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Tryptanthrin ameliorates atopic dermatitis through down-regulation of TSLP

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ABSTRACT

Atopic dermatitis (AD) is a common skin disease that greatly worsens quality of life. Thymic stromal lymphopoietin (TSLP) plays a decisive role in the development of AD. The purpose of this study is to examine whether tryptanthrin (TR) would suppress AD through the regulation of TSLP. We analyzed the effect of TR on the level of TSLP from phorbol myristate acetate/calcium ionophore A23187-activated human mast cell line, HMC-1 cells, in 2,4-dinitrofluorobenzene-induced AD-like skin lesions of NC/Nga mice, and in anti-CD3/anti-CD28-stimulated splenocytes. TR significantly suppressed the level of intracellular calcium and the production and mRNA expression of TSLP through the blockade of receptor-interacting protein 2/caspase-1/nuclear factor-κB pathway in the activated HMC-1 cells. TR also significantly suppressed the levels of histidine decarboxylase and IL-1β. Furthermore, TR ameliorated clinical symptoms in the AD model. TR significantly reduced the levels of TSLP, IL-4, IFN-γ, IL-6, TNF-α, thymus and activation-regulated chemokine, and caspase-1 in AD skin lesions. Also, TR significantly reduced the serum levels of histamine and IL-4 in the AD model. Finally, TR significantly inhibited the production of IL-4, IFN-γ, and TNF-α from the stimulated splenocytes. Taken together, TR exhibits the potential to be a therapeutic agent for AD through down-regulation of TSLP.

Introduction

Atopic dermatitis (AD)[1] is one of the most common inflammatory cutaneous diseases, characterized by dry, itchy skin, and relapsing eczematous skin lesions [1]. The etiology of this disease is incompletely understood, but the disease is manifested by complex interactions between various cells and molecular mediators. Mast cells as effector cells of allergic reactions are involved in the development of skin lesions in AD [2]. Mast cells secrete numerous cytokines that are relevant in chronic skin inflammation [3]. Thymic stromal lymphopoietin (TSLP), an interleukin 7-like cytokine, has been mainly known to trigger dendritic cell-mediated Th2 inflammatory responses [4]. However, the activated mast cells have expressed high levels of TSLP and triggered allergic inflammation [5]. And TSLP was highly expressed in skin lesions of patients with acute and chronic AD [6].

The activated inflammasome by an allergen causes the activation of caspase-1 in a cytosolic multi-adaptors complex, inflammasome-specific manner, which in turn drives maturation and secretion of the pro-inflammatory cytokines and regulates innate and adaptive immune responses [7]. Caspase-1 is responsible for activating the inactive precursor of interleukin (IL)-1β that are critical for inflammation [8]. Rereceptor-interacting protein 2 (Rip2) regulates caspase-1 which is activated within the inflammasome [9]. And Rip2 activates the transcription factor, nuclear factor (NF)-κB pathway that mediates immune and inflammation responses [10]. Caspase-1 is able to activate NF-κB via Rip2 [11]. NF-κB regulates the transcription of the TSLP gene by binding to its promoter [12].

The skin lesions in NC/Nga mice of murine AD models are characterized by infiltration of IL-4-producing $CD4^+$ T cells or mast cells as well as high expressions of Th2 cytokines and chemokines [13]. And repeated skin exposure to the haptens, such as 2,4-dinitrofluorobenzene (DNFB) causes AD-like skin lesions [14]. DNFB was reported to increase the caspase-1 activity in mouse skin dendritic cells [15].

Tryptanthrin (TR, Indolo(2,1-b)quinazoline-6,12-dione) is a natural product from the medicinal plant, Polygonum tinctorium (Naju Jjok) which is known to have anti-pyretic, anti-inflammatory, and detoxicant actions in traditional Korea medicine [16,17]. TR has been revealed to have therapeutic effects on

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[1] *Abbreviations used:* AD, atopic dermatitis; DNFB, 2,4-dinitrofluorobenzene; HMC-1, human mast cell line; PMACI, phorbol myristate acetate/calcium ionophore A23187; TR, tryptanthrin; TSLP, thymic stromal lymphopoietin.

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inflammatory bowel disease [17], tuberculosis [18], and *Escherichia coli* infection [19]. However, the effect of TR on AD has not yet been clarified. Therefore, we investigated the effect of TR on phorbol myristate acetate/calcium ionophore A23187 (PMACI)-stimulated human mast cell line (HMC-1) cells in vitro, DNFB-induced AD-like skin lesions of NC/Nga mice in vivo, and anti-CD3/anti-CD28-stimulated splenocytes ex vivo.

Materials and methods

HMC-1 cells culture

HMC-1 cells were incubated in IMDM supplemented with 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2 with 95% humidity.

Animals

Male NC/Nga mice were obtained from Charles River Laboratories International, Inc. (Yokohama, Japan). And the animals were maintained under conventional condition and performed under approval from the animal care committee of Kyung Hee University [Protocol Number. KHUASP (SE)-11–009]. Mice were sacrificed with CO_2 inhalation.

