

Inhibitory Effects of *Houttuynia cordata* Water Extracts on Anaphylactic Reaction and Mast Cell Activation

Guang Zhao LI,^c Ok Hee CHAI,^{a,b} Moo Sam LEE,^a Eui-Hyeog HAN,^a Hyoung Tae KIM,^a and Chang Ho SONG^{*,a,b}

^a Department of Anatomy, Chonbuk National University Medical School; ^b Research Center for Allergic Immune Diseases, Chonbuk National University; Jeonju, Jeonbuk, 561–756, Republic of Korea; and ^c Department of Anatomy, Yanbian University Medical School; Juzi Street 121, Yanji City, 133000, China.

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The present study was investigated the effect of *Houttuynia cordata* THUNB water extract (HCWE) on mast cell-mediated anaphylactic reactions. The mast cell-mediated anaphylactic reaction is involved in many allergic diseases such as asthma and allergic rhinitis. HCWE has been used as a traditional medicine in Korea and is known to have an antioxidant and anti-cancer activities. However, its specific effect of mast cell-mediated anaphylactic reactions is still unknown. We examined whether HCWE could inhibit compound 48/80-induced systemic anaphylaxis, IgE-mediated passive cutaneous anaphylaxis (PCA), and mast cell activation. The oral administration of HCWE inhibited compound 48/80-induced systemic anaphylaxis in mice. HCWE also inhibited the local allergic reaction, PCA, activated by anti-dinitrophenyl (DNP) IgE antibody in rats. HCWE reduced the compound 48/80-induced mast cell degranulation and colchicine-induced deformation of rat peritoneal mast cells (RPMC). Moreover, HCWE dose-dependently inhibited histamine release and calcium uptake of RPMC induced by compound 48/80 or anti-DNP IgE. HCWE increased the level of intracellular cAMP and inhibited significantly the compound 48/80-induced cAMP reduction in RPMC. These results suggest that HCWE may be beneficial in the treatment of mast cell-mediated anaphylactic responses.

Key words anaphylactic reaction; cAMP; calcium; histamine; *Houttuynia cordata*; mast cell

Houttuynia cordata THUNB, well known as ‘E-Sung-Cho’ in Korea, has been used for the treatment of Herpes simplex,¹⁾ chronic sinusitis and nasal polyps.²⁾ Recently, a few papers reported the antileukemic activity,³⁾ anticancer activity,⁴⁾ adjuvanticity,⁵⁾ and antioxidant action⁶⁾ of *Houttuynia cordata* THUNB water extract (HCWE).

Mast cell is well known as a critical participant in the various biologic processes including the allergic diseases, inflammatory reaction, cytotoxicity, angiogenesis, immunoregulation, and fibrosis.⁷⁾ Mast cells are secretory ones that store within their numerous cytoplasmic granules an array of biologically-potent inflammatory mediator such as histamine. Mast cells also express on their surface membrane receptors with high affinity and specificity for IgE. The interaction of multivalent antigens with surface-bound IgE initiates a series of biochemical events that culminates in the release of histamine and the production of cytokines.⁸⁾ Calcium ionophores, neuropeptides and compound 48/80 can also stimulate the mast cell degranulation.^{9–11)} The notable example in this category is compound 48/80, a mixed polymer of phenethylamine cross-linked by formaldehyde,¹²⁾ that has a hypotensive effect, resulting from histamine release, shown by Hong.¹³⁾

However, the effect of HCWE on mast cell-mediated anaphylactic reactions has not been studied. The aim of this study is to evaluate the effect of HCWE on the compound 48/80-induced systemic anaphylaxis, anti-DNP IgE-mediated PCA and to understand the inhibitory mechanism of HCWE on mast cell activation.

MATERIALS AND METHODS

Materials Our laboratory extracted *Houttuynia cordata*

THUNB. Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), bovine serum albumin (BSA) and HEPES were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Percoll solution was purchased from Pharmacia (Uppsala, Sweden).

Experimental Animals Male ICR mice and Sprague-Dawley rats were purchased from Damool Science (Daejeon, Korea). They were housed 3–5 per cages in laminar air-flow cabinet maintained at 23±2 °C and relative humidity of 55±10% throughout the study. Animal Research Committee of Chonbuk National University approved the animal study in accordance with the guidelines of the National Institutes of Health (NIH publication #85-23, 1985).

