Anti-Allergic Effects of *Artemisia iwayomogi* on Mast Cell–Mediated Allergy Model

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The discovery of drugs for the treatment of allergic disease is an important subject in human health. The *Artemisia iwayomogi* (Compositae) (AIE) has been used as a traditional medicine in Korea and is known to have an anti-inflammatory effect. However, its specific mechanism of action is still unknown. In this report, we investigated the effect of AIE on the mast cell–mediated allergy model and studied the possible mechanism of action. AIE inhibited compound 48/80–induced systemic reactions and plasma histamine release in mice. AIE decreased immunoglobulin E (IgE)–mediated local allergic reaction, passive cutaneous anaphylaxis (PCA) reaction. AIE dose dependently attenuated histamine release from rat peritoneal mast cells activated by compound 48/80 or IgE. AIE decreased the compound 48/80–induced intracellular Ca^{2+}. Furthermore, AIE decreased the phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187-stimulated tumor necrosis factor-α and interleukin-6 gene expression and production in human mast cells. The inhibitory effect of AIE on the proinflammatory cytokine was p38 mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) dependent. AIE attenuated PMA plus A23187-induced degradation of IκBα and nuclear translocation of NF-κB and specifically blocked activation of p38 MAPK but not that of c-jun N-terminal kinase and extracellular signal-regulated kinase. Our findings provide evidence that AIE inhibits mast cell–derived immediate-type allergic reactions and involvement of intracellular Ca^{2+}, proinflammatory cytokines, p38 MAPK, and NF-κB in these effects. Exp Biol Med 230:82–88, 2005

Key words: *Artemisia iwayomogi*; intracellular Ca^{2+}; tumor necrosis factor-α; interleukin-6; p38 mitogen-activated protein kinase; nuclear factor-κB; mast cells

The extract of *Artemisia iwayomogi* (Compositae) (AIE) has been used for centuries as an oriental traditional medicine. This crude drug is used for the treatment of various liver diseases. It has contents of esculetin-6-methylether, camphor, borneol, p-cymene, caryophyllene, methyleugenol, and bornyl acetate (1, 2).

Mast cells, which are constituents of virtually all organs and tissue, are important mediators of inflammatory responses such as allergy and anaphylaxis. Anaphylaxis is mediated by histamine released in response to antigen cross-linking of immunoglobulin E (IgE) bound to FcεRI on mast cells. Mast cell activation causes the process of degranulation that result in releasing of mediators, such as histamine and an array of inflammatory cytokines (3–5). Among the inflammatory substances released from mast cells, histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (6). 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 (7).

The signaling pathway leading to degranulation of mast cells has been extensively characterized (8, 9). Activation of mast cells leads to phosphorylation of tyrosine kinase and mobilization of internal Ca^{2+}. This is followed by activation of protein kinase C, mitogen-activated protein kinases (MAPKs), and nuclear factor-κB (NF-κB) and releasing of inflammatory cytokines. 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 (10–12). Mitogen-activated protein kinases and transcription factor NF-κB have important activities as mediators of cellular responses to extracellular signals. Some of the MAPKs important to mammalian cells include extracellular signal-regulated kinase, c-jun N-terminal kinase, and p38. p38 MAPK and NF-κB are thought to play an important role in the regulation of proinflammatory molecules on cellular responses, especially TNF-α, IL-1β, and IL-6 (13–15). The aim of this study is to evaluate the anti-allergic effect of AIE and to understand the mechanism of effect.

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Materials and Methods

Culture of HMC-1 Cells. HMC-1 cells, a human mast cell line, were grown in Iscove's media supplemented with 10% FCS and 2 m*M* glutamine.

