

***Amomum xanthiodes* Inhibits Mast Cell-Mediated Allergic Reactions Through the Inhibition of Histamine Release and Inflammatory Cytokine Production**

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In this study, we investigated the effect of *Amomum xanthiodes* (Zingiberaceae) extract (AXE) on the mast cell-mediated allergy model and studied the possible mechanism of action. We found that AXE inhibited compound 48/80-induced systemic reactions and plasma histamine release in mice. Additionally, AXE decreased immunoglobulin E (IgE)-mediated local allergic reactions and passive cutaneous anaphylaxis (PCA), and AXE dose-dependently attenuated the release of histamine from rat peritoneal mast cells (RPMC) activated by compound 48/80 or IgE. The amounts of AXE needed for inhibition of compound 48/80-induced plasma histamine release and PCA were similar to disodium cromoglycate, the known anti-allergic drug. We found that AXE increased the cAMP levels and decreased the compound 48/80-induced intracellular Ca^{2+} . Furthermore, AXE attenuated the phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore (A23187)-stimulated tumor necrosis factor- α (TNF- α) and interleukin (IL)-6 secretion in human mast cells. The inhibitory effect of AXE on the proinflammatory cytokines was nuclear factor- κ B (NF- κ B)-dependent. In addition, AXE decreased PMA plus A23187-induced degradation of κ B α and the nuclear translocation of NF- κ B. Our findings provide evidence that AXE inhibits mast cell-derived immediate-type allergic reactions, and that cAMP, intracellular Ca^{2+} , proinflammatory cytokines, and NF- κ B are involved in these effects. *Exp Biol Med* 230:681–687, 2005

Key words: *Amomum xanthiodes*; histamine; cAMP; intracellular Ca^{2+} ; tumor necrosis factor- α interleukin-6; nuclear factor- κ B; mast cells

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Introduction

Mast cells are important mediators of inflammatory responses, such as allergy and anaphylaxis. Anaphylaxis, an acute systemic allergic reaction, is mediated by histamine released in response to the antigen cross-linking of immunoglobulin E (IgE) bound to the Fc ϵ receptor I (Fc ϵ RI) on the mast cells. After activation via the Fc ϵ RI, the mast cells start the process of degranulation, which results in the releasing of mediators, such as the products of arachidonic acid metabolism and an array of inflammatory cytokines (1–3). Among the inflammatory substances released from the mast cells, histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (4). Mast cell degranulation also can be elicited by the synthetic compound 48/80, and it has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (5).

The signaling pathway leading to the degranulation of mast cells after engagement of the Fc ϵ RI has been extensively characterized (6–8). The activation of mast cells leads to the phosphorylation of tyrosine kinase and the mobilization of internal Ca^{2+} . This is followed by the activation of protein kinase C, an increase of mitogen-activated protein kinases (MAPKs) and nuclear factor- κ B (NF- κ B), and the release of inflammatory cytokines. In addition, mast cell-mediated histamine release correlates with decreased cAMP levels (9, 10). Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators, such as eicosanoids, proteoglycans, proteases, and several proinflammatory and chemotactic cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, IL-4, IL-13, and transforming growth factor- β (11–13). The transcriptional factor NF- κ B is important as a mediator of cellular responses to extracellular signals and NF- κ B is thought to play an important role in the regulation of proinflammatory molecules of cellular responses, especially TNF- α and IL-6 (14).

Amomum xanthiodes (Zingiberaceae) comes from the fruit of *Amomum villosum* Lour. The extract of *A. xanthiodes* (AXE) is used for the treatment of aromatic stomach and digestive disorders (15). The extract is comprised of borneol, linalool, camphene, and nerolidol (16). Recently, it has been reported that AXE inhibits free radical formation through the inhibition of NF- κ B activation (17). The aim of this study is to evaluate the anti-allergic effect of AXE and to understand the mechanism of its effect.

Materials and Methods

Animals. The original stock of male ICR mice and male Sprague-Dawley rats were purchased from the Dae-Han Experimental Animal Center (Daejeon, Korea). The animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed at 5–10 per cage in a laminar air flow room (conventional condition), maintained at a temperature of $22 \pm 2^\circ\text{C}$, with a relative humidity of $55 \pm 5\%$ throughout the study. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Reagents. Compound 48/80, anti-dinitrophenyl (DNP) IgE; DNP-human serum albumin (HSA); α -minimal essential medium (α -MEM); *o*-phthalaldehyde; phorbol 12-myristate 13-acetate (PMA); and the calcium ionophore, A23187 were purchased from the Sigma Chemical Co. (St. Louis, MO). The recombinant (r) cytokines rTNF- α and rIL-6 were purchased from R & D Systems Inc. (Minneapolis, MN).

