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Chrysophanol, an anthraquinone from AST2017-01, possesses the anti-proliferative effect through increasing p53 protein levels in human mast cells

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Abstract

Objective Natural products are well known as the source of drugs in the treatment of allergic inflammation. Chrysophanol, an anthraquinone from the AST2017-01 extract, showed a beneficial anti-inflammatory effect on activated human mast cells in our previous study. However, a regulatory effect of AST2017-01 and chrysophanol on mast cell proliferation induced by thymic stromal lymphopoietin (TSLP) remains unclear. The present study determined the anti-proliferative effect and the fundamental mechanism of AST2017-01 and chrysophanol in mast cells.

Methods We evaluated an anti-proliferative effect of AST2017-01 and chrysophanol in TSLP-stimulated human mast cell line, HMC-1.

Results Without cytotoxicity, AST2017-01 and chrysophanol decreased mast cells growth and Ki67 mRNA expression increased by TSLP. AST2017-01 and chrysophanol enhanced expressions of p53 and Bax, whereas inhibited expression of Bcl-2. AST2017-01 and chrysophanol restored caspase-3 activity which was decreased by TSLP. AST2017-01 and chrysophanol suppressed expressions of murine double minute-2 protein and phosphorylated-signal transducer and activator of transcription six which are associated with the regulation of p53 protein. AST2017-01 and chrysophanol decreased levels of interleukin (IL)-13, IL-6, and tumor necrosis factor-α. Moreover, AST2017-01 and chrysophanol reduced mRNA expressions of TSLP receptor and IL-7 receptor α.

Conclusions Therefore, this study proposes that AST2017-01 and chrysophanol may be promising candidates for the development of potent anti-inflammatory or health functional foods.

Keywords Chrysophanol · Thymic stromal lymphopoietin · p53 · Murine double minute-2 · Mast cell

Introduction

A pro-allergic cytokine, thymic stromal lymphopoietin (TSLP) is an interleukin (IL)-7-like cytokine that is mainly expressed in mast cells, fibroblasts, airway smooth muscle cells, and

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epithelial cells [1–4]. Produced TSLP binds to a unique heterodimeric receptor complex that consists of TSLP-specific TSLP receptor (TSLPR) and IL-7 receptor α (IL-7Rα) chain and directly act on several immune cell types including mast cells, basophils, dendritic cells, T cells, and B cells at the early phase of inflammation [2, 5]. Moreover, TSLP-induced Th2 reaction is associated with the pathogenesis of allergic diseases such as allergic rhinitis, atopic dermatitis, and asthma [2]. TSLP affects the differentiation, maturation, proliferation, and activation of mast cells [6]. Therefore, blockade of TSLP signaling pathway on mast cells has been considered as a strategy for immunological intervention in the treatment and prevention of allergic diseases.

Tumor suppressor protein p53 affects the induction of DNA repair, apoptosis, cell senescence, or cell growth arrest [7]. The p53-dependent apoptosis is regulated by activation of apoptotic proteins including caspase-3 and Bax that are important executors of apoptosis [8]. Bcl-2 is a cell survival protein and restrains the p53-depedent apoptosis [9]. The p53 is degraded by murine double minute 2 (MDM2)-mediated ubiquitin–proteasome system [7]. Our previous study has showed that TSLP reduces the expressions of p53, Bax, and caspase-3, but enhanced the expressions of Bcl-2 and MDM2 in mast cells [10].

AST2017-01 consists of processed-*Cordyceps militaris* and processed-*Rumex crispus* and has been commonly used in Korea. *Cordyceps militaris* extract possesses anti-inflammatory and anti-cancer activities [11, 12]. Kwon et al. [13] has reported that processed-*Cordyceps militaris* alleviates inflammatory reactions and contact dermatitis. *Rumex crispus* extract possesses anti-osteoclastogenic and anti-oxidant activities [14–16]. Park et al. [17] has reported that processed-*Cordyceps militaris* alleviates inflammatory reactions. In addition, AST2017-01 has been reported to have inhibitory effects on atopic dermatitis, allergic rhinitis, and inflammatory responses [18–21]. Chrysophanol is a member of the anthraquinone family and an active compound of AST2017-01 [22]. It has anti-obesity, anti-inflammatory, anti-allergic, and anti-tumor effects [18, 23, 24].

Mast cells affect many aspects of obesity, allergic inflammation, and cancer development [6, 25, 26]. Therefore, many studies on the down-regulation of proliferation and activation of mast cells have been carried out to treat various diseases. The aim of this study is to clarify the regulatory effects of AST2017-01 and chrysophanol on TSLP-induced mast cell proliferation and to identify the signal transduction pathways regulated by AST2017-01 and chrysophanol.

Materials and methods

Cell culture

HMC-1 cells were cultured in Isocove's modified Dulbecco's medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Welgene, Daegu, Republic of Korea) and 1% penicillin–streptomycin (Gibco BRL) at 37 °C under 5% CO_2 and 95% air.