Animals. The original stock of male ICR mice and male Sprague-Dawley rats were purchased from Dae-Han Experimental Animal Center (Daejeon, Korea), and the animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed 5–10 per cage in a laminar airflow room maintained at a temperature of 22 ± 2°C and relative humidity of 55 ± 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-DNP IgE, DNP-human serum albumin (HSA), α-minimal essential medium (α-MEM), o-phthaldialdehyde, phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187, pyrrolidine dithiocarbamate (PDTC), and metrizamide were purchased from Sigma Chemical Co. (St. Louis, MO). SB 203580 was purchased from Calbiochem (La Jolla, CA).

Preparation of AIE. The plants of *Artemisia iwayomogi* were purchased from the Bohwa Dang (Jeonbuk, Korea). A voucher specimen (number WSP-03-01) was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The plant sample was extracted with purified water at 70°C for 5 hrs. The extract was filtered and lyophilized. The dried extract was dissolved in saline or Tyrode buffer A (10 m*M* HEPES, 130 m*M* NaCl, 5 m*M* KCl, 1.4 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 5.6 m*M* glucose, 0.1% bovine serum albumin) before use.

Compound 48/80–Induced Systemic Reaction. Compound 48/80–induced systemic reaction was carried out as previously described (16). Briefly, the mice were given an intraperitoneal injection of 8 mg/kg body weight (BW) of the mast cell degranulator, compound 48/80. AIE was dissolved in saline and administered intraperitoneally at doses of 0.001–1 g/kg BW 1 hr before the injection of compound 48/80 (n = 10/group). In the time-dependent experiment, AIE (1 g/kg) was administered 5, 10, and 20 mins after injection of compound 48/80 (n = 10/group). Mortality was monitored for 1 hr after induction of anaphylactic shock.

Preparation of Plasma and Histamine Determination. The blood was centrifuged at 400 g for 10 mins. The plasma was withdrawn, and histamine content was measured by the o-phthaldialdehyde spectrofluorometric procedure of Shore *et al.* (17). The fluorescence intensity was measured at emission 438 nm and excitation 353 nm using spectrofluorometer (Shimadzu, RF-5301 PC, Japan).

PCA Reaction. The mice were injected intradermally with 0.5 μg of anti-DNP IgE. After 48 hrs, each mouse was received an injection of 1 μg of DNP-HSA in PBS containing 4% Evans blue (1:4) via tail vein. AIE (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 for measurement of the pigment area. The amount of dye was determined colorimetrically after extraction with 1 ml of 1 *M* KOH and 9 ml of a mixture of acetone and phosphoric acid (5:13). The intensity of absorbent was measured at 620 nm in a spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan).

Preparation of RPMC. Mast cells were separated from rat peritoneal cavity cells according to the method described by Yurt *et al.* (18). In brief, peritoneal cells were suspended in Tyrode buffer, layered on 2 ml of metrizamide (22.5 w/v%), and centrifuged for 15 mins at 400 g. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml of Tyrode 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 trypane blue uptake.

Inhibition of Histamine Release. Purified RPMC were resuspended in Tyrode buffer for the treatment with compound 48/80. The RPMC suspensions (2×10^5 cells/ml) were preincubated for 10 min at 37°C before the addition of compound 48/80 (5 μg/ml). The cells were preincubated with the AIE (0.001–1 mg/ml) preparations and incubated for 10 mins with compound 48/80. The RPMC suspensions (2×10^5 cells/ml) were sensitized with anti-DNP IgE (10 μg/ml) for 16 h. The cells were preincubated with the AIE (0.001–1 mg/ml) at 37°C for 10 mins prior to the challenge with DNP-HSA (1 μg/ml). The cells were separated from the released histamine by centrifugation at 400 g for 5 mins at 4°C.

Intracellular Ca^{2+}. Fura-2/AM (2 μ*M*, Molecular Probes, Eugene, OR) was used to determine the intracellular Ca^{2+} following the manufacturer's protocol briefly described as following. The RPMC were preincubated with Fura-2/AM for 30 mins at 37°C. After washing the dye from the cell surface, AIE was pretreated 10 mins prior to the compound 48/80 treatment. The fluorescent intensity was recorded using fluorescent plate reader (Molecular Devices, Sunnyvale, CA) at excitation of 340 nm and emission of 500 nm.

