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Original article

Tryptanthrin reduces mast cell proliferation promoted by TSLP through modulation of MDM2 and p53

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ABSTRACT

Background: Atopic dermatitis (AD) results from complex interactions between mast cells and inflammatory mediators. An inflammatory mediator, thymic stromal lymphopoietin (TSLP) is known to promote mast cell proliferation through up-regulation of mouse double minute 2 (MDM2, a negative regulator of p53) and aggravate AD. In this study, we investigated whether tryptanthrin (TR, an anti-inflammatory agent) would regulate TSLP-induced mast cell proliferation and TSLP-induced a pro-inflammatory cytokine, tumor necrosis factor (TNF)-α production from mast cells.
Methods: Human mast cell line (HMC-1) cells were treated with TR and stimulated with TSLP. Proliferation was measured with a bromodeoxyuridine incorporation assay. And pro- and anti-apoptotic factors were analyzed with quantitative real-time PCR, Western blot analysis, and ELISA. The mRNA expression and production of TNF-α were analyzed with quantitative real-time PCR and ELISA.
Results: TR significantly inhibited the proliferation of HMC-1 cells promoted by TSLP. TR inhibited MDM2 expression, whereas TR increased the expression of p53, poly ADP-ribose polymerase, and caspase-3 in the TSLP-stimulated HMC-1 cells. TR significantly inhibited Ki67 mRNA expression as well as mRNA expression and production of interleukin (IL)-13 in the TSLP-stimulated HMC-1 cells. Moreover, TR significantly suppressed mRNA expression and production of TNF-α in the TSLP-stimulated HMC-1 cells. Finally, the mRNA expression of IL-7 receptor α chain and TSLP receptor was inhibited by TR in the TSLP-stimulated HMC-1 cells.
Conclusion: Our results suggest that TR determined with new concept has intensive potential for the treatment of mast cell-mediated allergic diseases, such as AD.

1. Introduction

Atopic dermatitis (AD) is a multifactorial inflammatory skin disorder. The pathogenesis of AD has been ascribed to complex interactions between various immune cells and inflammatory factors [1]. In the inflammatory process, the cytokines recruit the activated immune cells to lesion site, thereby amplifying and maintaining this condition [2]. Especially, mast cell is a potent effector conducting a critical role in allergic inflammatory reactions, such AD [3]. Exogenous cytokines released from the activated immune cells influence the number of mast cells [4]. The increases in the mast cell number and mast cell activation in AD lesions contribute to development of AD [5]. The number of mast cells can be regulated by proliferation, migration, survival, and apoptosis from the inflammatory factors in inflamed tissue [6]. The inflammatory factors are critical for mast cell development although they are not mast cell growth factors, such as stem cell factor (SCF) and interleukin (IL)-3 [7]. The activated mast cells release various cytokines that are relevant in chronic skin inflammation [8]. Tumor necrosis factor (TNF)-α which is up-regulated in mast cells, acts as an inducer of cytokines, chemokines and adhesion molecules in the skin of AD lesions [9].

Thymic stromal lymphopoietin (TSLP) is a general biomarker for epidermal-barrier defects [10] and involved in the pathogenesis of AD [11]. TSLP signals through a receptor containing IL-7 receptor α chain (IL-7Rα) and a TSLP specific subunit, TSLP receptor (TSLPR) [12]. TSLP acts on various lineages, including dendritic cells, T cells, and mast cells via IL-7Rα/TSLPR [13]. Epithelial cell-derived TSLP

Abbreviations: AD, atopic dermatitis; BrdU, bromodeoxyuridine; FBS, fetal bovine serum; HMC-1, human mast cell line cells; IL, interleukin; IMDM, Isocove's Modified Dulbecco's Medium; MDM2, mouse double minute2; TR, tryptanthrin; TSLP, thymic stromal lymphopoietin.

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strongly activates dendritic cells and that exerts a profound influence on pro-inflammatory cytokines, such as TNF-α production from T cells or mast cells in many tissues [14]. In addition, TSLP functions as a factor in mast cell proliferation [15]. An E3 ubiquitin ligase, murine double minute 2 (MDM2) which induces cancer cell survival and growth by degrading cell cycle regulator p53, mediated mast cell proliferation promoted by TSLP [15,16]. MDM2 inhibitor inhibited cell proliferation which in turn leads to the activation of caspase-3 [17]. Increased activity of p53 resulted in the activation of caspase-3 and cleavage of poly-ADP-ribose polymerase (PARP) [18]. Recently, Hashimoto et al. [19] reported that MDM2 inhibition decreased NF-κB-dependent inflammation in vascular smooth muscle cells.

IL-13 is a vital stimulator of inflammation and AD [20]. TSLP resulted in a marked increase in IL-13 and enhanced lung inflammation [21]. In addition, TSLP promoted the mast cell proliferation, increasing IL-13 production [15]. IL-13 was reported to promote the mast cell proliferation as a growth factor of mast cell [22]. Therefore, pharmacologic regulation of the proliferation promoted by TSLP could be a hopeful strategy for mast cell-mediated allergic diseases, such as AD.

