Sesamin attenuates mast cell-mediated allergic responses by suppressing the activation of p38 and nuclear factor-κB

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Received October 22, 2014; Accepted September 22, 2015

DOI: 10.3892/mmr.2015.4546

Abstract. Establishing therapeutic agents for the treatment of allergic diseases is an important focus of human health research. Sesamin, a lignan in sesame oil, exhibits a diverse range of pharmacological properties. However, to the best of our knowledge, the effect of sesamin on mast cell-mediated allergic responses has not yet been investigated. Thus, the aim of the present study was to investigate the effect of sesamin on mast cell-mediated allergic responses and the underlying mechanisms by which it produces this effect. In rats, oral administration of sesamin inhibited passive cutaneous anaphylaxis. Sesamin exposure attenuated immunoglobulin E-induced histamine release from rat peritoneal mast cells, which was indicated to be mediated by the modulation of intracellular calcium. In human mast cells, sesamin reduced the stimulatory effects of phorbol 12-myristate 13-acetate and calcium ionophore A23187 on the production and secretion of pro-inflammatory cytokines, including tumor necrosis factor-α and interleukin-6. The inhibitory effect of sesamin on pro-inflammatory cytokine production was dependent on nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and p38 mitogen-activated protein kinase (MAPK). The present study demonstrates that sesamin inhibits mast cell-derived inflammatory allergic reactions by blocking histamine release, and pro-inflammatory cytokine production and secretion. In addition, the findings indicate that the effect of sesamin is mediated by its effect on p38 MAPK/NF-κB signaling. Furthermore, the *in vivo* and *in vitro* anti-allergic effects of sesamin reported in the present study suggest that it is a promising therapeutic agent for the treatment of inflammatory allergic diseases.

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Key words: sesamin, allergic response, mast cell, histamine, calcium, nuclear factor κ-light-chain-enhancer of activated B cells

Introduction

Mast cells are major effector cells of allergic inflammatory reactions with considerable influence on the pathogenesis of a number of disorders, including contact asthma, allergic rhinitis, tissue remodeling, rheumatoid arthritis and anaphylaxis (1,2). Mast cells are activated by the process of degranulation in response to antigen cross-linking of immunoglobulin E (IgE) bound to the high-affinity IgE receptor (Fcε RI), which results in phosphorylation of Syk tyrosine kinase, mobilization of internal calcium, activation of protein kinase C, mitogen-activated protein kinases (MAPKs), nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and release of inflammatory cytokines (3,4). Mast cell activation and degranulation releases inflammatory mediators, such as histamine, and chemotactic cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-6. Various acute and chronic allergic responses are induced by these mediators (5,6).

Lignans from *Sesamum indicum* (sesame) seeds are potent antioxidants, the most abundant of these lignans in sesame seed oil is sesamin (7). Sesamin inhibits lipopolysaccharide-induced IL-6 production by suppressing p38 MAPK and NF-κB activation in murine microglia and BV-2 cells (8). Sesame seed oil accelerates recovery from colon inflammation in rats with induced acute colitis by inhibiting inflammatory processes and sesamin suppresses macrophage-derived chemokine expression in human monocytes (9,10). However, the association between sesamin and mast cell-mediated anaphylactic reactions is poorly understood.

The aim of the present study was to assess the effect of sesamin on inflammatory allergic reactions, and to investigate the molecular mechanisms underlying the inhibitory effect of sesamin on histamine release and pro-inflammatory cytokine production in mast cells. In addition, the effect of sesamin on systemic and local allergic reactions was examined to assess its anti-allergic effect *in vivo*.

Materials and methods

Reagents and cell culture. Sesamin, anti-2,4-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187, pyrrolidine dithiocarbamate (PDTC), azelastine, anti-β actin antibodies, and HEPES were purchased from Sigma-Aldrich (St. Louis, MO, USA). Percoll solution was purchased from Pharmacia Biotech (Uppsala, Sweden). Sesamin was dissolved in a vehicle consisting of 0.5% (w/v) carboxy methylcellulose (Boster Biological Technology Co., Ltd., Wuhan, China) and 0.025% Tween-20 (Sigma-Aldrich) in distilled water.