Treatment with AST2017-01 or chrysophanol

AST2017-01 (Gahwa Well Food Co., Chungbuk, Republic of Korea) and chrysophanol (purity:≥98%, Sigma Chemical Co., St. Louis, MO, USA), as the chief constituent (0.13%) of AST2017-01 [22], were prepared according to our previous study [18]. AST2017-01 consists of 4:6 ratio of processed-*Cordyceps militaris* and processed-*Rumex crispus*. AST2017-01 was boiled in distilled water at 80 °C for 3 h. AST2017-01 extract was filtered, concentrated *in vacuo* at 60 °C, and lyophilized. The AST2017-01 was dissolved in distilled water and filtered for in vitro experiments. Chrysophanol was dissolved in dimethyl sulfoxide, diluted in distilled water, and filtered for in vitro experiments. The concentrations of AST2017-01 and chrysophanol were also determined according to our previous study [18]. HMC-1 cells were pretreated with AST2017-01 (0.5, 5, and 50 μg/ml) and chrysophanol (0.06 μg/ml) for 1 h prior to TSLP (20 ng/ml, R&D Systems, Inc., Minneapolis, MN, USA) stimulation for various times.

BrdU assay

A BrdU colorimetric immunoassay kit (Roche Diagnostics GmbH, Mannheim, Germany) was used to evaluate mast cell proliferation. Briefly, HMC-1 cells were treated with AST2017-01 or chrysophanol for 1 h prior to TSLP for 48 h. The cells were then incubated with BrdU for 4 h. Anti-BrdU antibody complex was treated for 90 min in the fixed cells. A colorimetric substrate solution was added to the washed cells. Absorbance was determined at 405 nm by automatic microplate reader.

RNA isolation and quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR)

RNA was isolated from harvested cells using an easy-BLUE™ RNA extraction kit (iNtRON Biotech, Sungnam, Republic of Korea) and chloroform. After centrifugation at 12,000 rpm for 10 min at 4 °C, the upper phase was treated with an equal volume (400 μl) of isopropanol for 3 min and

then centrifuged at 12,000 rpm for 5 min to form a pellet. Subsequently, the pellet was washed with 70% ethanol and then air-dried. The total RNA was then dissolved in RNase-free water (20 µl). The concentrations of total RNA were measured by NanoDrop spectrophotometry (Thermo Scientific, Worcester, MA, USA). The reverse transcription of first strand cDNA was performed with a cDNA synthesis kit (iNtRON Biotech) according to the manufacturer's instructions. As previously described [18], qRT-PCR was performed with primers (Table 1) through the ABI StepOne system instrument using the power SYBR Green PCR master mix (Applied Biosystems, Foster City, CA, USA). Quantification of targeted mRNA was done by the ΔΔCt method. For the qRT-PCR assay, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was tested as an internal control.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

As previously described [27], HMC-1 cells were treated with AST2017-01 or chrysophanol for 48 h in microplate wells and then incubated with 40 µl of MTT (5 mg/ml, Sigma Chemical Co.) for additional 4 h at 37 °C under 5% CO_2 and 95% air. Consecutively, 500 µl of dimethyl sulfoxide was added to dissolve MTT formazan. The absorbance was measured at 540 nm by an automatic microplate reader.

Western blot analysis

The lysate of HMC-1 cells was centrifuged at 12,000 rpm for 10 min to precipitate the debris. The protein content of the cell lysate was estimated using a bicinchoninic acid protein assay kit (Sigma Chemical Co.). The proteins were then subjected to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by transferred onto nitrocellulose membranes (Millipore, Bedford, MA, USA). After blocked in 6% bovine serum albumin (Sigma Chemical Co.) for 2 h, the membranes were incubated with anti-MDM2, phosphorylated (p)STAT6, STAT6, p53, Bcl-2, Bax, caspase-3, TSLPR, tubulin, and GAPDH antibodies (1:500, Santa Cruz Biotechnology, Dallas, TX, USA) overnight at 4 °C and incubated then with the secondary antibodies (1:3000, Santa Cruz Biotechnology) for 1 h. Tubulin and GAPDH were used as loading controls. Immunoreactive bands were visualized in enhanced chemiluminesence assay system (Amersham Co., Newark, NJ, USA).

Table 1 The sequence of primers used for real-time PCR analysis

Genes	Sequences
Ki67	
Sense	ATA AAC ACC CCA ACA CAC ACA A
Antisense	GCC ACT TCT TCA TCC AGT TAC
TSLPR	
Sense	CAG AGC AGC GAG ACG ACA TT
Antisense	GGT ACT GAA CCT CAT AGA GG
IL-7Rα	
Sense	GCT CAG GGG AGA TGG ATC CT
Antisense	GTC TTC TTA TGA TCG GGG AG
IL-13	
Sense	GCC CTG GAA TCC CTG ATC A
Antisense	GCT CAG CAT CCT CTG GGT CTT
GAPDH	
Sense	TCG ACA GTC AGC CGC ATC TTC TTT
Antisense	CCA AAT CCG TTG ACT CCG ACC TT

Caspase-3 assay

Caspase-3 activities were analyzed using a caspase-3 colorimetric assay kit (R&D Systems) according to the manufacturer's protocol. Briefly, the harvested cell pellet is lysed by lysis buffer. The protein content of the cell lysate was estimated using a bicinchoninic acid protein assay kit. 50 µl of 2× reaction buffer including DTT stock was added to 50 µl of cell lysate. Consecutively, 5 µl of caspase-3 colorimetric substrate (DEVD-pNA) was added to the each reaction well of microplate. The plate was incubated at 37 °C. Absorbance at 405 nm was detected by an automatic microplate reader.

Enzyme-linked immunosorbent assay (ELISA)

Secreted protein levels of IL-6, IL-13, and TNF-α were determined using a sandwich ELISA method according to the manufacturer's instructions (IL-6 and TNF-α, BD Biosciences Pharmingen, San Diego, CA, USA; IL-13, R&D Systems Inc.). Absorbance was measured at 405 nm by an automatic microplate reader. Each amount of cytokine were expressed in ng/ml.

