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Scutellaria baicalensis Inhibits Mast Cell-Mediated Anaphylactic Reactions

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Abstract : Mast cells play a critical role in the effector phase of immediate hypersensitivity and allergic diseases. *Scutellaria baicalensis* is a widely used herb in traditional oriental medicine with anticancer, antiviral, antibacterial and anti-inflammatory properties. However, the roles of *Scutellaria baicalensis* in mast cell-mediated anaphylactic reactions have not fully been investigated.

In this study, we examined the influences of the methanol extract of *Scutellaria baicalensis* (MESB) on compound 48/80- or anti-dinitrophenyl (DNP) IgE-induced anaphylaxis-like response *in vivo*. To further prove these *in vivo* results, the inhibitory effect of MESB on mast cell activation was evaluated, focusing on the histamine release from rat peritoneal mast cells (RPMC).

MESB inhibited compound 48/80-induced systemic anaphylaxis-like reaction, plasma histamine release and ear swelling response in mice. MESB also attenuated passive systemic and cutaneous anaphylaxis evoked by anti-DNP IgE. In *in vitro* experiments, MESB dose-dependently reduced histamine release from RPMC activated by compound 48/80 or anti-DNP IgE. Moreover, compound 48/80-elicited calcium uptake was suppressed in a concentration-dependent manner of MESB. Furthermore, MESB transiently increased the level of intracellular cAMP.

From these results, it is suggested that MESB possesses effective anti-anaphylactic activity.

Keywords : Mast cells, Anaphylactic reactions, *Scutellaria baicalensis*, Histamine, Calcium, cAMP

Introduction

Mast cells are tissue cells that are located preferentially at the host-environment interface. Mast cells are known mainly for their involvement in mediating various harmful inflammatory reactions in the host; the best known of these are immunoglobulin E (IgE)-mediated immediate-type hypersensitivity reactions (anaphylaxis). After activation, mast cells exert their biological effects by releasing preformed and *de novo*-synthesized mediators such as histamine, proteases (that is, tryptase), leukotrienes, prostaglandins and various cytokines (Galli and Tsai 2010). Among them, histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (Galli et al. 2008).

Mast cell degranulation can be elicited by the basic secretagogues. The most potent secretagogues include the synthetic compound 48/80 and polymers of basic amino acids. Compared with the natural process, a high concentration of compound 48/80 induces almost 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 (Nishikawa and Kitani 2008). The secretory response of mast

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cells can also be induced by aggregation of their cell surface-specific receptors for IgE by the corresponding antigen.

Scutellaria baicalensis is one of the most popular and multi-purpose herbal medicines or medicinal plants used in oriental countries. Historically, *Scutellaria baicalensis* has been used to treat respiratory tract infection, asthma, jaundice, hepatitis and cancer (Zhang et al. 2003). Recent investigations have shown that *Scutellaria baicalensis* has beneficial properties, including anti-oxidative, anti-tumor, anti-convulsant and anti-apoptotic effects (Ikemoto et al. 2000, Wang et al. 2000, Nemoto et al. 2002, Suh et al. 2003). In addition, it has been demonstrated that the flavonoids isolated from *Scutellaria baicalensis* have also various biological activities such as anti-oxidative, anti-inflammatory and anti-allergic effects (Lim et al. 1999, Lim 2002, 2003, Chi and Kim 2005). Especially, Lim et al. (1999, 2002, 2003) reported the anti-allergic effect of these flavonoid components using peritoneal exudate cells and mesenteric lymph node lymphocytes isolated from Sprague-Dawley rats. Recently, *Scutellaria baicalensis* was shown to inhibit mediator release from mast cells activated by anti-ovalbumin (OVA)/OVA binding (Kim et al. 2010). However, despite extensive study of the multiple effects of *Scutellaria baicalensis*, little is known about the effect of *Scutellaria baicalensis* on mast cell-mediated anaphylactic reactions.

The aim of this study is to evaluate the inhibitory effects of the methanol extract of *Scutellaria baicalensis* against mast cell-mediated anaphylactic reactions.

Materials and Methods

1. Materials

Compound 48/80, disodium cromoglycate (DSCG), anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA) and HEPES were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Percoll solution was purchased from Pharmacia (Uppsala, Sweden).