Preparation of *Houttuynia cordata* Water Extract (HCWE) *Houttuynia cordata* THUNB used in this study were purchased from Jangso Oriental Pharmacy (Chonbuk, Korea). A voucher specimen (number 2004-MS075) was deposited at the Herbarium of the Research Center for Allergic Immune Diseases, Chonbuk National University Medical School. The air-dried *Houttuynia cordata* (100 g) were immersed in 400 ml of distilled water, kept overnight in a refrigerator (10 °C), and boiled under reflux for 2 h. This hot-water extraction was conducted twice. The resulting extract was filtered through a 0.45 µm filter, and concentrated to approximately 100 ml under reducing pressure. The concentrated extract was finally lyophilized, yielded 16.3 g dried powder and kept at 4 °C until use. The dried extract was dissolved in saline or HEPES-Tyrode buffer (136 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 11 mM NaHCO₃, 0.6 mM NaH₂PO₄, 2.75 mM MgCl₂, 5.4 mM HEPES, 1.0 mg/ml bovine serum albumin, 1.0 mg/ml glucose, 0.1 mg/ml heparin) before use.

Compound 48/80-Induced Systemic Anaphylaxis Mice were given an intraperitoneal injection of 5, 10, or

* To whom correspondence should be addressed. e-mail: asch@chonbuk.ac.kr

15 mg/kg body weight (BW) of the mast cell degranulator compound 48/80.¹³ HCWE (10 or 100 mg/kg BW) was dissolved in saline and administered orally at 24, 12 and 1 h before the injection of compound 48/80 ($n=10$ /group). Mortality was monitored for 1 h after induction of anaphylactic shock.

Passive Cutaneous Anaphylaxis (PCA) 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 rat's penis vein. The DNP-HSA was diluted in phosphate-buffered saline (PBS). The rats were injected intradermally with 500 ng of anti-DNP IgE into each of 4 dorsal skin sites that had been shaved 24 h earlier. The sites were outlined with a water-insoluble red marker. After 48 h, each rat received an injection of 20 μ g DNP-HSA in PBS containing 0.5% Evans blue *via* the penis vein. HCWE was orally administered 24, 12 and 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 formamide based on the method of Fleming *et al.*¹⁴ The absorbent intensity of the extraction was measured at 620 nm in a spectrophotometer (Spectra MAX PLUS, Molecular Devices, CA, U.S.A.), and the amount of dye was calculated with the Evans blue measuring line.

Preparation of RPMC Suspension and Microscopic Observation RPMC were isolated as previously described.¹⁵ In brief, rats were anesthetized with ether and injected with 10 ml of calcium-free HEPES-Tyrode buffer into the peritoneal cavity, and the abdomen was gently massaged for about 90 s. The peritoneal cavity was opened, and the fluid was aspirated by a Pasteur pipette, and RPMC were purified by using a percoll density gradient as described in detail elsewhere.¹⁶ RPMC preparations were at least 95% pure and at least 98% of these cells were viable as assessed by trypan blue exclusion.¹⁷ Purified mast cells (1×10^6 cells/ml) were resuspended in HEPES-Tyrode buffer. Mast cells were observed under phase contrast microscope and photographed as described by Cochrane and Douglas.¹⁵

Mast Cell Viability Assay At time zero and subsequent time-points as indicated, the mast cells were counted in a hemacytometer and viability was assessed by trypan blue dye exclusion.¹⁷

Assay of Histamine Release Mast cell suspensions (2×10^5 cells in 200 μ l) were preincubated with HCWE (2.5–250 μ g) for 10 min at 37 °C and then incubated with compound 48/80 (125 ng). Mast cells were sensitized with anti-DNP IgE (250 ng) for 2 h and preincubated with HCWE at 37 °C for 10 min prior to the challenge with DNP-HSA (500 ng). The cells were separated from the released histamine by centrifugation at $150 \times g$ for 10 min at 4 °C. Residual histamine in the cells was released by disrupting the cells with boiling. After centrifugation, histamine content was measured by the radioenzymatic method described by Harvima *et al.*¹⁸ The inhibition percentage histamine release was calculated using the following formula: % inhibition = [(histamine release without HCWE – histamine release with HCWE)/histamine release without HCWE] $\times 100$.