Statistical analysis

All data were obtained from at least three independent experiments with duplicate. Quantitative data were expressed as mean ± standard errors of means (SEMs). Statistical differences were performed using independent *t* test between two groups (unstimulated cells and TSLP-stimulated cells) and one-way ANOVA followed by a Tukey post hoc test among multiple groups. Each statistical analysis was performed using SPSS 22.0 statistic software (SPSS Inc., Chicago, IL, USA). A p value of less than 0.05 ($p < 0.05$) means statistically significant.

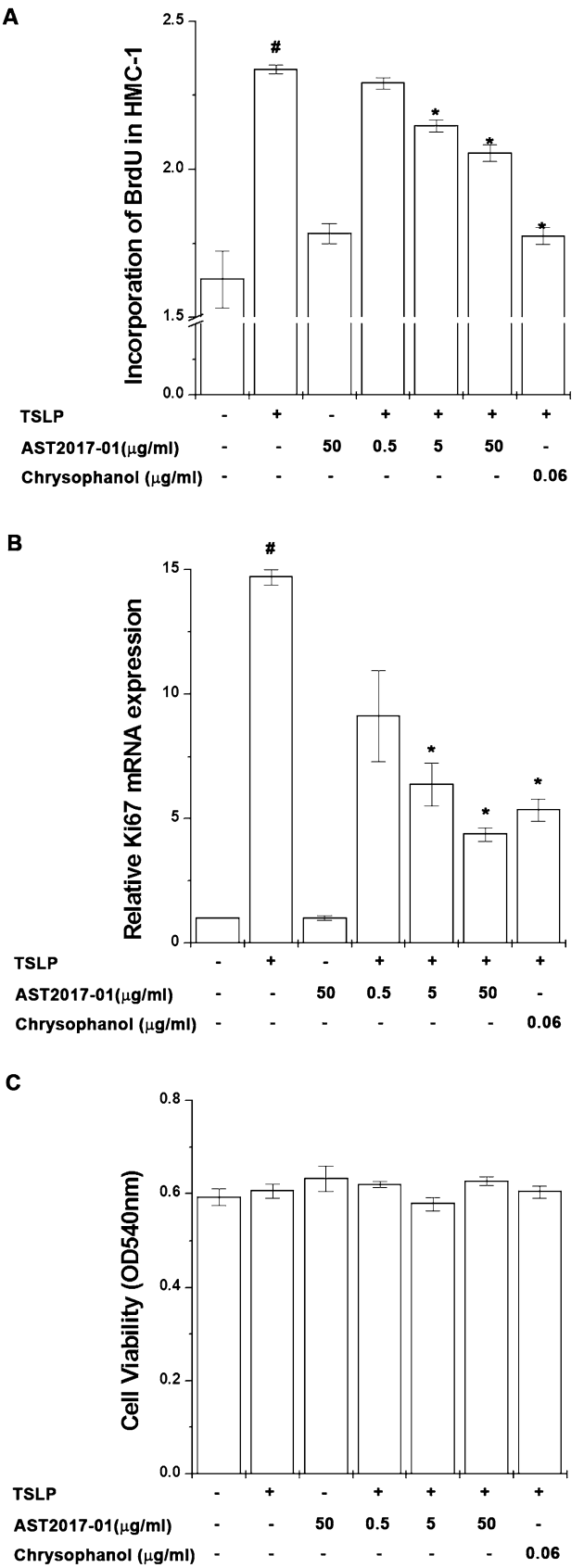

Fig. 1 Effect of AST2017-01 and chrysophanol on TSLP-induced growth of mast cells. Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 48 h. **a** A BrdU incorporation assay was performed. **b** Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 4 h. Ki67 mRNA expression was analyzed by qRT-PCR. **c** Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 48 h. Cell viability was analyzed by a MTT assay. Each datum represents the mean ± SEM of three independent experiments. $^{\#}p < 0.05$: significantly different from unstimulated cells. $^{*}p < 0.05$: significantly different from TSLP-stimulated cells

Results

Effect of AST2017-01 and chrysophanol on TSLP-induced growth of mast cells

Initially, we investigated whether AST2017-01 and chrysophanol could regulate the growth of HMC-1 cells. The inhibition of cell growth by AST2017-01 and chrysophanol was assessed by BrdU incorporation. As shown in Fig. 1a, AST2017-01 inhibited the TSLP-stimulated cell growth in a concentration-dependent manner. The significant inhibition of cell growth after TSLP stimulation was observed at 5 μg/ml of AST2017-01 (approximately 27%, $p < 0.05$) and 50 μg/ml (approximately 40%, $p < 0.05$). Chrysophanol also showed a significant decrease in the TSLP-stimulated cell growth ($p < 0.05$). Next, we investigated whether AST2017-01 and chrysophanol would regulate the mRNA expression of a cell growth marker, Ki67. TSLP significantly increased the Ki67 mRNA expression in HMC-1 cells (Fig. 1b, $p < 0.05$), but AST2017-01 (5 and 50 μg/ml) and chrysophanol significantly reduced the TSLP-induced Ki67 mRNA expressions (Fig. 1b, $p < 0.05$). To test cytotoxicity, we performed a MTT assay. AST2017-01 and chrysophanol had no effect on cytotoxicity at 48 h after TSLP stimulation (Fig. 1c).