Analysis of Cytokine mRNA Expression. Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to analyze the expression of mRNA for TNF-α, IL-6, and β-actin (internal control). The condition for reverse transcription and PCR steps were performed as previously reported (19). The respective primer sets were chosen by Primer3 program (Whithead Institute, Cambridge, MA). Optimization of cycle number was performed to ensure that product accumulation was in the linear range. Amplified products were separated by electrophoresis on 2% agarose gel containing ethidium bromide. The gels were documented using a Kodak DC 290 digital camera (Eastman Kodak, Rochester, NY) and digitized using UN-SCAN-IT software (Silk Scientific, Orem, UT).

Western Blot Analyses. Samples of protein were electrophoresed using 8%–12% sodium dodecyl sulfate–

polyacrylamide gel electrophoresis, as described elsewhere (20), and then transferred to nitrocellulose membrane. The TNF-α and IL-6 were assayed using anti-TNF-α and anti-IL-6 antibody (R&D Systems Inc., Minneapolis, MN). The p38 MAPK, ERK, and JNK activation was determined using anti-phospho-p38, -ERK, and -ERK antibodies (Cell Signaling, Beverly, MA). The nucleus and cytosolic p65 NF-κB was assayed using anti-NF-κB (p65) antibody (Santa Cruz Biotech, Santa Cruz, CA). Immunodetection was done using enhanced chemiluminescence detection kit (Amersham Pharmacia, Piscataway, NJ).

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, and $P < 0.05$ was used to indicate significance.

Results

Effect of AIE on Compound 48/80–Induced Systemic Anaphylaxis. To determine the effect of AIE on allergic reaction, an *in vivo* model of systemic reaction was used. Compound 48/80 (8 mg/kg BW) was used as a model of induction of a systemic fatal allergic reaction. After the intraperitoneal injection of compound 48/80, the mice were monitored for 1 hr, after which 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 AIE was intraperitoneally administered at concentrations ranging from 0.001 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 AIE (1 g/kg) 5, 10, and 20 mins after compound 48/80 injection increased time dependently (Table 2).

Table 1. Effect of *Artemisia iwayomogi* (Compositae) (AIE) on Compound 48/80–Induced Systemic Anaphylaxis[a]

AIE treatment (g/kg BW)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	+	100
0.01	+	100
0.05	+	80
0.1	+	20
0.5	+	0
1	+	0
1	–	0

[a] BW, body weight. Groups of mice (n = 10/group) were intraperitoneally pretreated with 200 μl of saline or AIE at various doses 1 h before the intraperitoneal injection of compound 48/80. Mortality (%) within 1 hr following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice.

Table 2. Time-Dependent Effect of *Artemisia iwayomogi* (Compositae) (AIE) on Compound 48/80–Induced Systemic Anaphylaxis[a]

AIE treatment (g/kg BW)	Compound 48/80 (8 mg/kg BW)	Time (mins)	Mortality (%)
None (saline)	+		100
1	+	0	0
	+	5	20
	+	10	80
	+	20	100

[a] BW, body weight. Mice (n = 10/group) were intraperitoneally pretreated with 200 μl of saline or AIE. AIE (1 g/kg) was given 5, 10, and 20 mins after the intraperitoneal injection of compound 48/80. Mortality (%) within 1 hr following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice.

Effect of AIE on Compound 48/80–Induced Plasma Histamine Release. The effect of AIE on compound 48/80–induced plasma histamine release was investigated. AIE was given at concentrations ranging from 0.01 to 1 g/kg BW 1 hr before (n = 10/group) compound 48/80 injection. The correlative results with those of the mortality test were shown when their plasma histamine contents were measured (Fig. 1). The inhibition rate due to treatment with AIE was significant at doses of 0.1–1 g/kg.

