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Monotropein mitigates atopic dermatitis-like skin inflammation through JAK/STAT signaling pathway inhibition

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ABSTRACT

Atopic dermatitis (AD) is a globally increasing chronic inflammatory skin disease with limited and potentially side-effect-prone treatment options. Monotropein is the predominant iridoid glycoside in *Morinda officinalis* How roots, which has previously shown promise in alleviating AD symptoms. This study aimed to systematically investigate the pharmacological effects of monotropein on AD using a 2, 4-dinitrochlorobenzene (DNCB)/*Dermatophagoides farinae* extract (DFE)-induced AD mice and tumor necrosis factor (TNF)-α/interferon (IFN)-γ-stimulated keratinocytes. Oral administration of monotropein demonstrated a significant reduction in AD phenotypes, including scaling, erythema, and increased skin thickness in AD-induced mice. Histological analysis revealed a marked decrease in immune cell infiltration in skin lesions. Additionally, monotropein effectively downregulated inflammatory markers, encompassing pro-inflammatory cytokines, T helper (Th)1 and Th2 cytokines, and pro-inflammatory chemokines in skin tissues. Notably, monotropein also led to a considerable decrease in serum immunoglobulin (Ig)E and IgG2a levels. At a mechanistic level, monotropein exerted its anti-inflammatory effects by suppressing the phosphorylation of Janus kinase / signal transducer and activator of transcription proteins in both skin tissues of AD-induced mice and TNF-α/IFN-γ-stimulated keratinocytes. In conclusion, monotropein exhibited a pronounced alleviation of AD symptoms in the experimental models used. These findings underscore the potential application of monotropein as a therapeutic agent in the context of AD, providing a scientific basis for further exploration and development.

1. Introduction

Atopic Dermatitis (AD) is a prevalent chronic inflammatory skin disorder, affecting approximately 20 % of children and 10 % of adults [1]. The profound impact of AD on the quality of life is evident through characteristic skin manifestations, including erythema, dryness, scaling, and pruritus [2,3]. Moreover, AD may lead to the subsequent development of other allergic conditions such as asthma, food allergy, and allergic rhinitis [4,5].

The multifaceted etiology of AD involves genetic, immunological, and environmental factors, alongside disruptions in the epidermal barrier function [6]. Immunologically, the involvement of T helper (Th) 2 cells, generating interleukins (IL)-4, IL-13, and IL-31 in acute lesions, contributes to barrier dysfunction and pruritus in AD [7,8]. As AD progresses, a broader immune response unfolds, encompassing not only Th2 cells but also Th1 cells, accompanied by elevated expression of tumor necrosis factor (TNF)-α and interferon (IFN)-γ in the lesions [9–11].

The crucial role of the epidermal barrier in preventing environmental insults is underscored by its impairment in AD, allowing allergens like house dust mites to penetrate the skin layers [12]. Keratinocytes, activated by various stimuli, further exacerbate AD lesions by producing pro-inflammatory cytokines (e.g., IL-1β and IL-6) and Th2-attracting chemokines (e.g., CC chemokine ligand (CCL) 17 and

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CCL22), leading to the recruitment of Th2 cells [13]. Thus, a comprehensive approach addressing both keratinocyte and immunological components is imperative for effective AD treatment.

While corticosteroids remain the primary treatment for AD, recent advancements include antagonists targeting specific cytokine receptors or inflammatory mediators like Janus kinase (JAK) inhibitors and dupilumab, marking a paradigm shift in AD management [14,15]. However, concerns over the prolonged use of these drugs due to potential side effects, such as weight gain, skin atrophy, ocular complications, and infections, underscore the urgent need for alternative agents balancing therapeutic efficacy with safety [16–18].

Monotropein, isolated from *Morinda officinalis* How, emerges as a significant candidate, being a major iridoid glycoside present in roots, stems, and leaves [19]. Iridoid glycosides, crucial in plant defense against infections and rapid recovery from damage, constitute over 2 % of *M. officinalis* roots, with monotropein being the predominant glycoside at 35.9 % [20,21]. Previous research has demonstrated the AD-alleviating effect of *M. officinalis* root extract, suggesting the importance of investigating the therapeutic impact of monotropein on AD [22]. With monotropein being a prominent component of iridoid glycosides in these roots, this study aims to comprehensively explore its therapeutic potential in the context of AD, anticipating significant contributions to the observed alleviation of AD symptoms. The outcomes of this research could shed light on monotropein's efficacy as a potential alternative or complementary therapeutic agent for AD management.

2. Materials and methods

2.1. Reagents and cell culture

Monotropein (GlpBio, Los Angeles, CA, USA) for cell experiments was dissolved in dimethyl sulfoxide (DMSO), and for animal experiments, it was prepared in phosphate-buffered saline (PBS). *Dermatophagoides farinae* extract (DFE; 1 mg/mL; Prolagen, Seoul, Republic of Korea) powder was diluted in PBS plus 0.5 % Tween 20. 2,4-dinitrochlorobenzene (DNCB) was diluted in an acetone/olive oil solution (4:1) and applied at concentrations of 2 % or 1 %. Recombinant human TNF-α and IFN-γ were acquired from R&D Systems (Minneapolis, MN, USA) and diluted in PBS at pH 7.4 plus 0.1 % bovine serum albumin (BSA).

The human keratinocyte cell line HaCaT was cultured in Dulbecco's modified Eagle medium (Gibco, Grand Island, NY, USA) including 10 % heat-inactivated fetal bovine serum (Gibco) and antibiotic/antimycotic Solution (100 U/mL of penicillin G, and 100 μg/mL of streptomycin) in 5 % CO_2 at 37 ℃.