Culture of Human Mast Cell (HMC)-1 Cells. The HMC-1 cells, a human mast-cell line, were grown in Iscove's media supplemented with 10% fetal calf serum (FCS) and 2 mM glutamine.

Preparation of AXE. The *A. xanthiodes* was purchased from the Bohwa Dang (Jeonbuk, Korea). A voucher specimen (No. WSP-04-04) was deposited at the Herbarium of the College of Pharmacy, Woosuk University. *Amomum xanthiodes* was ground (1000 rpm, 30 secs) at room temperature using a Micro Hammer-Cutter Mill (Culatti Co., Zurich, Switzerland). The particle size was 0.5–2 mm after grinding. The plant sample (60 g) was extracted twice with 500 ml of purified water at 70°C for 5 hrs in water bath. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was lyophilized using a 0.45 μm syringe filter. The yield of dried extract from starting crude material was approximately 5.2%. The dried extract was dissolved in saline or Tyrode's buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM CaCl_2 , 1 mM MgCl_2 , 5.6 mM glucose, and 0.1% bovine serum albumin) before use.

Compound 48/80-induced Systemic Reaction. Compound 48/80-induced systemic reaction was performed as previously described (18). Briefly, the mice

were given an intraperitoneal injection of 8 mg/kg body weight (BW) of the mast cell degranulator, compound 48/80. The AXE was dissolved in saline and administered intraperitoneally at doses of 0.005–1 g/kg BW 1 hr before the compound 48/80 injection ($n = 10$ per group). In the time-dependent experiment, 0.5 g/kg of AXE was administered at 5, 10, 20, and 30 mins after compound 48/80 injection ($n = 10$ per group). Mortality was monitored for 1 hr after induction of anaphylactic shock.

Passive Cutaneous Anaphylaxis (PCA) Reaction. The mice were injected intradermally with 0.5 μg of anti-DNP IgE. After 48 hrs, each mouse received an injection of 1 μg of DNP-HSA in phosphate-buffered saline (PBS) containing 4% Evans blue (1:4) via the tail vein. The AXE (0.01–1 g/kg BW) was administered 1 hr before the challenge. Thirty minutes after the challenge, the mice were killed and the dorsal skin was removed to measure the area of pigment. The amount of dye was determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of a 5:13 mixture of acetone and phosphoric acid, respectively. The intensity of the absorbance was measured at 620 nm in a spectrophotometer (UV-1201; Shimadzu, Kyoto, Japan).

Preparation of Plasma and Histamine Determination. The blood was centrifuged at 400 g for 10 mins. The plasma was withdrawn and the histamine content was measured by the *o*-phthalaldehyde spectrofluorometric procedure (19). The fluorescent intensity was measured at a 438 nm emission and a 353 nm excitation using a spectrofluorometer (RF-5301 PC; Shimadzu).

Preparation of Rat Peritoneal Mast Cells (RPMC). Mast cells were separated from the rat peritoneal cavity cells as previously described (18). In brief, the peritoneal cells were suspended in Tyrode's buffer, layered on 2 ml of metrizamide (22.5 w/v%), and centrifuged for 15 mins at 400 g. The cells that remained at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml of Tyrode's buffer. Mast cell preparations were about 95% pure, as assessed by toluidine blue staining. More than 95% of the cells were viable, as judged by trypan blue uptake.

Inhibition of Histamine Release. The RPMC were preincubated for 10 mins at 37°C before application of 5 μg /ml of the treatment, compound 48/80. The cells were preincubated with the 0.001–0.1-mg/ml AXE preparations, and incubated for 10 mins with compound 48/80. The RPMC were sensitized with 10 μg /ml of anti-DNP IgE for 16 hrs. The cells were preincubated with 0.001–0.1-mg/ml AXE at 37°C for 10 mins before the challenge with 1 μg /ml of DNP-HSA. The cells were separated from the released histamine by centrifugation at 400 g for 5 mins at 4°C .

cAMP Assay. The cAMP level was measured following the procedure of Peachell *et al.* (20). The cAMP level was determined by enzyme immunoassay using a commercial kit (Amersham Pharmacia Biotech, Piscataway, NJ).

