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Inhibitory effect of 5,6-dihydroergosteol-glucoside on atopic dermatitis-like skin lesions via suppression of NF-κB and STAT activation.

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ABSTRACT

Background: Atopic dermatitis (AD) is a Th2-type disease. Keratinocytes, a major type in the skin, produce Th2 chemokines such as thymus and activation-regulated chemokine (TARC)/CCL17 and macrophage-derived chemokine (MDC)/CCL22, which play pivotal roles in the development of Th2-dominant inflammatory skin diseases. Recently, it was reported that 5,6-dihydroergosterol-glucoside (DHE-Glc) was synthesized and exhibited strong anti-inflammatory activity.
Objective: We aimed to investigate the effects of DHE-Glc, a synthetic molecule derived from ergosterol, on AD-like skin lesions induced by 2,4-dinitrochlorobenzene (DNCB) in mice and to elucidate the effects of DHE-Glc on TNF-α/IFN-γ-induced production of CCL17 and CCL22 in human keratinocytes (HaCaTs) and DNCB induced skin inflammation mice model.
Method: Mice were sensitized and challenged on the skin of their backs with DNCB. At 30–60 days after sensitization, mice were treated with cutaneous administration of DHE-Glc by skin smear. HaCaT cells were used to evaluate the effects of DHE-Glc on production of CCL17 and CCL22 and investigate mechanisms of action by RT-PCR, ELISA, Western blot, and reporter assays.
Result: Topical administration of DHE-Glc attenuated AD-like skin inflammatory symptoms. DHE-Glc decreased infiltration of epidermal eosinophils and mast cells, and reduced levels of IgE, histamine, and mRNA expression and protein levels of CCL17/CCL22 in the plasma of DNCB-treated animals. In addition, DHE-Glc suppressed TNF-α/IFN-γ-induced expression of the Th2 chemokines CCL17 and CCL22 by inhibiting NF-κB and STAT activation in TNF-α/IFN-γ-induced HaCaT cells.
Conclusion: DHE-Glc improved AD-like skin inflammatory symptoms on the backs of DNCB-induced mice, partly by suppressing production of Th2 chemokines, CCL17 and CCL22 in inflamed skin. Therefore, DHE-Glc is a potential therapeutic agent for skin inflammatory diseases such as AD.

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by erythema, pruritic and eczematous skin lesions, edema, and thickening of the skin [1,2]. The pathogenesis of AD has been attributed to complex interactions between multiple factors such as genetic and environmental factors, altered skin barrier function, and the immune system [3,4]. Extrinsic AD caused by environmental factors or allergens induces Th2 cells to produce Th2 cytokines such as IL-4 and IL-13 and high serum IgE levels [5,6]. Th2 cell numbers are elevated during the acute and chronic stages of AD. Th2 cytokines have direct effects on skin cells such as epidermal keratinocytes, which produce pro-inflammatory cytokines and chemokines that induce infiltration of immune cells including T cells, monocytes, eosinophil and mast cells into inflammatory skin lesions [5].

Thymus and activation regulated chemokine (TARC/CCL17) is a CC chemokine that binds to the CC chemokine receptor 4 (CCR4), which is expressed selectively on Th2 cells [7–9]. CCL17 is constitutively expressed in the thymus and produced by various cells including monocyte-derived dendritic cells, endothelial cells and keratinocytes [10–12]. Macrophage-derived chemokine (MDC/

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CCL22) is also a CC chemokine that binds to CCR4. CCL22 is constitutively produced by dendritic cells, macrophages, and keratinocytes [13,14]. The interaction between CCR4 and its ligands is important for the migration of Th2 cells into inflamed tissue [15]. CCL17 and CCL22 are significantly elevated in AD patients and correlate with the severity in patients with AD [16–18]. Thus, the reduction of CCL17 and CCL22 in keratinocytes can be effective for treatment of the skin diseases.

A number of pharmacological controls for AD involve topical or systemic administration of steroids, antihistamines or specific inhibitors of NF-κB [19]. Steroids reduce all Th cell functions and are widely used as immunosuppressants [20]. However, prolonged use of steroid has side effects such as thinning of the skin, leading to cracking and bleeding [21]. Natural compounds from herbs and plants are potential sources of therapeutic agents for preventing and treating inflammatory skin diseases [22–24]. The phytosterol 3-O-β-D-glucopyranosylspinasterol (spinasterol-Glc) from *Stewartia koreana* leaf extracts is strongly anti-inflammatory and inhibits production of CCL17 in keratinocytes [25,26]. However, spinasterol-Glc is not readily available by isolation from plants. A derivative of spinasterol-Glc, 5,6-dihydroergosteol-glucoside (DHE-Glc), has been synthesized recently, which exhibited a strong anti-inflammatory activity [27]. In this study, we evaluated the effects of DHE-Glc on 2,4-dinitrofluorobenzene (DNCB)-induced AD mouse models and on production of CCL17 and CCL22 in HaCaT keratinocytes. We found that topical administration of DHE-Glc inhibited production of IgE and histamine and reduced epidermal thickness, infiltration of eosinophils, and lymph node and spleen size. In addition, we observed inhibitory effects by DHE-Glc on production of CCL17 and CCL22 in TNF-α/IFN-γ induced HaCaT keratinocytes.