Effects of AST2017-01 and chrysophanol on the expression of anti-apoptotic and apoptotic factors

Proteins p53, Bax, and Bcl-2 are important factors in cell cycle. The p53 and Bax affect the cell cycle arrest and apoptosis and high levels of Bcl-2 block the p53-induced apoptosis [28]. Thus, the regulatory effects of AST2017-01 and chrysophanol on the protein levels of p53, Bax, and Bcl-2 were analyzed by Western blotting. TSLP decreased the protein levels of p53 and Bax compared with the unstimulated cells, while increased the protein level of Bcl-2 (Fig. 2). However, AST2017-01 and chrysophanol recovered these changes induced by TSLP stimulation (Fig. 2).

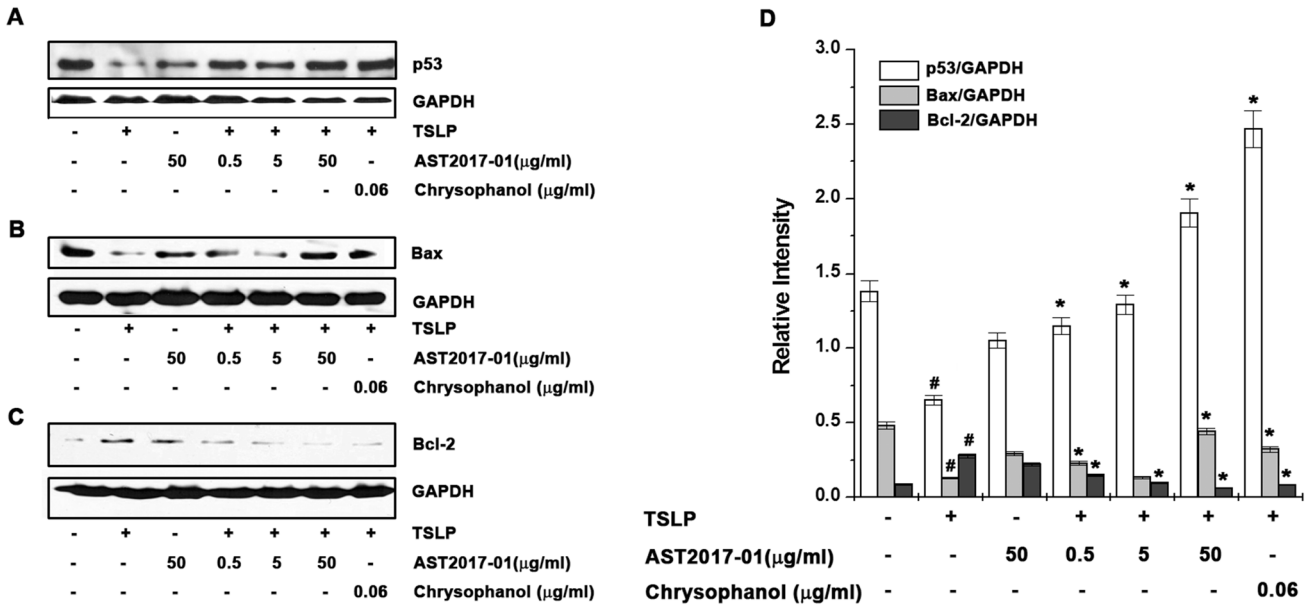

Fig. 2 Effect of AST2017-01 and chrysophanol on the expression of anti-apoptotic and apoptotic factors. Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 48 h. Levels of **a** p53, **b** Bax, and **c** Bcl-2 were analyzed by Western blotting. **d** The relative intensities quantified by densitometry mean p53/GAPDH, Bax/GAPDH, and Bcl-2/GAPDH. Results are representative of three independent experiments. Each datum represents the mean ± SEM of three independent experiments. $^{\#}p < 0.05$: significantly different from unstimulated cells. $^{*}p < 0.05$: significantly different from TSLP-stimulated cells

Effect of AST2017-01 and chrysophanol on activation of caspase-3

The activation of caspase-3 is an essential trait of cell cycle arrest. To determine whether AST2017-01 and chrysophanol could activate the caspase-3, we analyzed the caspase-3 activities by a caspase-3 assay and Western blotting. The results showed that AST2017-01 (5 and 50 μg/ml) increased the caspase-3 activities in HMC-1 cells when compared with the TSLP-stimulated cells in a concentration-dependent manner (Fig. 3a, $p < 0.05$). Chrysophanol also showed a significant increase in the caspase-3 activity (Fig. 3a, $p < 0.05$). Also, AST2017-01 and chrysophanol increased protein levels of caspase-3 (Fig. 3b).

Effects of AST2017-01 and chrysophanol on TSLP-induced MDM2 expression and STAT6 phosphorylation

Activations of MDM2 and STAT6 have been closely associated with TSLP-induced proliferation and survival of mast cells [10]. Thus, we investigated whether AST2017-01 and chrysophanol could regulate the expressions of MDM2 and pSTAT6. As a result, Fig. 4 showed that AST2017-01 and chrysophanol significantly reduced the protein levels of MDM2 and pSTAT6 increased by TSLP ($p < 0.05$).

Effects of AST2017-01 and chrysophanol on TSLP-induced IL-13 and inflammatory cytokine levels

IL-13 which is a Th2 cytokine and major effector in inflammatory reactions of allergic diseases was produced from TSLP-stimulated mast cells [29]. In the present study, TSLP significantly increased the production and mRNA expression of IL-13 in HMC-1 cells, but AST2017-01 and chrysophanol markedly reduced the TSLP-induced IL-13 levels (Fig. 5a, b, $p < 0.05$). In addition, we evaluated the inhibitory effect of AST2017-01 and chrysophanol on the productions of proinflammatory cytokines, IL-6 and TNF-α. The levels of IL-6 and TNF-α up-regulated by TSLP were significantly reduced by AST2017-01 and chrysophanol (Fig. 5c, d, $p < 0.05$).

