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Short communication

Down-regulation of thymic stromal lymphopoietin by curcumin

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Abstract:
Background: Thymic stromal lymphopoietin (TSLP) is a cytokine implicated in the pathogenesis of allergic diseases such as asthma, atopic dermatitis and allergic rhinitis. Curcumin has various effects such as antidepressant, antioxidant, antihyperglycemic, antitumor and anti-inflammatory. However, the effect of curcumin on the production of TSLP has not been clarified. Thus, we investigated how curcumin inhibits the expression and production of TSLP in the human mast cell line, HMC-1 cells.
Methods: We used enzyme-linked immunosorbent assay, reverse transcription-polymerase chain reaction, luciferase assay, and caspase-1 assay to investigate the effects of curcumin.
Results: The results show that curcumin inhibited the production and mRNA expression of TSLP in HMC-1 cells: the maximal inhibition rate of TSLP production by curcumin (50 μM) was 59.16 ± 4.20%. In addition, curcumin suppressed the nuclear factor-κB luciferase activity induced by phorbol myristate acetate plus A23187. In the activated HMC-1 cells, caspase-1 activity was increased, whereas caspase-1 activity was decreased by pretreatment with curcumin.
Conclusion: These results suggest that curcumin can be used to treat inflammatory and atopic diseases through the suppression of TSLP.

Key words: thymic stromal lymphopoietin, curcumin, nuclear factor-κB, caspase-1

Abbreviations: NF-κB – nuclear factor-κB, PMA – phorbol myristate acetate, TSLP – thymic stromal lymphopoietin

Introduction

Atopic dermatitis is a chronic and relapsing eczematous skin inflammation associated with epidermal barrier dysfunction, intense pruritus, and cutaneous hyperreactivity to environmental triggers [8]. The lifetime prevalence of atopic dermatitis is estimated to be 15–30% in children and 2–10% in adults, while the incidence of atopic dermatitis has increased 2- to 3-fold during the past 3 decades in industrialized countries [1]. Thus, atopic dermatitis has significant socioeconomic and personal impacts in these countries [21].

Thymic stromal lymphopoietin (TSLP) was found to enhance potently the maturation of $CD11c^+$ dendritic cells, and TSLP-primed and activated dendritic cells promoted the differentiation of naive $CD4^+$ T

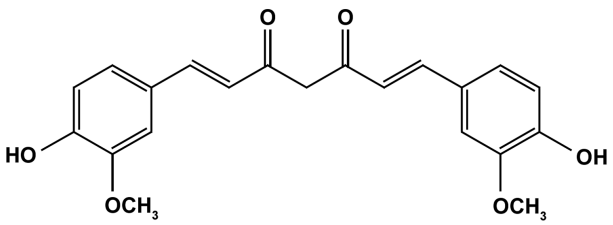

Fig. 1. Chemical structure of curcumin

cells into proinflammatory T_H2 cells [20]. A high expression of TSLP is a feature of keratinocytes in atopic dermatitis skin lesions, and the TSLP-priming of dendritic cells *in situ* may serve to induce or enhance T_H2 responses within the skin, as well as systemically. Consistent with this viewpoint, TSLP was originally reported to exert its T_H2-promoting properties through dendritic cell-mediated pathways in human beings that involved the induction of the OX40 ligand on dendritic cells [28]. TSLP has been implicated in the development of asthma and atopic dermatitis [13]. In atopic diseases such as asthma and atopic dermatitis, not only dendritic cells, epithelial cells, eosinophils, and T cells but also mast cells are important. A number of studies reported that mast cells are activated and infiltrated in the skin lesion of the atopic dermatitis animal model, suggesting the contribution of mast cells in atopic dermatitis [4, 9, 25, 30].

The cysteine protease caspase-1 is a member of the caspase family [6]. Quite unlike the role that most caspases have in apoptosis, caspase-1 mainly serves to cleave IL-1β and IL-18 from their inactive precursors to their active forms [2, 16]. In addition to the well-established roles of caspase 1 in the maturation of IL-1β and IL-18, caspase 1 is also capable of activating the nuclear factor (NF)-κB [15]. The activated caspase-1 activates NF-κB in HMC-1 cells [23]. NF-κB activated by caspase-1 mediates the induction of TSLP gene expression in airway epithelial cells [18].

