

Effect of isoliquiritigenin for the treatment of atopic dermatitis-like skin lesions in mice

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Abstract

Atopic dermatitis (AD) is a common chronic inflammatory skin disease characterized with high heterogeneity. Recent studies have suggested that it is driven by both terminal keratinocyte differentiation defects and type 2 immune responses. The mainstay steroid topical therapy has severe side effect and new treatment is in demand. Isoliquiritigenin (ISLG) is a small phenolic bioactive molecule from licorice that has shown multiple pharmacological effects against cancer, inflammatory disorder, and cardiovascular diseases. ISLG was evaluated in AD-like lesion model induced by the repetitive application of 2,4-dinitrochlorobenzene (DNCB) in BALB/c mice. Overall symptom score, serological and molecular changes of the skin lesions were evaluated. ISLG could ameliorate the overall manifestation of AD-like symptoms including scratching behavior incidence and skin lesion severity. At blood level, ISLG significantly suppressed the DNCB-induced IgE and Th2 cytokines up-regulation. At skin lesion site, ISLG also inhibited DNCB-induced pro-inflammatory cytokines like TNF- α , IL-6 as well as IL-4 expressions. In a human monocyte model THP-1, ISLG suppressed the up-regulation of CD86 and CD54 and abolished the DNCB-induced p38- α and ERK activation, suggesting a molecular mechanism for ISLG therapy. This study indicated that ISLG could be a potential therapeutic agent for the treatment of AD.

Keywords

Atopic dermatitis DNCB Isoliquiritigenin THP-1 Th2 cells

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Introduction

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease, which is characterized by complex pathophysiology and a wide spectrum of the clinical phenotypes [20]. It is often manifested as erythematous, thickened epidermal lesions that vary in intensity and affected body surface area [6, 34]. It is a major skin disorder that imposes considerable burdens upon patients' quality of life, health outcomes, and work productivity [30]. AD affects 11–30% of children and 2–10% of adults, depending on the population [5, 31]. In early models of etiology, AD was largely attributed to cutaneous inflammation at lesion sites, but recent studies have established that it is driven by both terminal keratinocyte differentiation defects and strong type 2 immune responses [27, 29]. Several observations suggested that AD was the cutaneous manifestation of a systemic disorder that also gave rise to asthma, food allergy, and allergic rhinitis [20, 21]. Elevated serum IgE levels and peripheral eosinophilia are commonly found in these conditions [7, 10]. In contrast to chronic plaque-type psoriasis, AD is now understood to be a heterogeneous disease, with additional activation of T_H22 , $T_H17/IL-23$, and T_H1 cytokine pathways depending on the subtype of the disease [25, 35]. The major components contributing to immune dysregulation in AD are Langerhans' cells, inflammatory dendritic epidermal cells, monocytes, macrophages, lymphocytes, mast cells, and keratinocytes, all of which interact through an intricate cascade of cytokines leading to a predominance of Th2 cells over Th1 cells. For instance, the Th2 cytokines, interleukin (IL)-4, IL-5, IL-10, and IL-13, are increased in the skin and blood, and there is a corresponding decrease in Th1 cytokines, mainly interferon- γ and IL-2. Noteworthy, although many immunopathologic features of AD have recently been elucidated, the fundamental etiology of these abnormalities remains to be elusive [9, 16, 36]. Topical steroid therapy remains the mainstay of treatment in AD. However, this treatment is associated with severe side effects, including atrophy, acne, cataracts, growth retardation, and red burning skin [2]. New therapeutic agent is in demand to tackle the challenge. For instance, candidates derived from natural products are becoming promising.

It has been a long time, since the roots and rhizomes of licorice (*Glycyrrhiza*) served as traditional Chinese medicines and natural sweeteners. Isoliquiritigenin (ISLG) is an important bioactive ingredient isolated from the roots of licorice, including *Glycyrrhiza uralensis*, *Mongolian glycyrrhiza*, and *Glycyrrhiza glabra*. In addition to widely used food additives, ISLG is also applied as therapeutic agent for many diseases based on its numerous pharmacological properties such as anti-inflammatory, anti-