2.2. Induction of AD in mouse model

The experimental scheme used to induce AD is presented in Fig. S1. Five-week-old BALB/c female mice were acclimatized for one week, and divided into the following seven groups ($n = 5$ per group): (1) vehicle (PBS) treated group, (2) monotropein 10 mg/kg treated group, (3) DNCB/DFE + vehicle treated group, (4) DNCB/DFE + monotropein 0.1 mg/kg treated group, (5) DNCB/DFE + monotropein 1 mg/kg treated group, (6) DNCB/DFE + monotropein 10 mg/kg treated group, (7) DNCB/DFE + dexamethasone (Dexa) 1 mg/kg treated group. After sensitizing each mouse ear twice a week with 20 μL of 2 % DNCB, for three weeks, we applied 20 μL of 1 % DNCB to each ear once a week and 20 μL of DFE to each ear twice a week. Vehicle, monotropein, or Dexa were administered orally 5 times a week for 2 weeks starting in week 3. Euthanasia with CO_2 was performed on day 28, and whole blood samples were taken from the abdominal aorta of the mice. After a 1 h coagulation period, the blood samples were centrifuged at 1000 × *g* for 15 min, and the serum was obtained. The mouse ears were excised for RT-qPCR, ELISA, and histopathological analysis.

2.3. Histological analysis

The mouse ear tissues were gathered after sacrifice and fixed for two days at room temperature using 10 % formaldehyde. After paraffin embedding, the tissues were sliced into sections measuring 5 μm. The sections were stained with hematoxylin and eosin (H&E) to measure eosinophil infiltration and epidermal and dermal thickness in tissues. In addition, sections were stained with toluidine blue (TB) to analyze mast cell infiltration. The thickness of the epidermis and dermis was measured at ×200 magnification by randomly selecting five areas from H&E-stained sections. To count the number of eosinophils and mast cells infiltrating the ear tissue, five areas were randomly selected from sections stained with H&E and TB and measured at ×400 magnification.

2.4. Immunohistochemistry (IHC)

The ear tissue sections underwent deparaffinization utilizing xylene. Subsequently, rehydration was achieved by exposing the sections to a range of graded ethanol solutions, followed by a final wash with deionized water. Antigen retrieval was accomplished by microwave treatment for 10 min using 0.01 M sodium citrate buffer. In order to quench endogenous peroxidase activity, the sections were subjected to treatment with 3 % hydrogen peroxide for 10 min. Sections were treated with a blocking buffer for 30 min at room temperature, followed by an overnight incubation at 4 °C with a primary antibody specific to CD4 (Invitrogen, Waltham, MA, USA). Afterward, the sections were treated with a biotinylated secondary antibody (Vector Laboratories, Newark, CA, USA) for 30 min, and this was succeeded by 30 min application of ABC-AP reagent (Vector Laboratories). The sections underwent a 5-min incubation with the AP substrate, followed by 1 min counterstaining with hematoxylin at room temperature. Observations of IHC-stained sections were conducted at a magnification of ×400.

2.5. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA from ear tissues or HaCaT cells was isolated with an RNAiso Plus kit (Takara Bio, Shiga, Japan). HaCaT cells were treated with monotropein (0.1, 1, or 10 μM) or Dexa (1 μM) for 1 h and then stimulated with TNF-α/IFN-γ (10 ng/mL) for 6 h. Homogenization of ear tissues was conducted three times, each at intervals of 30 s, employing the Tissue Lyser II (Qiagen, Hilden, Germany). Total RNA quantification was performed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and cDNA was synthesized with the RevertAid RT Kit (Thermo Fisher Scientific). After that, RT-qPCR was executed with a StepOnePlus Real-time PCR system (Thermo Fisher Scientific), adhering to the manufacturer's instructions. Each reaction tube contained a total of 20 μL of mixed sample consisting of 1 μL of cDNA (50 ng), 1 μL of sense and antisense primer (0.5 μM), 10 μL of QGreenBlue Master mix (Cellsafe, Yong-in, Republic of Korea) and 7 μL of nuclease free water. The sequences of the primer pairs used for qPCR are listed in Table S1. The gene expression was normalized to GAPDH.

2.6. Enzyme-linked immunosorbent assay (ELISA)

The secreted levels of IL-1β, IL-6, CCL17, and CCL22 in the conditioned media from stimulated HaCaT cells were determined by ELISA kits (R&D Systems) as per the manufacturer's instructions. After 1 h treatment with monotropein (0.1, 1, or 10 μM) or Dexa (1 μM), they were stimulated with TNF-α/IFN-γ (10 ng/mL) for 15 or 24 h. The supernatants were centrifuged at 1000 × *g* at 4 °C for 5 min. Levels of IgE and IgG2a in serum were determined by ELISA kits (BD Biosciences, Franklin Lakes, NJ, USA) as per the manufacturer's instructions. Ear tissues were homogenized with an extraction buffer added with protease/phosphatase inhibitor cocktail (Roche, Basel, Switzerland). The

homogenates were centrifuged at 20,000 × *g* for 15 min to remove debris and the supernatants were collected. IL-4, IL-6 (BD Biosciences), and IFN-γ (Invitrogen) protein levels in the homogenate supernatant were determined using dedicated ELISA kits. The absorbance was measured at 450 nm using the spectrophotometer.