Effect of AIE on PCA Reaction. Another way to test anaphylaxis is to induce PCA. As described in experimental procedures, local extravasation was induced by a local injection of IgE followed by an antigenic

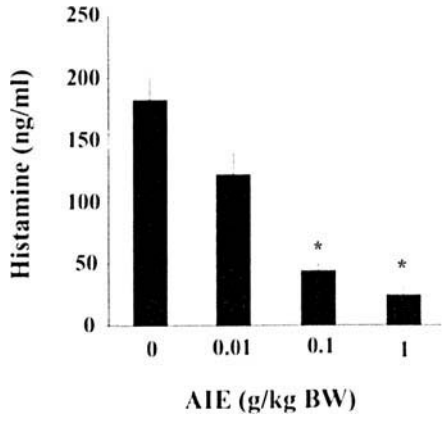

Figure 1. Effect of *Artemisia iwayomogi* (Compositae) (AIE) on compound 48/80–induced plasma histamine release. Groups of mice (n = 10/group) were intraperitoneally pretreated with 200 μl of saline or AIE. AIE was given at various doses 1 hr before the intraperitoneal injection of compound 48/80. Each bar represents the mean ± SEM of three independent experiments. *$P < 0.05$ (significantly different from the saline value).

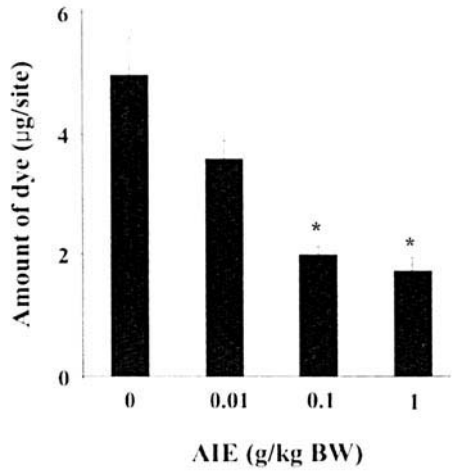

Figure 2. Effect of *Artemisia iwayomogi* (Compositae) (AIE) on the 48-hr PCA. AIE was intraperitoneally administered 1 hr prior to the challenge with antigen. Each bar represents the mean ± SEM of three independent experiments. $^*P < 0.05$ (significantly different from the saline value).

challenge. Administration of AIE (0.1 and 1 g/kg) showed a marked inhibition in PCA reaction (Fig. 2).

Effect of AIE on Histamine Release from RPMC. The inhibitory effect of AIE on compound 48/80–induced or IgE-mediated histamine release from RPMC is shown in Figure 3. AIE inhibited compound 48/80–induced or IgE-mediated histamine release from RPMC in a dose-dependent manner. AIE significantly inhibited the compound 48/80–induced or IgE-mediated histamine release at concentrations of 100–1000 μg/ml.

Effect of AIE on Intracellular Ca^{2+}. Calcium movements across membranes of mast cells are critical to histamine release (8). To investigate the mechanisms of AIE on the reduction of histamine release, we assayed the

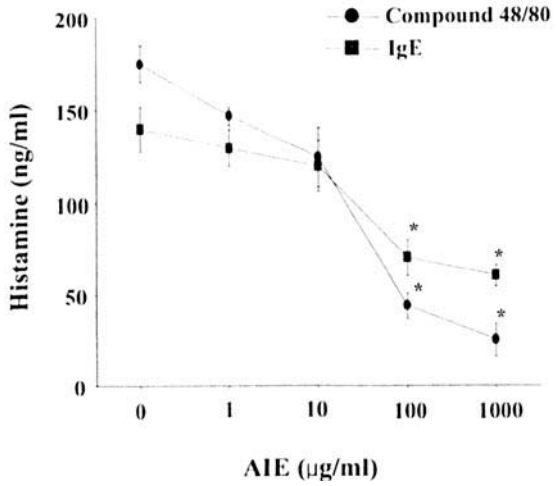

Figure 3. Effect of *Artemisia iwayomogi* (Compositae) (AIE) on compound 48/80– or IgE-induced histamine release from RPMC. The cells (2×10^5 cells/ml) were preincubated with AIE at 37°C for 10 mins prior to incubation with compound 48/80 or DNP-HSA. All data represent the mean ± SEM of three independent experiments. $^*P < 0.05$ (significantly different from the saline value).