Intracellular Ca²⁺. Following the manufacturer's protocol, 2 μ M Fura-2/AM (Molecular Probes, Eugene, OR) was used to determine the concentration of intracellular Ca²⁺. The RPMC were preincubated with Fura-2/AM for 30 mins at 37°C. After washing the dye from the cell surface, cells were pretreated with AXE for 10 mins before the compound 48/80 treatment. The fluorescent intensity was recorded using a fluorescence plate reader (Molecular Devices, Sunnyvale, CA) at an excitation of 340 nm and an emission of 500 nm.

Assay of TNF- α and IL-6 Secretion. Secretion of TNF- α and IL-6 was measured by modification of an enzyme-linked immunosorbent assay (ELISA). The HMC-1 cells were cultured with α -MEM plus 10% FBS and resuspended in Tyrode's buffer. The cells were sensitized with 20 nM PMA plus 1 μ M A23187 for 6 hrs in the absence or presence of AXE. The ELISA was performed by coating 96-well plates with 6.25 ng/well of monoclonal antibody with specificity for TNF- α and IL-6, respectively. Before use, and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween-20 and twice with PBS alone. For the standard curve, rTNF- α and rIL-6 were added to the serum, which was previously determined to be negative for endogenous TNF- α and IL-6. After exposure to the medium, the assay plates were exposed sequentially to biotinylated anti-human TNF- α and IL-6, and ATBS (2,2'-azino-bis[3-ethylbenzthiazoline-6-sulfonic acid]) tablet substrates. Optical density readings were made within 10 mins of the addition of the substrate with a 405-nm filter.

Nuclear Protein Extraction. Nuclear extract was prepared in a similar manner to other reports. Briefly, after cell activation for the times indicated, cells were washed in 1 ml of ice-cold PBS, centrifuged at 1200 rpm for 5 mins, resuspended in 400 μ l of ice-cold hypotonic buffer (10 mM HEPES/KOH; 2 mM MgCl₂; 0.1 mM EDTA; 10 mM KCl; 1 mM DTT; and 0.5 mM phenylmethylsulfonyl fluoride [PMSF], pH 7.9), left on ice for 10 mins, vortexed, and centrifuged at 15,000 g for 30 secs. Pelleted nuclei were gently resuspended in 50 μ l of ice-cold saline buffer (50 mM HEPES/KOH; 50 mM KCl; 300 mM NaCl; 0.1 mM EDTA; 10% glycerol; 1 mM DTT; and 0.5 mM PMSF, pH 7.9), left on ice for 20 mins, vortexed, and centrifuged at 15,000 g for 5 mins at 4°C. Aliquots of the supernatant that contained nuclear proteins were frozen in liquid nitrogen and stored at -70°C.

Western Blot Analyses. Electrophoresis was performed on samples using 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as described elsewhere (21), and samples were transferred to a nitrocellulose membrane. The I κ B α and p65 NF- κ B were assayed using antibodies to NF- κ B (p65) and I κ B α (Santa Cruz Biotech, Santa Cruz, CA). Immunodetection was performed using an enhanced chemiluminescence detection kit (Amersham Pharmacia).

Electrophoretic Mobility Shift Assays (EM-

SA). Nuclear protein (10 μ g) was incubated for 20 mins at room temperature with 20 μ g of bovine serum albumin, 2 μ g of poly dI-dC, from Pharmacia (Uppsala, Sweden), 2 μ l of Buffer C (20 mM HEPES/KOH; 20% glycerol; 100 mM KCl; and 0.5 mM PMSF, pH 7.9), 4 μ l of Buffer F (20% ficoll-400; 100 mM HEPES/KOH; 300 mM KCl; 10 mM dithiothreitol [DTT]; and 0.5 mM PMSF, pH 7.9), and 20,000 cpm of a ³²P-labeled probe that encoded the κ B consensus sequence (5'-CAG AGG GGA CTT TCC GAG AG-3') or activator protein (AP)-1 (5'-GCA TGA GTC AGA CAC AC-3') in a final volume of 20 μ l. DNA-protein complexes were resolved at 180 V for 4 hrs in a native 4% polyacrylamide gel, dried, and visualized (with autoradiography, using a Fuji x-ray film).