Curcumin (Fig. 1) is the main constituent of the spice turmeric (*Curcuma longa*) [7, 33]. A number of studies have reported that curcumin has antidepressant, antioxidant, antihyperglycemic, antitumor, and anti-inflammatory activities [5, 10, 19, 29, 32]. However, the effect of curcumin on the production of TSLP has not yet been clarified. Thus, we investigated how curcumin suppresses the production of TSLP in mast cells.

Materials and Methods

Reagents

Phorbol myristate acetate (PMA), A23187, and curcumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). We purchased IMDM from Gibco BRL (Grand Island, NY, USA); caspase-1 inhibitor, caspase-1 assay kit, and TSLP antibodies from R&D Systems (Minneapolis, MN, USA); TMB substrate from Pharmingen (San Diego, CA, USA).

Cell culture

The human mast cell line, HMC-1 cells, was grown in IMDM and supplemented with 100 units/ml of penicillin, 100 μg/ml of streptomycin and 10% fetal bovine serum at 37°C in 5% CO_2 with 95% humidity.

Cytokine assay

We used the enzyme-linked immunosorbent assay (ELISA) method to assay the culture supernatant for TSLP [22, 24]. A sandwich ELISA for TSLP was carried out in duplicate in a 96-well ELISA plate. First, we coated the plate with 100 μl aliquots of mouse anti-human TSLP monoclonal antibody at 1.0 μg/ml in PBS at pH 7.4 and incubated the plate overnight at 4°X. The plate was washed in PBS containing 0.05% Tween-20 (Sigma) and blocked with PBS containing 1% BSA, 5% sucrose and 0.05% NaN_3 for 1 h. After additional washes, the culture supernatant and TSLP standards were added and incubated at 37°C for 2 h. After 2 h incubation at 37°C, the wells were washed and then each of the 0.2 μg/ml of biotinylated anti-human TSLP was added and again incubated at 37°C for 2 h. After washing the wells, streptavidin-peroxidase was added and the plate was incubated for 20 min at 37°C. The wells were again washed and the TMB substrate (Pharmingen) was added. Color development was measured at 450 nm using an automated microplate ELISA reader. A standard curve was run on the plate using recombinant human TSLP in serial dilutions.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

We used the method of Moon et al. [22], using an easy-BLUE™ RNA extraction kit (iNtRON Biotech, Republic of Korea) and isolated the total RNA from HMC-1 cells in accordance with the manufacturer's specifications. The concentrations of total RNA in the final elutes were determined by a spectrophotometer. Total RNA (1 μg) was heated at 65°C for 10 min and then chilled on ice. Each sample was reverse-transcribed to cDNA for 90 min at 37°C using a cDNA synthesis kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The PCR was performed with the following primer for human TSLP (5'TAT GAG TGG GAC CAA AAG TAC CG3'; 5'GGG ATT GAA GGT TAG GCT CTG G3'). GAPDH (5'CAA AAG GGT CAT CAT CTC TG3'; 5'CCT GCT TCA CCA CCT TCT TG3') was used to verify if equal amounts of RNA were used for reverse transcription and PCR amplification from different experimental conditions. The annealing temperature was 62°C for TSLP and GAPDH. Amplified fragment sizes for TSLP and GAPDH were 97 bp and 446 bp, respectively. Products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide.

Transient transfection and luciferase assay

For the transfection, we seeded HMC-1 cells (1×10^7) in a 100 mm culture dish. We then used Lipofectamine™2000 purchased from Invitrogen (Carlsbad, CA, USA) to transiently transfect pNF-κB luciferase (LUC) and pSV40-LUC reporter gene constructs into HMC-1 cells. To measure the luciferase activity, we used a luminometer 1420 luminescence counter purchased from Perkin Elmer (Waltham, MA, USA) in accordance with the manufacturer's protocols. All the transfection experiments were performed in at least three different experiments, with similar results. The relative luciferase activity was defined as the ratio of *firefly* luciferase activity to *renilla* luciferase activity.

Caspase-1 assay

Caspase activity was measured according to the manufacturer's specifications by using a caspase assay kit. The whole-cell lysate was prepared in a cold lysis buffer on ice for 10 min and centrifuged at 12,000 × g for 1 min. An equal amount of total protein was quantified by a bicinchoninic acid protein quantification kit purchased from Sigma Chemical Co. (St. Louis, MO, USA) in each lysate. The catalytic activity of caspase-1 from the cell lysate was measured by the proteolytic cleavage of WEHD-p-nitroaniline (pNA, caspase-1 colorimetric substrate) for 24 h at 37°C. The plates were read at 405 nm. The recombinant caspase-1 enzyme was available as a positive control.

