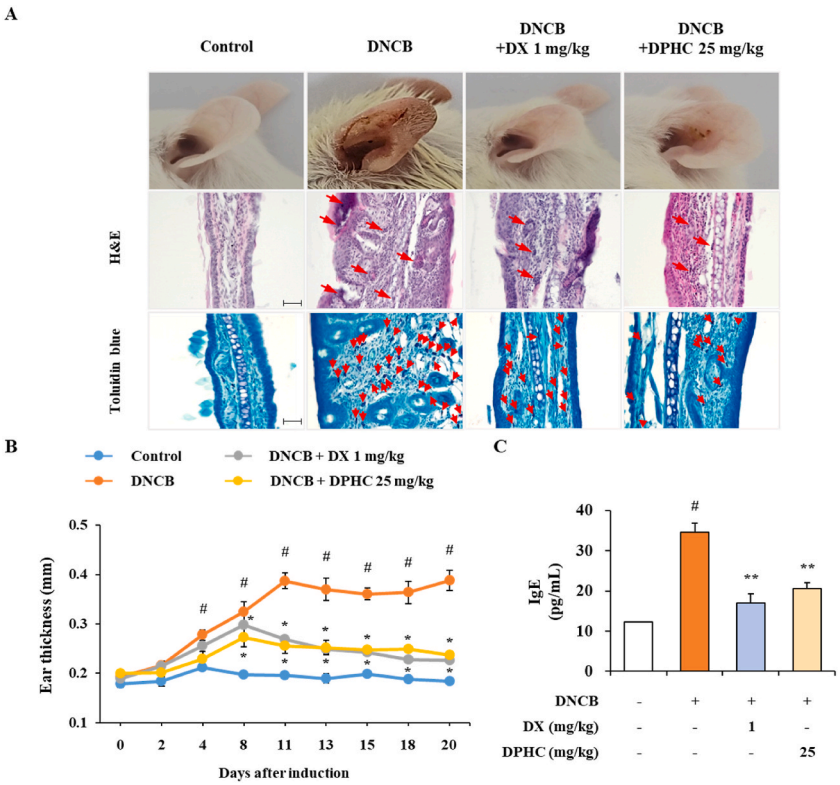

Fig. 9. Shows the effect of DPHC on skin lesions and IgE levels in a DNCB-induced atopic dermatitis mouse model. (A) Displays skin lesions on the ears of mice afflicted with DNCB-induced atopic dermatitis, with the image depicting the condition of the ear skin 24 h post the final DNCB treatment. (B) Demonstrates the measurement of ear thickness utilizing a dial thickness gauge 24 h following the initiation of DNCB. (C) Showcases the quantification of serum IgE levels through ELISA. Statistical analysis indicated a significant difference (*p < 0.05, **p < 0.01) when compared to the DNCB-treated group.

upregulating SPT expression, thereby enhancing skin barrier function and helping to alleviate inflammatory damage associated with atopic dermatitis. Th2 cytokines have been shown to induce JAK/STAT3 phosphorylation and activation, which in turn down-regulates barrier proteins in keratinocytes [35,36]. In our study, we examined whether it prevents JAK/STAT3 from being phosphorylated and activated. It was established that IOE and DPHC reduced the phosphorylation of JAK/STAT3. These findings indicate that IOE and DPHC suppress the JAK/STAT3 signalling pathway, improving skin barrier function (Figs. 3 and 8). Taken together, these findings suggest that IOE holds promise as a potential therapeutic agent for restoring skin barrier function compromised by atopic dermatitis. Furthermore, we propose that DPHC, derived from Ishige okamurae, is a promising option for the management and treatment of atopic dermatitis.

