

Artemisia iwayomogi Inhibits Immediate-Type Allergic Reaction and Inflammatory Cytokine Secretion

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The immediate-type allergic reaction is involved in many allergic diseases such as asthma and allergic rhinitis. The discovery of drugs for the treatment of immediate-type allergic diseases is a very important subject in human health. In this study, we investigated the effect of *Artemisia iwayomogi* (AIAE) on mast cell-mediated allergic reaction and inflammatory cytokine secretion. AIAE inhibited compound 48/80-induced systemic reactions in mice. AIAE decreased the passive cutaneous anaphylaxis (PCA) reaction activated by antidinitrophenyl (anti-DNP) IgE antibody. AIAE dose-dependently reduced histamine release from rat peritoneal mast cells activated by compound 48/80 or anti-DNP IgE. Furthermore, AIAE attenuated the phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated tumor necrosis factor- α and interleukin-6 secretion in human mast cells. These results provide evidence that AIAE may be beneficial in the treatment of allergic diseases.

Keywords Allergic Reaction, *Artemisia iwayomogi*, Interleukin-6, Mast Cells, Tumor Necrosis Factor- α .

INTRODUCTION

The aqueous extract of *Artemisia iwayomogi* (Compositae) (AIAE) has been used for centuries as traditional medicine. This crude drug is used for the treatment of various liver diseases.^(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, an acute systemic allergic reaction, is mediated by histamine released in response to the antigen cross-linking of

immunoglobulin E (IgE) bound to FcεRI on mast cells. Mast cell activation causes the process of degranulation that results 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 the 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⁾

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.^(8–11) Therefore, modulation of secretion of these cytokines from mast cells can provide a useful therapeutic strategy for allergic inflammatory disease. In this study, we evaluated the effect of AIAE on compound 48/80-induced systemic reaction, antidinitrophenyl (anti-DNP) IgE antibody-induced local allergic reaction and histamine release from rat peritoneal mast cells (RPMC). Additionally, the effect of AIAE on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187-induced TNF-α and IL-6 secretion in a human mast cell line (HMC-1) also was investigated.

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 mM glutamine.

Animals

The original stock of male ICR mice and male Sprague-Dawley rats were purchased from Dae-Han Biolink Co. Ltd. (Chungbuk, Korea), and the animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed 5 to 10 per cage in a laminar air flow room maintained at a temperature of $22 \pm 2^\circ\text{C}$ and 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-DNP IgE, DNP-human serum albumin (HSA), α-minimal essential medium (α-MEM), o-phthaldialdehyde, PMA, and calcium

ionophore A23187 were purchased from Sigma Chemical Co. (St Louis, MO, USA). rTNF- α and rIL-6 were purchased from R&D Systems. (Minneapolis, MN, USA).

Preparation of AIAE

Artemisia iwayomogi were purchased from the Bohwa Dang (Jeonju, 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 hr. The extract was filtered and lyophilized. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM CaCl₂, 1 mM MgCl₂, 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 examined as previously described.⁽¹²⁾ Mice were given an intraperitoneal injection of 0.008 g/kg body weight (BW) of the mast cell degranulator compound 48/80. AIAE was dissolved in saline and administered orally and anally at doses ranging from 0.005 to 1 g/kg BW 1 hr before the injection of compound 48/80 ($n = 10/\text{group}$). In the time-dependent experiment, AIAE (1 g/kg) was administered 5, 10, and 20 min after injection of compound 48/80 ($n = 10/\text{group}$). 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 into each of 2 dorsal skin sites that had been shaved 48 hr earlier. The sites were outlined with a water-insoluble red marker. After 48 hr, each mouse received an injection of 1 μg of DNP-HSA in PBS containing 4% Evans blue (1:4) via the tail vein. AIAE (0.001 to 1 g/kg BW) was orally and anally administered 1 hr before the challenge. Then 30 mins 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) based on the previous report.⁽¹³⁾ The absorbent intensity of the extraction was measured at 620 nm by using a spectrophotometer, and the amount of dye was calculated with Evans blue measuring-line.

Preparation of RPMC

Mast cells were separated from rat peritoneal cavity cells according to the method described by Yurt et al.⁽¹⁴⁾ In brief, rats were anesthetized by ether and injected with 20 ml of Tyrode buffer B (137 mM NaCl, 5.6 mM glucose, 12 mM NaHCO₃, 2.7 mM KCl, 0.3 mM NaH₂PO₄, and 0.1% gelatin), into the

peritoneal cavity and the abdomen was gently massaged for about 90 sec. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells were aspirated by Pasteur pipette. The peritoneal cells were sedimented at $150 \times g$ for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells, i.e., macrophages and small lymphocytes. Peritoneal cells were suspended in 1 ml of Tyrode buffer B, layered on 2 ml of metrizamide (22.5 w/v%), and centrifuged at room temperature for 15 min at $400 \times g$. 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 A. Mast cell preparations were ~95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypan blue uptake.