Tryptanthrin (indolo[2,1-b]quinazoline-6,12-dione; TR) is an alkaloid included in many plant species [23]. Studies reported that TR has multiple biological and pharmacological activities including anti-inflammatory [24], anti-allergic [25], and anti-tumor activity [26]. However, the precise regulatory mechanism of TR has not been elucidated in TSLP-induced mast cell proliferation and TSLP-induced inflammatory cytokine production from mast cells. Thus, we investigated anti-proliferative and anti-inflammatory effects of TR on TSLP-stimulated human mast cell line (HMC-1) cells.

2. Materials and methods

2.1. Materials

We purchased Isocove's Modified Dulbecco's Medium (IMDM) and fetal bovine serum (FBS) from Gibco BRL (Grand Island, NY, USA); TR (#SML0310, purity ≥98%) from Sigma Chemical Co. (St. Louis, MO, USA); recombinant TSLP and anti-IL-13 antibodies from R&D Systems (Minneapolis, MN, USA); anti-TNF-α antibodies from BD Pharmingen (San Diego, CA, USA); anti-MDM2, p53, PARP, caspase-3, actin, and GAPDH antibodies from Santa Cruz Biotechnology (Dallas, TX, USA). TR was dissolved with carboxylmethyl cellulose solution (0.5% w/v) containing 10% dimethylsulfoxide (DMSO).

2.2. Cell culture

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

2.3. Bromodeoxyuridine assay

The proliferation of HMC-1 cells (1×10^4) was determined using a colorimetric immunoassay based on the mensuration of bromodeoxyuridine (BrdU) incorporated by DNA synthesis (Roche Diagnostics GmbH, Mannheim, Germany).

2.4. MTT assay

Cell viability was measured by a MTT assay. HMC-1 cell (4×10^5) were treated with TR and incubated with MTT solution (5 mg/ml) at 37 °C. After washing the supernatant out, insoluble formazan product was dissolved in DMSO. The optical density was determined using an ELISA reader at 540 nm.

2.5. Quantitative real-time PCR

Total RNA was isolated from HMC-1 cells according to the manufacturer's specification using an easy-BLUE™ RNA extraction kit (iNtRON Biotech, Seongnam, Republic of Korea). The concentration of total RNA in the final elutes was determined by NanoDrop spectrophotometer (Thermo Scientific Inc., Wilmington, DE, USA). The cDNA synthesis reaction was performed for 60 min at 42 °C and 5 min at 94 °C using a cDNA synthesis kit (Bioneer Corporation, Daejeon, Republic of Korea). Quantitative Real-Time PCR was performed using a SYBR Green master mix (Applied Biosystems, Foster City, CA, USA). The mRNA detections of Ki67, IL-13, TNF-α, IL-7Rα and TSLPR were analyzed using an ABI StepOne real-time PCR System (Applied Biosystems). The primers are in Table 1. All mRNA levels were normalized to GAPDH levels compared with the control sample. All data were analyzed using the ΔΔCT method.

2.6. Cytokines assay

The production of IL-13 and TNF-α from HMC-1 cells was measured using a sandwich ELISA method according to the manufacturer's specifications (R & D system Inc. and BD Pharmingen).

2.7. Western blot analysis

Western blot analysis was performed with protein extracts from HMC-1 cells as described previously [27]. Briefly, TR-treated or TSLP-stimulated HMC-1 cells were lysed and separated through 12% SDS-PAGE. After electrophoresis, the proteins were transferred to nitrocellulose membranes. The membranes were blocked with phosphate-buffered saline with Tween 20 containing 5% skim milk and incubated with primary and secondary antibodies. Finally, Blots were visualized by an enhanced chemiluminesence assay according to the manufacturer's instructions (Amersham Co., Newark, NJ, USA). The relative intensity was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.8. Statistics

Experimental values are a summary from at least-three experiments and represented as the mean ± standard error of mean (SEM). Statistical significance was determined using an independent *t*-test and an ANOVA with a Tukey post hoc test. All statistical analyses were performed using IBM SPSS v21 statistics software (IBM, Armonk, NY, USA). Values of $p < 0.05$ were considered as significant results.

3. Results

3.1. Regulatory effect of TR on proliferation of HMC-1 cells

First of all, we clarified whether TR could regulate mast cell proliferation promoted by TSLP. The stimulation with TSLP significantly induced BrdU incorporation into HMC-1 cells as

Table 1
Primer sequences.

Gene	Forward primer	Reverse primer
Ki67	ATAAACACCCCAACACACACAA	GCCACTTCTTCATCCAGTTAC
IL-13	GCCCTGGAATCCCTGATCA	GCTCAGCATCCTCTGGGTCTT
TNF-α	AGGACGAACATCCAACCTTCCCAA	TTTGAGCCAGAAGAGGTTGAGGGT
IL-7Rα	GCT CAG GGG AGA TGG ATC CT	GTC TTC TTA TGA TCG GGG AG
TSLPR	CAG AGC AGC GAG ACG ACA TT	GGT ACT GAA CCT CAT AGA GG
GAPDH	ACCAAATCCGTTGACTCCGACCTT	TCGACAGTCAGCCGCATCTTCTTT