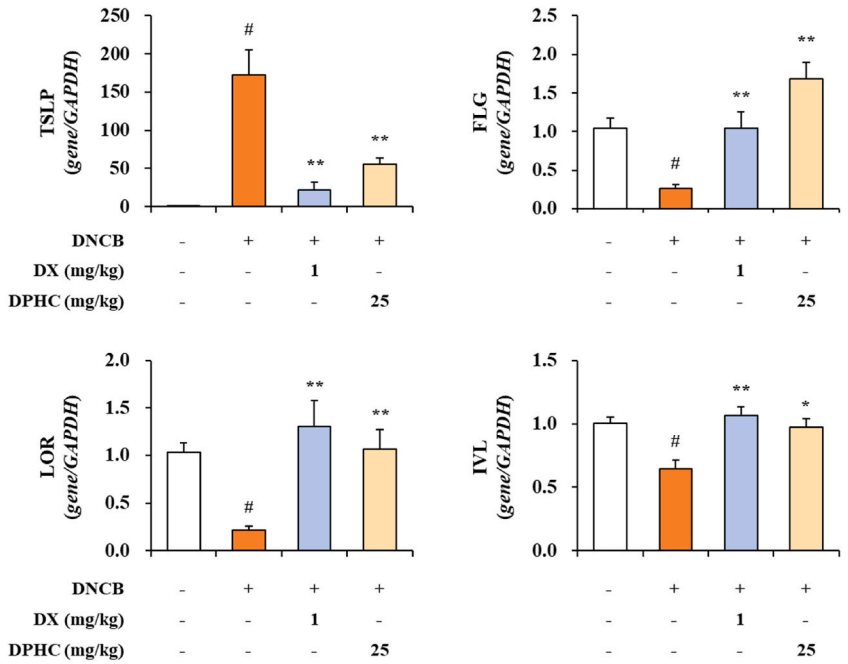

Fig. 10. Illustrates the impact of DPHC on skin barrier gene expression and production in a DNCB-induced atopic dermatitis mouse model. RNA was isolated from ear tissues, and the quantities of skin barrier proteins including TSLP, FLG, LOR, and IVL were assessed through real-time PCR. Statistical analysis indicated a significant difference (*$p < 0.05$, **$p < 0.01$) when compared to the DNCB-treated group.

CRediT authorship contribution statement

Seon Gyeong Bak: Writing – original draft, Formal analysis, Conceptualization. **Hyung Jin Lim:** Investigation, Formal analysis. **Yeong-Seon Won:** Investigation, Formal analysis. **Sang-Ik Park:** Methodology, Investigation, Formal analysis. **Sun Hee Cheong:** Methodology, Investigation, Formal analysis. **Seung Jae Lee:** Writing – original draft, Supervision, Conceptualization.

Data availability statement

Data included in article/supplementary material is referenced in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40227.

References

[1] B.E. Kim, D.Y. Leung, Epidermal barrier in atopic dermatitis, Allergy Asthma Immunol Res 4 (1) (2012) 12–16.