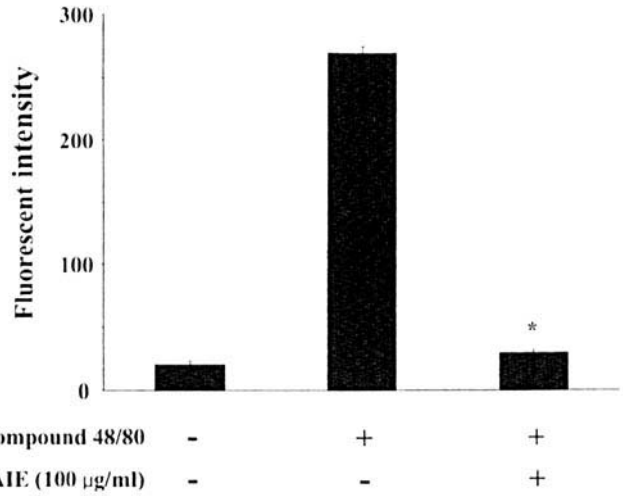

Figure 4. Effect of *Artemisia iwayomogi* (Compositae) (AIE) on the intracellular Ca^{2+} levels in RPMC. Cells were preincubated 10 mins with AIE (0.1 mg/ml) before adding compound 48/80 (2 μg/ml) and then another 10 mins with compound 48/80. Each bar represents the mean ± SEM of three independent experiments. $^*P < 0.05$ (significantly different from the compound 48/80 value).

intracellular Ca^{2+}. Figure 4 shows the stimulation of intracellular Ca^{2+} when the RPMC are treated with compound 48/80 (2 μg/ml). Preincubation of AIE (100 μg/ml) with RPMC decreased compound 48/80–induced intracellular Ca^{2+} levels.

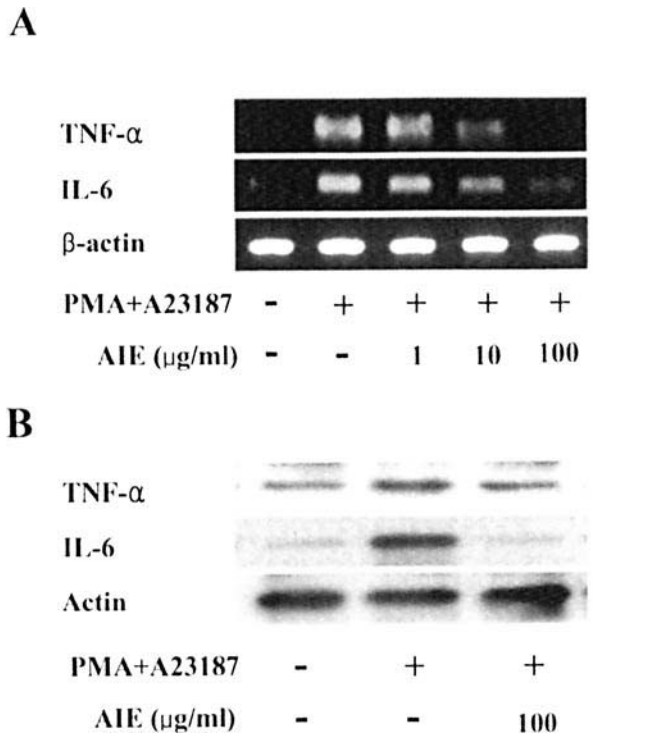

Figure 5. Effect of *Artemisia iwayomogi* (Compositae) (AIE) on the TNF-α and IL-6 expression (A) and production (B). HMC-1 cells were pretreated with AIE for 30 mins and stimulated by PMA (20 n*M*) plus A23187 (1 μ*M*) for 4 hrs for mRNA and 16 hrs for protein. TNF-α and IL-6 mRNA expression was quantified by RT-PCR. Extracts of cells (25 μg) were analyzed by Western blot.