Effects of AST2017-01 and chrysophanol on TSLP-induced TSLPR and IL-7Rα mRNA expression

Finally, we investigated whether the regulatory effect of AST2017-01 and chrysophanol on TSLP-induced mast cell proliferation would be associated with the regulation of TSLPR and IL-7Rα levels in HMC-1 cells. TSLP significantly increased TSLPR protein levels, but AST2017-01 and chrysophanol significantly reduced the TSLPR protein levels (Fig. 6a, b, $p < 0.05$). In addition, AST2017-01 and

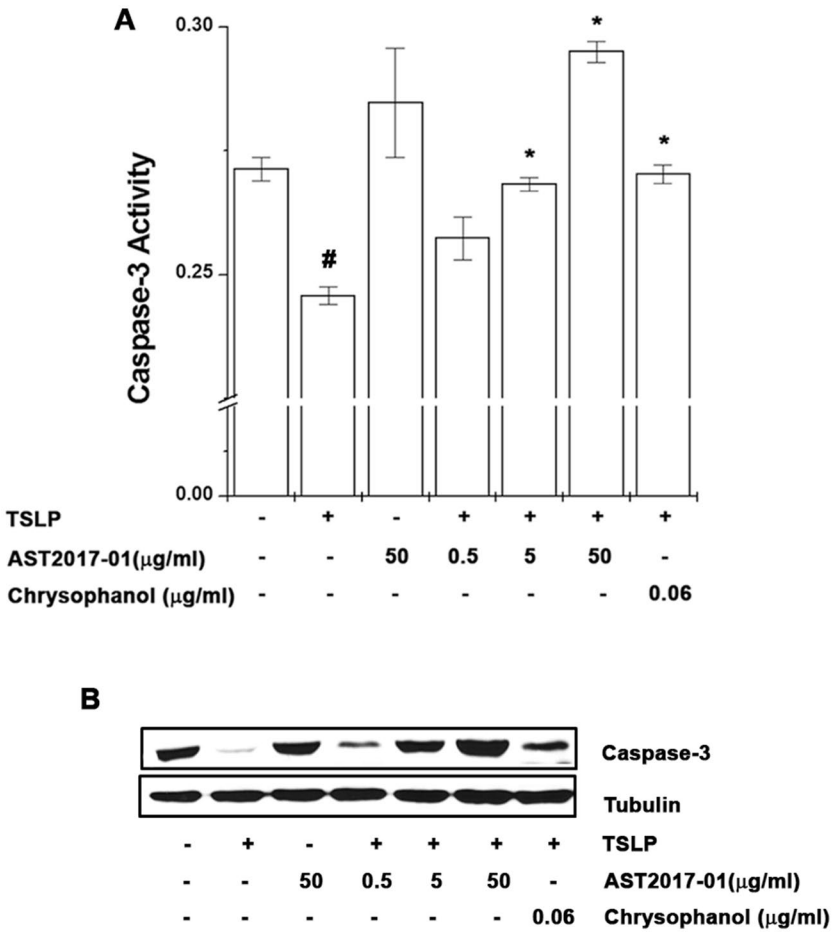

Fig. 3 Effect of AST2017-01 and chrysophanol on activation of caspase-3. Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 48 h. **a** Caspase-3 activity was analyzed using a caspase-3 colorimetric assay kit. **b** Caspase-3 protein levels were analyzed by Western blotting. Results are representative of three independent experiments. $^{\#}p < 0.05$; significantly different from unstimulated cells, $^{*}p < 0.05$, significantly different from TSLP-stimulated cells

chrysophanol significantly suppressed TSLPR and IL-7Rα mRNA levels (Fig. 6c, d, $p < 0.05$).

Discussion

Mast cell is predominantly considered as a key effector cell in allergic reaction and is considered to represent a variety of cells that may have an immuno-modulatory function in innate and adaptive immunity or activator [26]. In addition, diverse functions of mast cells have been proposed in various aspects of disease and health [26]. Especially, mast cells are known to have detrimental impacts on various diseases including autoimmune diseases, allergic diseases, and cancer [6, 25, 26, 30]. Mast cells are long-lived secretory cells derived from hematopoietic progenitor cells that generally complete their differentiation and maturation in the microenvironments of almost every vascular tissue [26]. Like monocyte lineage cells, the proliferation of mast cells can be induced by suitable stimulation of IL-3, stem cell factor, and TSLP [6, 31]. In addition, the maturation and survival of mast cells and the recruitment of mature mast cells contribute to the local accumulation of mast cells in tissues [6]. TSLP, stem cell factor, IL-3, and IL-13 signaling are essential for mast cells proliferation and development [6, 32]. Therefore, a pharmacological modulation targeting the proliferation of mast cell is a new strategy to prevent the development of various diseases including allergic diseases. Recently, we reported that rosmarinic acid reduced the allergen-induced allergic conjunctivitis via inhibiting TSLP-induced mast cell proliferation on in vivo and in vitro models [10]. The present study determined that AST2017-01 and chrysophanol suppressed the TSLP-induced HMC-1 cell proliferation. Therefore, we suggest that AST2017-01 and chrysophanol have an anti-allergic effect via blocking the mast cell proliferation.