2. Experimental animals

Male ICR mice (6-week-old, 25～30 g) and Sprague-Dawley rats (8-week-old, 230～280 g) were purchased from Damool Science (Daejeon, Korea). Animals were housed 3～5 per cages in laminar air-flow cabinet maintained at 22±1°C and relative humidity of 55±10% throughout the study. All experiments were performed in compliance with the guidelines approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School.

3. Preparation of the methanol extract of *Scutellaria baicalensis* (MESB)

The dried roots of *Scutellaria baicalensis* were purchased from Jangsu Oriental Pharmacy (Chonbuk, Korea). A voucher specimen (number 2005-SBMw 705023) was deposited at the Herbarium of the Research Center for Allergic Immune Diseases, Chonbuk National University. Dried roots of *Scutellaria baicalensis* (100 g) were immersed in 500 mL of 70% methanol, kept overnight in a refrigerator (10°C), and boiled under reflux for 2 h. The methanol extraction was repeated 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 15.2 g dried powder and kept at 4°C until use. The dried extract was dissolved in phosphate-buffered saline (PBS) or HEPES-Tyrode buffer (136 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 11 mM $NaHCO_3$, 0.6 mM NaH_2PO_4, 2.75 mM $MgCl_2$, 5.4 mM HEPES, 1.0 mg/mL BSA, 1.0 mg/mL glucose, 0.1 mg/mL heparin, pH 7.4) before use.

4. Compound 48/80-induced systemic anaphylaxis-like reaction in mice

Mice (n=10/group) intraperitoneally received compound 48/80 [8 mg/kg body weight (BW)] or saline injection as previously described (Choi et al. 2006). MESB or DSCG (0.01 to 1 g/kg BW) was dissolved in saline and administered orally 1 h before the injection of compound 48/80. DSCG was used as a positive control. Mortality was monitored for 1 h after the induction of anaphylactic shock. After the mortality test, blood was obtained from the heart of each mouse.

5. Preparation of plasma histamine determination

The blood was centrifuged at 150×g for 10 min at 4°C. The plasma was withdrawn and histamine content was measured by the radioenzymatic method (Carvalho et al. 2010). The inhibition percentage of plasma histamine

release was calculated using the following formula: % Inhibition=[(histamine release without MESB−histamine release with MESB)/histamine release without MESB]×100.

6. Compound 48/80-induced ear swelling response in mice

Ear swelling response was investigated by the method described previously (Choi et al. 2006). Compound 48/80 was freshly dissolved in saline (5 mg/mL) and injected intradermally in the ventral aspect of the left side of mouse ear (100 μg/site, 20 μL) using a 30-gauge hypodermic needle. Sham saline was injected intradermally in the ventral aspect of the right side of mouse ear. Ear thickness was measured with a digital micrometer (Mitutoyo, No. 7326, Japan) under mild anesthesia induced by intraperitoneal injection of 1 : 1 mixture (50 μL) of ketamin (1 mg/mL) and xylazine hydrochloride (23.32 mg/mL). Mice were kept in immobility state during the measurement. Ear swelling response represented an increment in thickness above baseline control values. Ear swelling response was determined 1 h after the injection of compound 48/80 or vehicle. MESB (0.01 to 1 g/kg BW) was orally administered 1 h before the injection of compound 48/80.

7. Passive systemic anaphylaxis (PSA) in mice

Anti-DNP IgE-mediated PSA was examined as follows. Mice (n=10/group) were intravenously injected with 3 μg anti-DNP IgE or PBS. Twenty-four hours later, mice were challenged with intravenously administration of 500 μg of DNP-HSA. After 1.5 min, mice were sacrificed by cervical dislocation and blood was immediately collected by cardiac puncture. Plasma was isolated from blood samples and tested for plasma histamine concentration by the radioenzymatic method (Carvalho et al. 2010). MESB (0.01 to 1 g/kg BW) was orally administered 1 h before the challenge.