[2] J.W. Lee, Q. Wu, Y.P. Jang, S.Y. Choung, Pinus densiflora bark extract ameliorates 2,4-dinitrochlorobenzene-induced atopic dermatitis in NC/Nga mice by regulating Th1/Th2 balance and skin barrier function, Phytother Res. 32 (6) (2018) 1135–1143.
[3] A. Sandilands, C. Sutherland, A.D. Irvine, W.H. McLean, Filaggrin in the frontline: role in skin barrier function and disease, J. Cell Sci. 122 (Pt 9) (2009) 1285–1294.
[4] M.J. Cork, S.G. Danby, Y. Vasilopoulos, J. Hadgraft, M.E. Lane, M. Moustafa, R.H. Guy, A.L. Macgowan, R. Tazi-Ahnini, S.J. Ward, Epidermal barrier dysfunction in atopic dermatitis, J. Invest. Dermatol. 129 (8) (2009) 1892–1908.
[5] B.E. Kim, D.Y. Leung, M. Boguniewicz, M.D. Howell, Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6, Clin Immunol 126 (3) (2008) 332–337.
[6] P.M. Steinert, T. Kartasova, L.N. Marekov, Biochemical evidence that small proline-rich proteins and trichohyalin function in epithelia by modulation of the biomechanical properties of their cornified cell envelopes, J. Biol. Chem. 273 (19) (1998) 11758–11769.
[7] M.R. Comeau, S.F. Ziegler, The influence of TSLP on the allergic response, Mucosal Immunol. 3 (2) (2010) 138–147.
[8] S.F. Ziegler, Thymic stromal lymphopoietin and allergic disease, J. Allergy Clin. Immunol. 130 (4) (2012) 845–852.
[9] T. Czarnowicki, J.G. Krueger, E. Guttman-Yassky, Skin barrier and immune dysregulation in atopic dermatitis: an evolving story with important clinical implications, J. Allergy Clin. Immunol. Pract. 2 (4) (2014) 371–379, quiz 380-371.
[10] P.M. Brunner, E. Guttman-Yassky, D.Y. Leung, The immunology of atopic dermatitis and its reversibility with broad-spectrum and targeted therapies, J. Allergy Clin. Immunol. 139 (4S) (2017) S65–S76.
[11] J.K. Gittler, A. Shemer, M. Suarez-Farinas, J. Fuentes-Duculan, K.J. Gulewicz, C.Q. Wang, H. Mitsui, I. Cardinale, C. de Guzman Strong, J.G. Krueger, E. Guttman-Yassky, Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis, J. Allergy Clin. Immunol. 130 (6) (2012) 1344–1354.
[12] I.P.S. Fernando, T.U. Jayawardena, K.K.A. Sanjeewa, L. Wang, Y.J. Jeon, W.W. Lee, Anti-inflammatory potential of alginic acid from Sargassum horneri against urban aerosol-induced inflammatory responses in keratinocytes and macrophages, Ecotoxicol. Environ. Saf. 160 (2018) 24–31.
[13] L. Wang, W. Lee, J.Y. Oh, Y.R. Cui, B. Ryu, Y.J. Jeon, Protective effect of sulfated polysaccharides from celluclast-assisted extract of hizikia fusiforme against ultraviolet B-induced skin damage by regulating NF-kappaB, AP-1, and MAPKs signaling pathways in vitro in human dermal fibroblasts, Mar. Drugs 16 (7) (2018).
[14] H.W. Yang, K.H.N. Fernando, J.Y. Oh, X. Li, Y.J. Jeon, B. Ryu, Anti-obesity and anti-diabetic effects of Ishige okamurae, Mar. Drugs 17 (4) (2019).
[15] Y. Zou, Z.J. Qian, Y. Li, M.M. Kim, S.H. Lee, S.K. Kim, Antioxidant effects of phlorotannins isolated from Ishige okamurae in free radical mediated oxidative systems, J. Agric. Food Chem. 56 (16) (2008) 7001–7009.
[16] L. Cao, S.G. Lee, K.T. Lim, H.R. Kim, Potential anti-aging substances derived from seaweeds, Mar. Drugs 18 (11) (2020).
[17] S.H. Lee, Y.J. Jeon, Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms, Fitoterapia 86 (2013) 129–136.
[18] H.-S. Kim, L. Wang, T.U. Jayawardena, E.-A. Kim, S.-J. Heo, I.S. Fernando, J.-H. Lee, Y.-J. Jeon, High-performance centrifugal partition chromatography (HPCPC) for efficient isolation of diphlorethohydroxycarmalol (DPHC) and screening of its antioxidant activity in a zebrafish model, Process Biochem. 88 (2020) 189–196.
[19] Y. Ding, Y. Jiang, S.T. Im, S. Myung, H.S. Kim, S.H. Lee, Diphlorethohydroxycarmalol inhibits melanogenesis via protein kinase A/cAMP response element-binding protein and extracellular signal-regulated kinase-mediated microphthalmia-associated transcription factor downregulation in α-melanocyte stimulating hormone-stimulated B16F10 melanoma cells and zebrafish, Cell Biochem. Funct. 39 (4) (2021) 546–554.