Measurement of ⁴⁵Ca Uptake The calcium uptake of mast cells was measured according to the method described

by Chai *et al.*⁹ Purified mast cells were resuspended in HEPES-Tyrode buffer containing ⁴⁵Ca (1.5 mCi/ml; 1 Ci = 3.7×10^{10} becquerels; PerkinElmer Life Sciences, MA, U.S.A.), and incubated for 10 min at 4 °C. Mast cell suspensions were preincubated with HCWE (2.5–250 μ g) for 10 min at 37 °C and then incubated with compound 48/80 (125 ng). The reaction was stopped by the addition of 1 mM lanthanum chloride and centrifuged 3 times at 4 °C for 10 min, then the cells in the pellet were disrupted with 10% Triton X-100 by vigorous shaking. Radioactivity of the solution was measured in a scintillation β -counter (Liquid scintillation Analyzer, A canberra company, Australia).

Measurement of cAMP Level The cyclic adenosine-3',5' monophosphate (cAMP) level was measured according to the method described by Holmegaard.¹⁹ In brief, mast cell suspensions were added to an equivalent volume (200 μ l) of prewarmed buffer containing the drug in an Eppendorf tube. The reaction was allowed to proceed for discrete time intervals, terminated by centrifugation, and then added 250 μ l of 50 mM sodium acetic buffer (pH 6.2) under vigour vortexing, followed by snap frozen in liquid nitrogen. The frozen samples were thawed and vortexed, the debris were sedimented by centrifugation $400 \times g$ at 4 °C for 10 min. The cAMP level in the supernatant was determined by radioimmunoassay, with the use of a Rianen assay system (PerkinElmer Life Sciences, MA, U.S.A.).

Statistical Analysis The results obtained were expressed as mean \pm S.E.M. for the number of experiments. 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 HCWE on Compound 48/80-Induced Systemic Anaphylaxis To investigate the effects of HCWE in anaphylactic reactions, we first used the *in vivo* model of systemic anaphylaxis. After the injection of compound 48/80 (concentration ranging from 5, 10, 15 mg/kg BW), the mice were monitored for 1 h to determine their mortality. As shown in Table 1, compound 48/80 induced fatal shocks in ranging from 80 to 100% of some groups. When HCWE (10 or 100 mg/kg BW) was orally administrated at 24, 12 and 1 h before the injection of compound 48/80, the mortality with compound 48/80 was dose-dependently reduced. At the dose

Table 1. Inhibitory Effect of *Houttuynia cordata* Water Extract (HCWE) on the Compound 48/80-Induced Systemic Anaphylaxis

HCWE (mg/kg BW)	Compound 48/80 (mg/kg BW)	Mortality (%)
0	5	0
0	10	80
0	15	100
10	10	0
10	15	10
100	10	0
100	15	0

Groups of mice ($n=10$ /group) were orally administrated with 500 μ l saline or HCWE at 24, 12 and 1 h before the injection of various doses of compound 48/80. The compound 48/80 was intraperitoneally given to the group of mice. Mortality (%) within 1 h following the compound 48/80 injection was presented as the number of dead mice $\times 100$ / total number of experimental mice.

of 100 mg/kg BW, HCWE inhibited the compound 48/80-induced systemic anaphylaxis completely.

Effect of HCWE on Anti-DNP IgE-Mediated PCA

Another way to test the anaphylactic reaction is to induce PCA. As described in Materials and Methods, local extravasation was induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Oral administration of HCWE (10 or 100 mg/kg BW) inhibited anti-DNP IgE-mediated PCA (Table 2).

Effect of HCWE on Mast Cell Degranulation of RPMC

To investigate the inhibitory mechanism of HCWE on the anaphylactic reactions, we observed the compound 48/80-induced or anti-DNP IgE-mediated mast cell activation. The inhibitory effects of HCWE on the compound 48/80-induced mast cell degranulation and colchicine-induced mast cell deformation were shown (Fig. 1). The normal rat peritoneal

mast cells (RPMC) were generally spherical, or oval in shape, and had many fine granules surrounding a prominent nucleus (Fig. 1A). After stimulation with 125 ng compound 48/80 for 5 min, RPMC were degranulated (Fig. 1B). The characteristics of mast cell degranulation were the cell swelling, cytoplasmic vacuoles, and extruded granules near the cell surface and in the surrounding medium. After stimulation with 10^{-5} M colchicine for 60 min, RPMC became polymorphic and its nucleus was displaced peripherally. Also some RPMC appeared as a snowman or dumbbells with hyaloplasmic veils (Fig. 1C). When RPMC were incubated with HCWE alone, RPMC were similar to ones as seen in Fig. 1A (Fig. 1D). After a stimulation of HCWE-preincubated RPMC with 125 ng compound 48/80 for 5 min, RPMC showed cell swelling without degranulation (Fig. 1E). After a stimulation of HCWE-preincubated RPMC with 10^{-5} M colchicine for 60 min, RPMC were similar to normal ones, but some cells showed the irregularity (Fig. 1F). The results suggest that HCWE significantly inhibit the compound 48/80-induced mast cell degranulation and colchicine-induced mast cell deformation.