8. Passive cutaneous anaphylaxis (PCA) in rats

Anti-DNP IgE-mediated PCA was examined as previously reported (Neel et al. 2004). Rats were sensitized in the right dorsal skin by intradermal injection of 500 ng anti-DNP IgE in 20 μL PBS and were given a sham PBS injection in the left dorsal skin. Forty-eight hours later, the rats received into the penile vein an injection of 200 μL of PBS containing 100 μg DNP-HSA with 2% Evans blue. MESB was orally administered 1 h before the challenge. Thirty minutes after the challenge, the rats were sacrificed, tissue sections around the intradermal injection site excised and weighed, followed by extraction of extravasated Evan's blue dye by incubation of biopsies in 1 mL formamide at 55°C for 24 h and measurement of absorbance at 620 nm. Tissue Evans blue concentrations were quantified by interpolation on a standard curve of dye concentrations in the range of 0.01 to 30 μg/mL.

9. Preparation of rat peritoneal mast cells (RPMC) and microscopic observation

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 using a Pasteur pipette, and RPMC were purified by using a Percoll density gradient as described in detail elsewhere (Martynova et al. 2005). RPMC preparations were about 95% pure as assessed by toluidine blue staining and at least 98% of these cells were viable as assessed by trypan blue exclusion. Purified RPMC (1×10^6 cells/mL) were resuspended in HEPES-Tyrode buffer and observed under phase contrast microscope and photographed.

10. RPMC viability assay

To test the viability of RPMC, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was performed as previously described (Yoshimura et al. 2004). Briefly, RPMC (2×10^5 cells/well) were incubated with various concentrations (0.01～1 mg/mL) of MESB at 37°C for 2 h. After addition of MTT (100 μg in 100 μL PBS), RPMC were incubated at 37°C for 1 h. The crystallized MTT was dissolved and the absorbance was measured at 570 nm with a spectrophotometer (Spectra MAX PLUS, Molecular Devices, CA, USA).

11. Histamine assay

RPMC suspensions (2×10^5 cells in 200 μL) were preincubated with MESB (0.01～1 mg/mL) or DSCG at 37°C for 5 min and then incubated with compound 48/80 (0.25 μg/mL) for 15 min. RPMC were sensitized with 10 μg/mL anti-DNP IgE for 6 h and preincubated with MESB or

DSCG at 37˚C for 5 min prior to challenge with DNP-HSA (100 ng/mL). Following centrifugation at 150 × g for 10 min at 4˚C, the amount of histamine in the supernatant was determined by the radioenzymatic method (Carvalho et al. 2010). The inhibition percentage of histamine release was calculated using the following formula: % Inhibition= [(histamine release without MESB − histamine release with MESB)/histamine release without MESB] × 100.

12. Measurement of ^{45}Ca uptake

Purified RPMC were resuspended in HEPES-Tyrode buffer containing ^{45}Ca (1.5 mCi/mL; 1 Ci=3.7×10^{10} becquerels; PerkinElmer Life Sciences, MA, USA), and incubated at 4˚C for 10 min. Mast cell suspensions were pre-incubated with MESB (0.01 ~ 1 mg/mL) at 37˚C for 5 min and then incubated with compound 48/80 (0.25 μg/mL) at 37˚C for 15 min. The reaction was stopped by the addition of 1 mM lanthanum chloride. The samples were centrifuged 3 times at 150 × g for 10 min at 4˚C, and then RPMC were lysed with 10% Triton X-100 and vigorous shaking. Radioactivity of the solution was measured in a scintillation β-counter (Liquid Scintillation Analyzer, A Canberra Company, Australia).

13. Cyclic adenosine-3′, 5′ monophosphate (cAMP) assay

The cAMP level was measured by the method described below. In brief, RPMC suspensions were added to an equivalent volume (200 μL) of prewarmed buffer containing MESB (1 mg/mL) in an Eppendorf tube. The reaction was allowed to proceed for discrete time intervals, terminated by centrifugation at 150 × g for 10 min at 4˚C, and then each sample was added to 250 μL of 50 mM sodium acetate buffer (pH 6.2) under vigorous vortexing, followed by snap frozen in liquid nitrogen. The frozen samples were thawed and vortexed, and then the debris were sedimented by a centrifugation at 1,200 × g for 10 min at 4˚C. The cAMP level in the supernatant was determined by radioimmunoassay using a Rianen assay system (PerkinElmer Life Sciences, MA, USA).

14. Statistical analysis

The results obtained were expressed as mean ± SEM for the number of experiments. Statistical evaluation of the results was performed using one-way ANOVA, followed by Duncan's multiple range tests. Results with $p < 0.05$ were considered statistically significant.

