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Effect of *Salvia plebeia* on IgE antibody mediated allergic reaction in rats

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SUMMARY

The effect of aqueous extract of *Salvia plebeia* R. Br. (Labiatae)(AESP) on immunoglobulin (IgE) antibody mediated allergic reactions in rats was investigated. AESP inhibited passive cutaneous anaphylaxis (PCA) when intravenously, intraperitoneally, and orally administered. AESP dose-dependently inhibited histamine release from rat peritoneal mast cells activated by anti-DNP IgE antibody. Moreover, AESP had an inhibitory effect on anti-DNP IgE antibody induced TNF-α production from RPMC. These results suggest that AESP inhibit the IgE-mediated allergic reaction in rats.

INTRODUCTION

Salvia plebeia R. Br. (Labiatae) well known 'Baem-cha-zu-ki' in Korea, has been used for cardiac. Mast cells and basophils are primary target cells for IgE and the reaction of cell-bound IgE antibodies with multivalent antigen results in the release of a variety of inflammatory mediators (Ishizaka, 1981). In general, IgE-mediated allergic reaction is mediated by various chemical mediators released from mast cells (Wasserman and Marquardt, 1998).
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). The secretory response of mast cells can be induced by aggregation of their cell surface-specific receptors for IgE by the corresponding antigen (Segal *et al.*, 1977; Metzger *et al.*, 1986; Alber *et al.*, 1991). It has been established that the anti-IgE antibody induces passive cutaneous anaphylaxis (PCA) reactions as a typical *in vivo* model for immediate hypersensitivity in allergic cutaneous reactions. Rats or guinea pig skins are useful sites 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 their production of tumor necrosis factor-α (TNF-α) IL-6 and other cytokines

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within 30 min after their surface FcεRI are crosslinked with specific antigen (Burd *et al.*, 1989; Plaut *et al.*, 1989; Wodnar-filipowicz *et al.*, 1989; Gurish *et al.*, 1991). In this paper, we have evaluated the effect of AESP on the anti-DNP IgE antibody-induced allergic reaction in rats. We also investigated the influence of AESP on anti-DNP IgE antibody-induced TNF-α production from rat peritoneal mast cells.

MATERIALS AND METHODS

Reagents

Anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), Evans blue, o-phthaldialdehyde (OPA), α-ninimal essential medium (α-MEM) and metrizamide were purchased from Sigma Chemical Co. (St. Louis, MO). TNF-α was obtained from R & D Systems Inc. (USA).

Animals

The original stock of male Sprague-Dawley rats (250-300 g) was purchased from Dae-Han Experimental Animal Center(Taejeon), and the animals were maintained in the College of Pharmacy, Woosuk University. The rats were housed five per cage in a laminar air flow room maintained under a temperature of 22 ± 2℃ and relative humidity of 55 ± 5% throughout the study.

Preparation of AESP

The plants of *Salvia plebeia* were collected in Wanju, Chonbuk, South Korea, on the 19th of July, 1999. The plant sample was extracted twice distilled water at 70℃ for 5 hrs. The extract was filtered through a 0.45 ㎛ filter, lyophilized, and kept at 4℃. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.6 mM glucose, 0.1% bovine serum albumin) before use.

Induction of PCA

An IgE-dependent cutaneous reaction was generated by sensitizing the skin with an intradermal injection of anti-DNP IgE, followed 48 hrs later with an injection of DNP-HSA into the rats tail vein. The anti-DNP IgE antibody and DNP-HSA were diluted in PBS. The rats were injected intradermally with 0.5 ㎍ (50 ㎕) of anti-DNP IgE into each of four dorsal skin sites that had been shaved 48 hrs earlier, The sites were outlined with a water-insoluble red marker. Each rat, 48 hrs later, received an injection of 100 ㎍ (100 ㎕) of DNP-HSA in PBS containing 4% Evans blue (1 : 4) via the tail vein. A total of 200 ㎕ of AESP solution dissolved in sterile saline was administered by various routes 1 hrs before the challenge. Then, 30 min after the challenge, the rats were sacrificed 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 1 N KOH and 9 ml of a mixture of acetone and phosphoric acid (5 : 13) based on the method of Katayama *et al.* (1978). The absorbant intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan).

