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A comparative study on the inhibitory effects of mast cell-mediated allergic reactions by artificially cultured and wild *Acanthopanax senticosus*

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SUMMARY

We compared the effect between CAS and WAS(root, stem) on mast cell-mediated allergic reaction. CAS, WAS-root and WAS-stem, significantly inhibited compound 48/80-induced systemic allergic reaction(1g/kg) and histamine release from RPMC(1mg/ml). CAS, WAS-root and WAS-stem also inhibited passive cutaneous anaphylactic reaction. In addition, IgE-induced TNF-α secretion from RBL-2H3 was inhibited by pretreatment of CAS, WAS-root or WAS-stem(0.01μg/ml). Taken together, inhibitory effect on mast cell-mediated allergic reactions of WAS-root is greater than those of WAS-stem but less than those of CAS.

INTRODUCTION

The mast cells have been thought to play a major role in the development of many physiologic changes during allergic responses. Among the preformed and newly synthesized inflammatory substances released on degranulation of mast cells, histamine remains the best characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (Petersen et al., 1996).

Key words: *Acanthopanax senticosus;* Mast cell-mediated immediate type allergic reactions; Peritoneal mast cell

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Mast cell degranulation can be elicited by a number of positively charged substances, collectively known as the basic secretagogues of mast cells (Lagunof *et al.*, 1983). The most potent secretagogues include the synthetic compound 48/80 and polymers of basic amino acids (Ennis *et al.*, 1980). An appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylactic reactions (Allan smith *et al.*, 1989). The secretary response of mast cells can also be induced by aggregation of their cell surface-specific receptors for immunoglobulin E (IgE) by the corresponding antigen (Alber *et al.*, 1991; Metzger *et al.*, 1986; Segal *et al.*, 1977). It has been established that the anti-IgE antibody induces passive cutaneous anaphylaxis (PCA) as a typical model for

mast cell-dependent immediate type allergic reactions. The skin of rat is a useful site for studying PCA (Saito and Nomura, 1989). Although mast cells also store small amounts of cytokines in their granules (Gordon and Galli, 1990), these cells dramatically increase the production of tumor necrosis factor-α (TNF-α), interleukin 6 and other cytokines within 30min after their surface FcεRI are cross-linked with specific antigen (Burd *et al.*, 1989; Gurish *et al.*, 1991; Plaut *et al.*, 1989; Wodnar-Filipowicz *et al.*, 1989).

Acanthopanax senticosus AS is a typical Oriental herb. The roots and stems of which have been used as a tonic, anti-rheumatic and prophylactic for chronic bronchitis, hypertension, anti-stress and ischemic heart disease (Nishibe *et al.*, 1990). It has also been used for treatment of various allergic diseases in Korea. But it is still unclear how AS inhibits these diseases in experimental animal models.

In the present study, we have compared the effect on mast cell-mediated allergic reaction of WAS with those of artificially cultured *Acanthopanax senticosus* (CAS).

MATERIALS AND METHODS

Reagents

Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), *o*-phthaldialdehyde (OPA), Evans blue, fetal bovine serum, α-minimum essential medium and metrizamide (density, 1.120 g/㎖) were purchased from Sigma Chemical Co. (St. Louis, MO). Recombinant TNF-α, biotinylated TNF-α, anti-murine TNF-α was purchased from R & D system Inc, USA. Phosphatase-labeled anti-rabbit IgG was purchased from Serotec (Oxford, England).

Animals

The original stock of ICR mice and Wistar rats were purchased from the Dae Han Experimental Animal Center (Taejon, Korea) and the animals were maintained at the College of Pharmacy, Wonkwang University. The animals were housed five to ten per cage in a laminar air flow room maintained at a temperature of 22±2°C and relative humidity of 55±10% throughout the study.