Effect of HCWE on Histamine Release from RPMC

The inhibitory effects of HCWE on the compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC were shown (Fig. 2). HCWE dose-dependently inhibited the compound 48/80-induced or anti-DNP IgE-mediated histamine release at concentrations of 2.5 to 250 μ g (Fig. 2). These results indicate that HCWE may be an activity to inhibit the compound 48/80-induced or anti-DNP IgE-mediated anaphylactic reactions by blocking histamine release from mast cells.

Table 2. Inhibitory Effect of *Houttuynia cordata* Water Extract (HCWE) on the Anti-DNP IgE-Mediated Passive Cutaneous Anaphylaxis

HCWE (mg/kg)	Anti-DNP IgE	Dose of Evans blue (μ g/g)	Inhibition (%)
0	—	5.2 \pm 0.4	
0	+	53.5 \pm 7.5	
10	—	6.0 \pm 1.1	
10	+	23.0 \pm 1.0	68
100	—	7.1 \pm 1.5	
100	+	16.0 \pm 4.5	79

HCWE was administered orally 24, 12 and 1 h prior to the challenge with antigen. Each dose of Evans blue represents the mean \pm S.E.M. of five experiments. Inhibition (%)=[(dose of Evans blue without HCWE–dose of Evans blue with HCWE)/dose of Evans blue without HCWE] \times 100.

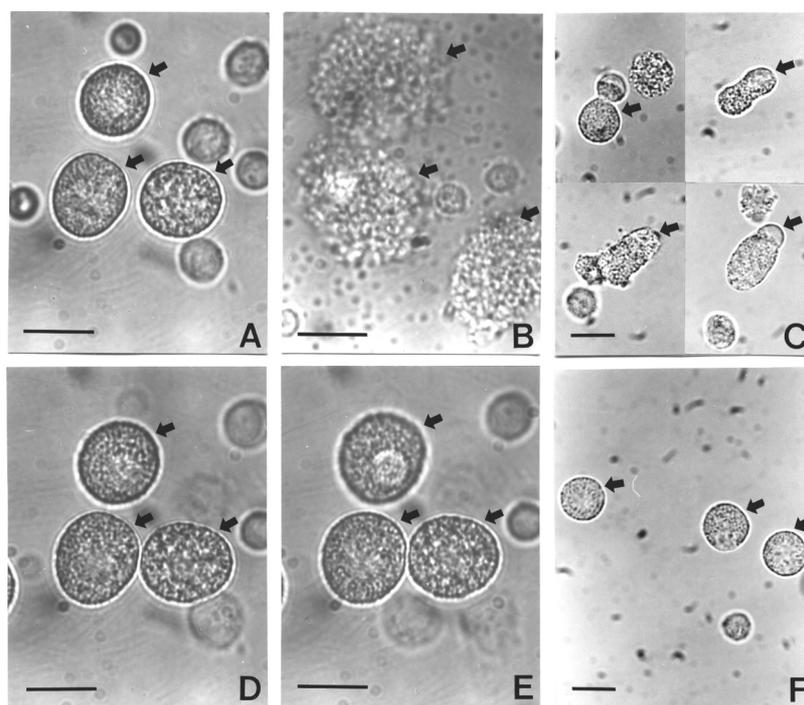


Fig. 1. Light Microphotographs Using Inverted Microscopy of Rat Peritoneal Mast Cells (Arrows, RPMC) in HEPES-Tyrode Buffer (A), after Stimulation with 125 ng Compound 48/80 (B), 10^{-5} M Colchicine (C), and 250 μ g *Houttuynia cordata* Water Extract (HCWE) (D), after Being Pretreated with 250 μ g HCWE Prior to the Stimulation with 125 ng Compound 48/80 (E), after Being Pretreated with 250 μ g HCWE Prior to the Stimulation with 10^{-5} M Colchicine (F)

Normal RPMC are round shape with fine granules and regular surface. Compound 48/80-induced degranulated RPMC appear the cell swelling, many cytoplasmic vacuoles, an irregular surface and extruded granules. Colchicine-induced RPMC are polymorphic with hyaloplasmic veils and its nucleus is displaced peripherally. However, HCWE significantly inhibited the compound 48/80-induced mast cell degranulation and colchicine-induced mast cell deformation. Bar=10 μ m.