Preparation of RPMC

RPMC were harvested from the abdominal cavity of rats by the method of Shin *et al.* (1999). Briefly, rats were anesthetized by ether, and infected 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) containing 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 was

aspirated by a Pasteur pipette. Thereafter, the peritoneal cells were sedimented at 150 ×g for 10 min and resuspened 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 Tyrode buffer B were layered on 2 ml of metrizamide (22.5 w/v%) and centrifuged 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 containing calcium. 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.

Inhibition of histamine release

RPMC suspensions (2×10^5 cells/mℓ) were sensitized with 10 μg/mℓ anti-DNP IgE for 16 hrs. The cells were pre-incubated with the AESP for 10 min at 37℃ (CO_2 incubator) before the challenge with DNP-HSA (1 μg/mℓ). 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

Histamine content was assayed using the OPA spectrofluorometric procedure of Shore *et al.* (1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectro-fluorometer (Shimadzu, RF-5301, Japan).
The inhibition percentage of histamine release was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A-B}{A} \times 100$$

A: Histamine release without AESP.
B: Histamine release with AESP.

Assay of TNF-α production

TNF-α production was measured with the quantitative sandwich enzyme immuno-assay technique, using a commercial kit (R & D Systems, U.S.A.). RPMC (3×10^5 cells/mℓ) were sensitized with anti-DNP IgE (1 μg/mℓ) and incubated for 18 hrs in the absence or presence of AESP (0.1 mg/mℓ) before the challenge DNP-HAS (0.1 μg/mℓ). TNF-α production was measured by ELISA. The ELISA was performed by coating 4-well plates with murine polyclonal antibody with specificity for murine TNF-α Standard, controls, and samples are pipetted into the wells and any mouse TNF-α present is bound by the immunobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse TNF-α is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution (100 μℓ) is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop solution (100 μℓ) is added. The intensity of the color measured is in proportion to the amount of mouse TNF-α bound in the initial step. Optical density readings were made on a Titertek Multiscan (Flow Laboratories) with a 405 nm filter. The sample values are then read off the standard curves.

Statistical analysis

The results obtained were expressed as mean ± SEM. The student's t-test was used to make a statistical comparison between the groups. Results with $p < 0.05$

were considered statistically significant.

RESULTS

Effect of AESP on PCA

AESP dose-dependently inhibited PCA when intraperitoneally, or orally administered. On the other hand, it showed weak inhibitory activity when intravenously administered (Fig. 1). Treatment with AESP (1 mg/g) detected no physiological differences by appearance.

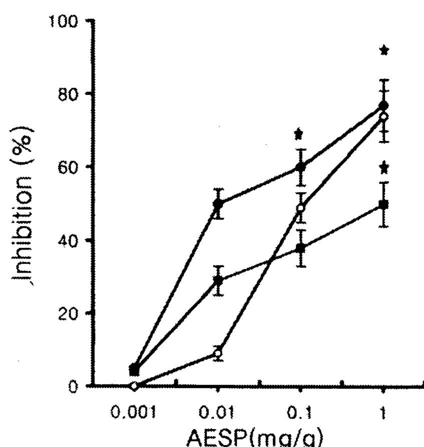

Fig. 1. Effect of AESP following various administration routes on PCA. AESP was administered orally(●), intraperitoneally (○) and intravenously(■) 1 hr prior to the challenge with antigen. Each data represents the mean ± SEM of three independent experiments.
*$p<0.05$, significantly different from the control value.

Effect of AESP on IgE-mediated histamine release from RPMC

The inhibitory effects of AESP on anti-DNP IgE-mediated histamine release from RPMC are shown in Fig. 2. AESP dose-dependently inhibited anti-DNP IgE-mediated histamine release at concentrations from 0.001 to 1 mg/㎖. The inhibition rate was significant at the doses of 1 mg/㎖.