Statistical Analysis. Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC). Treatment effects were analyzed using one-way ANOVA, followed by Duncan's multiple range tests. Significance was set at $P < 0.05$.

Results

Effect of AXE on Compound 48/80-Induced Systemic Reaction. To determine the effect of AXE on allergic reaction, an *in vivo* model of a systemic reaction was used. Compound 48/80 (0.008 g/kg) was used as a model of induction for a systemic fatal allergic reaction. After the intraperitoneal injection of compound 48/80, the mice were monitored for 1 hr, after which time, the mortality rate was determined. As shown in Table 1, injection of compound 48/80 into mice induced fatal shock in 100% of animals. When AXE was intraperitoneally administered at a concentrations ranging from 0.005 to 1 g/kg BW for 1 hr, the mortality with compound 48/80 was dose-dependently reduced. In addition, the mortality of mice administered with 0.5 g/kg BW of AXE, 5, 10, 20, and 30 mins after compound 48/80 injection increased depending on time (Table 2). We evaluated the effect of AXE treatment on compound 48/80-induced plasma histamine release, comparing the effects to those found with the known anti-allergic drug, disodium cromoglycate (DSCG; Ref. 22). Our results indicated that AXE dose-dependently decreased the compound 48/80-induced plasma histamine release. The effect of AXE on the level of compound 48/80-induced plasma histamine release was similar to DSCG at 1 g/kg BW (Fig. 1).

Effect of AXE on Anti-DNP IgE-Mediated PCA. Another way to test anaphylaxis is to induce PCA. As described in "Materials and Methods," local extravasation was induced by a local injection of IgE followed by an antigenic challenge. The AXE dose-dependently inhibited the PCA reaction (Fig. 2).

Effect of AXE on Histamine Release from RPMC. The inhibitory effects of AXE on compound 48/80-mediated and anti-DNP IgE-mediated histamine release from RPMC are shown in Figure 3. Treatment with AXE

Table 1. Effect of AXE on Compound 48/80–Induced Systemic Anaphylaxis^a

AXE treatment (g/kg BW)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	+	100
0.005	+	100
0.01	+	80
0.05	+	60
0.1	+	40
0.5	+	0
1	+	0
1	–	0

^a Groups of mice ($n = 10$ per group) were intraperitoneally pretreated with 200 μ l of saline or AXE at various doses 1 hr before intraperitoneal injection of compound 48/80. Percent mortality within 1 hr after compound 48/80 injection is represented as the number of dead mice times 100, divided by the total number of experimental mice.

dose-dependently inhibited compound 48/80–mediated and anti-DNP IgE-mediated histamine release at concentrations of 0.001–0.1 mg/ml.

Effect of AXE on cAMP and $[Ca^{2+}]_i$ in RPMC. To investigate the mechanisms of AXE on the reduction of histamine release, we assayed the cAMP levels and $[Ca^{2+}]_i$. When RPMC were incubated with AXE at 0.1 mg/ml, the cAMP content increased at 1 min and decreased to basal levels at 3 mins (Fig. 4A). Calcium movements across membranes of mast cells are critical to histamine release (8). To further investigate the mechanisms of AXE on the reduction of histamine release, we assayed the $[Ca^{2+}]_i$. Figure 4B shows the stimulation of intracellular Ca^{2+} when the RPMC are treated with 2 μ g/ml of compound 48/80. Preincubation of 0.1 mg/ml of AXE with RPMC decreased compound 48/80–induced $[Ca^{2+}]_i$.

Effect of AXE on TNF- α and IL-6 Secretion from HMC-1 Cells. The most important proinflammatory cytokines are TNF- α and IL-6. Therefore, we tested the effect of

Table 2. Time-Dependent Effect of AXE on Compound 48/80–Induced Systemic Anaphylaxis^a

AXE treatment (g/kg BW)	Compound 48/80 (8 mg/kg BW)	Time (min)	Mortality (%)
None (saline)	+		100
0.5	+	0	0
0.5	+	5	0
0.5	+	10	60
0.5	+	20	90
0.5	+	30	100

^a Mice ($n = 10$ per group) were intraperitoneally pretreated with 200 μ l of saline or AXE. The 0.5 g/kg of AXE was given 5, 10, 20, and 30 mins after the intraperitoneal injection of compound 48/80. Percent mortality within 1 hr after compound 48/80 injection is represented as the number of dead mice times 100, divided by the total number of experimental mice.