microbial, anti-oxidative, anti-cancer activities, immunoregulatory, hepatoprotective, and cardioprotective effects [26]. As a potent modulator of inflammatory response, ISL was reported to suppress the expression of VCAM-1 (vascular cell adhesion molecule) and E-selectin on activated human umbilical vein endothelial cells (HUVEC); markedly interfere with THP monocyte adhesion to TNF- α -activated endothelial cells and attenuate platelet endothelial cell adhesion molecule-1 expression induced by TNF- α . At the molecular level, ISLG abolished TNF- α -induced mRNA accumulation of VCAM-1 and E-selectin and downregulated cell adhesion molecule proteins in TNF- α -activated HUVEC at the transcriptional levels by blocking degradation of I κ B α and nuclear translocation of NF κ B [14, 18, 19]. ISLG could act as anti-asthma herbal medicine with inhibitory effects on memory Th2 responses in vitro and antigen-induced Th2 inflammation in vivo via significant down-regulation of IL-4 and IL-5 production in a dose-dependent manner [37].

In this study, we aimed to investigate the therapeutic effect of topical application of ISLG on BALB/c mice induced with AD-like skin lesion, a well-established model induced by 2,4-dinitrochlorobenzene (DNCB).

Materials and methods

Reagents

ISTG, DNCB (99% purity) were purchased from Sigma; anti-human CD54 (clone; 6.5B5) from DAKO (Glostrup, Denmark) and anti-human CD86 (clone; Fun-1) from BD PharMingen (San Diego, CA, USA); p38-p (#4511), p38-total (#9212), ERK-p (197G2), ERK total (#9102), and β -actin (8H10D10) antibodies from cell signaling.

Cell culture

THP-1 cells were purchased from ATCC (VA, USA). Cells were cultured in RPMI 1640 medium (GIBCO BRL, Grand Island, NY, USA) containing 1% (v/v) 100 U/mL penicillin G, and 100 mg/mL streptomycin (GIBCO BRL, USA), 10% (v/v) heat-inactivated fetal bovine serum medium (Hyclone) and 2 mM L-glutamine (GIBCO BRL, USA). All the cells were then maintained in 25-cm² culture flasks at 37 °C in humidified cell incubator supplemented with 5% CO₂.

Mouse strain

All experimental protocols involving the use of live animals were approved by the Institutional Animal Care and Use Committee (IACUC). The 6–8-week-old male and female BALB/c mice were obtained from Jackson Laboratory

(ME, USA) and were housed with five mice per cage at room temperature of 23 ± 3 °C with a relative humidity of $55 \pm 10\%$ throughout the study. Animal treatment and maintenance complied with guidelines issued by the ethics committee for animal welfare at The Affiliated Qingdao Municipal Hospital of Qingdao University.

DNCB-induced AD model

To induce AD-like symptoms, DNCB (Sigma-Aldrich, MO, USA) was used to induce the skin lesion. The design of the experiment is shown in Fig. 1b. Briefly, the dorsal hair of the mice was removed using an electronic clipper 2 days before DNCB treatment. Then shaved dorsal skin of BALB/c was applied with 200 µl of 1% DNCB (in acetone:olive oil = 4:1) for sensitization. The repeated challenge was performed on the same site with 0.2% DNCB once every 3 days for about 2 weeks. The mice were divided into four groups ($n = 6$ per group): (1) vehicle-treated control; (2) DNCB-treated only; (3) ISLG-treated only; and (4) DNCB + ISLG-treated group. 1% ISLG was prepared in the same vehicle as DNCB solution. After the animals were shaved at back on day 0, the DNCB model group was sensitized with 1% DNCB on days 1 and 3, followed by further challenge with 0.2% DNCB every 3 day from day 6 to day 18. The vehicle control group was stimulated with vehicle only in the same manner, while the ISLG only group and DNCB plus ISLG treatment group were treated with 1% ISLG daily from day 6 to day 18, as shown in Fig. 1b, the latter group was subject to the same treatment scenario of DNCB solution as described for DNCB model group.

Fig. 1

Experimental design. a Chemical structure of Isoliquiritigenin (ISLG). **b** Shaved dorsal skin was applied with 200 µl of 1% DNCB (in acetone:olive oil = 4:1) for sensitization. The repeated challenge was performed on the same site with 0.2% DNCB once every 3 days for about 2 weeks. The mice were divided into four groups ($n = 6$ per group): (1) vehicle-treated control; (2) DNCB-treated only; (3) ISLG-treated only (in the same vehicle as DNCB); and (4) DNCB + ISLG-treated group

Evaluation of scratching behavior in mice with AD-like skin lesions

Scratching behavior was observed after the completion of all treatments. Briefly, the mice were placed in cages and allowed to acclimatize for 15 min, and the number of scratching behaviors was counted for the next 10 min. This was repeated six times (60 min in total). Scratching behavior was defined in this experiment as movement with the hind paws. Scratching of the nose, ears, and dorsal skin with hind paws was recorded, while licking of the belly and dorsal skin during grooming was not recorded. All evaluations were performed in a blinded manner.