The p53 which regulates the apoptosis pathway, is mainly regulated by interaction with regulatory proteins, translational regulation, and post-translational modifications [7]. Activation of p53 modulates expressions of its downstream effectors such as Bax and Bcl-2 and promotes cell death [7, 9]. Caspases, proteolytic enzymes that are largely known for their role in controlling cell death and inflammation, are activated via p53 signaling pathway [33]. The p53 was stabilized and activated by decreasing MDM2 expression [7]. Shangary et al. [34] have reported

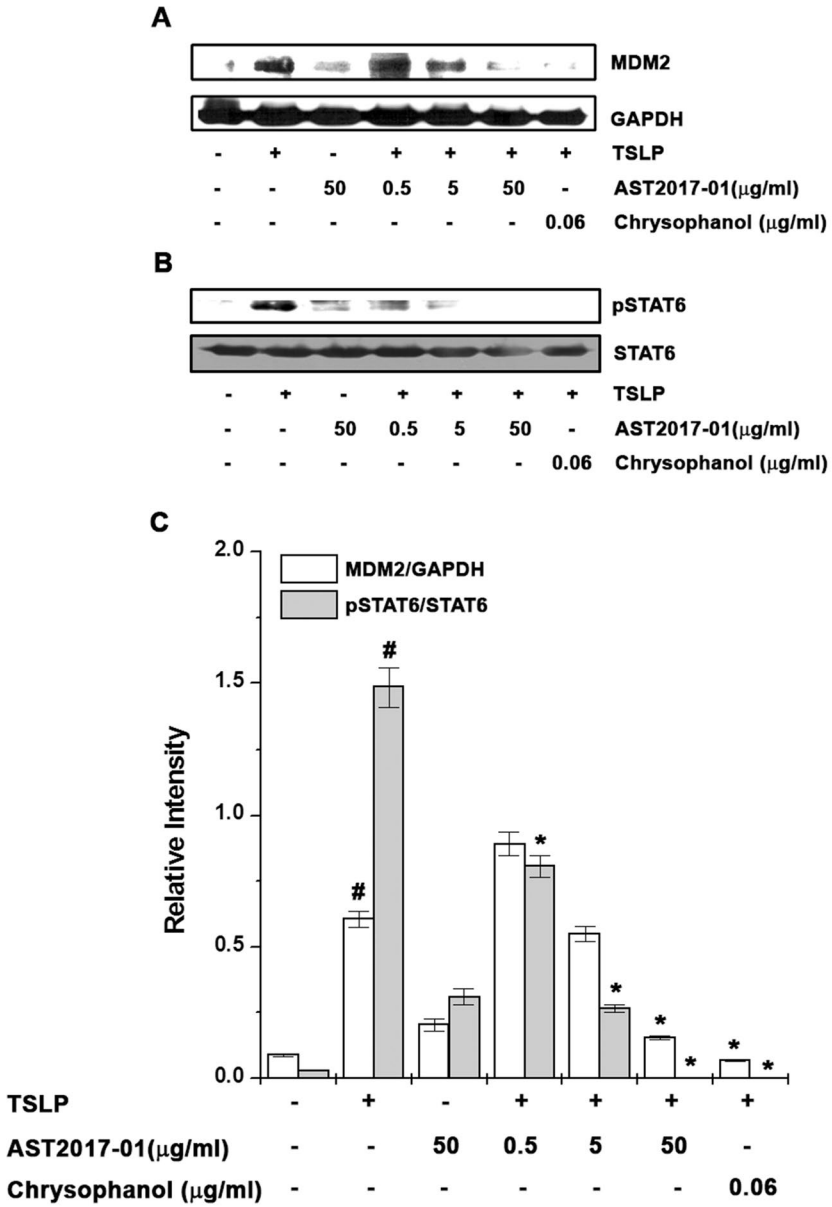

Fig. 4 Effect of AST2017-01 and chrysophanol on TSLP-induced MDM2 expression and STAT6 phosphorylation. Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 8 h. Expression of **a** MDM2 and **b** phosphorylation of STAT6 were analyzed by Western blotting. **c** The relative intensities quantified by densitometry mean MDM2/GAPDH and pSTAT6/STAT6. Results are representative of three independent experiments. Each datum represents the mean ± SEM of three independent experiments. $^{\#}p < 0.05$: significantly different from unstimulated cells. $^{*}p < 0.05$: significantly different from TSLP-stimulated cells

that a MDM2 inhibitor reduces cell proliferation by disrupting p53-MDM2 interaction. The MDM2 which is an E3 ligase regulates the degradation of p53 [35]. The p53-MDM2 pathway regulates cell proliferation and apoptosis [36]. In human mast cell, TSLP increased the cell proliferation via decreasing p53 levels and increasing MDM2 levels [10]. Furthermore, TSLP activates STAT6 which is a proliferator or differentiator of mast cells [37]. STAT6 activated by TSLP increased the MDM2 expression [6]. In this study, we showed that AST2017-01 and chrysophanol activated the p53 signaling pathway and blockaded the MDM2 signaling pathway. Therefore, we suggest that AST2017-01 and chrysophanol have an anti-proliferative effect via the modulation of p53/MDM2/STAT6 pathway.