Animals. Male ICR mice (n=60; 6 weeks old; 25-30 g) and male Sprague-Dawley rats (n=10; 8 weeks old; 230-280 g) were obtained from the in-house animal facility of Yanbian University Health Science Center (Yanji, China). The animals were housed 3-5 per cage in a laminar air-flow cabinet that was maintained at a temperature of 22±1˚C and relative humidity of 55±10%, under a 12-h light/dark cycle for 1 week prior to the experiments. Water and a standard diet were provided *ad libitum* throughout the study. The experiments were performed in compliance with the guidelines approved by the Institutional Animal Care and Use Committee of Yanbian University School of Basic Medical Sciences (Yanji, China).

Anti-DNP IgE-mediated passive cutaneous anaphylaxis (PCA). Anti-DNP IgE-mediated PCA was examined as reported previously (11). The PCA reaction was generated by sensitizing skin with an intradermal injection of 500 ng anti-DNP IgE in 50 ml phosphate-buffered saline (PBS). After 24 h, each mouse received an injection of 20 ml PBS containing 100 mg antigen, DNP-HSA, and 1% Evans blue (Sigma-Aldrich) via the tail vein. Sesamin (50, 100 or 200 mg/kg) was administered orally 1 h prior to the antigen challenge. Azelastine, an anti-histamine and mast cell stabilizing agent, was orally administered at a dose of 10 mg/kg 1 h prior to the antigen challenge. The mice were sacrificed by terminal anesthesia via intraperitoneal injection of pentobarbital (50 mg/kg; Boster Biological Technology Co., Ltd.) 30 min subsequent to the antigen challenge and the dorsal skin around the intradermal injection site was removed to weigh the pigmented area, which was followed by extraction of the extravasated Evans blue dye by incubation of the biopsies in 1 ml formamide at 55˚C for 24 h. The absorbance of the dye was measured at 620 nm using a Spectra Max Plus spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA, USA). The concentrations of Evans blue dye were quantified by interpolation on a standard curve of dye concentrations in the range of 0.01-30 mg/ml.

Preparation of rat peritoneal mast cells (RPMC). RPMCs were isolated as described previously (12). The rats were anesthetized with 5 ml/L ether, 10 ml calcium-free HEPES-Tyrode buffer (137 mM NaCl, 5.6 mM glucose, 12 mM $NaHCO_3$, 2.7 mM KCl, 0.3 mM NaH_2PO_4 and 0.1% gelatin; Sigma-Aldrich) was then injected into the peritoneal cavity, and the abdomen was gently massaged for ~90 sec. The peritoneal cavity was opened carefully and the fluid that contained peritoneal cells was collected by Pasteur pipette. RPMCs were purified using a Percoll density gradient, as described previously (13). RPMC preparations were ~95% pure, as assessed by toluidine blue staining (Sigma-Aldrich), and >98% of the cells were viable, as determined by Trypan blue uptake. Purified mast cells ($1x10^6$ cells/ml) were resuspended in HEPES-Tyrode buffer.

Mast cell viability assay. Mast cell viability was determined and the MTT colorimetric assay was performed, as described previously (14). RPMCs were incubated with various concentrations of sesamin (25-100 μg/ml) at 37˚C for 2 h. Following the addition of MTT (100 mg in 100 ml PBS), RPMCs were incubated at 37˚C for 1 h, and absorbance was measured at 570 nm with the spectrophotometer.

Histamine release assay. The histamine content of RPMCs was evaluated according to a radioenzymatic method, as previously described (15). Purified RPMCs were sensitized with 10 μg/ml anti-DNP IgE for 6 h and preincubated with sesamin (25, 50 and 100 μM) or azelastine (100 μM) at 37˚C for 30 min prior to challenge with DNP-HSA (100 ng/ml). Following centrifugation at 150 x g for 10 min at 4˚C, the supernatant was harvested for measurement of histamine content. The percentage inhibition of histamine release was calculated according to the following formula: Inhibition (%) = [1-(T-B)/(C-N)]x100, where C is the control (IgE without sesamin), N is the normal group (no IgE and no sesamin), T is the test group (IgE and sesamin) and B is the blank group (sesamin without IgE).

Measurement of ^{45}Ca uptake. The calcium uptake of the mast cells was measured using the procedure of Choi *et al* (16). RPMCs were resuspended in HEPES-Tyrode buffer containing ^{45}Ca (1.5 mCi/ml; 1 Ci = $3.7x10^{10}$ Bq; Perkin-Elmer Inc., Waltham, MA, USA) at 4˚C for 10 min. Mast cell suspensions were sensitized with 10 μg/ml anti-DNP IgE for 6 h and preincubated with prewarmed buffer containing sesamin. The reaction proceeded for 30 min at 37˚C prior to the challenge with DNP-HSA (100 ng/ml) and was terminated by the addition of 1 mM lanthanum chloride (Sigma-Aldrich). The samples were centrifuged three times at 150 x g for 10 min, and mast cells were lysed with 10% Triton X-100 (Sigma-Aldrich) with vigorous agitation. Sample radioactivity was determined with a scintillation β-counter (B1600/1600 Tri-Carb Liquid Scintillation Analyzer; Canberra Industries, Inc., Meriden, CT, USA).