Table 1. Inhibitory effect of the methanol extract of *Scutellaria baicalensis* (MESB) on compound 48/80-induced systemic anaphylaxis-like reaction in mice

Treatment (g/kg BW)		Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)		+	100
MESB	0.01	+	90
	0.1	+	60
	1	+	40
	1	−	0
DSCG	0.01	+	80
	0.1	+	60
	1	+	20

Groups of mice (n=10/group) were orally administrated with 300 μL of saline or drugs {MESB or disodium cromoglycate (DSCG)} 1 h before the injection of compound 48/80. The compound 48/80 was intraperitoneally given to the group of mice. Mortality (%) within 1 h following compound 48/80 injection was presented as the number of dead mice × 100/total number of experimental mice.

Results

1. Methanol extract of *Scutellaria baicalensis* (MESB) inhibits compound 48/80-induced systemic anaphylaxis-like reaction in mice

To investigate the effect of MESB in anaphylaxis-like reactions, we first employed an *in vivo* model of systemic anaphylaxis-like reaction using compound 48/80. After the intraperitoneal injection of compound 48/80 (8 mg/kg BW) into mice, a mortality rate was examined for 1 h. As shown in Table 1, the injection of compound 48/80 resulted in 100% death of mice. Oral administration of MESB (0.01 to 1 g/kg BW) reduced compound 48/80-induced mortality in a dose-dependent manner. Disodium cromoglycate (DSCG: positive control) also inhibited compound 48/80-induced mortality in a dose-dependent fashion.

2. MESB reduces compound 48/80-induced plasma histamine release

The effect of MESB on compound 48/80-induced plasma histamine release was examined. MESB was given at doses ranging from 0.01 to 1 g/kg BW 1 h before the injection of compound 48/80. The histamine content of plasma samples was 278.5 ± 32.9 ng/mL in mice treated with

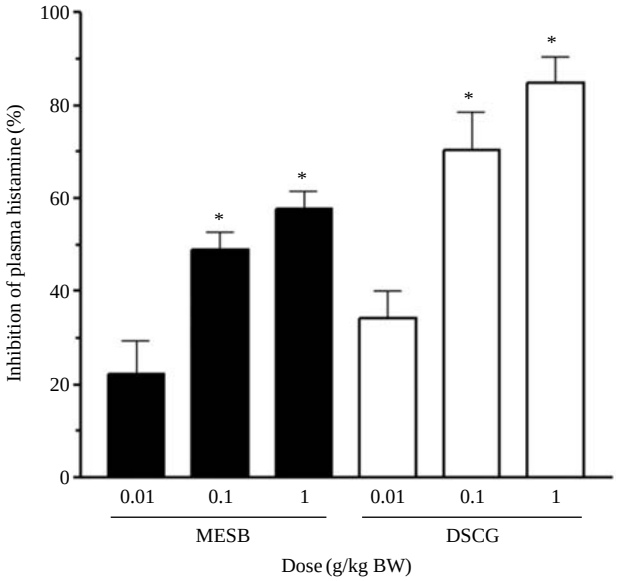

Fig. 1. Effect of the methanol extract of *Scutellaria baicalensis* (MESB) on compound 48/80-induced plasma histamine release in mice. Groups of mice (n=10/group) were orally administrated with 300 μL of saline or drugs {MESB or disodium cromoglycate (DSCG)} 1 h before the injection of compound 48/80. The compound 48/80 solution was intraperitoneally given to the group of mice. Each bar represents the mean±SEM of five independent experiments. *$p<0.05$, significantly different from the control value.

compound 48/80 alone. The inhibition rate due to treatment with MESB was significant at doses of 0.1～1.0 g/kg BW (Fig. 1).

3. MESB attenuates compound 48/80-induced ear swelling response in mice

Ear swelling was induced by the injection of compound 48/80 (100 μg/site) as described (Choi et al. 2006). Oral administration of MESB reduced the ear swelling response induced by compound 48/80 in a dose-dependent way (Table 2).

