

Inhibition of IgE-mediated anaphylactic reaction by *Mentha arvensis* in rats

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Background : It has been reported that *Mentha arvensis* water extract (MAWE) inhibited systemic anaphylaxis and histamine release from the rat peritoneal mast cells (RPMC) by compound 48/80.

Objective and method : This study was undertaken to determine the inhibitory effects of immunoglobulin E (IgE)-mediated anaphylactic reaction by MAWE. This paper deals with an evaluation of the effect of MAWE on the anti-dinitrophenyl (DNP) IgE antibody induced anaphylactic reaction in rats. We also investigated the influence of MAWE on anti-DNP IgE antibody-induced tumor necrosis factor- α (TNF- α) production.

Results : MAWE inhibited passive cutaneous anaphylaxis (PCA) when intravenously, intraperitoneally, and orally administered. MAWE dose-dependently inhibited histamine release from RPMC activated by anti-DNP IgE antibody. Moreover, MAWE had an inhibitory effect on anti-DNP IgE antibody induced TNF- α production from RPMC.

Conclusion : These results suggest that MAWE inhibits the IgE-mediated anaphylactic reaction in rats. (J Asthma Allergy Clin Immunol 20: 601-608, 2000)

Key words : *Mentha arvensis*, anaphylactic reaction, TNF- α , anti-dinitrophenyl IgE, mast cells.

Introduction

Mentha arvensis var. *piperascens* Malinvaud (Labiatae), known as 'Bak-ha' in Korea, has been used as analgesic, local vasodilator, skin irritant and antispasmodic agent. It still occupies an important place in traditional Oriental medicine. The herba has a rich content of essential oils. More than 70 compounds have been identified from the oil and the main constituents include menthol and its glucosides, menthyl glucoside and

thone, menthyl acetate, pulegone, piperitone, piperitenone, menthofuran, piperitone oxide, piperitenone oxide, linalool, linalyl acetate, 1,8-cineole, carvone and dihydrocarvone. The herba also contains about 20 flavonone compounds, including luteolin-7-rutinoside, hesperidine, luteolin-7- β -D-glucoside, acaciin, hesperetin-7- β -D-glucoside, eriodictyol, eriodictyol-7-glucoside, acacetin, apigenin, luteolin, apigenin-7-glucoside, 3'-hydroxy-4', 5, 6, 7-tetramethoxyflavone, petunidin, pelar-

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gonidin, delphinidin and isoraifolia¹⁻³⁾.

The mast cell has long been thought to play a major role in the development of many physiological changes during anaphylactic and allergic responses.

In general, IgE-mediated anaphylactic reaction is mediated by various chemical mediators released from mast cells¹⁾. 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 hypersensitivity⁵⁾. The secretory response of mast cells can be induced by aggregation of their cell surface-specific receptors for IgE by the corresponding antigen⁶⁻⁸⁾. 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 PCA⁹⁾. Although mast cells also store small amounts of cytokines in their granules¹⁰⁾, these cells dramatically increase their production of tumor necrosis factor- α (TNF- α), IL-6 and other cytokines within 30 min after their surface Fc ϵ RI are crosslinked with specific antigen¹¹⁻¹⁴⁾. The compound 48/80 is one of the most potent secretagogues of the mast cells¹⁵⁾. An appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylactic reaction¹⁶⁻¹⁸⁾. Shin et al.¹⁹⁾ reported that *Mentha arvensis* inhibited systemic anaphylaxis induced by compound 48/80. In experimental allergic models, disodium cro-

moglycate (DSCG : a mast cell stabilizer) showed an obvious inhibitory effect on the immediate-type allergic reactions. The effect of DSCG is thought to be based on the inhibition of the mediator release from mast cells²⁰⁾. We evaluated the inhibitory effects of MAWE in comparison with those of DSCG.

In this paper, we evaluated the effect of *Mentha arvensis* water extract (MAWE) on the anti-DNP IgE antibody-induced anaphylactic reaction in rats. We also investigated the influence of MAWE on anti-DNP IgE antibody-induced TNF- α production from rat peritoneal mast cells.

Materials and methods

1. Reagents and animals

1) Reagents

Anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), Evans blue, o-phthalaldehyde (OPA), α -minimal essential medium (α -MEM), disodium cromoglycate (DSCG) and metrizamide were purchased from Sigma Chemical Co. (St. Louis, MO); Fetal calf serum (FCS) from Life Technologies (Grand Island, NY); Recombinant TNF- α (rTNF- α) (1×10^5 U/ml), rabbit anti-murine TNF- α antibody from Genzyme (Munich, Germany) and Phosphatase-labeled anti-rabbit IgG from Serotec (Oxford, UK).

2) Animals

The original stock of male Sprague-Dawley rats (250-300g) was purchased from Dae-Han Experimental Animal Center (Taejeon), and the animals were maintained in

the College of Pharmacy, Woosuk University. Five to ten rats were kept in each cage in a laminar air flow room maintained under a temperature of $22 \pm 1^\circ\text{C}$ and relative humidity of $55 \pm 10\%$ throughout the study.