Assay of TNF-α and IL-6 secretion. The HMC-1 human mast cell line (American Type Culture Collection, Manassas, VA, USA) was used. The cells were treated with 20 nM PMA and 1 μM A23187 for 4 h in the absence or presence of various concentrations of sesamin (25, 50 and 100 μM). TNF-α and IL-6 concentrations in the supernatant were determined using Biosource ELISA kits according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA, USA).

Western blot analysis. HMC-1 cells were treated with 20 nM PMA and 1 μM A23187 for 4 h in the absence or presence of sesamin (25, 50 or 100 μM). Cell extracts were prepared by using a detergent lysis procedure, as described previously (17). Samples of protein (30 μg) were loaded per lane and subject to 12% SDS-PAGE (Bio-Rad Laboratories, Inc., Hercules,

CA, USA) at 120 V for 90 min. Separated proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA). p38 MAPK, extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinase (JNK) activation were determined using rabbit monoclonal anti-p38 MAPK (D13E1; cat. no. 8690s; 1:1000), phosphorylated (p)-p38 MAPK (Thr180/Tyr182; 3D7; cat. no. 4092s; 1:1,000), p-p44/42 MAPK (Erk1/2; Thr202/Tyr204; 197G2; cat. no. 14227s; 1.1000), p44/42 MAPK (Erk1/2; 137F5; cat. no. 4348s; 1:1,000), P-SAPK/JNK (Thr183/Tyr185; 81E11; cat. no. 4668s; 1:1,000) and SAPK/JNK (cat, no, 9252s; 1:1,000) antibodies (Cell Signaling Technology, Inc., Beverly, MA, USA). TNF-α and IL-6 were measured using rabbit polyclonal anti-TNF-α (h-156; cat. no. sc-8301; 1:1,000) and anti-IL-6 (H-183; cat. no. sc-7920; 1:1,000) antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) incubated at 4˚C for 2 h. Immunodetection was performed using an enhanced chemiluminescence detection kit (Amersham ECL Select Detection System; Amersham Pharmacia Biotech).

Nuclear protein extraction for analysis of NF-κB. Nuclear protein was extracted according to the procedure of Choi and Yan (18). Cells were harvested, washed three times and lysed in 2 volumes of lysis buffer A [50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% glycerol, 0.5 mM dithiothreitol, 5 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride (PMSF) and protease inhibitor cocktail; Sigma-Aldrich] for 5 min at 4˚C. Subsequently, the cell suspension was centrifuged at 1,000 x g for 15 min at 4˚C. The supernatant fraction was incubated on ice for 10 min and centrifuged at 100,000 x g for 1 h at 4˚C to obtain cytosolic protein extracts. The pelleted nuclei were resuspended in buffer B (1.3 M sucrose, 1.0 mM $MgCl_2$ and 10 mM potassium phosphate buffer; pH 6.8; Sigma-Aldrich) and centrifuged at 1,000 x g for 15 min. The pellets were suspended in buffer B with a final sucrose concentration of 2.2 M and centrifuged at 100,000 x g for 1 h. The resulting nuclear pellets were washed once with a solution containing 0.25 M sucrose, 0.5 mM $MgCl_2$ and 20 mM Tris-HCl (pH 7.2) and centrifuged at 1,000 x g for 10 min. The pellets were solubilized with a solution containing 50 mM Tris-HCl (pH 7.2), 0.3 M sucrose, 150 mM NaCl, 2 mM EDTA, 20% glycerol, 2% Triton X-100, 2 mM PMSF and protease inhibitor cocktail. The mixture was maintained on ice for 1 h with gentle stirring and centrifuged at 12,000 x g for 30 min. The resulting supernatant served as the soluble nuclear protein sample. For western blot analysis, samples (30 μg protein per lane) were subjected to 10% SDS-PAGE at 120 V for 90 min, and separated proteins were transferred to PVDF membranes using the wet transfer method (250 mA; 90 min). Nonspecific sites were blocked with 5% non-fat dry milk in PBS for 1 h, and the blots were incubated with antibodies against NF-κB (Upstate Biotechnology, Inc., Lake Placid, NY, USA) overnight at 4˚C. Horseradish peroxidase-conjugated goat anti-rabbit IgG (cat. no. sc-2004; 1:1,000) was used to detect bound antibodies. Protein bands were visualized by exposing the membranes to photographic film (X-OMAT BT photographic film; Kodak, Rochester, NY, USA) following treatment with the enhanced chemiluminescence system reagents.