Evaluation of the severity of AD-like skin lesions

The severity of the dermatitis in the dorsal skin of DNCB-induced AD mice was evaluated on day 18. The severity of four symptoms (erythema/hemorrhage, edema, excoriation/erosion, and scarring/dryness) was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). Dermatitis score was defined as the sum of these individual scores in accordance with the method of Matsuda et al. [23].

Measurement of cytokine levels in serum by ELISA

Whole blood was collected from mice in each of the treatment groups on the last day of the study (day 19). The serum was then collected by centrifugation for 20 min at 12,000 rpm and stored at -80°C , until further use. The level of serum IgE was measured with ELISA kits: IgE ELISA (ab157718, Abcam), IL-4, and IL-13 ELISA (R&D Systems, Minneapolis, MN, USA), following the instruction of manufacturer.

RNA extraction and real-time PCR analysis

Total RNA in skin tissue was isolated using Trizol[®] reagent according to the protocol from the manufacturer on day 45. The equal amount of RNA was reversely transcribed into cDNA using Prime Script[™] RT reagent Kit with gDNA Eraser[®]. Quantitative real-time PCR was performed using Agilent Technologies Stratagene M × 3000 P (Agilent Technologies Inc., Waldbronn, Germany) with SYBR[®] premix Ex Taq[™] and specific primers of TNF- α , IL-4, IL-6, and GAPDH. The amplification program is consisted of 1 cycle of 95°C for 30 s, 40 cycles of 95°C for 5 s, and 60°C for 31 s, and the last cycle of 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. Fluorescence intensity was measured in real time using the optical module. Melt curves were used to determine product specificity. Results were normalized to the level of gapdh gene expression. Primer sequences were listed as below:

TNF- α

5'-TACTGAACTTCGGGGTGATTGGTCC-3'

5'-CAGCCTTGTCCTTGAAGAGAACC-3'

IL-6

5'-CCGGAGAGGAGACTTCACAG-3'

5'-GGAAATTGGGGTAGGAAGGA-3'

IL-4

5'-AGATGGATGTGCCAAACGTCCTCA-3'

5'-AATATGCGAAGCACCTTGGAAGCC-3'

GAPDH

5'-CACCACCCTGTTGCTGTAGCC-3'

5'-ACCACAGTCCATGCCATCAC-3'.

Flow cytometry

THP-1 cells were stimulated with DMSO (vehicle control), DNCB (5 µg/ml) or DNCB plus ISLG (10 µM) for 24 h. Then, equal amount of cells were stained with antibodies specific for human CD86, CD54 for 30 min followed with FITC-conjugated secondary antibody incubation in a staining buffer (PBS containing 1% fetal bovine serum and 0.01% sodium azide) for 30 min on ice and analyzed by fluorescence-activated cell sorting (FACS) on a FACScan analyzer, using the Cell-Quest software (both from BD Biosciences, Franklin Lakes, NJ, USA).

Western blotting analysis

Lysates from the THP-1 cells after treatment were prepared by incubating the cells in lysis buffer (125 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol (v/v) at 4 °C for 30 min. Protein concentration was determined using the BCA protein assay kit (Bio-Rad Laboratories). 40 µg of cell lysates were resolved on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a PVDF membrane (Millipore, Temecula, CA, USA). After being blocked with 10% milk in TBST at room temperature for 1 h, the membrane was then probed with primary antibodies against p38-p, ERK-p, p38, and ERK total protein at 4 °C for 12 h.

Statistical analysis

All data were presented as mean ± SD and were analyzed by paired and unpaired two-tailed Student's *t* tests. Difference between the groups was considered statistically significant when $p < 0.05$.