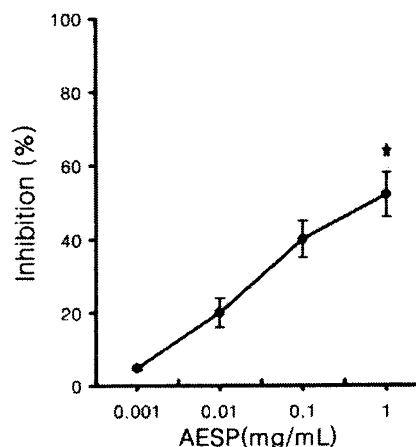

Fig. 2. Effect of AESP on anti-DNP IgE-mediated histamine release from RPMC. RPMC (2×10^5 cells/ml) were pre-incubated with drugs at 37℃ for 10 min prior to challenge with antigen (DNP-HSA) for 10 min. Each data represents the mean ± SEM of three independent experiments. *$p<0.05$; significantly different from the control value.

Effect of AESP on IgE-mediated TNF-α production from RPMC

We determined the amount of TNF-α production from RPMC. RPMC (3×10^5 cells/㎖) were sensitized with anti-DNP IgE and incubated for 18 hrs in the absence or presence of AESP before stimulation with DNP-HSA. AESP (0.1 mg/㎖) significantly inhibited IgE-mediated TNF-α production (Table 1). The cytotoxicity of AESP on the culture was not observed in the concentration used in the experiment, as assessed by trypan blue uptake.

Table 1. Effect of AESP on IgE-mediated TNF-α production from RPMC

AESP addition (mg/mℓ)	Anti DNP IgE plus DNP-HSA	TNF-α production (pg/mℓ)
None (saline)	-	69 ± 5.8
None (saline)	+	209 ± 18.7
0.1	+	147 ± 11.5*

Each data represents the mean ± SEM. of three independent experiments.
*p <0.05, significantly different from the control value.

DISCUSSION

The present study showed that AESP inhibited anti-DNP IgE antibody-induced PCA reactions when administered intraperitoneally, intravenously, and orally. AESP inhibited the anti-DNP IgE-induced histamine release from RPMC.

In spite of the increasing evidence of the role of several other mediators, histamine is still regarded as the principal mediator of antigen-induced skin reactions (Rafferty and Holgate, 1989; Rimmer and Church, 1990). The AESP-administered rats are protected from IgE-mediated local anaphylaxis. It suggests that AESP might be useful in the treatment of allergic skin reactions. Our data demonstrated that AESP inhibited anti-DNP IgE-mediated TNF-α production from RPMC. The effect of AESP on mast cell cytokine production *in vivo* and the relative importance of mast cells as a source of TNF-α during inflammatory and immune responses are important areas for future studies. In the present study, AESP inhibited histamine release activated by anti-DNP IgE antibody. The results do demonstrate that AESP inhibit the mast cell-dependent allergic reaction in rats and suggest that the activity may be due to the inhibition of histamine release from the mast cells. The results obtained in the present study provided that AESP inhibited the IgE-mediate allergic reactions *in vivo* and *in vitro* in rats. Koda *et al.* (1977) reported that disodium baicalein 6-phosphate (constituent of Scutellaria baicalensis) inhibited not only reaginic antibody-mediated reactions including antigen-induced mediator release from monkey lung, homologous PCA in rats, and reaginic antibody-mediated degranulation of mast cell, but also non-reaginic antibody-mediated reactions, such as mediator release from guinea pig lung sensitized with ovalbumin and that from human lung caused by anti-IgE. The agent, however, did not affect the mediator release from lung of rats sensitized with dinitrophenylated ascaris extract plus Bordetella pertusis. Further work should address the possibility that AESP may also be active in the inhibition of human mast cell degranulation. The results obtained suggest that AESP may contain compounds with actions that suppress mast cell degranulation in the rat. Therefore, further investigation is necessary to clarify the unknown antianaphylactic plant constituents which may be more active than AESP itself.