2.7. Western blot

HaCaT cells were treated with monotropein (0.1, 1, or 10 μM) or Dexa (1 μM) for 1 h and then stimulated with TNF-α/ IFN-γ (10 ng/mL) for 15 min. Total protein extracts were prepared by scraping cells using lysis buffer added with protease/phosphatase inhibitor cocktail (Roche). The lysate was centrifuged at 18,000 × *g* at 4 °C for 20 min. Homogenization of ear tissues was carried out in RIPA buffer with the addition of a protease/phosphatase inhibitor cocktail (Roche). Protein quantification was conducted with a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific). Protein samples (15–30 μg) were separated by molecular weight through electrophoresis on 8–10 % sodium dodecyl sulfate-polyacrylamide gel and subsequently transferred to BioTrace™ NT nitrocellulose membranes (Pall Corporation, Ann Arbor, MI, USA). After blocking the membrane with 4 % BSA in Tris-buffered saline with Tween 20, it was incubated with the primary antibody overnight. The membrane was reacted with SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific) and analyzed using G: BOX Chemi XRQ (Syngene, Cambridge, UK).

2.8. Statistical analysis

Statistical analysis was executed with GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA). Statistical significance was assessed using Dunnett's multiple comparison test and one-way ANOVA. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of monotropein on the phenotypes in AD-induced mice

To clarify the effects of monotropein on AD phenotypes, we constructed an AD-induced mouse model (Fig. S1). After inducing the AD phenotype with DNCB and DFE for 2 weeks, vehicle, monotropein (0.1, 1, or 10 mg/kg), or Dexa (1 mg/kg) was orally administered for an additional 2 weeks. Throughout the experiment, there were no noticeable changes in body weight and liver weight in the monotropein-treated group, indicating the non-toxicity of monotropein up to 10 mg/kg (Fig. S2A, B). Edema, scaling, and erythema are well-known phenotypes of AD [23]. As DNCB and DFE were applied repeatedly, these AD phenotypes became more severe. However, monotropein alleviated these phenotypes in a dose-dependent manner (Fig. 1A). Ear thickness increased significantly in the AD group, but was decreased by oral administration of monotropein (Fig. 1B). These results show that monotropein effectively mitigates the AD phenotype without inducing any toxicity.

3.2. Effects of monotropein on the histopathological alterations in AD-induced mice

Histopathological observations show that AD lesions exhibit increased epidermal and dermal thickness and infiltration of immune cells [24,25]. To confirm the effect of monotropein on histological changes, ear tissues were stained with H&E and TB. Layer thickness and eosinophil number were measured by H&E staining, and mast cell was measured by TB staining. The thickness of the epidermis and dermis decreased in the monotropein-treated group compared to the AD group (Fig. 2A, B). Simultaneously, eosinophil infiltration was also suppressed (Fig. 2A, C and Fig. S3). TB-stained tissue sections demonstrated that monotropein reduced the elevated mast cell count caused by DNCB/DFE stimulation (Fig. 2A, C). In addition, IHC staining results showed that the increased infiltration of $CD4^+$ immune cells in the AD group was significantly reduced by oral administration of monotropein (Fig. 2A, C). These observations suggest that monotropein can alleviate the pathological symptoms of AD by effectively blocking the infiltration of the two major cells involved in allergic immune responses into the lesion area.

3.3. Effects of monotropein on inflammatory responses in tissues and serum immunoglobulin levels

In AD, the release of cytokines and chemokines by activated immune cells exacerbates the skin lesion [25]. We analyzed gene expression levels of pro-inflammatory cytokines (IL-1β and IL-6), pro-inflammatory chemokine (CCL17), Th2 cytokines (IL-4, IL-13 and IL-31) and Th1 cytokines (TNF-α and IFN-γ) in skin lesions using RT-qPCR. Topical application of DNCB/DFE increased the expression of all genes. However, oral administration of monotropein reduced all increased gene expression in a dose-dependent manner (Fig. 3A). Subsequently, the