Results

ISLG ameliorated DNCB-induced scratching incident and dermatitis severity

ISLG is a phenolic chemical compound found in licorice (Fig. 1a) and has shown multiple pharmacological effects in various diseases including cancer and inflammatory disorders. Here, we evaluated its function in murine AD model induced by DNCB, a widely accepted murine AD model. First, the

overall disease progression and manifestation were assessed by evaluation of scratching behavior and severity of skin lesions in DNCB-induced AD-like model. Compared to vehicle control in which scratching activity was barely seen, DNCB model group showed about average 12 times per 10 min of scratching behavior within 1 h time frame. ISLG only group was similar to vehicle control, suggesting that it has no irritating effect. DNCB plus ISLG treatment group showed significantly reduced scratching frequency to about eight times/10 min, as shown in Fig. 2a. Dorsal skin lesion score showed the similar pattern. From Fig. 2b, we could clearly see that vehicle control and ISLG only groups showed very low severity at about 1.5 score, while DNCB model group had score of about 10 and DNCB plus ISLG treatment group had score at about 7, which was significantly decreased compared to DNCB group. These results demonstrated that DNCB-induced AD-like model was successful and ISLG treatment could markedly reduce the overall scratching behavior incidence as well as severity of skin lesions.

Fig. 2

Effects of ISLG on DNCB-induced AD-like symptoms in mice. **a** Scratching behavior was assessed on the last day of the study (day 19). Briefly, the number of scratching behaviors was counted for 10 min. Measurement was repeated for six times (60 min in total). **b** Severity of clinical symptoms (erythema/hemorrhage, edema, excoriation/erosion, and scarring/dryness) of the AD-like skin lesions was evaluated macroscopically as the dermatitis scores. The data are presented as the mean $\pm$ SD ($n = 6$). $^{##}p < 0.01$, significantly different from control group; $^{**}p < 0.01$, significantly different from DNCB-treated group

ISLG suppresses DNCB-induced IgE and Th2 cytokines (IL4

and IL13) elevation in blood

IgE and Th2 cytokines have been indicated as major drivers for the pathological symptoms of AD [8, 15, 22]. We next examined the overall level of IgE and Th2 cytokines like IL4 and IL13 in the different groups of murine AD-like model. The whole blood was collected at the end of experiment (Day 19) and ELISA against IgE, IL4, and IL13 was performed. As shown in Fig. 3a, IgE level in peripheral blood was low in vehicle and ISLG only group, about 2–3 ng/ml. However, DNCB stimulation readily increased IgE to about 12 ng/ml. Strikingly, DNCB plus ISLG treatment group showed about 50% reduction of IgE level compared to DNCB model group. Th2 cytokines IL-4 and IL-13 were induced by DNCB treatment to about two times the level of vehicle group, which were 60 pg/ml and 420 pg/ml, respectively. ISLG only group showed the similar level of IL-4 and IL-13 as vehicle control group, while these two cytokines in DNCB plus ISLG treatment group were reduced by 1/3 compared to DNCB model group, as shown in Fig. 3b. These ELISA results indicated that ISLG treatment could suppress DNCB mediated induction of IgE and Th2 cytokines.

Fig. 3

Effects of ISLG on DNCB-induced increase in serum IgE and Th2 cytokines in mice. **a**

Sample blood was collected on the last day of the study (day 19). The level of serum IgE was measured with ELISA. **b** Th2 cytokines (IL-4 and IL-13) in serum was measured with

ELISA. The data are presented as the

mean $\pm$ SD ($n = 6$). $^{##}p < 0.01$, significantly different from control group; $^{*}p < 0.05$,

$^{**}p < 0.01$, significantly different from DNCB-treated group

ISLG inhibits DNCB-induced pro-inflammatory cytokines (TNF- α and IL-6) and IL-4 expression in skin lesion

AD is a multifactorial disorder associated with Th2-polarized CD4 T cells that

overproduce IL-6, IL-5, IL-4, and IL-13. IL-6 is mainly secreted by activated T cells and macrophages [15, 21, 32]. It regulates the immune response, inflammation and pathogen responses, bone metabolism, as well as hematopoiesis. TNF- α is also an important pro-inflammatory cytokine involved in the immunopathology of AD [12]. To investigate the effect of ISLG on the skin lesion induced by DNCB, we next measured the local mRNA level in the lesion tissue by real-time PCR. As shown in Fig. 4a, DNCB stimulation group showed enhanced level of TNF- α and IL-6 to about 2.5–3 times of the vehicle control group which was normalized as one. ISLG only group showed similar level of TNF- α and IL-6 as vehicle control. DNCB plus ISLG treatment group showed significantly reduced level of TNF- α and IL-6 at about 66–70% of the amount seen in DNCB model group. We also measured IL-4 level in the same setting. IL-4 is a key cytokine to promote Th2 lineage differentiation and induce B cell class switching to IgE. As shown in Fig. 4b, DNCB plus ISLG treatment showed significant decrease (50%) in IL-4 level compared to DNCB model group. These results were consistent with the previous ELISA data about overall serum IL-4 level and suggested that ISLG could suppress the immune response elicited by DNCB at local skin tissue.