Table I. Inhibitory effect of sesamin on anti-DNP IgE-mediated passive cutaneous anaphylaxis in rats.

Treatment (mg/kg)	Anti-DNP IgE	Evans blue (μg/g)	Inhibition (%)
Sesamin			
Group N			
0	-	46.51±4.21	-
Group B			
50	-	47.23±3.42	-
100	-	48.34±5.25	-
200	-	45.75±4.34	-
Azelastine			
10	-	44.92±3.89	-
Sesamin			
Group C			
0	+	263.82±13.64	-
Group T			
50	+	235.49±11.28	13.37
100	+	186.54±10.81[a]	36.40
200	+	162.56±13.26[a]	46.25
Azelastine			
10	+	150.63±9.83[a]	51.36

Sesamin was administered orally 1 h prior to the antigen challenge (DNP-human serum albumin). Each result for Evans blue represents the mean ± standard error of five independent experiments. The following formula was used to calculate percentage inhibition: Inhibition (%) = [1-(T-B)/(C-N)]x100, where C is control (IgE without sesamin), N is normal (no IgE and no sesamin), T is test (IgE and sesamin) and B is blank (sesamin without IgE). Azelastine (10 mg/kg) served as a positive anti-allergic control. [a]P<0.05 vs. the 0-mg/kg sesamin treatment. DNP, 2,4-dinitrophenyl; IgE, immunoglobulin E.

Statistical analysis. Results are expressed as the mean ± standard error of the mean. Statistical evaluation of the results was performed using one-way analysis of variance followed by Duncan's multiple range test. P<0.05 was considered to indicate a statistically significant difference.

Results

Sesamin inhibits PCA induced by anti-DNP IgE. To confirm the anti-allergic effects of sesamin *in vivo*, extravasation was induced by a local injection of anti-DNP IgE into the dorsal skin followed by an intravenous antigenic challenge in PCA model rats. After 24 h, the animals were injected intravenously with DNP-HSA and Evans blue dye. Sesamin was administered orally 1 h prior to the antigen challenge. Sesamin (50-200 mg/kg) inhibited dye extravasation in a dose-dependent manner (Table I). Similarly, azelastine significantly inhibited the PCA reaction at a dose of 10 mg/kg compared with the control (P<0.05).

Effect of sesamin on mast cell viability. The viability of RPMCs exposed to sesamin was determined by MTT assay.

RPMC viability was ~100% following exposure to 100 μM sesamin for 2 h (data not shown). Thus, sesamin was not cytotoxic in RPMCs.

Sesamin inhibits the release of histamine induced by IgE in RPMCs. RPMCs were preincubated with sesamin at concentrations of 25-100 μM. As demonstrated in Fig. 1, sesamin inhibited anti-DNP IgE-mediated histamine release from RPMCs in a concentration-dependent manner (13, 43 and 62% inhibition at 25, 50 and 100 μM, respectively). Similarly, azelastine significantly inhibited histamine release at a dose of 100 μM (75% inhibition; P<0.05). These results suggest that sesamin inhibits IgE-induced anaphylactic reactions by blocking histamine release from mast cells.

Sesamin inhibits calcium uptake into RPMCs. Calcium movement across mast cell membranes is critical to histamine release (19). Thus, to investigate the mechanisms by which sesamin inhibits histamine release, calcium uptake was measured. As presented in Fig. 2, antigen-elicited calcium uptake was inhibited in a concentration-dependent manner by sesamin, and significant effects were produced at concentrations of 50-100 μM (P<0.05). These results suggest that sesamin downregulates IgE-mediated histamine release by reducing calcium uptake by RPMCs.