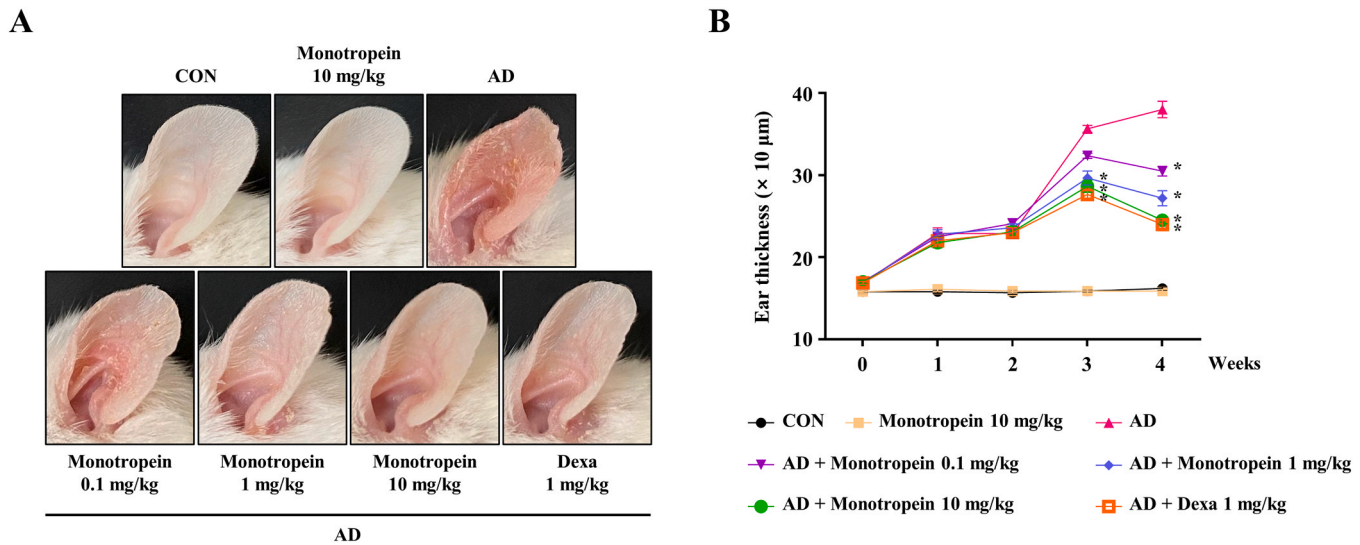

Fig. 1. Effects of monotropein on symptoms in AD-induced mice. (A) Photographs of AD lesions in each experimental group. (B) Ear thickness was measured with a dial gauge 24 h after DNCB/DFE application. Data are depicted as mean values ± SEM ($n = 5$). *$p < 0.05$ compared with AD group. Dexa: dexamethasone.

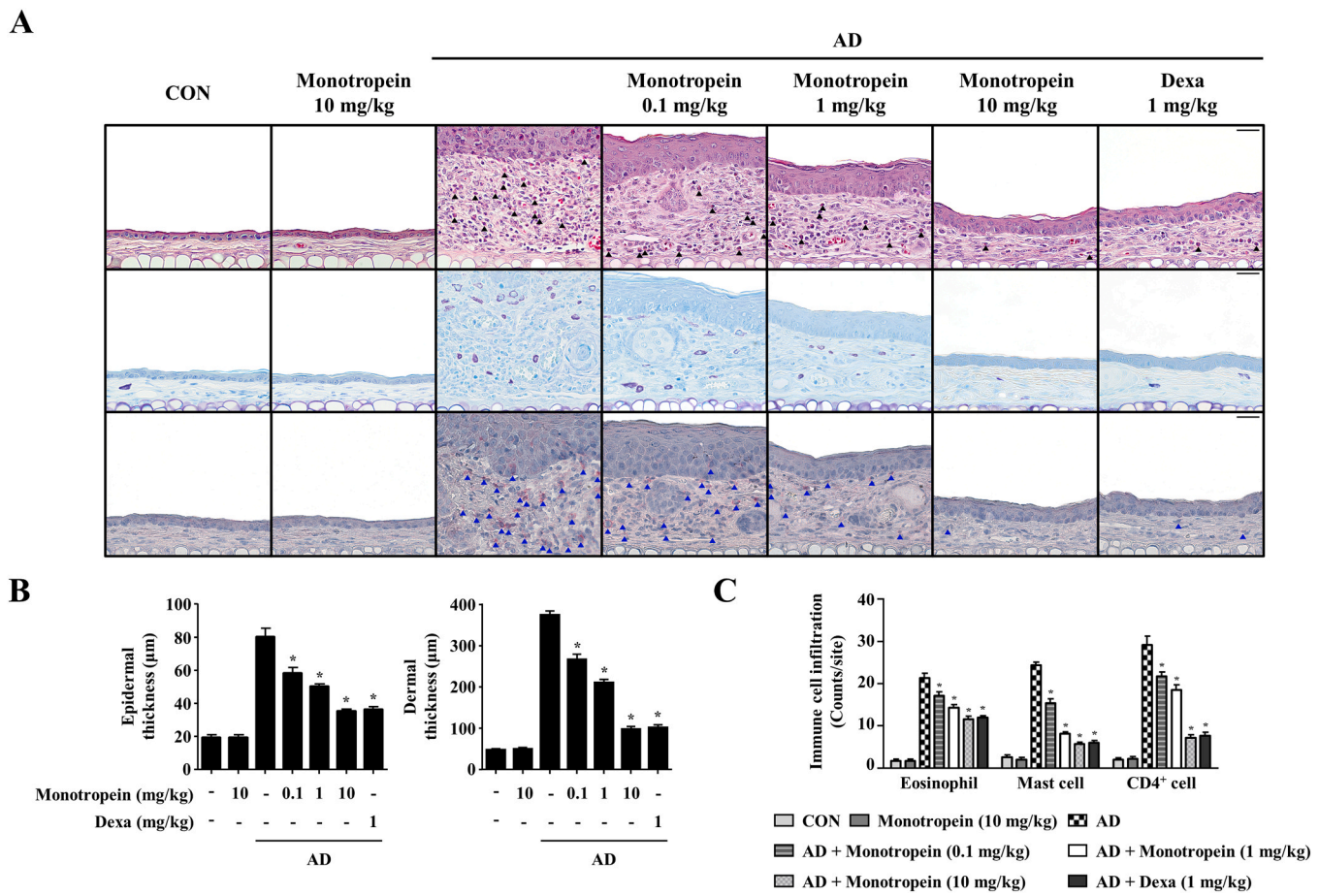

Fig. 2. Histological observation of AD-induced mice. (A) Photomicrographs of ear tissues stained with H&E (1st panel, eosinophils indicated by black arrows), TB (2nd panel), and CD4 antibody (3rd panel, $CD4^+$ cells indicated by blue arrows) at ×400 magnification. Scale bar: 50 μm. (B) Epidermal and dermal thickness was assessed in H&E-stained skin sections at an identical magnification. (C) The counting of eosinophils, mast cells, and $CD4^+$ immune cells that infiltrated the dermal layer was performed on slides stained with H&E, TB, and CD4 antibody, respectively. Data are depicted as mean values ± SEM ($n = 5$). *$p < 0.05$ compared with AD group. Dexa: dexamethasone.

protein level of IL-6, IL-4, and IFN-γ was confirmed using ELISA. Ultimately, it exhibited a similar trend to gene expression (Fig. 3B). To investigate whether monotropein affects Th1 and Th2 responses, we measured serum levels of IgG2a and IgE using an ELISA. DNCB/DFE treatment raised the serum levels of these immunoglobulins. However, monotropein dose-dependently reduced IgE and IgG2a levels compared to the AD group (Fig. 3C). These findings indicate that monotropein effectively suppressed AD-like skin inflammation.