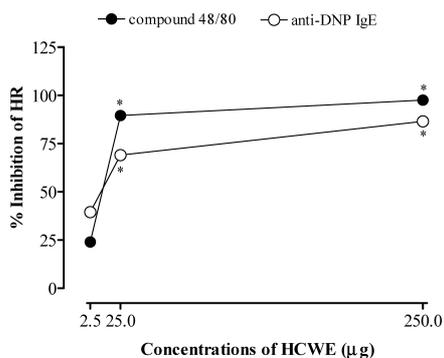


Fig. 2. Inhibitory Effect of *Houttuynia cordata* Water Extract (HCWE) on the Compound 48/80- or Anti-DNP IgE-Mediated Histamine Release (HR) from Rat Peritoneal Mast Cells (RPMC)

RPMC were preincubated with HCWE at 37 °C for 10 min prior to the incubation with compound 48/80 or to the challenge with antigen. HCWE dose-dependently inhibited the compound 48/80-induced or anti-DNP IgE-mediated histamine release. * $p < 0.01$; significantly different from the saline value.

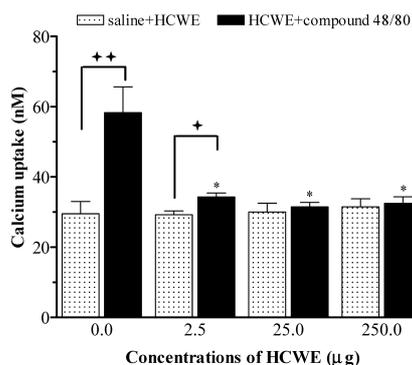


Fig. 3. Inhibitory Effect of *Houttuynia cordata* Water Extract (HCWE) on the Compound 48/80-Induced Calcium Uptake in Rat Peritoneal Mast Cells (RPMC)

RPMC were preincubated with HCWE at 37 °C for 10 min prior to the incubation with compound 48/80. HEMA dose-dependently inhibited the compound 48/80-induced calcium uptake. * $p < 0.05$ and ** $p < 0.01$; when compared between saline plus HCWE and HCWE plus compound 48/80, * $p < 0.01$; significantly different from the saline value.

Effect of HCWE on Calcium Uptake into RPMC To investigate the mechanisms of HCWE on the reduction of histamine release, we measured the calcium uptake. HCWE alone did not affect the calcium uptake into RPMC, while compound 48/80 increased the calcium uptake into RPMC. The calcium uptake into RPMC induced by 0, 2.5, 25, or 250 μg of HCWE amounted to 29.5 ± 2.5 , 29.5 ± 0.6 , 30.0 ± 1.8 , or 31.5 ± 1.6 μM, respectively. However, HCWE inhibited the compound 48/80-induced calcium uptake increment into RPMC in a concentration-dependent manner (Fig. 3). The calcium uptake into the compound 48/80-treated RPMC after pretreatment of 0, 2.5, 25, or 250 μg of HCWE amounted to 58.3 ± 5.2 , 35.7 ± 0.7 , 31.5 ± 0.9 , or 32.5 ± 1.3 μM, respectively. These results suggest that HCWE may be inhibited histamine release by blocking of calcium uptake into mast cells.

Effect of HCWE on cAMP Level of RPMC To investigate the mechanisms of HCWE on the reduction of histamine release, we also measured the intracellular cAMP level. HCWE itself increased the cAMP level in RPMC six-times, compared with those in control cells. The cAMP level in RPMC induced by 0, 2.5, 25, or 250 μg of HCWE amounted

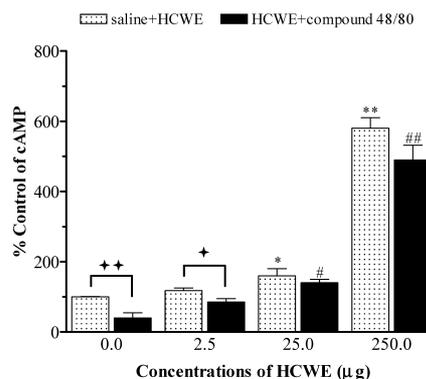


Fig. 4. Inhibitory Effect of *Houttuynia cordata* Water Extract (HCWE) on the Compound 48/80-Induced Decrease of cAMP Level in Rat Peritoneal Mast Cells (RPMC)