Fig. 4

Effects of ISLG on the expression cytokines in the skin of DNCB-treated mice. **a** Dorsal skin was collected on the last day of the study (day 19). Total RNA was extracted from skin tissue with TRIzol reagent. The pro-inflammatory cytokines TNF- α and IL-6 were determined by real-time PCR. **b** IL-4 expression level in was also measured by real-time PCR. The data are presented as the mean $\pm$ SD ($n = 6$).

$^{##}p < 0.01$, significantly different from control group; $^{*}p < 0.05$, $^{**}p < 0.01$, significantly different from DNCB-treated group

ISLG can antagonize DNCB-induced co-stimulatory signal molecules CD86 and CD54 and suppress p38 and ERK

phosphorylation in THP-1 cells

To understand the molecular mechanism of ISLG in treating AD and to extend the murine model results to human, we next used THP-1 as a human antigen-presenting cell (APC) model to investigate the effects of ISLG in DNCB-induced APC activation, which is a critical step for the priming and activation of downstream immune cells such as Th2 and mast cells [1] and in MAPK signaling, an essential pathway for pro-inflammatory response. In addition, THP-1 is a human leukemia monocytic cell line, which has been extensively used to study the role and function of monocyte/macrophage in regulating the immune response [4]. Macrophages have been shown to play an important role in the AD immunopathogenesis [15, 21]. CD86 is an important co-stimulatory molecule on antigen-presenting cells and helps prime T cell differentiation and activation. CD54 is a cell adhesion molecule expressed on macrophages, lymphocytes, and endothelial cells. It promotes stable interaction and facilitates the leukocyte endothelial transmigration. CD86 and CD54 expressions on THP-1 cells were used as in vitro skin sensitization assay to monitor the activity of sensitizer such as DNCB [3, 28]. As shown in Fig. 5a, CD86 and CD54 levels on THP-1 cell surface were induced by DNCB stimulation to about threefold of that in DMSO-treated control. DNCB plus ISLG treatment could significantly reduce their levels by 30% compared to DNCB stimulation group. Similarly, TNF- α level was also induced by DNCB treatment to about 20-fold of that in control group and reduced to about 12-fold of control level by ISLG treatment.

Fig. 5

Effects of ISLG on the biological response of THP-1 to DNCB. **a** THP-1 cells were treated with the indicated chemicals for 24 h before harvesting. Cell surface markers CD86 and CD54 were measured by flow cytometry. The MFI was used as indicator of surface marker expression. **b** Cell supernatants were also collected for the measurement of TNF- α production. **c** Cell lysate of THP-1 after treatments was subjected to SDS-PAGE to determine p38 and ERK1/2 phosphorylation. Actin was used as loading control. Data are

representatives of at least three independent experiments. $^{##}p < 0.01$, significantly different from DMSO treatment; $^{*}p < 0.05$, $^{**}p < 0.01$, significantly different from DNCB treatment

We next investigated the impact of ISLG treatment on MAPK pathway activation by western blotting [17]. As shown in Fig. 5c, DNCB-treated samples showed strong activation of p38 α and ERK kinases compared to DMSO-treated controls, while ISLG treatment completely abolished the phosphorylation of p38 α and ERK. This result revealed that ISLG could suppress DNCB-induced key cytokines and immune cell surface factors as well as pro-inflammatory MAPK-signaling pathways.

