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Anti-allergic Effects of *Artemisia iwayomogi* on Animal Models of Allergic Reactions

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Abstract – The effects of aqueous extract of *Artemisia iwayomogi* (Compositae) (AIAE) on the mast cell-dependent allergic and inflammatory reactions were investigated. AIAE (0.05 to 1 g/kg) dose-dependently inhibited systemic allergic reaction induced by compound 48/80 in mice. AIAE (0.1 and 1 g/kg) also significantly inhibited local allergic reaction activated by anti-DNP IgE. AIAE (0.001 to 1 mg/ml) dose-dependently inhibited the histamine release from rat peritoneal mast cells (RPMC) activated by compound 48/80. Moreover, AIAE inhibited the secretion of interleukin (IL)-6 in phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187-stimulated human mast cell line (HMC-1) cells. These results provide evidence that AIAE may be beneficial in the treatment of allergic diseases.
Keywords – *Artemisia iwayomogi*, allergic reaction, compound 48/80, anti-DNP IgE, IL-6

Introduction

The *Artemisia iwayomogi* (Compositae), well known as "Haninjin or Dowijigi" in Korea, has been used for centuries as traditional medicine. This crude drug is used for treatment of various liver diseases (Lee *et al.*, 1993). It has contents of esculetin-6-methylether, esculetin-7-methylether, camphor, borneol, p-cymene, caryophyllene, δ-cadinene, methyleugenol and bornyl acetate (Kang *et al.*, 1993), The mast cell has long been thought to play a crucial role in the development of many physiological changes during immediate allergic responses (Kim and Lee, 1999). In general, immediate hypersensitivity, which involves urticaria, allergic rhinitis and asthma, is mediated by various chemical mediators released from mast cells. Among the preformed and newly synthesized inflammatory substances released from the degranulation of mast cells, histamine remains the best characterized and most potent vasoactive mediator implicated in the acute phase of immediated-type allergic reactions (Petersen *et al.*, 1996). Mast cell degranulation can be elicited by a number of positively charged substances, collectively known as the basic secretagogues of mast cells (Lagunoff *et al.*, 1983). Compound 48/80 and polymers of basic amino acids, such as substance P, are some of the most potent secretagogues of mast cells (Ennis *et al.*, 1980). Compared with the natural process, a high concentration of compound 48/80 induces almost a 90% release of histamine from mast cells. Thus, an appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (Allansmith *et al.*, 1989; Shin, 2003). The secretory response of mast cells can also be induced by aggregation of their cell surface-specific receptors for immunoglobulin E (IgE) by the corresponding antigen (Segal *et al.*, 1977; Metzger *et al.*, 1986; Alber *et al.*, 1991; Beaven and Metzger, 1993). It has been established that the anti-IgE antibody induces passive cutaneous anaphylaxis (PCA) reactions as a typical *in vivo* model for the immediate hypersensitivity. Although mast cells also store small amounts of cytokines in their granules (Gordon and Galli, 1990; Gordon *et al.*, 1990), dramatically increase the production of tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and other cytokines after stimulated with phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187 (Shin *et al.*, 2003). The finding that the activated mast cells are also a source of several cytokines suggests an additional role of mast cells in late-phase reactions and other persistent inflammatory processes (Galli, 1993). Both TNF-α and IL-6 activate inflammatory cells and stimulate them to synthesize other cytokines. This paper deals with an evaluation of the effects of AIAE on the compound

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48/80-induced systemic anaphylaxis, anti-dinitrophenyl (DNP) IgE antibody-induced PCA, and histamine release from rat peritoneal mast cells (RPMC). The effect of AIAE on PMA plus calcium ionophore A23187-stimulated IL-6 secretion in human mast cell line (HMC-1) cells was also investigated

Materials and Methods

Reagents – Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), α-minimal essential medium (α-MEM), *o*-phthaldialdehyde, evans blue, phorbol 12-myristate 13-acetate, calcium ionophore A23187 and metrizamide were purchased from Sigma Chemical Co. The rIL-6 was obtained from R&D Systems Inc. (USA).

Animals – The original stock of ICR mice and SD rats were purchased from Dae-Han Biolink (Korea), and the animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed five to ten per cage in a laminar air flow room maintained under a temperature of 22±2°C and relative humidity of 55±5% throughout the study.

Cell culture – The human mast cell line, HMC-1, was established from a patient with mast cell leukaemia, and exhibited a phenotype of immature mast cells. HMC-1 cells were cultured in a α-MEM with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, 50 μg/ml streptomycin, and 1.2 mM α-thioglycerol. Cells were passaged every three to four days.