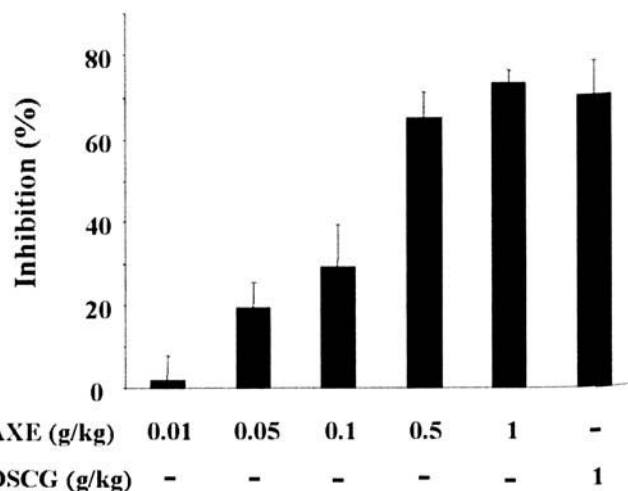


Figure 1. Effect of AXE on compound 48/80–induced plasma histamine release. Groups of mice ($n = 10$ per group) were intraperitoneally pretreated with 200 μ l of saline or AXE or DSCG. Various doses of AXE or DSCG were given 1 hr before the injection of compound 48/80. The compound 48/80 solution was given intraperitoneally to the group of mice. Each bar represents the mean \pm SEM of three independent experiments.

AXE on TNF- α and IL-6 secretion from HMC-1 cells. Culture supernatants were assayed for the level of each cytokine by ELISA. As shown in Figure 5, AXE inhibited the secretion of TNF- α and IL-6 in PMA plus A23187–stimulated HMC-1 cells.

Effect of AXE on NF- κ B Activation. To evaluate the mechanisms of effect of AXE on the cytokine secretion, we examined the effect of AXE on NF- κ B activation. Expression of proinflammatory cytokines, such as TNF- α and IL-6, is regulated by a transcription factor, NF- κ B. We previously showed that treatment with NF- κ B regulates PMA plus A23187–induced TNF- α and IL-6 secretion in HMC-1 cells (18). Stimulation of HMC-1 cells with PMA

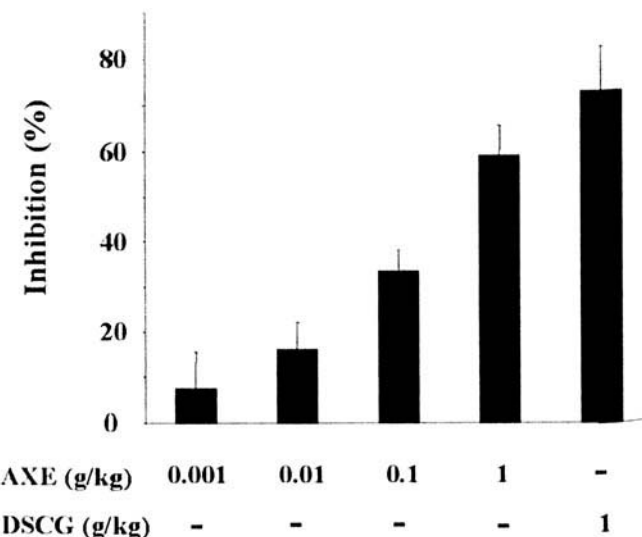


Figure 2. Effect of AXE on 48-hr PCA. One hour before the antigen challenge, AXE or DSCG was intraperitoneally administered. Each bar represents the mean \pm SEM of three independent experiments.