3.4. *Effects of monotropein on phosphorylation of JAK/STAT signaling molecules in the skin tissue of AD-induced mice*

The JAK/STAT pathway is a significant signaling pathway regulated by cytokines and has recently emerged as a focus for AD therapeutic interventions [26–28]. IFN-γ, the hallmark Th1 cytokine, utilizes STAT1 for signal transduction [29]. Additionally, STAT6 not only regulates Th2 differentiation but also promotes the switch of immunoglobulin classes to IgE [30]. To unravel the inhibitory mechanism of monotropein, we evaluated its impact on JAK1, STAT1 and STAT6 activation in ear tissue. Oral administration of monotropein inhibited the phosphorylation of those molecules in the lesional tissues of AD-induced mice (Fig. 4). These findings imply that monotropein exerts anti-inflammatory effects by suppressing of the JAK/STAT signaling pathway.

3.5. *Effects of monotropein on keratinocytes activated by TNF-α/IFN-γ*

Activated keratinocytes release pro-inflammatory cytokines and chemokines, exacerbating AD in skin lesions [31]. The effect of monotropein on keratinocytes was confirmed using TNF-α/IFN-γ, which stimulates the activation of keratinocytes by promoting an inflammatory response [13]. Initially, the cell viability of monotorpein was evaluated using the MTT assay. Monotropein showed no toxicity in HaCaT cells up to 100 μM (Fig. S4). We investigate the effect of monotropein on gene expression and secretion of pro-inflammatory cytokines (IL-1β and IL-6) and chemokines (CCL17 and CCL22) in TNF-α/IFN-γ-stimulated keratinocytes. The gene expression levels of IL-1β, IL-6, CCL17, and CCL22 were heightened by TNF-α/IFN-γ but decreased by monotropein in a concentration-dependent manner (Fig. 5A). The secretory protein levels also showed similar patterns to the gene expression levels (Fig. 5B).

The activation of various inflammatory cytokines and chemokines-mediated cells is regulated through the JAK/STAT signaling pathway [26]. In particular, CCL17 and CCL22 are regulated in a STAT6-dependent manner [32]. Therefore, we investigated the inhibitory effect of monotropein on the activation of the signaling molecules using Western blot analysis. TNF-α/IFN-γ treatment increased phosphorylation of JAK1, STAT1, and STAT6 in HaCaT cells. However, monotropein inhibited the activation of these signaling pathways (Fig. 5C). These results indicate that monotropein reduces the production of pro-inflammatory cytokines and chemokines by inhibiting the JAK/STAT signaling pathway.