2. Preparation of MAWE

The *Mentha arvensis* from the oriental drug store, Bohwa Dang (Chonju) were extracted twice with distilled water at 70°C for 5 hrs. The extract was filtered through a $0.45 \mu\text{m}$ filter, lyophilized, and kept at 4°C . 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.

3. 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 $0.5 \mu\text{g}$ ($50 \mu\text{l}$) 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 $100 \mu\text{g}$ ($100 \mu\text{l}$) of DNP-HSA in PBS containing 4% Evans blue (1:4) via the tail vein. A total of $200 \mu\text{l}$ of MAWE solution dissolved in sterile saline was administered by various routes 1 hr 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 1.0 ml of 1.0 N KOH and 9 ml of a mixture of acetone and phosphoric acid (5:13) based on the method of Katayama et al.²¹⁾. The absorbant intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan).

4. Preparation of RPMC

RPMC were harvested from the abdominal cavity of rats by the method of Shin et al.²²⁾. Briefly, rats were anesthetized with 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 $150 \times g$ for 10 min 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.²³⁾. In brief, peritoneal cells suspended in 1.0 ml Tyrode buffer B were layered on 2 ml of metrizamide (22.5 w/v%) and centrifuged 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 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.

5. 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 sensitized with 10 $\mu\text{g/ml}$ anti-DNP IgE for 16 hrs. The cells were pre-incubated with the MAWE for 10 min at 37°C (CO₂ incubator) before the challenge with DNP-HSA (1 $\mu\text{g/ml}$). The reaction was stopped by cooling the tubes in ice. The cells were separated from the released histamine by centrifugation at $400 \times g$ for 5 min at 4°C. Residual histamine in the cells was released by disrupting the cells with perchloric acid and centrifugation at $400 \times g$ for 5 min at 4°C.

6. Assay of histamine release

Histamine content was assayed using the OPA spectrofluorometric procedure of Shore et al.²¹⁾ The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer (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 MAWE.

B: Histamine release with MAWE.

7. Assay of TNF- α production

RPMC (2×10^5 cells/ml) were resuspended

in Tyrode buffer A. The cells were sensitized with anti-DNP IgE (1 $\mu\text{g/ml}$) and incubated for 6 hrs in the absence or presence of MAWE (0.001 to 0.1 $\mu\text{g/ml}$) before the challenge with DNP-HSA (0.1 $\mu\text{g/ml}$). TNF- α production was measured by modification of an enzyme linked immunosorbent assay (ELISA) as described²⁵⁾. The ELISA was sensitive to TNF- α concentrations in the medium above 40 pg/ml. The ELISA was devised 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 (PBS-Tween) and twice with PBS alone. All reagents used in this assay and the coated wells were incubated for 1 hr. For the standard curve, TNF- α was added to serum previously determined to be negative for endogenous TNF- α . After exposure to the medium, the assay plates were sequentially exposed to rabbit anti-TNF- α , phosphatase-conjugated goat anti-rabbit IgG, and 2, 2'-azinobis. 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.

8. Statistical analysis

The results obtained were expressed as mean $\pm$ 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

1. Effect of MAWE on PCA

MAWE 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 MAWE (1 mg/g) detected no physiological differences by appearance.

2. Effect of MAWE on IgE-mediated histamine release from RPMC

The inhibitory effects of MAWE or DSCG on anti-DNP IgE-mediated histamine release from RPMC are shown in Fig. 2. MAWE dose-dependently inhibited anti-DNP IgE-mediated histamine release at

concentrations from 0.001 to 1.0 mg/ml. The inhibition rate was significant at the doses of 0.1 - 1.0 mg/ml. DSCG significantly inhibited IgE-mediated histamine release at concentrations from 0.01 to 1.0 mg/ml.

3. Effect of MAWE on IgE-mediated TNF- α production from RPMC.

We determined the amount of TNF- α production from RPMC. RPMC (2×10^5 cells/ml) were sensitized with anti-DNP IgE and incubated for 6 hrs in the absence or presence of MAWE before stimulation with DNP-HSA. MAWE (0.1 mg/ml) significantly inhibited IgE-mediated TNF- α production (Table 1). The cytotoxicity of MAWE on the culture was not observed in the con-