2. Materials and methods

2.1. Cell culture

Immortal human keratinocyte (HaCaT) cells were cultured in Dulbecco's modified Eagles medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin at 37 °C in a humidified 95% and 5% (v/v) mixture of air and CO_2, respectively.

2.2. Cell viability assay

Cells were seeded in 96-well plates at 5×10^4 cells/well and allowed to attach for 18 h. After discarding the growth medium, HaCaT cells were treated with indicated concentrations of DHE-Glc in serum-free medium for 24 h. After incubation, cells were treated with 100 μg/ml of 3-(4,5-dimetnythiazol-2-yl)-2,5-diphenyl-thetazolium bromide (MTT) for 1 h. Formazan precipitates were dissolved in 200 μl of DMSO and absorbance at 560 nm was determined spectrophotometrically. Analyses were repeated three times, and the results were expressed as means of three independent experiments.

2.3. Animals and maintenance

Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC), Kyung Hee University (KHUASP (SU) 11-012). Male BALB/c mice were from Orient Bio. Inc. (Gyeonggi-do, Korea) and were 6 weeks old at initiation of experiments. All mice were maintained at the animal facility of Kyung Hee University (Yoinin-si, Korea) and were housed in an environmentally controlled room with a 12 h light/dark cycle and allowed free access to water. Room temperature was maintained at 22 ± 1 °C with a relative humidity of 50 ± 10%. Animals were fed with a sterilized pelleted diet (Orient Bio. Inc.). DNCB (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in vehicle (3:1 acetone:

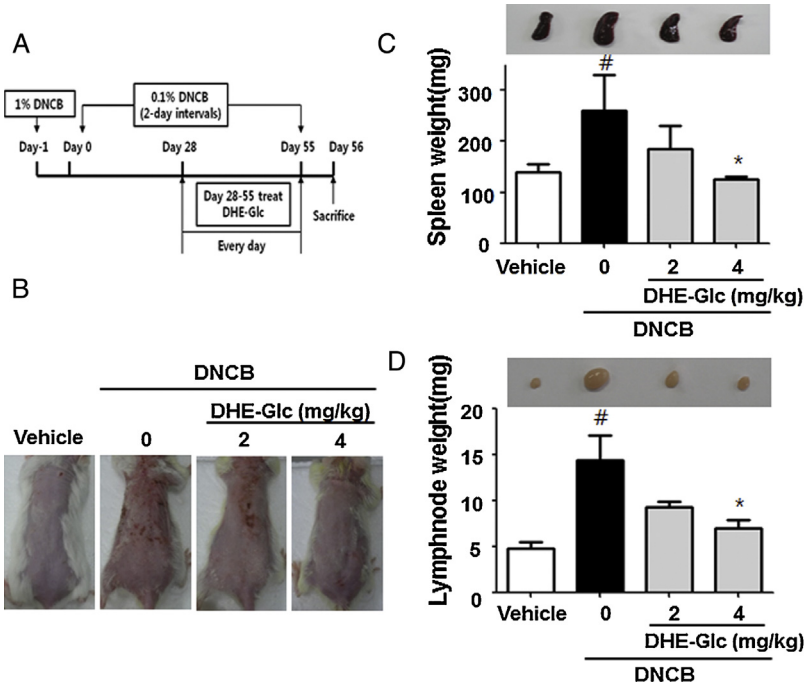

Fig. 1. Effects of DHE-Glc on DNCB-induced AD skin severity. (A) Sensitization of DNCB mice ($n = 5$): stimulation was with DNCB for 1 month, and after 1 month, with cream containing 2 mg/kg and 4 mg/kg of DHE-Glc for 1 month. Mice were sacrificed on day 60. (B) Clinical severity of inflammatory skin lesions. Photographs were taken on the day before mice were sacrificed. (C, D) Organ sizes were compared by photographic images. Organs and whole-body weights of five mice per group were measured. #$p < 0.05$ vs. negative control, *$p < 0.05$ vs. positive control.

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