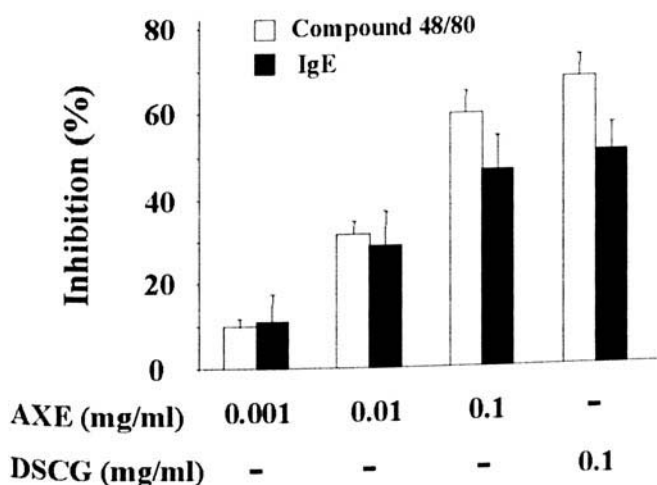


Figure 3. Effect of AXE on compound 48/80-induced or IgE-induced histamine release from RPMC. The cells (2×10^5 cells/ml) were preincubated with AXE or DSCG at 37°C for 10 mins before incubation with compound 48/80 or DNP-HSA. Each data represents the mean \pm SEM of three independent experiments.

plus A23187 induced the degradation of I κ B α and the nuclear translocation of p65 NF- κ B after 4 hrs of incubation. We used pyrrolidine dithiocarbamate (PDTC), a potent inhibitor of NF- κ B, as a positive control. Treatment with AXE inhibited the PMA plus A23187-induced degradation of I κ B α and the nuclear translocation of p65 NF- κ B (Fig. 6A). To investigate whether NF- κ B/DNA binding was inhibited by AXE, we performed EMSA. Cells pretreated with either AXE or PDTC were subsequently stimulated by PMA plus A23187, and the effect of AXE and PDTC on binding activity of transcription factors was examined. Although 0.1 mg/ml of AXE, similar to 10 μ M PDTC, significantly inhibited the PMA plus A23187-induced NF- κ B binding activity, no significant effect was observed on the binding activity of activator protein (AP)-1.

Discussion

Immediate-type allergic reaction (anaphylaxis) is a life-threatening syndrome induced by the sudden systemic release of inflammatory mediators, such as histamine, heparin, lipid-derived mediators, and various cytokines from mast cells (23). Stimulation of mast cells with compound 48/80 or IgE initiates the activation of a signal-transduction pathway, which leads to a histamine release. Several recent studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G proteins (24, 25). Compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation in the membrane. Thus, the increase in membrane permeability may be an essential trigger for the release of the mediator from mast cells. The AXE might stabilize the lipid bilayer membrane, thus preventing the perturbation from being induced by compound 48/80. In addition, we found that AXE-treated mice were protected

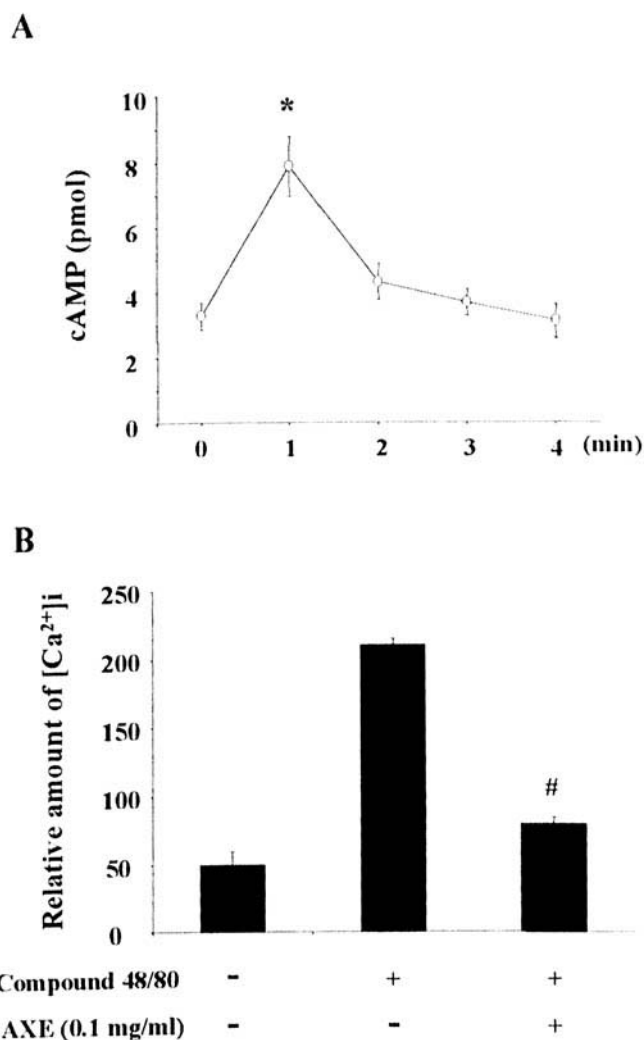


Figure 4. Effect of AXE on cAMP and intracellular calcium in RPMC. (A) RPMC were treated with 0.1 mg/ml of AXE at 37°C. (B) RPMC were preincubated for 10 mins with 0.1 mg/ml of AXE before adding 2 μ g/ml of compound 48/80, and then incubated for another 10 mins with compound 48/80. Each data represents the mean \pm SEM of three independent experiments. * Statistically significant difference from the control. # Significantly different from the compound 48/80 value.