Inhibition of Histamine Release

Purified RPMC were resuspended in Tyrode buffer A for the treatment with compound 48/80. 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 AIAE (0.001 to 1 mg/ml) preparations, and then incubated for 10 min with compound 48/80. RPMC suspensions (2×10^5 cells/ml) also were sensitized with anti-DNP IgE (10 µg/ml) for 16 hr. The cells were preincubated with the AIAE (0.001 to 1 mg/ml) at 37°C for 10 min prior to the challenge with DNP-HSA (1 µg/ml). The cells were separated from the released histamine by centrifugation at $400 \times g$ for 5 min at 4°C. The histamine content was measured by the o-phthaldialdehyde spectrofluorometric procedure.⁽¹⁵⁾ The fluorescence intensity was measured at emission of 438 nm and excitation of 353 nm using a spectrofluorometer.

The inhibition percentage of histamine release was calculated using the following equation:

$$\% \text{ Inhibition} = (A - B) \times 100 / A \quad (1)$$

where A is histamine release without AIAE and B is histamine release with AIAE.

Assay of TNF- α and IL-6 Secretion

TNF- α and IL-6 secretion was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as described previously.⁽¹⁶⁾ HMC-1 cells were cultured with α -MEM plus 10% FBS and resuspended in Tyrode buffer A. The cells were sensitized with PMA (20 nM) plus A23187 (1 µM) for 6 hr in the absence or presence of AIAE. 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 serum previously determined to be negative to endogenous TNF- α and IL-6. After exposure to the medium, the assay plates were exposed sequentially to biotinylated antihuman TNF- α and IL-6, and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablets substrates. Optical density readings were made within 10 min of the addition of the substrate with a 405 nm filter.

Statistical Analysis

Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC, USA). Treatment effects were analyzed using one-way ANOVA, followed by Duncan's multiple range tests. $p < 0.05$ was used to indicate significance.

RESULTS

AIAE Attenuates the Compound 48/80-Induced Systemic Reaction

To determine the effect of AIAE on allergic reaction, an in vivo model of systemic reaction was used. Compound 48/80 (0.008 g/kg) 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 AIAE was administered orally and anally at a concentrations ranging from

Table 1: Effect of AIAE on compound 48/80-induced systemic reaction.

AIAE treatment (g/kg BW)	Compound 48/80 (0.008 g/kg BW)	Mortality(%)	
		Orally	Anally
None (saline)	+	100	100
0.005	+	100	100
0.01	+	100	80
0.05	+	90	40
0.1	+	60	20
0.5	+	50	0
1	+	20	0
1	—	0	0

Groups of mice ($n = 10/\text{group}$) were orally and anally pretreated with 200 μl saline or AIAE. AIAE was given at various doses 1 hr before the compound 48/80 injection. The compound 48/80 solution was given intraperitoneally to the group of mice. Mortality (%) within 1 hr following compound 48/80 injection is represented as the number of dead mice $\times 100/\text{total number of experimental mice}$.

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 AIAE (1 g/kg) 5, 10, and 20 min after compound 48/80 injection increased time-dependently (Table 2).

AIAE Inhibits IgE-Mediated PCA

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 challenge. AIAE dose-dependently inhibited PCA reaction (Figure 1).

Table 2: Time-dependent effect of AIAE on compound 48/80-induced systemic reaction.

AIAE treatment (g/kg BW)	Time (min)	Compound 48/80 (0.008 g/kg BW)	Mortality(%)	
			Orally	Anally
None (saline)		+	100	100
1	5	+	50	40
1	10	+	60	60
1	20	+	100	100

Groups of mice ($n = 10/\text{group}$) were orally and anally pretreated with 200 μl saline or AIAE. AIAE (1 g/kg) was given at 5, 10, and 20 min after the compound 48/80 injection. The compound 48/80 solution was given intraperitoneally to the group of mice. Mortality (%) within 1 hr following compound 48/80 injection is represented as the number of dead mice $\times 100/\text{total number of experimental mice}$.

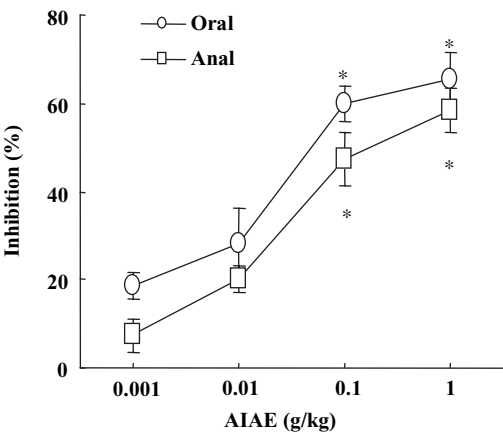


Figure 1: Effect of AIAE on the PCA. AIAE was administered orally and anally 1 hr prior to the challenge with antigen. Each date represents the mean $\pm$ SEM of three independent experiments. * $p < 0.05$; significantly different from the saline value.