Statistical analysis

All results are expressed as the mean ± SEM. The statistical evaluation of the results was performed by an independent *t*-test and an ANOVA with a Tukey *post-hoc* test. The results were significant with a value of $p < 0.05$.

Results

Effect of curcumin on the production of TSLP in HMC-1 cells

To investigate the inhibitory effect of curcumin on the production of TSLP, we stimulated HMC-1 cells with PMA plus A23187 for 7 h, and we used the ELISA to analyze the supernatants for TSLP. The stimulation with PMA plus A23187 increased TSLP production from HMC-1 cells (Fig. 2A). The levels of TSLP which had increased due to PMA plus A23187 were significantly decreased by curcumin (50 μM) (Fig. 2A, $p < 0.05$). The maximal inhibition rate of TSLP production by curcumin (50 μM) was 59.16 ± 4.20%. In previous study, we reported that caspase-1 is involved in the production of TSLP [23]. Thus, we confirmed the inhibition of TSLP production by the caspase-1 inhibitor in HMC-1 cells (Fig. 2A). The caspase-1 inhibitor reduced TSLP production up to 62.26 ± 0.82%. When curcumin was given as a pretreatment at various concentrations ranging from 0.5 to 50 μM, the cytotoxicity due to curcumin was not shown (data not shown).

Effect of curcumin on the mRNA expression of TSLP in HMC-1 cells

To examine whether curcumin can modulate PMA plus A23187-induced mRNA expression of TSLP, we pretreated the cells with curcumin for 2 h before the

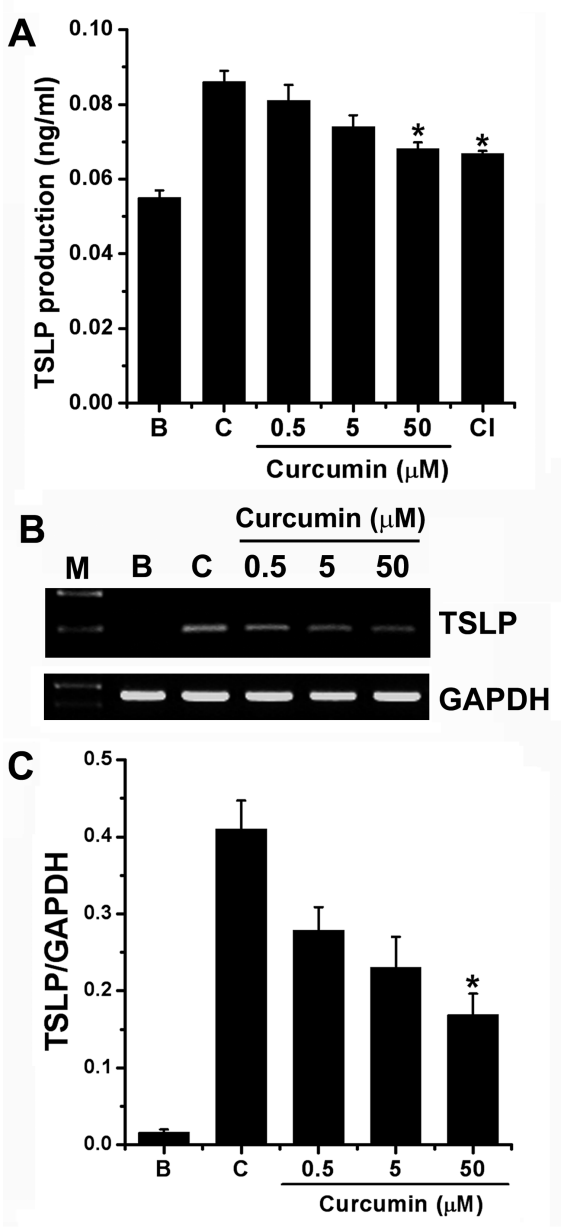

Fig. 2. Effects of curcumin on the production and mRNA expression of TSLP in HMC-1 cells. (**A**) HMC-1 cells (4×10^5) were treated with various concentrations (0.5 to 50 μM) of curcumin for 2 h, and then stimulated with PMA plus A23187 for 7 h. The levels of TSLP in the supernatant were measured with the ELISA method. B, unstimulated cells; C, vehicle-treated, and then PMA plus A23187-stimulated cells. CI, caspase-1 inhibitor (500 nM)-treated, and then PMA plus A23187-stimulated cells. (**B**) HMC-1 cells (1×10^6) were stimulated with PMA plus A23187. The mRNA was measured with the RT-PCR method. M, marker. (**C**) The TSLP mRNA expression levels were quantified by densitometry