from IgE-mediated PCA, which is one of the most important *in vivo* models of anaphylaxis in a local allergic reaction. This finding suggests that AXE might be useful in the treatment of allergic skin reactions.

Although functional compartments in mast cells for cAMP and calcium on histamine release are controversial, the cAMP and [Ca²⁺]_i pathways are critical to the degranulation of mast cells. Agents that stimulate an intracellular cAMP level have been shown to inhibit mast cell degranulation. An increase of cAMP is believed to precede the inhibition of histamine release from RPMC in response to stimulation of IgE receptors or compound 48/80 (26–28). In addition, calcium movements across the membranes of mast cells represent a major target for effective anti-allergic drugs, because the movement of calcium across the membrane is an essential event that links

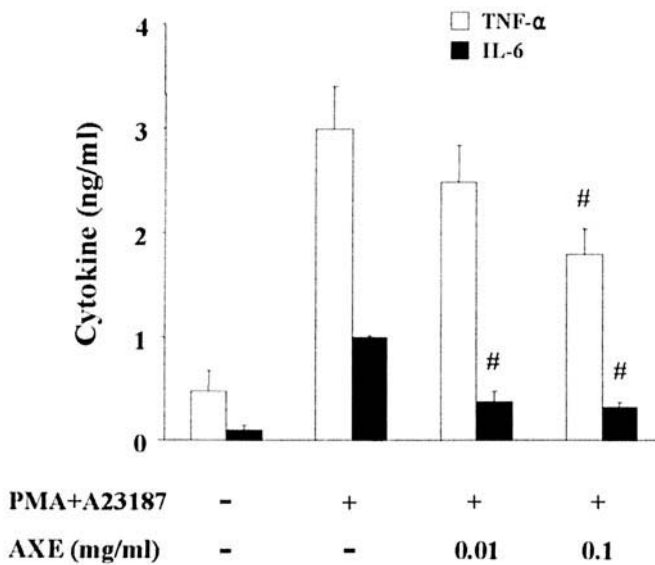


Figure 5. Effect of AXE on proinflammatory cytokine secretion. Human mast cell-1 cells stimulated with 20 nM PMA plus 1 μ M A23187 were incubated for 6 hrs in either the absence or presence of AXE. The TNF- α and IL-6 secreted into the medium are presented as the mean \pm SEM of three independent experiments. # Significantly different from the PMA+A23187 value.

stimulation to secretion (7). The transduction pathways modulating cAMP and $[Ca^{2+}]_i$ are modified by ADP-ribosylated G-protein-binding proteins (6). Our results, which show an enhancement of cAMP and an attenuation of $[Ca^{2+}]_i$ in mast cells with AXE treatment is consistent with other reports. According to these observations, we strongly suggest that increased cAMP and decreased $[Ca^{2+}]_i$ might be involved in the inhibitory effect of AXE on histamine release, and that AXE might have membrane-stabilizing activity through G proteins.

The HMC-1 cell line is a useful cell line for studying cytokine activation pathways (29, 30). The spectrum of cytokines produced by HMC-1 cells with PMA plus A23187 stimulation supports the well-recognized role of mast cells in immediate-type hypersensitivity reactions. Proinflammatory cytokines, including TNF- α and IL-6, play a major role in triggering and sustaining the allergic inflammatory response in mast cells. Mast cells are a principal source of TNF- α in human dermis, and degradation of mast cells in the dermal endothelium is abrogated by the antibodies to TNF- α (31). IL-6 is also produced from mast cells and its local accumulation is associated with the PCA reaction (32). This report indicates that the reduction of proinflammatory cytokines from mast cells is one of the key indicators of reduced allergic symptom. The AXE inhibited the secretion of TNF- α and IL-6 in PMA plus A23187-stimulated HMC-1 cells.