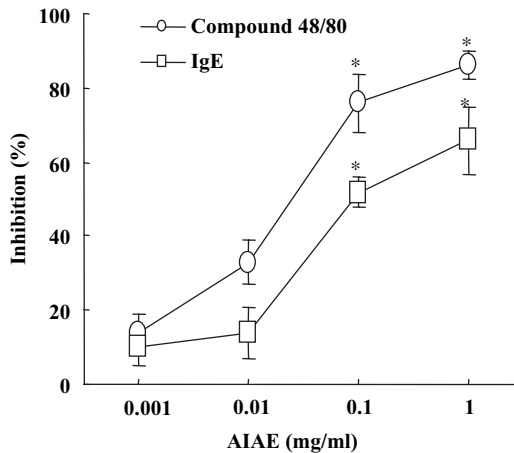


Figure 2: Effect of AIAE on compound 48/80- or IgE-induced histamine release from RPMC. The cells (2×10^5 cells/ml) were preincubated with AIAE at 37°C for 10 min prior to incubation with compound 48/80 or DNP-HSA. Each data represents the mean $\pm$ SEM of three independent experiments. * $p < 0.05$; significantly different from the saline value.

AIAE Inhibits Compound 48/80- or anti-DNP IgE-Mediated Histamine Release

The inhibitory effects of AIAE on compound 48/80- or anti-DNP IgE-mediated histamine release from RPMC are shown in Figure 2. AIAE dose-dependently inhibited compound 48/80- or anti-DNP IgE-mediated histamine release at concentrations of 0.001–1 mg/ml.

AIAE Inhibits Proinflammatory Cytokine Secretion in HMC-1 cells

We examined whether AIAE could regulate proinflammatory cytokines such as TNF- α and IL-6 in HMC-1 cells. Stimulation of HMC-1 cells with PMA plus A23187 induced the secretion of both cytokines. Pretreatment of AIAE dose-dependently inhibited PMA plus A23187-induced TNF- α and IL-6 secretion (Table 3).

DISCUSSION

Immediate-type allergic reaction 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 upon allergen exposure, they are stimulated via the IgE receptor.⁽¹⁷⁾ The results of this study demonstrated that AIAE has antiallergic properties. AIAE inhibited compound 48/80-induced systemic reaction and anti-DNP IgE-mediated local allergic reaction. AIAE attenuated compound 48/80-induced or anti-DNP IgE-mediated histamine release from

Table 3: Effect of AIAE on PMA plus A23187-stimulated proinflammatory cytokine secretion from HMC-1 cells.

Treatment	Concentration (mg/ml)	Content (ng/ml)	
		TNF- α	IL-6
None (saline)		0.271 $\pm$ 0.065	0.621 $\pm$ 0.034
PMA + A23187		1.252 $\pm$ 0.562	1.663 $\pm$ 0.146
PMA + A23187 + AIAE	0.01	0.839 $\pm$ 0.143	1.623 $\pm$ 0.169
PMA + A23187 + AIAE	0.1	0.584 $\pm$ 0.023*	1.303 $\pm$ 0.475*
PMA + A23187 + AIAE	1	0.593 $\pm$ 0.081*	1.070 $\pm$ 0.206*

PMA plus A23187-stimulated HMC-1 cells were incubated for 8 hr in the absence or presence of AIAE. Proinflammatory cytokines secreted into the medium are presented as the mean $\pm$ SEM of three independent experiments. * p < 0.05; significantly different from the PMA + A23187 value.

RPMC. These results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by AIAE. Numerous reports established that stimulation of mast cells with compound 48/80 or IgE initiates the activation of a 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.⁽¹⁸⁾ 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, antiallergic agents having a membrane-stabilizing action may be desirable. AIAE might stabilize the lipid bilayer membrane, thus preventing perturbation being induced by compound 48/80. Additionally, the AIAE-administered mice are protected from anti-DNP IgE-mediated PCA, one of the most important in vivo models of anaphylaxis in local allergic reaction. This finding suggests that AIAE might be useful in the treatment of allergic skin reactions.

Mast cell-derived 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.⁽¹⁹⁾ IL-6 also is produced from mast cells and its local accumulation is associated with a PCA reaction.⁽²⁰⁾ These reports indicate that reduction of proinflammatory cytokines from mast cell is a one of the key indicators of reduced allergic symptom. In the present study, AIAE inhibited the secretion of TNF- α and IL-6 in PMA plus A23187-stimulated HMC-1 cells. This result suggests that the antiallergic effect of AIAE results from its reduction of TNF- α and IL-6 release from mast cells.

In conclusion, the results obtained in the present study provide evidence that AIAE contributes importantly to the prevention or treatment of mast cell-mediated allergic diseases.

ACKNOWLEDGMENT

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (the Center for Healthcare Technology Development, Woosuk University, Republic of Korea).

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