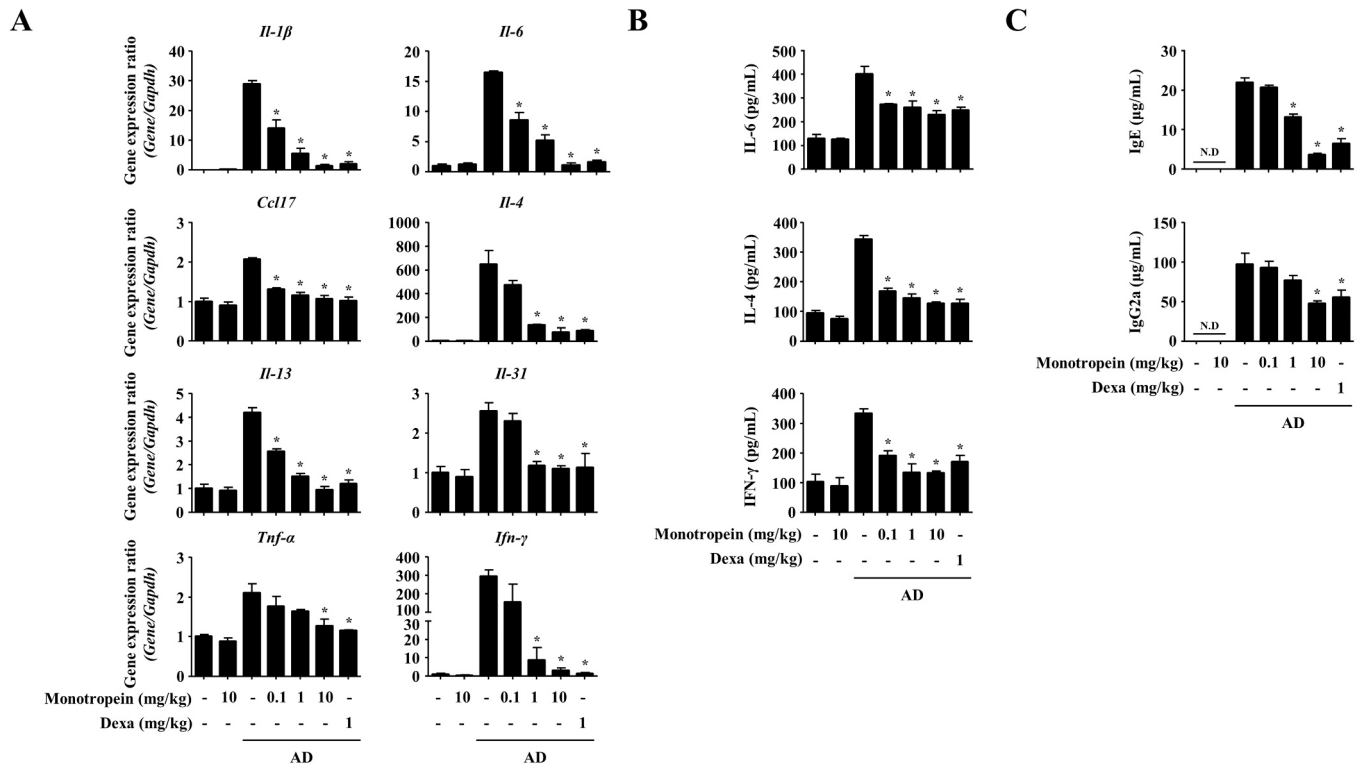

Fig. 3. Effects of monotropein on inflammatory responses in tissues and serum immunoglobulin levels. (A) After sacrifice, both ears of mice were collected and gene expression was measured by RT-qPCR. (B) Homogenization of mouse ears with a tissue extraction buffer was followed by ELISA analysis to determine cytokine levels in the ear tissue homogenate supernatants. (C) After the mice were sacrificed, blood was collected from the abdominal aorta. The levels of serum IgE and IgG2a were detected by ELISA. Data are depicted as mean values ± SEM ($n = 5$). *$p < 0.05$ compared with AD group. Dexa: dexamethasone; ND: not detected.

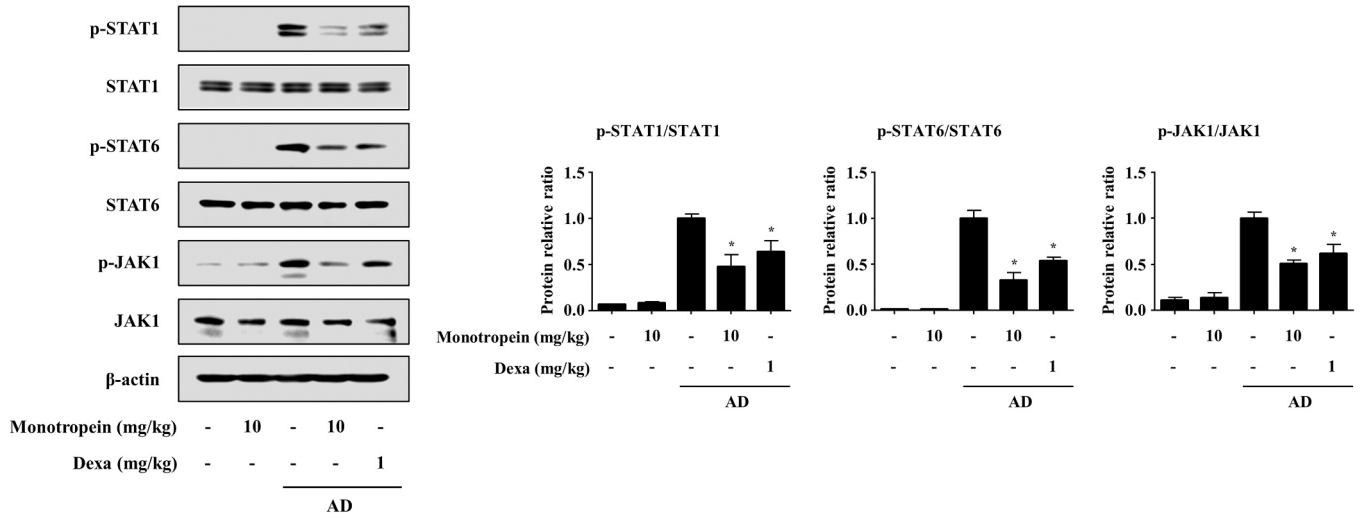

Fig. 4. Monotropein inhibits the phosphorylation of JAK/STAT signaling molecules in the skin tissue of AD-induced mice. Phosphorylation of JAK1, STAT1 and STAT6 was analyzed by Western blot. Total forms were used as loading controls. The intensity of bands was quantified using Image J software. Data are depicted as mean values ± SEM ($n = 3$). *$p < 0.05$ compared with AD group. Dexa: dexamethasone.

4. Discussion

AD, a chronic inflammatory skin disorder, has witnessed a steady increase in prevalence over recent decades, necessitating the exploration of alternative therapeutic approaches that balance efficacy, safety, and cost-effectiveness [1]. In this pursuit, phytochemicals derived from medicinal plants present a compelling avenue. *M. officinalis* is a member of the Morinda species, which is commonly found in India. Various Morinda-based ingredients are presently commercialized in products such as capsules, cosmetics, and juice, targeting health promotion and treatment. *M. officinalis* contains various phytochemicals, such as iridoid glycosides, flavonoid glycosides, and anthraquinones, and has been studied to have various biological activities, such as anti-oxidant and anti-inflammatory properties [33]. Moreover, the safety of *M. officinalis* root extract has been proven through clinical studies [33]. *M. officinalis* roots, is known for its diverse pharmacological properties, specifically its potential to alleviate AD symptoms [19,21,22,33]. Hence, this study reports the pivotal role of monotropein, a key component of *M. officinalis*