4. MESB attenuates passive systemic anaphylaxis (PSA) and passive cutaneous anaphylaxis (PCA)

To evaluate the effect of MESB on IgE-mediated anaphylaxis, *in vivo* models of PSA and PCA were chosen. IgE-mediated PSA is dependent upon passive transfer of anti-DNP IgE followed by intravenous administration of the antigen. MESB dose-dependently inhibited anti-DNP IgE-mediated PSA in mice (Fig. 2). Meanwhile, local extravasation is also induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Oral administration of MESB reduced anti-DNP IgE-mediated PCA in a dose-dependent fashion (Fig. 3).

Table 2. Inhibitory effect of the methanol extract of *Scutellaria baicalensis* (MESB) on compound 48/80-induced ear swelling response in mice

MESB (g/kg BW)	Compound 48/80 (100 μg/site)	Increment in ear thickness (×100 μm)	Inhibition (%)
0	+	2.057±0.183	–
0.01	+	1.565±0.332	23.92*
0.1	+	1.122±0.117	45.45*
1	+	0.986±0.154	52.06*
1	–	0.583±0.069	–

Groups of mice (n=10/group) were orally administrated with 300 μL of saline or MESB 1 h before compound 48/80 injection. Twenty microliters of compound 48/80 (100 μg/site) were injected to the ears of mice. Each value represents the mean±SEM of five independent experiments. Inhibition (%)=[(increment in ear thickness without MESB−increment in ear thickness with MESB)/increment in ear thickness without MESB]×100. *$p<0.05$ (significantly different from the control value).

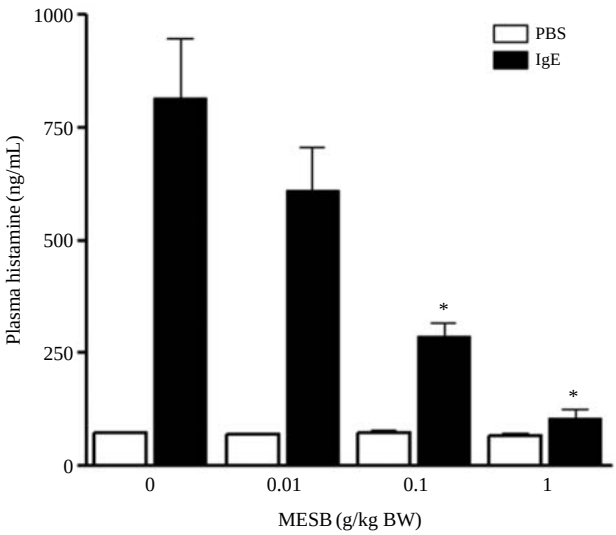

Fig. 2. Effect of the methanol extract of *Scutellaria baicalensis* (MESB) on anti-DNP IgE-mediated passive systemic anaphylaxis in mice. MESB was orally administered 1 h prior to the challenge with antigen. Each bar represents the mean±SEM of five independent experiments. *$p<0.05$, significantly different from the control value.

5. MESB has no cytotoxicity on rat peritoneal mast cells (RPMC)

MTT conversion assay was used to determine the viability of RPMC exposed to MESB. The viable cells were

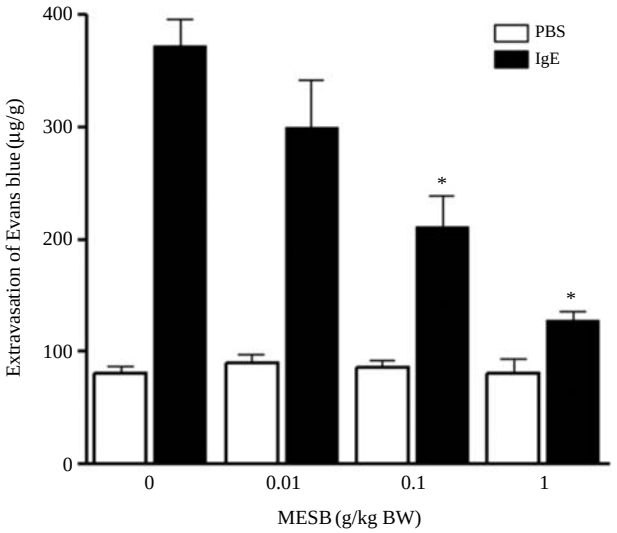

Fig. 3. Effect of the methanol extract of *Scutellaria baicalensis* (MESB) on anti-DNP IgE-mediated passive cutaneous anaphylaxis in rats. MESB was orally administered 1 h prior to the challenge with antigen. Each bar represents the mean±SEM of five independent experiments. *$p<0.05$, significantly different from the control value.