Sensitization with DNFB

For active sensitization, 100 μl 0.15% DNFB dissolved in acetone was topically challenged to the shaved abdominal skins of NC/Nga mice. A week later, the shaved dorsal skins of NC/Nga mice was challenged with 50 μl 0.15% DNFB. At that time, TR (10 μM), DEX (3 μg/ml), or saline (control group) were orally administrated to DNFB-challenged mice (Supplementary Fig. S1). The same volume of acetone was challenged to the shaved dorsal skin and saline was orally administrated as an unchallenged vehicle group. Dorsal skin samples, serum, and spleen were obtained 4 h after the last DNFB challenge. After anesthetization, blood was withdrawn from the heart of mouse into syringes. And then, serum was prepared by centrifugation at 3400 rpm at 4 °C for 10 min.

Statistical analysis

The results shown are a summary of the data from at least-three experiments and are presented as the mean ± SD. Statistical evaluation of the results was performed by an independent *t*-test and ANOVA with Tukey post hoc test. The results were considered significant at a value of $P < 0.05$.

Results

TR suppresses the level of TSLP from the activated HMC-1 cells

An increase in concentration of intracellular calcium is a sufficient condition for activation of mast cells and secretion of mediators from the mast cells [20]. Thus, first we examined the regulatory effect of TR on the level of intracellular calcium in the PMACI-activated HMC-1 cells. BAPTA-AM (calcium chelator) was used as a positive control. The activation with PMACI increased the calcium release from intracellular stores (in 0.5 mM EGTA containing media), whereas TR suppressed the level of intracellular calcium increased by PMACI (Fig. 1a). Because increased intracellular calcium induces the production of TSLP from the activated mast cells [21], we examined the regulatory effect of TR on the production of TSLP. TR (1 and 10 μM) significantly suppressed the production of TSLP from the activated HMC-1 cells ($P < 0.05$; Fig. 1b). TR (1 and 10 μM) also significantly reduced the mRNA expression of TSLP in the activated HMC-1 cells ($P < 0.05$; Fig. 1c).TR alone had no effect on the mRNA expression and production of TSLP (Fig. 1b and c). TR (0.1, 1, and 10 μM) did not show cytotoxicity in the HMC-1 cells (Fig. 1d).

TR suppresses the activations of Rip2/caspase-1/NF-κB in the activated HMC-1 cells

Next, we examined that the regulatory effect of TR on the level of TSLP that would be mediated through Rip2/caspase-1/NF-κB pathways. As shown in Fig. 2a and b, TR significantly suppressed the expressions of Rip2 and caspase-1 that were increased by activation with PMACI ($P < 0.05$). And TR significantly suppressed the caspase-1 enzymatic activity in a dose-dependent manner ($P < 0.05$; Fig. 2c). In addition, TR significantly suppressed the cleavage of the caspase-specific peptide (tetrapeptide WEHD-*p*NA, substrate of caspase-1) through inhibition of binding reaction between the recombinant caspase-1 and substrate during the indicated time ($P < 0.05$; Fig. 2d). Furthermore, TR suppressed the NF κB translocation to the nuclei and IκBα phosphorylation in cytosol ($P < 0.05$; Fig. 2e and f). Caspase-1 was known as IL-1β-converting enzyme and NF-κB was a transcription factor of IL-1β [8]. Therefore, we analyzed whether TR also would regulate the level of IL-1β. TR significantly suppressed the production and mRNA expression of IL-1β in the activated HMC-1 cells ($P < 0.05$; Fig. 3).

TR suppresses the level of HDC in the activated HMC-1 cells

Mast cells induce histamine release by increasing intracellular calcium [22]. Histamine was formed from L-histidine by activation of HDC [23]. Thus, we examined the effect of TR on HDC activity. Activation with PMACI increased the expression of HDC, whereas TR decreased the expression of HDC ($P < 0.05$; Supplementary Fig. S2a and b). And TR significantly suppressed the activity of HDC in the activated HMC-1 cells ($P < 0.05$; Supplementary Fig. S2c).

TR ameliorates clinical symptoms in DNFB-induced AD murine model

Furthermore, we examined the regulatory effect of TR on the TSLP level of the DNFB-induced AD murine model. DEX was used as a positive control [24]. The noticeable erythema, hemorrhage, excoriation, dryness, and erosion were present in DNFB-induced AD-like skin lesions (control group), whereas the administration of TR (10 μM) or DEX (3 μg/ml) markedly ameliorated these phenotypes in AD skin lesions (Fig. 4a). And we examined the thickness of epidermis and infiltration of inflammatory cells by H&E staining. The epidermis thickness was reduced in skin lesions of TR or DEX-administered AD murine model (Fig. 4a). As shown in Fig. 4a and Supplementary Fig. S3, the number of inflammatory cells was lower in skin lesions of TR or DEX-administered AD murine model than that of the skin lesions of the control group ($P < 0.05$). In addition, TR or DEX significantly suppressed scratching behavior (Fig. 4b).

TR suppresses the level of TSLP in skin lesions of DNFB-induced AD murine model

Next, we examined whether TR would suppress the levels of TSLP in skin lesions of the AD murine model. The mRNA expression of TSLP was up-regulated by DNFB challenge, but the up-regulated mRNA expression was suppressed by TR (10 μM) or DEX (3 μg/ml) (Fig. 5a). The protein expression of TSLP was also significantly suppressed by TR or DEX ($P < 0.05$; Fig. 5b). In in vitro study, TR significantly suppressed the level of TSLP through the inhibition of