Preparation of CAS and WAS water extract

The CAS somatic embryos were allowed to grow in an MS medium (2% sugar) without growth regulators in bio-reactors equipped with an airlift (Kim *et al.*, 1998). The culturing for period of about 10-15 days led the matured somatic embryos to development of plantlets. The WAS were collected from south-dukyu mountain of Korea. All extract of these AS was prepared by decocting the dried prescription of herbs with boiling distilled water (100 g/L). The duration of decoction was about 3 h. The decoction was filtered, lyophilized, and kept at 4°C. The yield of extraction was about 14% (w/w). The AS water extract powder was dissolved in sterile saline (50 mg/㎖). CAS were obtained from J.W. Kim, Microplants Co. Ltd.

Compound 48/80-induced systemic anaphylactic shock

Mice were given an ip injection of 8 mg/kg compound 48/80. AS was dissolved in saline and administered orally 1 h before the injection of compound 48/80. Mortality was monitored for 1 h after induction of anaphylactic shock

Preparation of rat peritoneal mast cells (RPMCs)

RPMCs were isolated as previously described (Kim et al., 1998). In brief, rats were anesthetized by ether and injected with 20 ml of Tyrode buffer B (137 mM/L

NaCl, 5.3 mM/L glucose, 12 mM/L $NaHCO_3$, 2.7 mM/L KCl, 0.3 mM/L NaH_2PO_4) containing 0.1% gelatin (Sigma Chemical Co.) into the peritoneal cavity and the abdomen was gently massaged for about 90 s. 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.* (Yurt *et al*, 1977). In brief, peritoneal cells suspended in 1 ml of Tyrode buffer B were layered on 2 ㎖ of 22.5% w/v metrizamide (density, 1.120 g/㎖, Sigma Chemical Co.) 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 of Tyrode buffer A (10 mM/L HEPES, 130 mM/L NaCl, 5 mM/L KCl, 1.4 mM/L $CaCl_2$, 1 mM/L $MgCl_2$, 5.6 mM/L glucose) containing 0.1% bovine serum albumin (Sigma Chemical Co.). Mast cell preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypan blue uptake.

Compound 48/80-Induced Histamine Release

Purified RPMCs were resuspended in Tyrode buffer A containing 0.1% bovine serum albumin for the treatment of stimulators. Mast cell suspensions (2×10^5 cells/㎖) were pre-incubated for 10 min at 37℃ before the addition with compound 48/80 (5 ㎍/㎖). The cells were preincubated with the AS and then incubated (20 min) with compound 48/80. The reaction was stopped by cooling the tubes in ice. The cells were separated from the released histamine by centrifugation at 400 × g for 5 min at 4℃. Residual histamine in the cells was released by disrupting the cells with perchloric acid and centrifugation at 400 × g for 5 min at 4℃.

Assay of histamine release

The histamine content was measured by the OPA spectrofluorometric procedure of Shore et al. (Shore *et al*, 1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer. The inhibition percentage of histamine release was calculated using the following equation:

$$\% \text{ Inhibition} = (a - b) \times 100/a$$

Where a is histamine release without AS and b is histamine release with AS.

PCA reaction

An IgE-dependent cutaneous reaction was generated by sensitizing the skin with an id injection of anti-DNP IgE followed 24 h later with an injection of DNP-HSA into the rat's tail vein. The DNP-HSA was diluted in PBS. The rats were injected intradermally with 100 ng of anti-DNP IgE (a mean value) into each of 4 dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Twenty-four hours later each rat received an intravenous injection of 1 mg of DNP-HSA in PBS containing 4% Evans blue (1:1) via the tail vein. AS was orally administered 1 h before the challenge Thirty minutes after the challenge, the rats were killed and the dorsal skin was removed for measurement of the pigment area. The amount of dye was then determined colorimetrically after extraction with 1 ml of 0.1N KOH and 9 ml of a mixture of acetone and phosphoric acid (5:13) based on the method of Katayama *et al.* (Katayama, 1978). The absorbent

intensity of the extraction was measured at 620 nm in spectrofluorometer and the amount of dye was calculated with the Evans blue measuring-line.