Preparation of AIAE – The plants of *Artemisia iwayomogi* were purchased from the oriental drug store, 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 distilled water at 70°C for 5 h. The extract was filtered through Whatman No.1 filter paper, and the filtrate was lyophilized. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 0.1% bovine serum albumin) before use.

Compound 48/80-induced systemic anaphylaxis – Compound 48/80-induced systemic anaphylaxis was carried out according to the previous method (Shin, 2003). Briefly, mice were given an intraperitoneal injection of 8 mg/kg body weigh (BW) of compound 48/80. AIAE was dissolved in saline and administered intraperitoneally at doses of 0.01 to 1 g/kg, I h before the injection of compound 48/80 (n=10/group). In time dependent experiment, AIAE (1 g/kg BW) was administered intraperitoneally at 0, 5, 10, and 20 min after compound 48/80 injection (n=10/group). Mortality was monitored for 1 h after the induction of anaphylactic shock.

PCA reaction – An IgE-dependent cutaneous reaction was generated by sensitizing the skin with an intradermal injection of anti-DNP IgE followed 48 h later with an injection of DNP-HSA into the tail vein. The mice were injected intradermally with 0.5 μg of anti-DNP IgE into each of two dorsal skin sites that had been shaved 48 h earlier. Each mouse, 48 h later, received an injection of 100 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 administered 1 h before the challenge. Then 30 min after the challenge, the mice were sacrificed and the dorsal skin was removed for measurement of pigment area. The amount of dye was then determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of mixture of acetone and phosphoric acid (5:13) based on the method of Katayama *et al.* (1978). The absorbent intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan).

Preparation of RPMC – RPMC were isolated as previously described (Kanemoto *et al.*, 1993). 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 $NaHCO_3$, 2.7 mM KCl, 0.3 mM NaH_2PO_4 and 0.1% gelatin) into the peritoneal cavity and the abdomen was gently massaged for about 90 seconds. The peritoneal cavity was carefully opened and the fluid containing peritoneal cells was aspirated by a Pasteur pipette. Thereafter, the peritoneal cells were sedimented at 150×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, according to the method described by Yurt *et al.* (1977). In brief, peritoneal cells suspended in 1 ml of Tyrode buffer B were layered on 2 ml of metrizamide (22.5 W/V%) and centrifuged at room temperature for 15 min 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 Tyrode buffer A.

Inhibition of histamine release-Purified RPMC were resuspended in Tyrode buffer A for the treatment of 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 (10 min) with the compound 48/80. The cells

were separated from the released histamine by centrifugation at 400×g for 5 min at 4°C. Residual histamine in cells was released by disrupting the cells with perchloric acid and centrifugation at 400×g for 5 min at 4°C.

Assay of histamine release – The histamine content was measured by the *o*-phthaldialdehyde spectrofluorometric procedure of Shore *et al.* (1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer (Shimadzu, RF-5301 PC, Japan).

Assay of IL-6 secretion – IL-6 secretion was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as described previously (Kim and Lee, 1999). HMC-1 cells were resuspended in Tyrode buffer A. The cells were sensitized with PMA (20 nM) plus A23187 (1 μM) for 6-9 h in the absence or presence of AIAE. The ELISA was performed by coating 96-well plates with 6.25 ng/well of murine monoclonal antibody with specificity for murine IL-6. For the standard curve, rIL-6 was added to serum previously determined to be negative to endogenous IL-6. After exposure to the medium, the assay plates were exposed sequentially to biotinylated anti-human 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 on a Titertek Multiscan (Flow Laboratories) with a 405 nm filter.

Statistical analysis – The results obtained were expressed as mean±SEM. Student's *t*-test was used to make a statistical comparison between the groups. Results with $p<0.05$ were considered statistically significant.

Result

Effect of AIAE on compound 48/80-induced systemic anaphylaxis – After the intraperitoneal injection of compound 48/80, the mice were monitored for 1 h, after which the mortality rate was determined. As shown in Table 1, an intraperitoneal injection of 0.2 ml saline as a control induced a fatal shock by 100%. When the AIAE was intraperitoneally administered at a concentrations ranging from 0.01 to 1 g/kg BW 1 h before the compound 48/80 injection, the mortality was reduced dose-dependently. In addition, the mortality of mice administered intraperitoneally with AIAE (1 g/kg) at 0, 5, 10, and 20 min after compound 48/80 injection increased time-dependently (Table 2).

Effect of AIAE on anti-DNP IgE-mediated PCA reaction – As described in this experimental procedures, local extravasation was induced by a local injection of anti-DNP IgE followed by an antigenic challenge. Oral administration of AIAE (0.1 and 1 g/kg) showed a marked inhibition rate in PCA reaction (Table 3).