Sesamin inhibits pro-inflammatory cytokine expression in human mast cells. The HMC-1 cell line has been established as a useful cell type in which to study signaling events leading to cytokine activation (20). As mast cell activation stimulates cytokine release, the present study investigated whether sesamin exposure regulated the release of pro-inflammatory cytokines, such as TNF-α and IL-6 in HMC-1 cells. Western blotting indicated that stimulation of HMC-1 cells with 20 nM PMA and 1 μM A23187 for 4 h induced TNF-α and IL-6 production. Sesamin inhibited protein expression of TNF-α and IL-6 in stimulated HMC-1 cells in a concentration-dependent manner (Fig. 3A). The ability of sesamin to suppress the secretion of TNF-α and IL-6 was investigated by evaluating their abundance in the HMC-1 cell medium. Consistent with the western blot analysis, ELISA analysis demonstrated that treatment with sesamin inhibited TNF-α and IL-6 secretion induced by PMA and A23187 in stimulated HMC-1 cells (Fig. 3B).

Sesamin inhibits p38 MAPK activation. The MAPK signaling cascade is an important pathway in the regulation of pro-inflammatory molecules that mediate cellular responses (21). Sesamin inhibited the activation of p38 MAPK in HMC-1 cells (Fig. 4A), however, it had no effect on phosphorylation of ERK and JNK (data not shown). Furthermore, western blot analysis and ELISA (Fig. 4B and C) demonstrated that exposure to the selective p38 MAPK inhibitor, SB 203580 (5 μM) decreased TNF-α and IL-6 production that had been induced by PMA and A23187 stimulation in HMC-1 cells.

Sesamin inhibits NF-κB activation. Previous studies have demonstrated that NF-κB is critical in immune and inflammatory responses, and identified that it is an important transcriptional regulator of inflammatory cytokines (22-24).

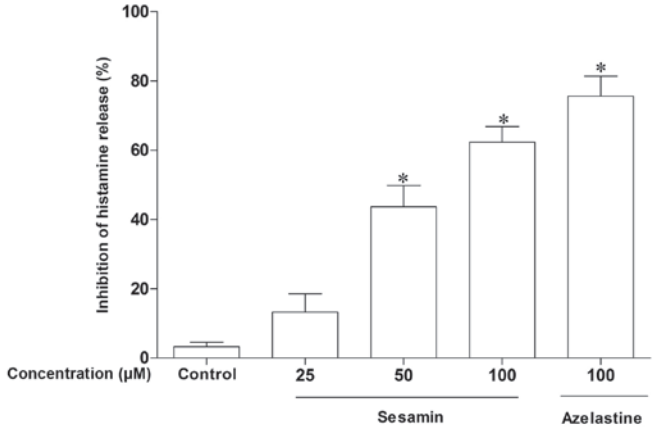

Figure 1. Inhibitory effect of sesamin on anti-DNP IgE-mediated histamine release from RPMCs. Purified RPMCs were sensitized with 10μg/ml anti-DNP IgE for 6 h and preincubated with sesamin (25, 50 or 100 μM) or azelastine (100 μM) at 37˚C for 30 min prior to the challenge with DNP-human serum albumin (100 ng/ml). Each result represents the mean ± standard error of the mean of five independent experiments. *P<0.05, vs. control. IgE, immunoglobulin E; RPMC, rat peritoneal mast cell; DNP, 2,4-dinitrophenyl.

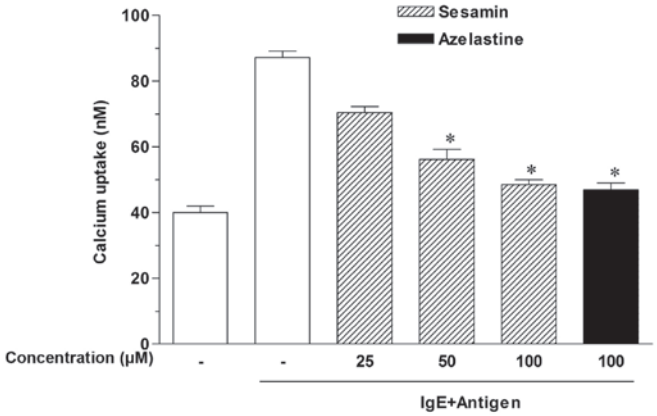

Figure 2. Inhibitory effect of sesamin on anti-DNP IgE-mediated calcium uptake in RPMCs. Purified RPMCs were sensitized with 10 μg/ml anti-DNP IgE for 6 h and preincubated with sesamin (25, 50, or 100 μM) or azelastine (100 μM) at 37˚C for 30 min prior to the challenge with DNP-human serum albumin (100 ng/ml). The treatment group on the far left (white bar) is the normal RPMC group; the white bar to its right indicates the group of RPMCs sensitized with 10 μg/ml anti-DNP IgE for 6 h without sesamin or azelastine). Each result represents the mean ± standard error of the mean of five independent experiments. *P<0.05, vs. 10 μg/ml anti-DNP IgE without sesamin or azelastine. RPMCs, rat peritoneal mast cells; DNP, 2,4-dinitrophenyl.