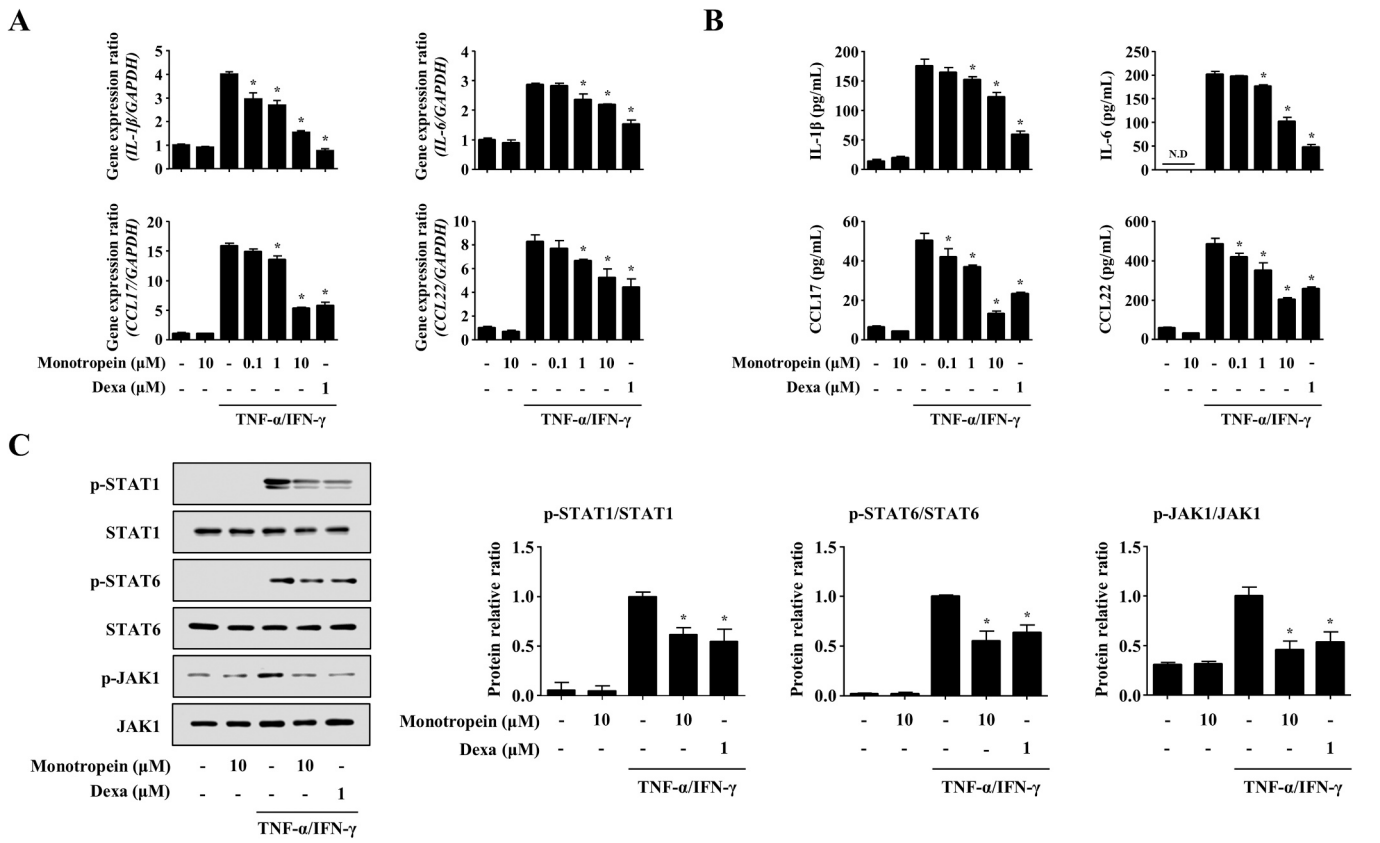

Fig. 5. Effect of monotropein on keratinocytes activated by TNF-α/IFN-γ. (A) Gene expression levels of cytokines and chemokines were quantified using RT-qPCR. After 1 h pretreatment with monotropein (0.1, 1, 10 μM) or Dexa (1 μM), cells were stimulated with TNF-α/IFN-γ (10 ng/mL) for 6 h. (B) Protein secretion was assessed using ELISA. After 1 h pretreatment with monotropein (0.1, 1, 10 μM) or Dexa (1 μM), cells were stimulated with TNF-α/IFN-γ (10 ng/mL) for 15 or 24 h. (C) Phosphorylation of JAK1, STAT1 and STAT6 was analyzed by Western blot. After 1 h pretreatment with monotropein (10 μM) or Dexa (1 μM), cells were stimulated with TNF-α/IFN-γ (10 ng/mL) for 15 min. Data are depicted as mean values ± SEM ($n = 3$). *$p < 0.05$ compared with AD group. Dexa: dexamethasone.

roots, in alleviating AD symptoms and its therapeutic potential.

A variety of house dust mite species, including *D. farinae*, can cause pathology through direct skin contact [34]. Mite extracts affect keratinocytes, inducing the release of pro-inflammatory and Th2 cytokines [35]. Indeed, IgE to mite allergens warrants attention as a trigger, given its detection in up to 95 % of AD patients [36]. Therefore, our experimental framework employed *D. farinae*, a major house dust mite species, as a pertinent environmental trigger for AD. The established AD model, utilizing DNCB for sensitization and DFE as an allergen, effectively replicated AD-like phenotypes, including scaling, erythema, and increased skin thickness. In this context, monotropein demonstrated pronounced efficacy in mitigating these phenotypes, highlighting its potential as a therapeutic agent for AD management.