REFERENCES

Alber G, Miller L, Jelsema C, Varin-Blank N, Metzger H. (1991) Structure-function relationships in the mast cell high affinity receptor for IgE (FcεRl). Role of cytoplasmic domains and of the beta subnit. *J. Biol. Chem.* **266**, 22613-22620.

Burd PR, Rogers HW, Gordon JR, Martin CA, Jayarama S, Wilson SD, Dvork AM, Galli SJ, Dorf ME. (1989) Interleukin 3-dependent and-independent mast cells stimulated with IgE and antigen express multiple cytokines. *J. Exp. Med.* **170**, 245-257.

Gordon JR, Galli SJ. (1990) Mast cell as a source of both preformed and immunologically inducible TNF-α /cachectin. *Nature* **346**, 274-276.

Gurish MF, Ghildyal N, Arm J, Austen KF, Avraham S, Reynolds DS, Stevens RL. (1991) Cytokine mRNA are preferentially increased relative to secretory granule protein mRNA in mouse bone marrow-derived mast cells that have undergone IgE-mediated activation and degranulatlon. *J. Immunol.* **146**, 1527-1533.

Ishizaka T. (1981) Analysis of triggering events in mast cells for immunoglobulin E mediated histamine release. *J. Allergy Clin. Immunol.* **67**, 90-96.

Katayama S, Shionoya H, Ohtake S. (1978) A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. *Microbiol. Immunol.* **22**, 89-101.

Koda A, Watanabe S, Yanagihara Y, Nagai H, Sakamoto K. (1977) A comparative study of the anti-allergic effects of disodium baicallein 6-phosphate (BPS) and disodium cromoglycate (DSCG). *Jpn. J. Pharmacol.* **27**, 31-38.

Metzger H, Alcaraz G, Hohman R, Kinet JP, Pribluda V, Quarto R. (1986) The receptor with high affinity for immunoglobulin E. *Annu. Rev. Immunol.* **4**, 419-470.

Petersen LJ, Mosbech H, Skov P. (1996) Allergen-induced histamine release in intact human skin in vivo assessed by skin microdialysis technique: Characterization of factors influencing histamine release ability. *J. Allergy Clin. Immunol.* **97**, 672-679.

Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordon RP, Paul WE. (1989) Mast cell lines produce lymphokines in response to cross-linkage of FcεRl or to calcium ionophores. *Nature* **339**, 64-67.

Rafferty P, Holgate ST. (1989) Histamine and its antagonists in asthma. *J. Allergy Clin. Immunol.* **84**, 144-151.

Rimmer SJ, Church MK. (1990) The pharmacology and mechanisms of action of histamine H1-antagonists. *Clin. Exp. Allergy* **20**, 3-17.

Saito H, Nomura Y. (1989) Screening methods for drug evaluation, 3. In: Suzuki L, Tanaka H, Yajima H, Fukuda H, Sezaki H, Koda K et al.: Pharmaceutical Research and Development. p 22, Hirokawa, Tokyo.

Segal DM, Taurog J, Metzger H. (1977) Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc. Natl. Acad. Sci.* 74, 2993-2997.

Shin TY, Park JH, Kim HM. (1999) Effect of Cryptotympana atrata extract on compound 48/80-induced anaphylactic reactions. *J. Ethnopharmacol.* **66**, 319-325

Shore PA, Burkhalter A, Cohn VH. (1959) A method for fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.* **127**, 182-186.

Wasserman SI, Marquardt DL. (1998)

Anaphylaxis in Allergy; Principles and Practice. 3rd ed, p 1365-1370, Mosby Co., St. Louis.

Wodnar-filipowicz A, Heusser CH, Moroni C. (1989) Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. *Nature* **339**, 150-152.

Yurt RW, Leid RW Jr, Austen KF. (1977) Native heparin from rat peritoneal mast cells. *J. Biol. Chem.* **252** 518-521.