To investigate the intracellular mechanism responsible for the inhibitory effect of sesamin on TNF-α and IL-6 expression levels, the effect of sesamin on NF-κB activity was examined using western blotting. Stimulation of HMC-1 cells with PMA and A23187 induced nuclear translocation of NF-κB p65. Sesamin inhibited nuclear translocation of NF-κB p65 induced by PMA and A23187, and increased the expression levels of NF-κB p65 in the cytosol preparations (Fig. 4A). These results indicate that sesamin inhibits NF-κB activity by preventing its translocation into the nucleus. In addition, western blotting (Fig. 4B) and ELISA (Fig. 4C) demonstrated that TNF-α and IL-6 production induced by PMA and A23187 stimulation were decreased by treatment with PDTC, a potent NF-κB inhibitor.

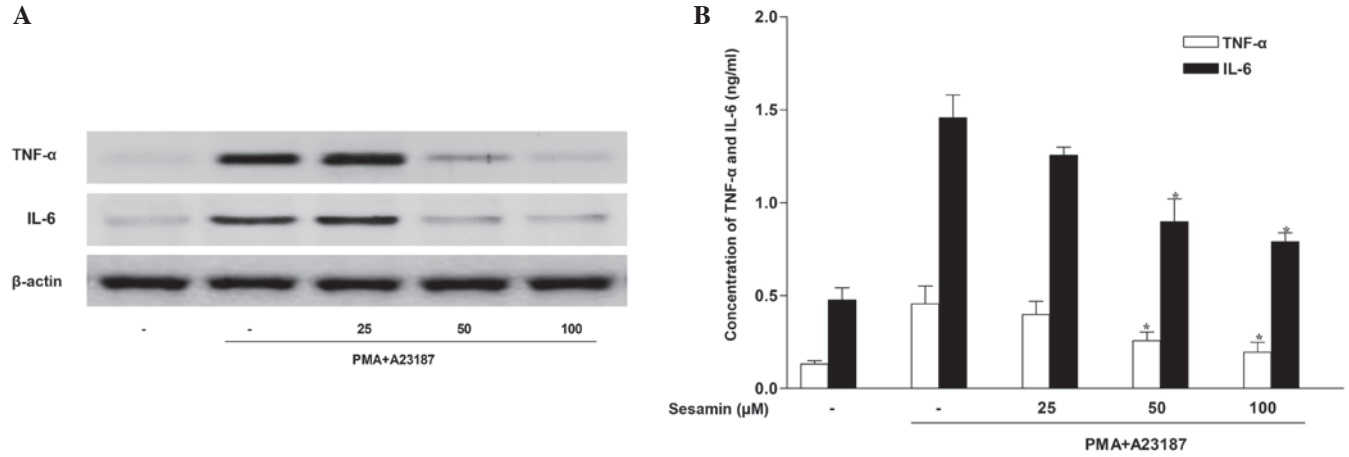

Figure 3. Inhibitory effect of sesamin on protein expression and production of cytokines (TNF-α and IL-6) in HMC-1 cells. HMC-1 cells were stimulated with 20 nM PMA and 1 μM calcium ionophore A23187 for 4 h in the absence or presence of sesamin (25, 50 or 100 μM). (A) Protein expression of cytokines TNF-α and IL-6 was evaluated by western blot analysis. The result is representative of at least five separate experiments with comparable results. (B) The abundance of TNF-α and IL-6 in the supernatant was measured using ELISA. Each result represents the mean ± standard error of the mean of five independent experiments. *P<0.05. PMA, phorbol 12-myristate 13-acetate; TNF, tumor necrosis factor; IL-6, interleukin-6.