In the present study, we found that AST2017-01 and chrysophanol suppressed the proliferation of mast cell and increased the activation of caspase-3 without affecting cell viability and apoptosis. Interestingly, Racke et al. [38] have demonstrated that activation of caspase-3 alone is insufficient for apoptosis of human neuroblastoma cells and activation of another caspase is required for apoptosis. The proliferation or activation of T lymphocytes was regulated with increase in activation of caspase-3 in the absence of any detectable cell death [39–41]. In addition, a cell apoptosis assay of our previous study showed no significant difference in cell death between unstimulated group and TSLP-stimulated group in HMC-1 cells [42]. We also found that AST2017-01 and chrysophanol had no effect on

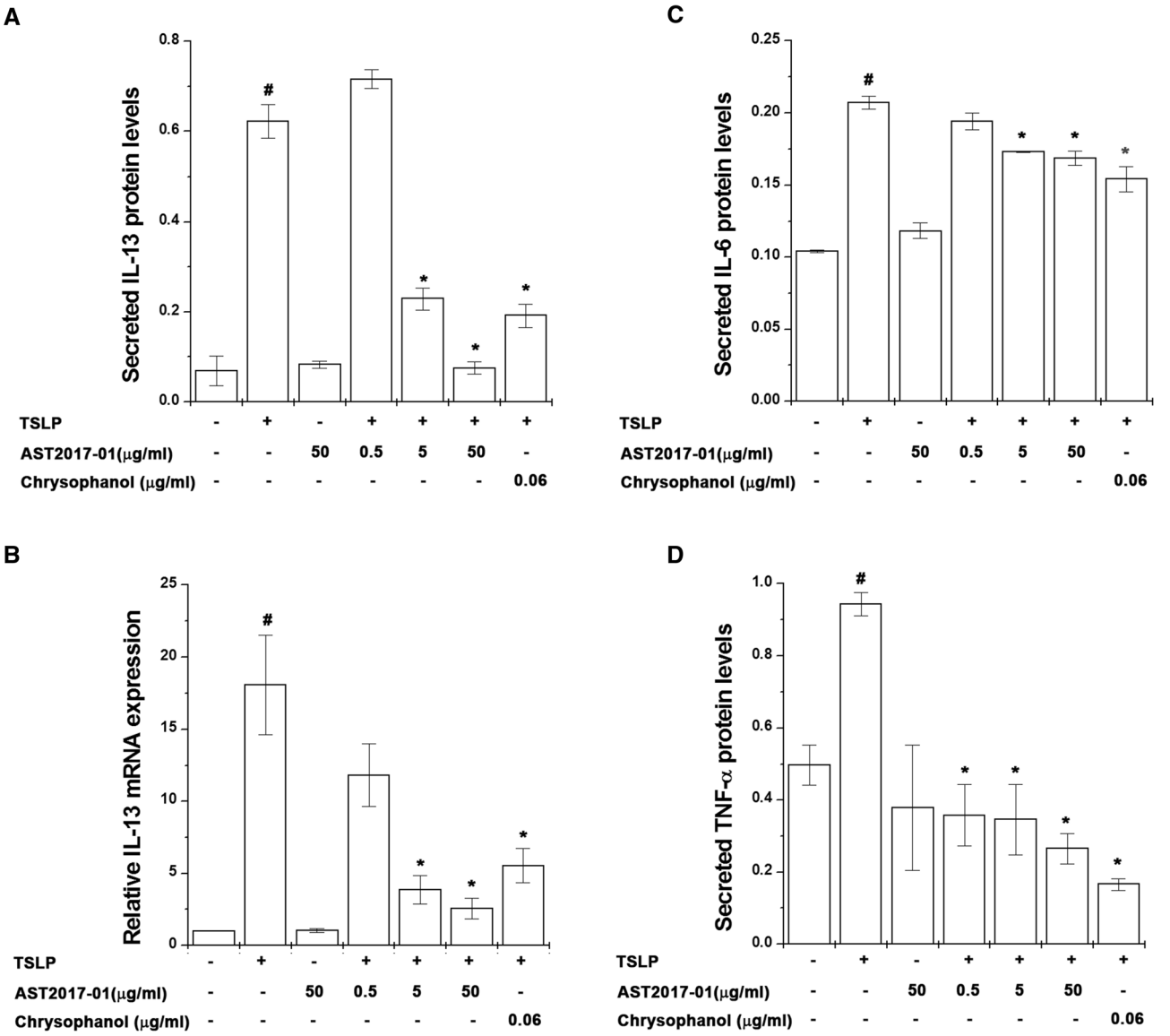

Fig. 5 Effects of AST2017-01 and chrysophanol on TSLP-induced IL-13 and inflammatory cytokine levels. Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 8 h. **a** The level of IL-13 secreted in the culture supernatant of the cells was measured by ELISA. **b** Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 4 h. The total mRNA was analyzed by qRT-PCR. **c**, **d** Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 8 h. The levels of IL-6 and TNF-α secreted in the culture supernatant of the cells were measured by ELISA. Each datum represents the mean ± SEM of three independent experiments. $^{\#}p < 0.05$: significantly different from unstimulated cells. $^{*}p < 0.05$: significantly different from TSLP-stimulated cells

cytotoxicity at 72 h after TSLP stimulation in HMC-1 cells (data not shown). Thus, we suggest that AST2017-01 and chrysophanol could down-regulate the proliferation of mast cell via activation of caspase-3 without detectable cell death. However, further research is needed to clarify the regulatory effects of AST2017-01 and chrysophanol on apoptosis.