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

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

Mice (n=10/group) were intraperitoneally pretreated with 0.2 ml saline or AIAE at various doses 1 h before intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead mice×100/total number of experimental mice.

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

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

Mice (n=10/group) were intraperitoneally pretreated with 0.2 ml saline or AIAE (1 g/kg) at 0, 5, 10, and 20 min after intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead mice×100/total number of experimental mice.

Table 3. Effect of AIAE on PCA

AIAE treatment (g/kg BW)	Anti-DNP IgE plus DNP-HSA	Amount of dye (μg/site)
None (saline)	+	4.965±0.631
0.001	+	4.033±0.398
0.01	+	3.572±0.312
0.1	+	1.985±0.145*
1	+	1.730±0.217*

AIAE was administered orally 1 h prior to the challenge with antigen. Each datum represents the mean±SEM of three independent experiments. *$p<0.05$; significantly different from the saline value.

Effect of AIAE on compound 48/80-induced histamine release from RPMC – As shown in Table 4. AIAE dose-dependently inhibited compound 48/80-induced histamine release at concentrations of 0.001 to 1 mg/ml. In particular, AIAE significantly inhibited the compound 48/80-induced histamine release at the concentrations of 0.1 and 1 mg/ml.

Effect of AIAE on IL-6 secretion from HMC-1 cells – To assess the effect of AIAE in PMA plus A23187-stimulated IL-6 secretion, HMC-1 cells were pretreated with AIAE for 30 min prior to PMA plus A23187-stimulation. As shown in Table 5, AIAE (0.1 mg/ml)

Table 4. Effect of AIAE on compound 48/80-induced histamine release from RPMC

AIAE treatment (mg/ml)	Compound 48/80 (5 μg /ml)	Amount of histamine (μg/ml)
None (saline)	+	0.182±0.021
0.001	+	0.147±0.035
0.01	+	0.122±0.017
0.1	+	0.044±0.005*
1	+	0.025±0.005*

The cells (2×10^5 cells/ml) were preincubated with AIAE at 37°C for 10 min prior to incubation with compound 48/80. Each datum represents the mean±SEM of three independent experiments. *$p<0.05$; significantly different from the saline value.

Table 5. Effect of AIAE on PMA plus A23187-stimulated IL-6 secretion from HMC-1 cells

Treatment	Concentration (mg/ml)	Content (ng/ml)
None (saline)	–	0.310±0.071
PMA+A23187	–	1.716±0.414
PMA+A23187+AIAE	0.01	1.407±0.561
	0.1	0.624±0.070*

PMA plus A23187-stimulated HMC-1 cells (3×10^5) were incubated for 8 h in the absence or presence of AIAE. IL-6 secreted into the medium are presented the mean±SEM of three independent experiments. *$p<0.05$; significantly different from the saline value.

significantly inhibited the secretion of IL-6 in PMA plus A23187-stimulated HMC-1 cells.

Discussion

This study has shown that AIAE inhibited compound 48/80-induced systemic allergic reaction and anti-DNP IgE-mediated local allergic reaction. AIAE also inhibited compound 48/80-induced histamine release from RPMC. These results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by AIAE. There is no doubt that stimulation of mast cells with compound 48/80 initiates the activation of signal-transduction pathway, which leads to histamine release. There have been some reports that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (Mousli *et al.*, 1990a; Mousli *et al.*, 1990b). The evidence indicates that the protein is G inhibitory-like and that the activation is inhibited by benzalkonium chloride (Bueb *et al.*, 1990). Tasaka *et al.* (1986) reported that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. This result indicates that the permeability increase of the cell membrane 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. AIAE might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80. PCA is one of the most important *in vivo* models of anaphylaxis in local allergic reaction. The AIAE-administered mice are protected from IgE-mediated local allergic reaction. It is conceivable that AIAE inhibits the initial phase of immediate-type allergic reactions, probably through interference with the mast cell/histamine system. It is believed that cytokines, including TNF-α and IL-6 play a major role in triggering and sustaining the allergic inflammatory response. AIAE inhibited the secretion of IL-6 in PMA plus A23187-stimulated HMC-1 cells. Many signals for anaphylactic reaction, including protein kinase C and Ca^{2+}, are also involved in production in mast cells distinct from that for degranulation. Further work should address the possibility that AIAE may also be active in the inhibition of human mast cell degranulation and in the treatment of human allergic disorders.

In conclusion, the results obtained in the present study provide evidence that AIAE importantly contributes to the prevention or treatment of allergic diseases. The studies on the isolation and characterization of the active chemical constituents are in progress.

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