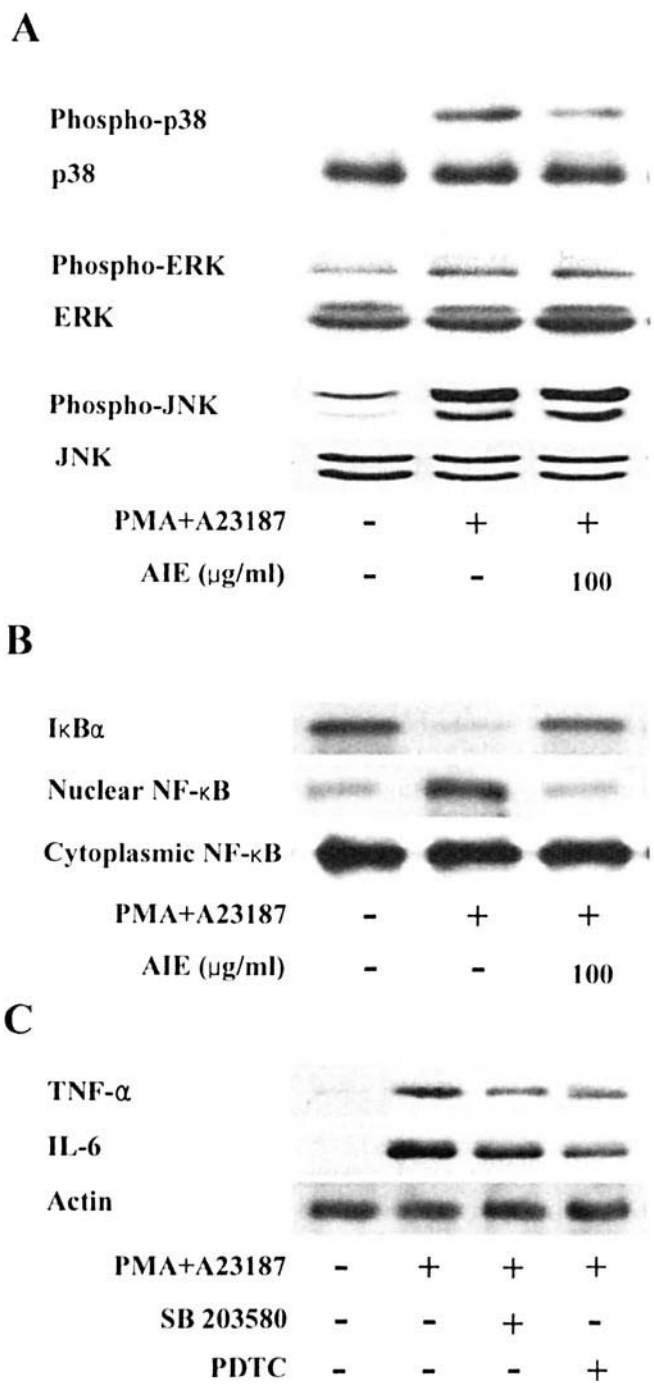

Figure 6. Effect of *Artemisia iwayomogi* (Compositae) (AIE) on the MAPKs and NF-κB. After pretreatment of AIE (100 μg/ml) for 30 mins, HMC-1 cells were stimulated by PMA (20 n*M*) plus A23187 (1 μ*M*) for 15 mins for MAPKs activation or 4 hrs for NF-κB activation. (A) Phosphorylation of MAPKs was analyzed by Western blot. (B) Cell extract was analyzed by Western blot for IκBα in cytoplasmic and translocation of NF-κB in nuclear extracts. (C) HMC-1 cells were pretreated with SB 203580 (5 μ*M*) or PDTC (10 μ*M*) for 30 mins prior to PMA plus A23187 stimulation. Production of TNF-α and IL-6 was analyzed by Western blot.