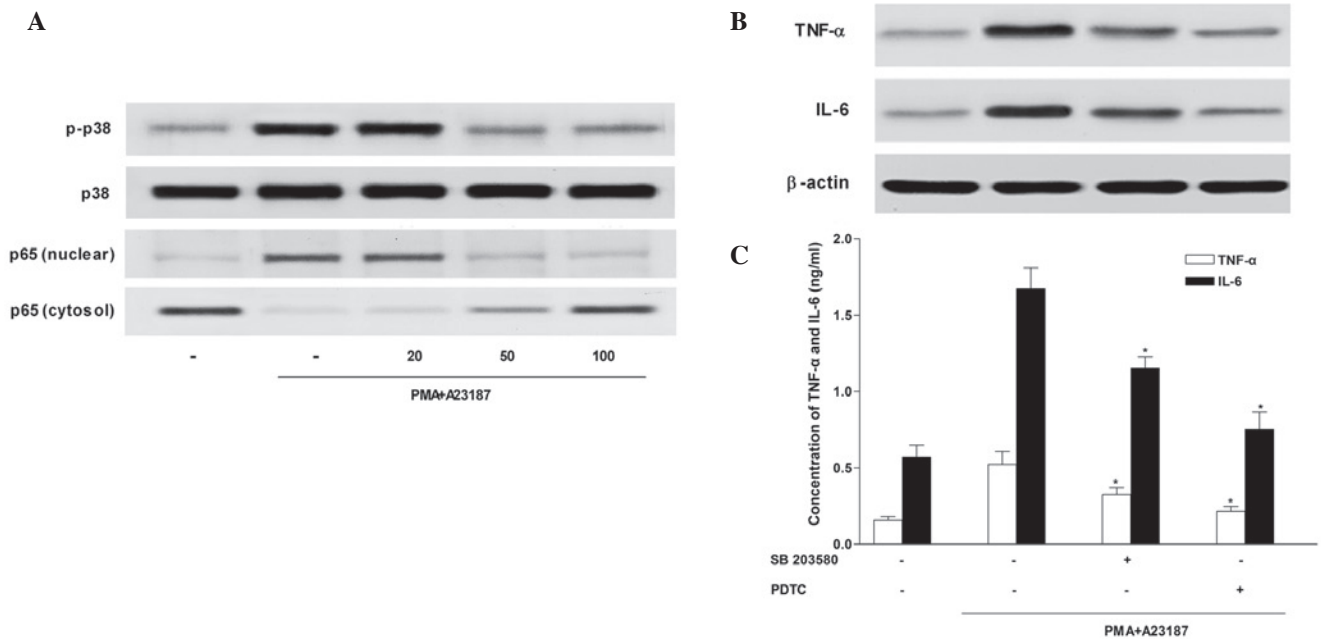

Figure 4. Inhibitory effect of sesamin on p38 MAPK and NF-κB p65 activation in HMC-1 cells. HMC-1 cells were stimulated with 20 nM PMA and 1 μM calcium ionophore A23187 for 4 h in the absence or presence of sesamin (25, 50 or 100 μM). Western blot analysis was used to determine (A) p38 MAPK, extracellular signal-regulated kinases (data not shown), and c-Jun N-terminal kinase activation (data not shown). HMC-1 cells were pretreated with inhibitors of p38 MAPK and NF-κB (SB 203580 and PDTC, respectively) for 30 min prior to stimulation with PMA (20 nM) and A23187 (1 μM), and production and secretion of TNF-α and IL-6 were evaluated by (B) western blotting and (C) ELISA. *P<0.05. p38 MAPK, p38 mitogen-activated protein kinase; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells TNF, tumour necrosis factor; IL-6, interleukin-6; PMA, phorbol 12-myristate 13-acetate; PDTC, pyrrolidine dithiocarbamate.

Discussion

Mast cell activation leads to the release of inflammatory mediators including, histamine, heparin and various cytokines, and is significant in allergic inflammation (25-27). Previous studies have demonstrated that stimulation of mast cells with IgE initiates the activation of the cell signaling, which leads to histamine release and in turn produces local effects, including increased cutaneous blood flow and enhanced vascular permeability, resulting in tissue swelling and itching due to stimulation of cutaneous sensory nerves (28). In the present study, sesamin effectively suppressed histamine release from mast cells. In addition, the experiments conducted in the present study using the PCA animal model [a well-established model for evaluating localized mast cell-mediated allergic reactions *in vivo* (29)], were consistent with the *in vitro* experiments, and indicated that sesamin significantly inhibited mast cell membrane

perturbation in PCA rats. This finding suggests that sesamin may inhibit IgE-mediated anaphylaxis by inhibiting mast cell activation.