To evaluate the mechanisms of effect of AXE on the proinflammatory cytokines, we examined the effect of AXE on NF- κ B activation. Expression of TNF- α and IL-6 genes is also dependent on the activation of the transcription factor, NF- κ B (33). Activation of NF- κ B requires phos-

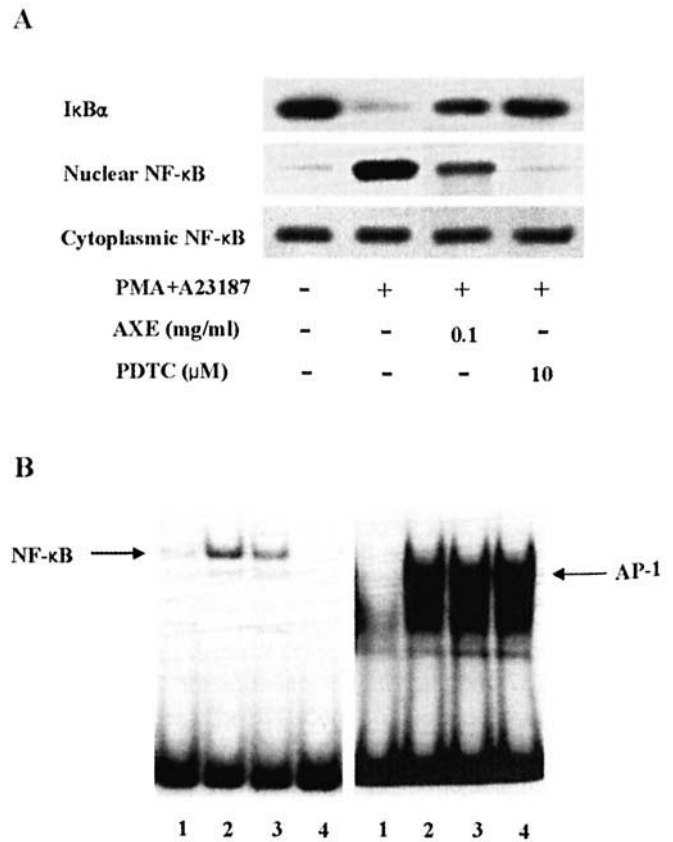


Figure 6. Effect of AXE on the activation of NF- κ B. Human mast cell-1 cells were pretreated with AXE or PDTC for 30 mins before stimulation with 20 nM PMA plus 1 μ M A23187. (A) Western blot analysis of cytoplasmic I κ B α and p65 NF- κ B, and of nuclear p65 NF- κ B. (B) Nuclear extracts prepared and incubated with 32 P-labeled oligonucleotides corresponding to NF- κ B and AP-1 were analyzed by EMSA. Lane 1, no treatment; Lane 2, PMA + A23187; Lane 3, PMA + A23187 + 0.1 mg/ml of AXE; and Lane 4, PMA + A23187 + 10 μ M PDTC.

phorylation and proteolytic degradation of the inhibitory protein, I κ B α , an endogenous inhibitor that binds to NF- κ B in the cytoplasm (14). In PMA plus A23187-stimulated mast cells, AXE decreased the degradation of I κ B α and the nuclear translocation of p65 NF- κ B. The AXE specifically inhibited the DNA binding of NF- κ B but not of AP-1. In addition, PDTC, the potent inhibitor of NF- κ B, reduced PMA plus A23187-induced TNF- α and IL-6 production. These data demonstrate that gallic acid specifically attenuates activation of NF- κ B, and of downstream TNF- α and IL-6 production. The MAPK cascade is also one of the important signaling pathways in immune responses (34). In our experiments, PMA plus A23187 simultaneously activated all three MAPKs in HMC-1 cells. However, AXE had no effect on the PMA plus A23187-activated MAPK phosphorylation (data not shown). These data suggest that the inhibitory effect of AXE on proinflammatory cytokine secretion is specifically mediated by NF- κ B.

In the present report, we provided evidence that AXE inhibits a model of mast cell-mediated allergic reactions,

and indicated possible mechanisms for this inhibition, such as through cAMP, $[Ca^{2+}]_i$, NF- κ B, and downstream proinflammatory cytokines. The results obtained in the present study show that AXE contributes to the prevention or treatment of mast cell-mediated allergic diseases.

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