Effect of AIE on Proinflammatory Cytokines. We examined whether AIE could regulate proinflammatory cytokines such as TNF-α and IL-6 in HMC-1 cells. The HMC-1 cell line is a useful cell for studying the cytokine activation pathway (21). Stimulation of HMC-1 cells with PMA (20 n*M*) plus A23187 (1 μ*M*) during 4 hrs induced the gene expression of both cytokines. Pretreatment of AIE dose dependently inhibited PMA plus A23187-induced TNF-α and IL-6 gene expression (Fig. 5A). Pretreatment of AIE (100 μg/ml) also decreased PMA plus A23187-induced TNF-α and IL-6 production in HMC-1 at 16 hrs (Fig. 5B).

Effect of AIE on MAPKs Activation and Translocation of NF-κB. Stimulation of HMC-1 cells with PMA plus A23187 resulted in increased phosphorylation of all three MAPKs peaked at 15–30 mins (data not shown). Treatment of AIE attenuated the PMA plus A23187-induced p38 MAPK activation but did not affect the phosphorylation of ERK and JNK (Fig. 6A). Stimulation of HMC-1 cells with PMA plus A23187 induced the degradation of IκBα and nuclear translocation of p65 NF-κB after 4 hrs of incubation. AIE inhibited the PMA plus A23187-induced degradation of IκBα and nuclear translocation of p65 NF-κB (Fig. 6B). Treatment of cells with specific p38 MAPK inhibitor, SB 203580 (5 μ*M*), and NF-κB inhibitor, PDTC (10 μ*M*) decreased PMA plus A23187-induced TNF-α and IL-6 production.

Discussion

Anaphylaxis is a life-threatening syndrome induced by the sudden systemic release of inflammatory mediators, such as histamine, heparin, and various cytokines, from mast cells. Mast cells are located throughout the human body, and on allergen exposure, they are stimulated via the IgE receptor (22). The results of this study demonstrated that AIE has anti-allergic properties. AIE inhibited compound 48/80–induced systemic allergic reaction and IgE-mediated local allergic reaction. AIE attenuated compound 48/80–induced or IgE-mediated histamine release from RPMC. These results indicate that mast cell–mediated immediate-type allergic reactions are inhibited by AIE.

Numerous reports established that stimulation of mast cells with compound 48/80 or IgE initiates the activation of signal-transduction pathway, which leads to histamine release. Several recent studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (23). Compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation in the membrane. This result indicates that the increase in membrane permeability may be an essential trigger for the release of the mediator from mast cells. In this sense, anti-allergic agents having a membrane-stabilizing action may be desirable. AIE might stabilize the lipid bilayer membrane, thus preventing the perturbation being induced by compound 48/80.

The intracellular calcium pathways are critical to the degranulation of mast cells. Agents that stimulate an intracellular calcium level have been shown to inhibit mast cell degranulation (24). Calcium movements in mast cells

represent a major target for effective anti-allergic drugs, as this is an essential events linking stimulation to secretion (9). The transduction pathways modulating intracellular Ca^{2+} are modified by ADP-rybosylates G-protein binding protein (25). Our results showing an attenuation of compound 48/80–induced intracellular Ca^{2+} in mast cells with AIE treatment is consistent with other reports. From these observations, we strongly speculate that the decreased intracellular Ca^{2+} is involved in the inhibitory effect of AIE on histamine release, and AIE might have membrane-stabilizing activity through G-protein.