Various concentrations of HEMA were added into the rat peritoneal mast cell suspension for 10 min, and cAMP levels were measured. HCWE inhibited the compound 48/80-induced cAMP reduction of RPMC in a dose-dependent manner. * $p < 0.05$ and ** $p < 0.01$; when compared between saline plus HCWE and HCWE plus compound 48/80, * $p < 0.01$ and ** $p < 0.001$; significantly different from the saline, # $p < 0.01$ and ## $p < 0.001$; significantly different from the saline plus compound 48/80.

to 2.5 ± 0.1 , 2.9 ± 0.2 , 4.0 ± 0.5 , or 14.5 ± 0.7 pmol, respectively. RPMC treated with compound 48/80 showed a less than 3-fold decrease in the cAMP level as compared to those treated with buffer only. HCWE inhibited the compound 48/80-induced cAMP reduction of RPMC in a dose-dependent manner (Fig. 4). The cAMP level in the compound 48/80-treated RPMC after pretreatment of 0, 2.5, 25, or 250 μg of HCWE amounted to 1.0 ± 0.4 , 2.1 ± 0.3 , 3.5 ± 0.3 , or 12.3 ± 1.1 pmol, respectively. These results suggest that HCWE may be reduced histamine release by increasing the cAMP level in mast cells.

DISCUSSION AND CONCLUSIONS

The antiallergic effect of *Houttuynia cordata* THUNB (Saurauaceae) was analyzed, as part of our continuing search for biologically active antiallergic agents from the medicinal sources. In this study, HCWE inhibited the compound 48/80-induced systemic anaphylaxis and anti-DNP IgE-mediated PCA. The release of histamine and other pharmacological mediator from mast cells is a prominent feature of acute inflammatory processes including the immediate type anaphylactic reactions.⁷⁾ Histamine released from mast cells is stored in secretory granules. There are various agents to induce the mast cell degranulation, which are commonly associated with the anaphylactic shocks in human and other mammals. Compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation of the cell membrane, and elicits mast cell activation that is mediated by the mast cell degranulation, and by the change of free cytoplasmic calcium concentration.²⁰⁾

HCWE inhibited the compound 48/80-induced mast cell degranulation and colchicine-induced mast cell deformation. Also HCWE inhibited the compound 48/80 or anti-DNP IgE-induced histamine release from RPMC in concentration-dependent manner. The close correlation among the concentration of the histamine-releasing stimulus, the calcium ion influx, and the amount of released histamine suggest a cause-and-effect-relationship between the influx of calcium and the

release of histamine.¹⁶⁾ Compound 48/80 caused the release of histamine from RPMC following calcium uptake, but the pretreatment with HCWE inhibited the compound 48/80-induced calcium uptake into RPMC.

We also examined the relationship between the cAMP production and the releases of histamine. The release of histamine from mast cells was also inhibited by the increase of the intracellular cAMP. According to another study, agents increasing cAMP levels inhibited mediator release.²¹⁾ The inhibitory mechanism on the histamine release from mast cells may be related to the increase of adenylate cyclase activity or a subsequent increase in intracellular cAMP.²²⁾ Disodium cromoglycate, one of anti-anaphylactic agents, especially inhibited the chemical-mediator release by increasing intracellular cAMP *via* the inhibition of cAMP phosphodiesterase.²³⁾ The intracellular cAMP level of RPMC, when incubated with HCWE, increased more than six-times compared with that of control cells. The inhibitory effect of HCWE on the mast cell degranulation and histamine release from RPMC may be related with preventing the increase of intracellular calcium content, which increase the intracellular cAMP level *via* the inhibition of cAMP phosphodiesterase.

In conclusion, these results suggest that HCWE may contain the bioactive compounds that inhibit the compound 48/80- or anti-DNP IgE-induced mast cell activation and anaphylactic reactions by blocking of calcium uptake into mast cells, or increasing the cAMP level in mast cells. The purification of the exact anti-anaphylactic components from HCWE may be beneficial in the treatment of mast cell-mediated allergic diseases.

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