PMA plus A23187 stimulation. We stimulated the cells with PMA plus A23187 for 5 h and then performed the RT-PCR analysis. The mRNA expression of TSLP was up-regulated by PMA plus A23187; however, the up-regulated TSLP mRNA expression was decreased by the treatment with curcumin (Fig. 2B). The inhibitory effect of 50 μM of curcumin was greater than 0.5 and 5 μM, thus, we evaluated the effect of 50 μM of curcumin in the next set of experiments: luciferase assay and caspase-1 assay.

Effect of curcumin on the activation of NF-κB in HMC-1 cells

To determine whether curcumin could modulate the luciferase expression specifically *via* NF-κB activation, we performed a dual-luciferase assay. As shown in Figure 3, the PMA plus A23187 stimulation increased the reporter gene activity. However, the increased NF-κB luciferase activity was significantly decreased by curcumin (50 μM, $p < 0.05$). The relative luciferase activity at the dose of 50 μM was 22.31 ± 0.80. The control and spontaneous values were 32.75 ± 1.20 and 1.24 ± 0.01, respectively.

Effect of curcumin on the activation of caspase-1 in HMC-1 cells

Finally, to examine the effect of curcumin on the activation of caspase-1, we performed a caspase-1 assay on HMC-1 cells. In the control group that was stimulated by PMA plus A23187, the levels of caspase-1 activity were increased in HMC-1 cells. However, the levels of caspase-1 activity were decreased by pretreatment with curcumin (Fig. 4).

Discussion

Natural product-based compounds seem better than synthetic compounds because natural product-based compounds are generally supposed to be devoid of severe side effects [12]. Curcumin is the main constituent of the spice turmeric (*Curcuma longa*) [7, 33]. Therefore, we investigated the effect of the natural product-based compound curcumin on the expression and production of TSLP.

The PKC activator PMA is generally a substitute for diacylglycerol and A23187 is a widely used ionophore. Our previous study showed that TSLP is produced by PMA plus A23187 stimulation [23]. Lesional, but not unaffected, skin from patients with atopic dermatitis expresses high levels of TSLP [34]. In the present study, curcumin suppressed the production and mRNA expression of TSLP (Fig. 2). To our

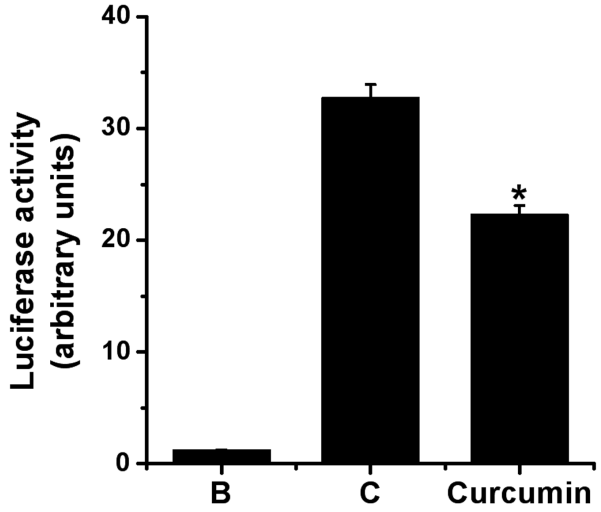

Fig. 3. Effects of curcumin on the activation of NF-κB in HMC-1 cells. HMC-1 cells (1×10^7) were transiently transfected with pNF-κB-LUC and pSV40-LUC and treated with curcumin (50 μM) for 2 h, and then stimulated with PMA plus A23187 for 48 h. The NF-κB activity was assessed with a luciferase assay. B, unstimulated cells; C, vehicle-treated, and then PMA plus A23187-stimulated cells; Curcumin, 50 μM of curcumin-treated, and then PMA plus A23187-stimulated cells. Each datum represents the mean ± SEM of three independent experiments. * $p < 0.05$; significantly different from the control (vehicle-treated, and then PMA plus A23187-stimulated cells) value