Mast cell-derived proinflammatory cytokines, especially TNF-α and IL-6, have a critical biological role in the allergic reaction. 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 anti-TNF-α antibody (26). IL-6 is also produced from mast cells, and its local accumulation is associated with a PCA reaction (27). These reports indicate that reduction of proinflammatory cytokines from mast cells is a one of the key indicators of reduced allergic symptoms. In our present study, AIE inhibited the gene expression and production of TNF-α and IL-6 in PMA plus A23187-stimulated HMC-1 cells. This result suggests that the anti-allergic effect of AIE results from its reduction of TNF-α and IL-6 generation from mast cells. Intracellular Ca^{2+} plays an important role in the expression of TNF-α and IL-6. Depletion of intracellular Ca^{2+} blocked the IgE-induced TNF-α and IL-6 expression through the NF-κB signaling pathway in RBL-2H3 mast cells (28). Because of the reducing effect of AIE on the intracellular Ca^{2+}, we suggest that one possible pathway of the inhibitory effect of AIE on the TNF-α and IL-6 expression is mediated by the reduction of intracellular Ca^{2+} in HMC-1 cells.

To evaluate the mechanisms of effect of AIE on the proinflammatory cytokine expression, we examined the effect of AIE on the MAPKs activation. The MAPK cascade is one of the important signaling pathways in immune responses. The exact signaling pathways among three types of MAPKs, such as p38, ERK, and JNK, are still unclear; however, p38 MAPK is thought to play an important role in regulation of inflammatory responses. Phosphorylation of p38 MAPK is essential for the expression of the proinflammatory cytokines (29, 30). According to the present results, PMA plus A23187 simultaneously activated all three MAPKs in HMC-1 cells. Among the MAPKs, AIE inhibited the activation only of p38 MAPK but not of ERK or JNK. Furthermore, the specific p38 MAPK inhibitor, SB 203580, decreased TNF-α and IL-6 production. These data suggest that AIE has the inhibitory activity on p38 MAPK activation and downstream TNF-α and IL-6 production. Expression of TNF-α and IL-6 gene is also dependent on the activation of transcription factor NF-κB. Activation of NF-κB requires phosphorylation and proteolytic degradation of the inhibitory protein IκBα (15). In PMA and A23187-stimulated mast cells, AIE decreased the degradation of IκBα and nuclear translocation of p65 NF-κB. Additionally, the specific NF-κB inhibitor, PDTC, reduced PMA plus A23187-induced TNF-α and IL-6 production. These data demonstrate that AIE attenuates activation of NF-κB and downstream TNF-α and IL-6 production.

In the present study, we used the whole water extract of *Artemisia iwayomogi*; hence, the active components that are responsible for the biological effects are not clear at this time. The effort to identify active components from AIE in the immediate-type allergic reaction is ongoing in our laboratory. It has been reported that *Artemisia iwayomogi* has an inhibitory effect on nitric oxide synthase. Recently active components of *Artemisia iwayomogi*, such as genkwanin, jaceosidin, and quebrachitol, have been shown to have an $ONOO^-$ scavenging property (31). Additionally several essential oils, such as camphor, borneol, caryophyllene, and eugenol, have been identified from *Artemisia iwayomogi* (32). It has been reported that the derivative of eugenol, methyeugenol, suppresses compound 48/80–induced histamine release from mast cells and inhibits the gene expression of L-histidine decarboxylase,which catalyzes the formation of histamine from its precursor (33).

PCA is one of the most important *in vivo* models of anaphylaxis in local allergic skin reaction. The mice receiving AIE were protected from IgE-mediated PCA. This finding suggests that AIE might be useful in the treatment of allergic skin reactions. In conclusion, the results obtained in the present study provide evidence that AIE contributes importantly to the prevention or treatment of mast cell–mediated allergic diseases.

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