Assay of TNF-α secretion

RBL-2H3 cells were resuspended in Tyrode buffer A. The cells were sensitized with anti-DNP IgE (1 μg/mℓ) and incubated for 16 h in the absence or presence of AS before the challenge with DNP-HSA (0.1 μg/mℓ) for 4 h. TNF-α secretion was measured by a modified ELISA as described. The ELISA was sensitive to TNF-α concentrations in the medium above 0.01 ng/ml. The ELISA was performed by coating 96-well plates with 6.25 ng/well of murine monoclonal antibody with specificity for murine TNF-α. 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. All reagents used in this assay and the coated wells were incubated for 1 h at room temperature. For the standard curve, rTNF-α were added to serum previously determined to be negative for endogenous TNF-α. After exposure to the medium, the assay plates were exposed sequentially to rabbit anti-TNF-α, phosphatase-conjugated goat anti-rabbit IgG, and *p*-nitrophenyl phosphate. 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. Appropriate specificity controls were included.

$$\% \text{ Inhibition} = (a - b) \times 100/a$$

Where "a" is cytokine release without AS and "b" is cytokine release with AS.

Statistical analysis

Each datum represents the mean and standard error of the mean (SEM) of the different experiments under the same conditions. The Student's *t*-test was used to make a statistical comparison between the groups. Results with $P<0.01$ were considered statistically significant

RESULTS

Effect of AS on compound 48/80-induced systemic allergic reaction and histamine release

To assess the contribution of AS in anaphylactic reactions, we first used the *in vivo* model of systemic allergic reaction. We used compound 48/80 (8 mg/kg) as a systemic fatal allergic inducer. After the 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 orally injection of 200 μℓ saline as a control induced fatal shock in 100% of each group. When the AS was orally administered at a concentration ranging from 0.01 to 1 g/kg for 1 h before compound 48/80 injection, the mortality was dose-dependently reduced. The inhibitory effects of AS on compound 48/80-induced histamine release from RPMCs is shown in Fig. 1. AS dose-dependently inhibited compound 48/80-induced histamine release. Inhibitory effects on compound 48/80-induced systemic allergic reaction and histamine release of CAS is larger than those of WAS (root) and WAS (stem).

Table 1. Effect of CAS and WAS on compound 48/80-induced systemic allergic reaction

Dose[a] (g/kg)	Compound 48/80 [b] (8mg/kg)	Mortality (%)[c]		
		CAS	WAS(root)	WAS(stem)
None(Saline)	+	100	100	100
0.01	+	100	100	100
0.1	+	75	100	100
1	+	25	50	50
1	-	0	0	0

[a]200 ㎕ saline or AS was orally given at various doses 1 h before (n=8 group) the compound 48/80 injection.
[b]The compound 48/80 solution was intraperitoneally given to mice.
[c]Mortality (%) within 1 h following the compound 48/80 injections is presented as the No. of dead mice × 100/total No. of experimental mice.

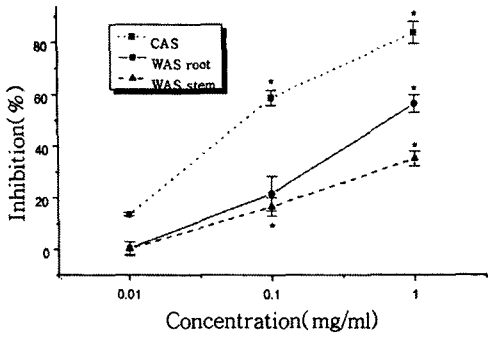

Fig. 1. Effects of CAS and WAS on compound 48/80-induced histamine release from RPMCs. RPMCs (2×10^5 cells/㎖) were preincubated with drug at 37℃ for 10 min prior to incubation with compound 48/80. Each data is presented as the mean ± SEM of three independent experiments. *P<0.01; significantly different from the control value.