The intracellular calcium response induced by Fcε RI cross-linking on mast cells is critical to mast cell degranulation (30,31). Agents that decrease intracellular calcium levels reduce mast cell degranulation, and histamine release is inhibited by decreases in intracellular calcium content due to the activation of mast cells (32). Changes in calcium homeostasis occur as a result of calcium release from internal stores or calcium uptake from the external environment; calcium uptake is essential for Fcε RI-induced degranulation (33). Therefore, in the present study, calcium uptake was measured using a radiotracer assay. The intracellular calcium content of RPMCs was increased by incubation with IgE in comparison with that of the basal cells. However, as expected based upon the inhibition of histamine production by sesamin, sesamin markedly inhibited antigen-induced calcium influx into RPMCs. These results indicate that the effects of sesamin on allergic reactions may be associated with a decrease in mast cell intracellular calcium content. Furthermore, these findings demonstrate that sesamin inhibits histamine release by suppressing calcium uptake into mast cells.

The HMC-1 cell line is suitable for the study of cytokine activation pathways (20,34). The variety of cytokines produced by HMC-1 cells following stimulation with PMA and A23187 supports the well-established role of mast cells in immediate-type hypersensitivity. TNF-α is a key mediator in a number of cytokine-dependent inflammatory events, which induce chemotaxis of neutrophils and T cells, enhance macrophage cytotoxicity, and stimulate the expression of adhesion molecules in mast cells (35). IL-6 is one of the most important mediators of fever and acute phase responses, and it is predominantly secreted by T cells, macrophages and mast cells (36). Triggering and sustaining allergic inflammation in mast cells are significant functions of TNF-α and IL-6, and local accumulation of these two cytokines is associated with the PCA reaction (37). In the present study, sesamin inhibited the PMA- and A23187-induced secretion of TNF-α and IL-6, indicating that sesamin may suppress acute allergic inflammation by decreasing pro-inflammatory cytokine production.

The MAPK cascade is one of the important signaling pathways in the immune response (38). Although expression of TNF-α and IL-6 is regulated by MAPKs, the precise signaling pathways involved and the contributions of the three primary types of MAPKs (p38 MAPK, ERKs and JNKs) remain unclear; however, p38 MAPK is considered to be an important regulator of inflammatory responses. Activation of p38 MAPK is essential for the expression of pro-inflammatory cytokines (39,40). In the current study, stimulation with PMA and A23187 activated p38 MAPK in HMC-1 cells, and sesamin specifically inhibited p38 MAPK activation. Furthermore, sesamin and SB 203580, a specific inhibitor of p38 MAPK, reduced TNF-α and IL-6 production. These data suggest that sesamin inhibits p38 activation and downstream TNF-α and IL-6 production.

Previous reports have indicated that NF-κB is critical in immune and inflammatory responses (22). Activation of NF-κB has also been observed following IgE-induced TNF-α and IL-6 production in mast cells (41). NF-κB is present in the cytoplasm, where it is associated with nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor α (IκBα), an NF-κB inhibitory protein. Upon stimulation, IκBα is phosphorylated and undergoes proteolytic degradation, which results in the release of NF-κB from IκBα and its translocation into the nucleus, where it promotes the transcription of genes, including TNF-α and IL-6 (42). In the present study, sesamin reduced NF-κB p65 levels in the nucleus and increased levels of NF-κB p65 protein in cytosolic extracts from mast cells stimulated with PMA and A23187. Furthermore, sesamin and PDTC, a potent NF-κB inhibitor, downregulated TNF-α and IL-6 production. These results indicate that sesamin prevents translocation of NF-κB into the nucleus and, thus, inhibits its transcriptional activity, as well as its downstream TNF-α and IL-6 production.

In conclusion, sesamin inhibits IgE-mediated anaphylactic reactions *in vivo* and *in vitro*. Inhibition of pro-inflammatory mediator release by sesamin appears to be involved in the inhibition of p38 MAPK and NF-κB activity, in addition to the suppression of calcium uptake. The present study provides insight into the underlying mechanisms by which sesamin produces anti-allergic effects, and indicates that the administration of sesamin may contribute to the prevention and treatment of mast cell-mediated allergic diseases.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (grant nos. 81260665 and 81260016), the Project of Research and Innovation of Jilin Youth Leader and Team (grant no. 20140519013JH), the Natural Science Research Foundation of Jilin Province for Sciences and Technology (grant no. 20130101121JC) and the Natural Science Research Foundation of the Education Department of Jilin Province (grant no. 2014-21).

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