Discussion

AD is a chronic or relapsing inflammatory skin disease that often precedes asthma and allergic disorders. It imposes considerable burdens on patients' quality of life, health outcome, and work productivity. Noteworthy, standard steroid treatment has intrinsic side effect and AD is often associated with microbial organism infections [20, 21, 34]. AD pathology is characterized with skin barrier abnormalities and immune dysregulation at local and systemic levels with high heterogeneity [5, 6]. ISLG is a small phenolic bioactive molecule from licorice. It has shown multiple pharmacological activities against cancer, inflammatory disorder and cardiovascular diseases [26]. In this study, we tested the effect of ISLG in DNCB-induced AD-like murine model. Our results demonstrated that ISLG could ameliorate the overall manifestation of AD-like symptoms such as scratching behavior incidence and skin lesion severity. At systemic level, ISLG significantly suppressed the DNCB-induced IgE and Th2 cytokines in peripheral blood. At local skin lesion site, ISLG also inhibited DNCB-induced pro-inflammatory cytokine like TNF- α and IL-6 as well as IL-4 mRNA production. Using a human monocyte model THP-1, we revealed that ISLG could suppress the up-regulation of CD86 and CD54 at cell surface and abolish the DNCB-induced p38- α and ERK activation, suggesting the underlying molecular mechanism of treating AD-like lesion by ISLG.

As mentioned previously, AD is a highly heterogeneous and complex disease featured with abnormal immunological and inflammatory pathways that include defective skin barrier, exposure to environmental agents, and neuropsychological factors [34]. Approximately 70–80% of AD patients present with the “extrinsic” form of AD. They have elevated levels of serum IgE and IgE antibodies to environmental and/or food allergens. The

remaining 20–30% present with the “intrinsic” form of AD and have low serum IgE levels with no evidence of IgE antibodies [21]. However, a number of these individuals develop allergic IgE-mediated sensitization later in life. Acute AD skin lesions exhibit Th2-dominant inflammation characterized by dermal infiltration of CD4⁺ T cells and eosinophils with the deposition of eosinophil products and increased skin expression of Th2 cytokines. Subsequently, the chronic phase demonstrates a local Th1 IFN- γ response and tissue remodeling with increased deposition of collagen and dermal thickening [25, 27]. In DNCB-induced murine AD-like model, we clearly observed the up-regulation of serum IgE and Th2 cytokines such as IL-4 and IL-13 (Fig. 3), suggesting that the model mimics the AD manifestation. The down-regulation of IgE and IL-4/IL-13 serum levels by ISLG treatment was well correlated to the ameliorated severity of AD-like lesions and animal scratching behavior (Fig. 2). IL-4 is a pleiotropic cytokine product of Th2 cells regulating immunoglobulin isotype switching (IgE, IgG4). Arguably, the most important effect of IL-4 is its role in T cell growth, especially the development of Th2 lineage via the positive feedback loop. It also has B cell stimulating activities and can suppress Th1-mediated immune responses [24]. Our result is supported by the reports that IL-4-deficient mouse model showed inhibitory effect to DNCB-induced dermatitis [33], while IL-4 overexpressing transgenic mice developed spontaneous pruritus and chronic dermatitis at the age of 4 months [11]. ISLG has been shown to inhibit IL-4 synthesis and memory Th2 response in asthma model in vitro and in vivo [37].

Epidermal cytokines such as IL-1 β , TNF- α , and IL-6 have been described to play a crucial role in the induction and elicitation phases of allergic contact dermatitis upon exposure to sensitizer [15, 16]. Following antigen uptake and in the presence of a danger signal generated by microbial antigens or from mechanical injury, immature dendritic cells (DC) acquire the phenotype of functional antigen-presenting cells (APC), including the up-regulation of major histocompatibility complex class II molecules and of key co-stimulatory molecules such as CD80 and CD86 [13]. DCs play a critical role in the polarization of T cells into Th1, Th2, or Th17 cells depending on their specific expression of cytokines and co-stimulatory molecules and the influence of the individual tissue microenvironment from which they originate [7, 8, 21]. In our study, we chose THP-1, a widely used monocyte and macrophage model, to investigate the molecular mechanism of ISLG effect on DNCB caused allergic immune response. MAPK including p38 α and ERK kinases are important downstream signaling factors of TNF- α and IL-1 stimulation [17]. However, the inhibitory effect of ISLG on MAPK was much more dramatic than on TNF- α production, suggesting that ISLG-mediated down-regulation of TNF- α and other cytokines might not be the sole reason for the suppression of DNCB-induced MAPK activation in THP-1 cell model. This result should be validated in a more physiological relevant system in the future, given the fact that AD pathology involves multiple cell types and pathways.

In conclusion, we have established the potent anti-inflammatory role of ISLG in AD-like symptoms. As a bioactive natural product, it could be a promising therapeutic agent for chronic AD and goes beyond the modulation with

topical administration of mainstream steroid therapy.

Notes

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Research involving human participants and/or animals

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent

Not applicable.

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