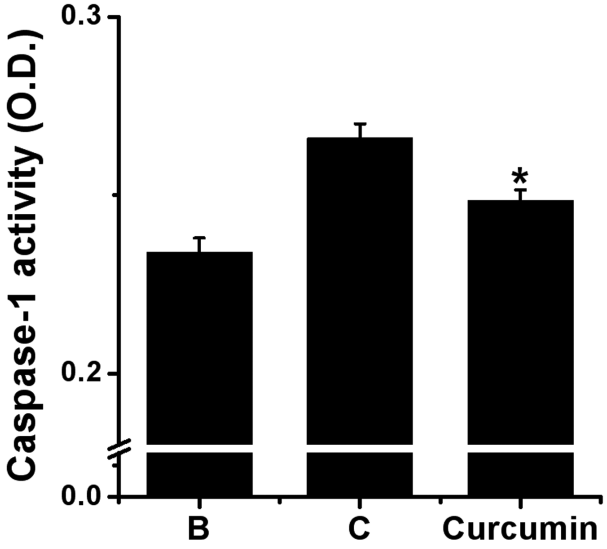

Fig. 4. Effects of curcumin on the activation of caspase-1 in HMC-1 cells. HMC-1 cells (5×10^6) were treated with 50 μM of curcumin for 2 h, and then stimulated with PMA plus A23187 for 1 h. B, unstimulated cells; C, vehicle-treated, and then PMA plus A23187-stimulated cells; Curcumin, 50 μM of curcumin-treated, and then PMA plus A23187-stimulated cells. Each datum represents the mean ± SEM of three independent experiments. * $p < 0.05$; significantly different from the control (vehicle-treated, and then PMA plus A23187-stimulated cells) value

knowledge, this is the first study showing an inhibition of TSLP by curcumin in mast cells. Thus, we presume that curcumin might have a potential in the treatment of inflammation and atopic dermatitis.

NF-κB is a transcription factor that regulates the expression of the genes involved in the immune response and inflammation [3]. It has been reported that the expression of human TSLP mRNA was controlled by NF-κB in various cells such as fibroblasts and epithelial cells [17, 18, 26]. The expression and production of TSLP was controlled by NF-κB in mast cells [23]. Rafiee et al. [27] reported that curcumin inhibits NF-κB activation in acid-activated HET-1A cells. In addition, curcumin inhibited NF-κB binding activity in mice [31]. Our results also showed that curcumin inhibited the NF-κB luciferase activity in mast cells (Fig. 3). From previous reports and our results, we could confirm that NF-κB is a general transcription factor in fibroblasts, epithelial cells, and mast cells. In this study, curcumin inhibited NF-κB luciferase activity about 33%. However, curcumin inhibited TSLP production about 59%. Kouzaki et al. [14] reported that proteases induce TSLP production through the protease-activated receptor-2 (PAR-2). Thus, we can presuppose that curcumin inhibits TSLP production by the inhibition of not only NF-κB but also of the other mechanisms such as PAR-2.

Upon receipt of a pro-inflammatory stimulus, caspase-1 is activated [11]. Our results also showed that caspase-1 activity was increased by pro-inflammatory stimulus in HMC-1 cells (Fig. 4). In previous study, we reported that caspase-1 is involved in the production of TSLP [23]. Thus, we confirmed the inhibition of TSLP production by the caspase-1 inhibitor in this study (Fig. 2A). Pretreatment of curcumin inhibited the PMA plus A23187-induced activation of caspase-1 (Fig. 4). To our knowledge, this is also the first study showing an inhibition of caspase-1 activity by curcumin in mast cells. Thus, we can assume that curcumin inhibits the expression and production of TSLP through the blocking of caspase-1 in mast cells.

In conclusion, we have shown that curcumin can regulate the inflammatory responses induced by PMA plus A23187 in mast cells. Curcumin suppressed the expression and production of TSLP through the blocking of caspase-1 and NF-κB pathways. Overall, this study suggests that curcumin has potential in the treatment of inflammatory and atopic diseases through the suppression of TSLP.

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