The AD pathology is closely associated with the numbers of activated eosinophils and mast cells infiltrated in skin lesions [24]. Eosinophils, by aggregating in inflamed skin areas, exacerbate lesions through the secretion of cytokines and chemokines [37]. Elevated eosinophil levels in peripheral blood and skin lesions are a characteristic risk factor for AD and serve as clinical biomarkers for AD evaluation [38–40]. Mast cells amplify the inflammatory response by forming cross-links with IgE through high-affinity IgE receptors (FcεRI) on the cell surface and producing various cytokines and chemokines [24]. Thus, it is imperative to manage histopathological changes, including the infiltration of these immune cells, for effective AD treatment. In the present study, monotropein effectively inhibited the infiltration of eosinophils and mast cells, thereby reducing pathological changes, highlighting the potential of monotropein to modulate important cellular components involved in AD pathogenesis.

The activation of naïve $CD4^+$ T cells leads to their differentiation into specific T helper subsets, producing cytokines that play crucial roles in shaping immune responses [41,42]. In the acute phase of AD, naïve $CD4^+$ T cells are activated and their differentiation into Th2 cells is promoted by increasing CCL17, a chemotactic chemokine for Th2 cells [43]. Differentiated Th2 cells contribute to symptom aggravation by producing cytokines, including IL-4, IL-13, and IL-31 [7,8]. In particular, IL-4 and IL-13 are crucial in regulating the migration of immune cells and facilitating IgE class switching in B cells [44]. As the chronic phase progresses, differentiation into Th1 cells also becomes predominant, and production of TNF-α and IFN-γ increases [9–11]. The intricate interplay of $CD4^+$ T cells and cytokine production is crucial in AD progression, and the dense infiltration of activated $CD4^+$ T cells in the lesions underscores their involvement in the pathology [45,46]. Our study demonstrated the comprehensive anti-inflammatory effects of monotropein by confirming curtailed $CD4^+$ T cell infiltration and downregulated Th2 and Th1 cytokines expression.

A hallmark of AD progression is the shift from elevated serum IgE levels, driven by Th2 cytokines, in the acute phase to increased IgG2a production, associated with the Th1 response, in the chronic phase [47,48]. In the acute phase, a compromised skin barrier enhances vulnerability to external antigens and allergens, driving a promoted Th2 immune response that leads to increased IgE production [49]. FcεRI-mediated binding of IgE to diverse immune cells contributes to AD by serving dual roles, acting as an effector for the release of chemical mediators and as a regulator for cytokine production [49]. Upon the transition of AD to the chronic stage, Th1 cells become activated and produce IFN-γ, thereby inducing the production of IgG2a [48]. To effectively treat AD, it is imperative to regulate both Th2 and Th1 responses, thereby addressing the shifts in immune activity throughout the

progression of the disease. Monotropein administration effectively attenuated both IgE and IgG2a levels, indicating its capability to regulate Th1- and Th2-mediated immune responses throughout different phases of AD.

Consisting mainly of keratinocytes, the epidermis acts as the frontline of defense, forming the outermost protective barrier of the skin [50]. In AD, disruptions in the skin barrier activate keratinocytes, prompting the production of inflammatory cytokines (IL-1β and IL-6) and chemokines (CCL17 and CCL22) [13]. In particular, CCL17 and CCL22 induce a Th2 immune response in skin lesions by attracting CCR4-expressing cells, such as Th2 cells [51]. Monotropein exhibited a significant reduction in these mediators in TNF-α/IFN-γ-stimulated keratinocytes, emphasizing its role in mitigating AD-associated inflammation at the cellular level.

The pivotal role of the JAK/STAT signaling pathway in immune responses and AD pathogenesis positions it as an attractive target for therapeutic intervention [27,28]. STAT6, acknowledged as a potent transducer and activator in allergic diseases, serves as a critical mediator through which IL-4 and IL-13, recognized as key markers of AD, modulate Th2 responses [52]. Additionally, STAT1 is activated by various cytokines, including TNF-α, IFN-γ, and IL-6, to modulate immune responses [52]. Monotropein showcased its anti-inflammatory potential by inhibiting the phosphorylation of JAK1, STAT1 and STAT6 in AD-induced mice and TNF-α/IFN-γ-stimulated keratinocytes. This modulation aligns with contemporary approaches involving JAK inhibitors for AD treatment, highlighting monotropein's potential as a safer and cost-effective alternative, given the need for further research on the potential side effects of JAK inhibitors and the cost burden they may impose on patients.

In summary, the oral administration of monotropein exhibited significant alleviation of AD symptoms in a mouse model. Monotropein demonstrated a dual effect by mitigating both Th1 and Th2 immune responses. This therapeutic impact was achieved through the inhibition of the JAK/STAT signaling pathway, addressing inflammatory mediators in AD-affected skin tissue and activated keratinocytes. The findings suggest that monotropein holds promise as a functional ingredient or novel therapeutic agent for AD-like skin inflammation, paving the way for potential advancements in AD treatment strategies. Although clinical investigations have been conducted on *M. officinalis* root, monotropein alone has not been investigated, so it is important to verify efficacy and safety in human subjects.

5. Conclusions

In this study, oral administration of monotropein alleviated the symptoms of AD and simultaneously reduced Th1 and Th2 immune responses. Monotropein inhibited the expression of inflammatory mediators by blocking the JAK/STAT signaling pathway in the skin tissue of AD mice model and in activated keratinocytes. Therefore, monotropein provides important implications for the development of functional ingredients or novel therapeutic agent related to AD-like skin inflammation.

CRediT authorship contribution statement

Sang-Hyun Kim: Writing – review & editing. **Dongwoo Khang:** Validation. **Soyoung Lee:** Investigation. **Taeg Kyu Kwon:** Project administration. **Young-Ae Choi:** Methodology. **Na-Hee Jeong:** Writing – review & editing. **Inyoung Yang:** Writing – original draft.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2024.116911.

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