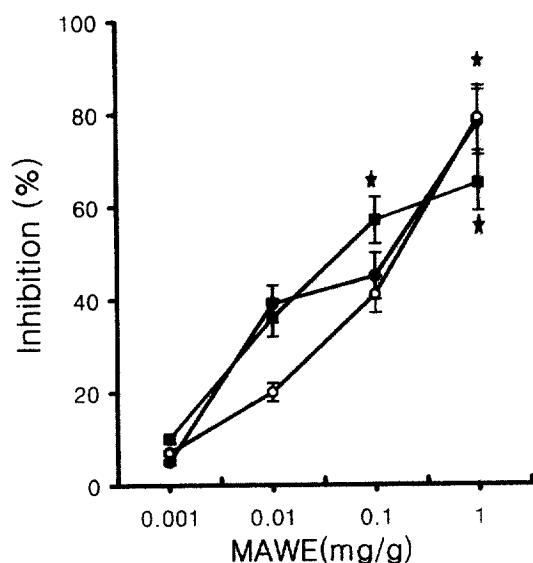


Fig. 1. Effect of *Mentha arvensis* water extract following various administration routes on PCA. *Mentha arvensis* water extract was administered orally (●), intraperitoneally (○) and intravenously (■) 1 hr prior to the challenge with antigen. Each data represents the mean $\pm$ SEM of three independent experiments.

* $p < 0.05$, significantly different from the control value.

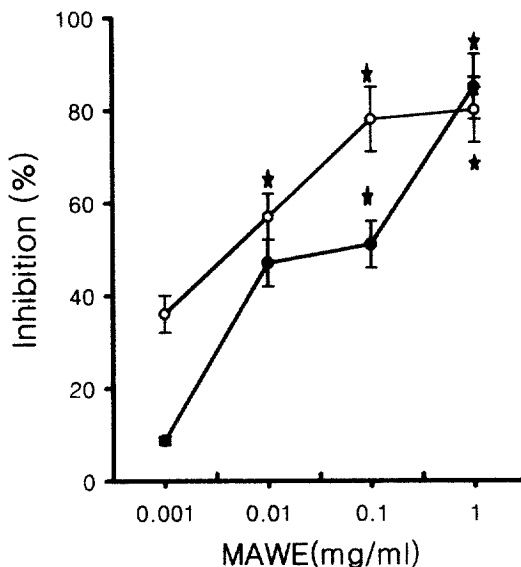


Fig. 2. Effect of *Mentha arvensis* water extract on anti-DNP IgE-mediated histamine release from RPMC. RPMC (2×10^5 cells/ml) were pre-incubated with drugs at 37°C for 10 min prior to challenge with antigen (DNP-HSA) for 10 min. (●), MAWE + anti-DNP IgE; (○), DSCG + anti-DNP IgE. Each data represents the mean $\pm$ SEM of three independent experiments. * $p < 0.05$; significantly different from the control value.

centration used in the experiment, as assessed by trypan blue uptake.

Discussion

The present study showed that MAWE inhibited anti-DNP IgE antibody-induced PCA reactions when administered intraperitoneally, intravenously, and orally. MAWE 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^{26, 27)}. The MAWE-administered rats are protected from IgE-mediated local anaphylaxis. It suggests that MAWE might be useful in the treatment of allergic skin reactions. Our data demonstrated that MAWE inhibited anti-DNP IgE-induced TNF- α production from RPMC. The effect of MAWE 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. The inhibitory effects of DSCG on anaphylactic reactions in rats are reported to derive from inhibition of mediator release from the mast cells^{28, 29)}. In the present study, MAWE and DSCG inhibited histamine release acti-

vated by anti-DNP IgE antibody. The results demonstrate that MAWE inhibit the mast cell-dependent anaphylactic 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 showed that MAWE inhibited the IgE-mediated anaphylactic reactions *in vivo* and *in vitro* in rats. Koda et al³⁰⁾ reported that disodium baicalin 6-phosphate (constituent of *Scutellaria baicalensis*) inhibited reagenic antibody-mediated reactions including antigen-induced mediator release from monkey lung, homologous PCA in rats, and reagenic antibody-mediated degranulation of mast cell, and also non-reagenic antibody-mediated reactions such as mediator release from guinea pig lung sensitized with ovalbumin and 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 pertussis*. Further work should address the possibility that MAWE may also be active in the inhibition of human mast cell degranulation. The results obtained suggest that MAWE may contain compounds with actions that suppress mast cell degranulation in the rat. Therefore, further investigation is necessary to

Table 1. Effect of *Mentha arvensis* water extract on IgE-mediated TNF- α production from RPMC

MAWE addition (mg/ml)	Anti DNP IgE plus DNP-HSA	TNF- α production (ng/ml)
None (saline)	-	7.176 $\pm$ 0.1
None (saline)	+	15.277 $\pm$ 0.8
0.1	+	10.453 $\pm$ 0.5*

Each data represents the mean $\pm$ SEM, of five independent experiments.

* $p < 0.05$, significantly different from the control value.

clarify the unknown antianaphylactic plant constituents which may be more active than MAWE itself.

Conclusion

These results suggest that MAWE inhibit the IgE-mediated anaphylactic reaction *in vivo* and *in vitro* in rats. The studies on the isolation and characterization of the active chemical constituents are in progress. Further work should address the possibility that MAWE may also be active in the inhibition of human mast cell degranulation and in the treatment of human allergic disorders.

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