Mast cells are considered to play an important role in allergic inflammatory reactions. In patients with allergic inflammatory diseases, the number of mast cells and levels of mast cells-mediated mediators were increased. [43]. The binding of TSLP to TSLPR in mast cells causes the secretion of IL-1β, IL-6, IL-13, IL-8, and TNF-α [10]. Inflammatory cytokines induced by TSLP trigger and maintain allergic inflammation [44]. Administration of IL-6 and TNF-α to humans caused inflammation, tissue destruction, shock, and death [45]. Our previous studies has showed that AST2017-01 and chrysophanol relieve clinical severity in atopic dermatitis-like lesions [19] and suppress inflammatory cytokine

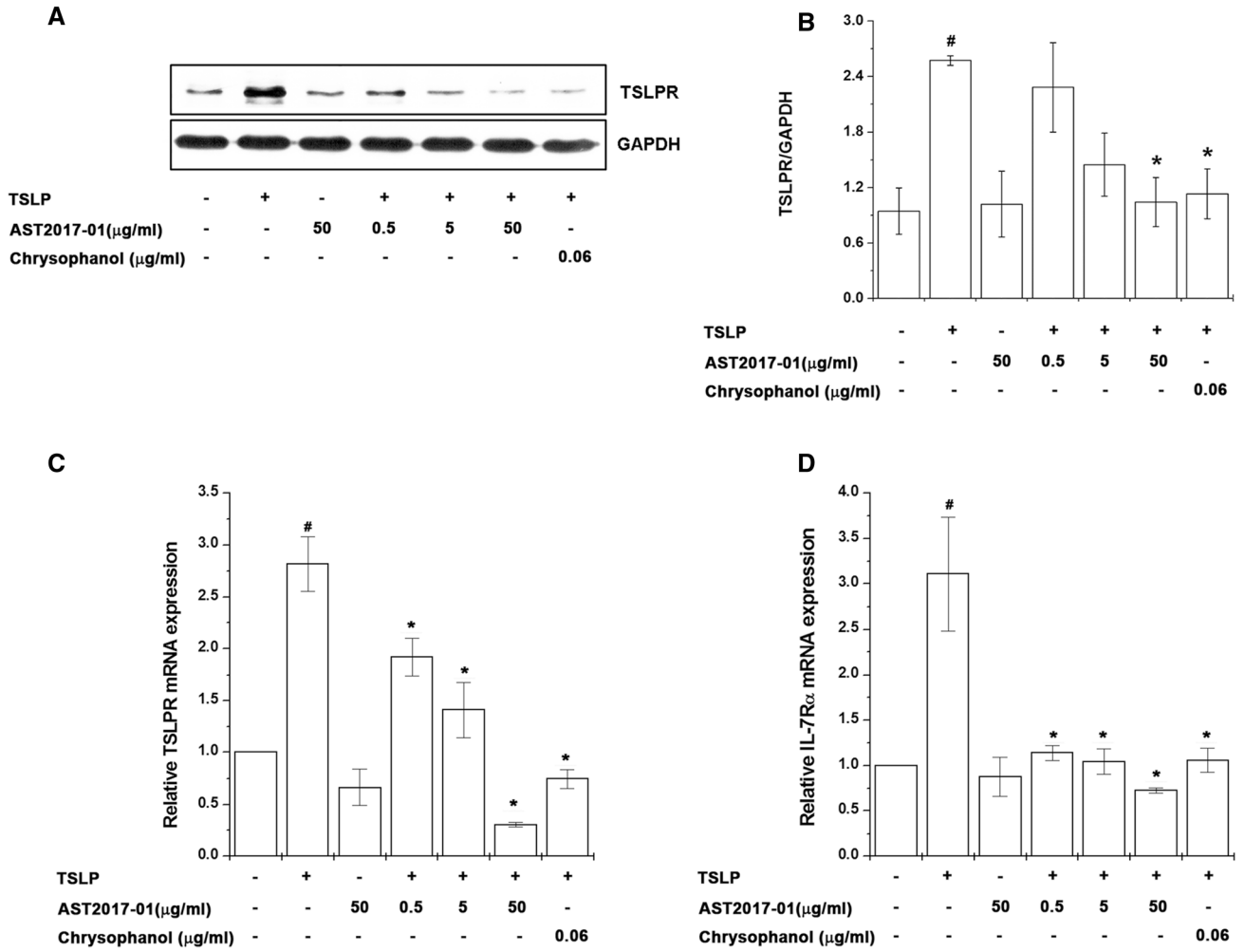

Fig. 6 Effect of AST2017-01 and chrysophanol on TSLP-induced TSLPR and IL-7Rα transcription levels. **a** Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 8 h. Expression of TSLPR was analyzed by Western blotting. **b** The relative intensities quantified by densitometry mean TSLPR/GAPDH. Results are representative of three independent experiments. **c**, **d** Cells were pretreated with AST2017-01 and chrysophanol for 1 h prior to TSLP (20 ng/ml) stimulation for 4 h. The total mRNA was analyzed by qRT-PCR. Each datum represents the mean ± SEM of three independent experiments. $^{\#}p < 0.05$: significantly different from unstimulated cells. $^{*}p < 0.05$: significantly different from TSLP-stimulated cells

production from activated HMC-1 cells [18]. The present study determined that AST2017-01 and chrysophanol abrogated the IL-6, IL-13, and TNF-α levels in the TSLP-stimulated HMC-1 cells. In addition, AST2017-01 and chrysophanol diminished the TSLPR and IL-7Rα levels. Therefore, we propose that AST2017-01 and chrysophanol might inhibit inflammatory reaction by blocking expression of TSLP receptor.

Conclusions

These results show that AST2017-01 and chrysophanol alleviate the allergic inflammatory reactions through suppressing the TSLP signaling pathway. These finding propose that AST2017-01 and chrysophanol would be helpful in preventing and treating allergic diseases.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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