[20] S.G. Bak, H.J. Lim, E.J. Park, Y.-S. Won, S.W. Lee, B. Ryu, H.J. Ha, S.-H. Cheong, S.J. Lee, Ishige okamurae extract: diphlorethohydroxycarmalol with effect of atopic dermatitis-like skin inflammation, Revista Brasileira de Farmacognosia 34 (2) (2024) 338–349.
[21] F. Yang, J. Hyun, D. Nagahawatta, Y.M. Kim, M.-S. Heo, Y.-J. Jeon, Cosmeceutical effects of Ishige okamurae Celluclast extract, Antioxidants 11 (12) (2022) 2442.
[22] Z. Xiao, S. Yang, Y. Liu, C. Zhou, P. Hong, S. Sun, Z.-J. Qian, A novel glyceroglycolipid from brown algae Ishige okamurae improve photoaging and counteract inflammation in UVB-induced HaCaT cells, Chem. Biol. Interact. 351 (2022) 109737.
[23] L. Wang, H.S. Kim, J.Y. Oh, J.G. Je, Y.-J. Jeon, B. Ryu, Protective effect of diphlorethohydroxycarmalol isolated from Ishige okamurae against UVB-induced damage in vitro in human dermal fibroblasts and in vivo in zebrafish, Food Chem. Toxicol. 136 (2020) 110963.
[24] A.T. Vu, T. Baba, X. Chen, T.A. Le, H. Kinoshita, Y. Xie, S. Kamijo, K. Hiramatsu, S. Ikeda, H. Ogawa, K. Okumura, T. Takai, Staphylococcus aureus membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like receptor 2-Toll-like receptor 6 pathway, J. Allergy Clin. Immunol. 126 (5) (2010) 985–993, 993 e981-983.
[25] J. Brandner, S. Kief, E. Wladykowski, P. Houdek, I. Moll, Tight junction proteins in the skin, Skin Pharmacol. Physiol. 19 (2) (2006) 71–77.
[26] G. Egawa, K. Kabashima, Barrier dysfunction in the skin allergy, Allergol. Int. 67 (1) (2018) 3–11.
[27] L. Shan, N.S. Redhu, A. Saleh, A.J. Halayko, J. Chakir, A.S. Gounni, Thymic stromal lymphopoietin receptor-mediated IL-6 and CC/CXC chemokines expression in human airway smooth muscle cells: role of MAPKs (ERK1/2, p38, and JNK) and STAT3 pathways, J. Immunol. 184 (12) (2010) 7134–7143.
[28] E.B. Brandt, U. Sivaprasad, Th2 cytokines and atopic dermatitis, J. Clin. Cell. Immunol. 2 (3) (2011).
[29] M. Furue, T. Chiba, G. Tsuji, D. Ulzii, M. Kido-Nakahara, T. Nakahara, T. Kadono, Atopic dermatitis: immune deviation, barrier dysfunction, IgE autoreactivity and new therapies, Allergol. Int. 66 (3) (2017) 398–403.
[30] M. Yokouchi, A. Kubo, H. Kawasaki, K. Yoshida, K. Ishii, M. Furuse, M. Amagai, Epidermal tight junction barrier function is altered by skin inflammation, but not by filaggrin-deficient stratum corneum, J. Dermatol. Sci. 77 (1) (2015) 28–36.
[31] S. Hönzke, L. Wallmeyer, A. Ostrowski, M. Radbruch, L. Mundhenk, M. Schäfer-Korting, S. Hedtrich, Influence of Th2 cytokines on the cornified envelope, tight junction proteins, and β-defensins in filaggrin-deficient skin equivalents, J. Invest. Dermatol. 136 (3) (2016) 631–639.
[32] M. Rabionet, K. Gorgas, R. Sandhoff, Ceramide synthesis in the epidermis, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1841 (3) (2014) 422–434.
[33] A. Di Nardo, P. Wertz, A. Giannetti, S. Seidenari, Ceramide and cholesterol composition of the skin of patients with atopic dermatitis (1998).
[34] D. Sajić, R. Asiniwasis, S. Skotnicki-Grant, A look at epidermal barrier function in atopic dermatitis: physiologic lipid replacement and the role of ceramides, Skin therapy letter 17 (7) (2012) 6–9.
[35] W. Amano, S. Nakajima, H. Kunugi, Y. Numata, A. Kitoh, G. Egawa, T. Dainichi, T. Honda, A. Otsuka, Y. Kimoto, The Janus kinase inhibitor JTE-052 improves skin barrier function through suppressing signal transducer and activator of transcription 3 signaling, J. Allergy Clin. Immunol. 136 (3) (2015) 667–677, e667.
[36] L. Bao, H. Zhang, L.S. Chan, The involvement of the JAK-STAT signaling pathway in chronic inflammatory skin disease atopic dermatitis, JAK-STAT 2 (3) (2013) e24137.