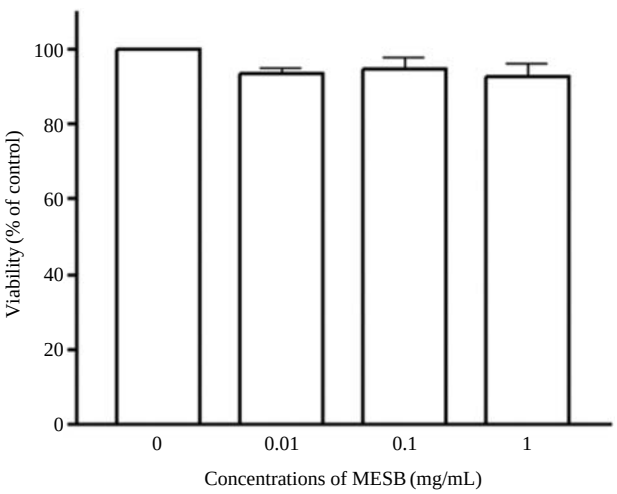

Fig. 4. Effect of the methanol extract of *Scutellaria baicalensis* (MESB) on rat peritoneal mast cells (RPMC) viability. RPMC were treated with the various concentrations of MESB for 2 h. RPMC viability was determined by MTT assay and the percentage of viability was calculated as a ratio of A_{570} of control cells (treated with HEPES-Tyrode buffered solution). Each bar represents the mean±SEM of five independent experiments.

almost 100% after exposure to various concentrations (0.01～1 mg/mL) of MESB for 2 h. Thus, MESB had no cytotoxicity on RPMC (Fig. 4).

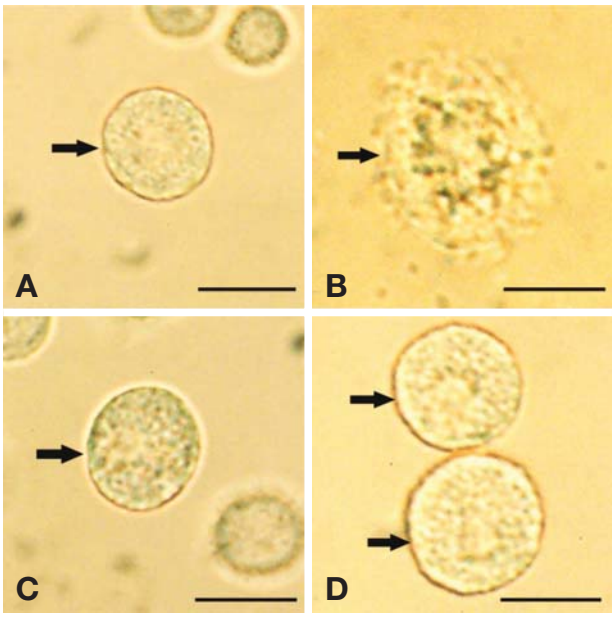

Fig. 5. Inverted light microscope of rat peritoneal mast cells (RPMC, arrows). (A) The normal RPMC in HEPES-Tyrode buffered solution. (B) The degranulated RPMC after the addition of compound 48/80. (C) The RPMC observed within 5 min after the addition of *Scutellaria baicalensis* (MESB; 1 mg/mL) show similar findings as seen in Fig. 5A. (D) The RPMC pretreated with MESB observed within 5 min after the addition of compound 48/80, show similar findings as seen in Fig. 5C. Bar=10 μm.

6. MESB inhibits compound 48/80-induced degranulation of RPMC

To investigate the inhibitory mechanism of MESB on the anaphylaxis-like reaction, we evaluated compound 48/80-induced mast cell activation. The inhibitory effect of MESB on the compound 48/80-induced mast cell degranulation was shown (Fig. 5). The normal RPMC were generally spherical or oval in shape and contained many fine granules surrounding a prominent nucleus (Fig. 5A). After stimulation with compound 48/80, RPMC were degranulated (Fig. 5B). Characteristics of mast cell degranulation were the cell swelling, cytoplasmic vacuoles, and extruded granules near the cell surface and in the surrounding medium. When RPMC were incubated with MESB alone, RPMC were similar to ones as seen in Fig. 5A (Fig. 5C). Pretreatment of MESB inhibited degranulation of RPMC stimulated with compound 48/80, and the cell sizes appeared to be somewhat larger than the control (Fig. 5D). However, there was no significant difference in size between the two groups (data not shown). These results pro-