Effect of AS on PCA

Another way to test allergic reactions is to induce PCA (Wershil *et al*, 1987). As described in the experimental procedures, local extravasation is induced by skin injection of anti-DNP IgE followed by an intravenous antigenic challenge. Anti-DNP IgE was injected in the right dorsal skin sites. As a control, the left dorsal skin sites of these rats were injected with saline alone After 24h, rats were injected intravenously with DNP-HSA plus Evans blue. The cutaneous anaphylactic reaction was best visualized by the extravasation of the dye. Oral administration of AS (1 g/kg) showed a significant inhibition rate in PCA reactions (Table 2). Inhibition rate on PCA of CAS is also larger than that of WAS-root or WAS-stem.

Table 2. Effects of CAS and WAS on the 24h PCA in rats

Dose[a] (g/kg)	Inhibition (%)[b]		
	CAS	WAS (root)	WAS (stem)
None (Saline)	-	-	-
1	51*	42.3*	36.7*

[a]CAS and WAS were administered orally 1 h prior to the challenge with antigen.
[b]Each value is presented as the mean of four independent experiments.
***P< 0.01; significantly different from the saline value.**

Table 3. Effect of CAS and WAS on IgE-induced TNF-α secretion from RBL-2H3 cells

AS concentration [a] (㎎/㎖)	Inhibition of TNF-α secretion (%)[b]		
	CAS	WAS (root)	WAS (stem)
0.01	39.5 *	20.9*	25.5*
0.1	20.0 *	24.4*	30.9*

[a]IgE-stimulated RBL-2H3 cells (3×10^5) were incubated for 30 min in the absence or presence of AS before challenge with DNP-HSA (100 ㎎/㎖).
[b]Each data represents the mean of three independent experiments.
* $P<0.01$; significantly different from the saline value.

Effect of AS on TNF-α secretion from RBL-2H3 cells

To assess the effect of AS on IgE-induced TNF-α secretion, RBL-2H3 cells were pretreated with various concentration of AS for 30 min prior to antigenic stimulation. Our results showed that pretreatment of cells with AS resulted in inhibition of TNF-α secretion (Table 3). At the dose of 0.01 ㎎/㎖, inhibitory effect of CAS on IgE-induced TNF-α secretion was greatest. In contrast, at the dose 0.1 ㎎/㎖, those of WAS (stem) was greatest.

DISCUSSION

We have demonstrated that AS pretreatment profoundly affected compound 48/80-induced systemic allergic reaction and histamine release from RPMCs. Stimulation of mast cell with compound 48/80-initiates the activation of signal-transduction pathway which leads to histamine release. Some recent studies have shown that compound 48/80 and other polybasic compounds are able to activate G-proteins (Mousli *et al.*, 1990 a; Mousli *et al.*, 1990 b) Compound 48/80 increased the permeability of the lipid bilayer membrane by cause a perturbation of the membrane (Tasaka *et al.*, 1986) The membrane permeability increase may be essential trigger for the release of the mediators from mast cell. CAS and WAS might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80.

CAS or WAS also inhibited IgE-mediated cutaneous allergic reaction and TNF-α secretion. Antigen stimulation of mast cells via the IgE receptor elicits release of numerous mediators containing histamine in minutes and cytokines in hours. Consequently, these mediators induce immediate or late hypersensitive reaction. It is likely that CAS and WAS regulate the degranulation of the mast cell in rat skin by stabilizing membrane fluidity. In addition, CAS and WAS may inhibit late hypersensitivity reaction, inflammation, by blocking TNF-α secretion.

WAS has been used both root and stem for regulation of immune response and it still occupies an important place in traditional oriental medicine. WAS-root rather than WAS-stem has clinically been used. Our results show that inhibitory effect on mast cell-mediated allergic reactions of WAS-root is larger than those of WAS-stem. However, although CAS is most stems, antiallergic effect is large rather than those of WAS-root.

Taken together, both CAS and WAS

inhibited the mast cell-mediated allergic reactions in murine models. Antiallergic effects of CAS were greater than those of WAS-root or WAS-stem. This culture technique may be available for massive production of valuable herbs.

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