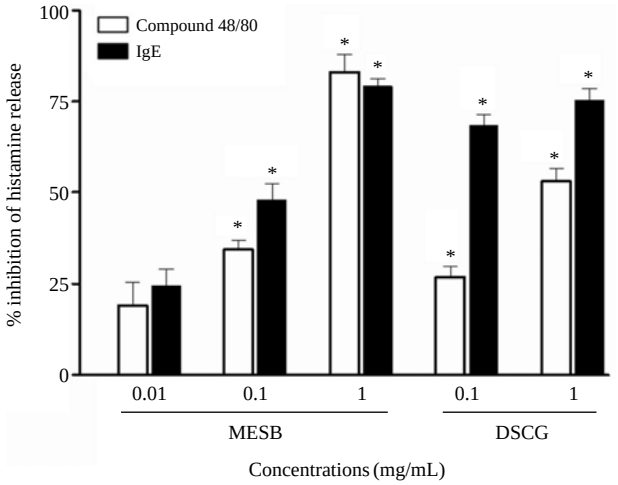

Fig. 6. Effect of the methanol extract of *Scutellaria baicalensis* (MESB) on compound 48/80- or IgE-induced histamine release from rat peritoneal mast cells (RPMC). RPMC were preincubated with MESB at 37˚C for 5 min prior to the incubation with compound 48/80 or DNP-HSA. MESB dose-dependently inhibited compound 48/80- or IgE-induced histamine release. Each bar represents the mean±SEM of five independent experiments. $*p<0.05$, significantly different from the control value. DSCG: disodium cromoglycate.

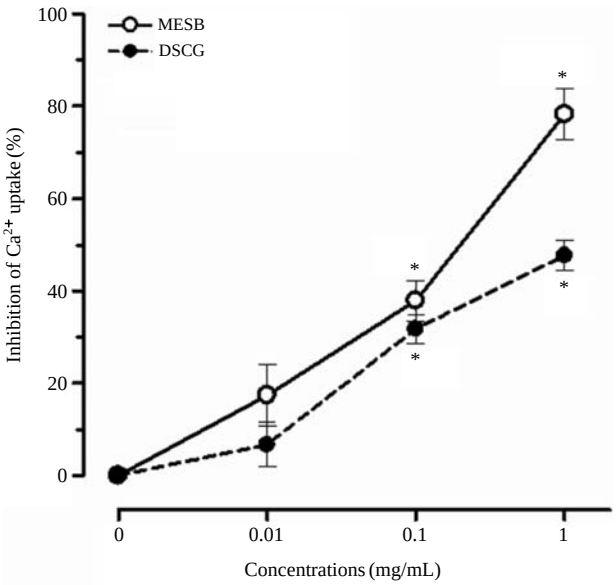

Fig. 7. Effect of the methanol extract of *Scutellaria baicalensis* (MESB) on compound 48/80-induced calcium uptake into rat peritoneal mast cells (RPMC). RPMC were preincubated with MESB at 37˚C for 5 min prior to the incubation with compound 48/80. MESB dose-dependently inhibited compound 48/80-induced calcium uptake into RPMC. Each data value represents the mean± SEM of five independent experiments. $*p<0.05$, significantly different from the control value. DSCG: disodium cromoglycate.

pose that MESB suppresses the compound 48/80-induced mast cell degranulation.

7. MESB inhibits compound 48/80-induced or IgE-mediated histamine release from RPMC

In view of the inhibitory effects of MESB on these *in vivo* experiments, we examined its effect on RPMC triggered by compound 48/80 or anti-DNP IgE. The effect of MESB on compound 48/80-induced or IgE-mediated histamine release from RPMC was shown (Fig. 6). The histamine release from compound 48/80-treated RPMC was reduced in a dose-dependent manner of MESB. MESB also dose-dependently inhibited IgE-mediated histamine release from RPMC. DSCG showed a significant inhibition rate at the dose of 0.1 and 1 mg/mL. These results indicate that MESB contains the active compound(s), which inhibit compound 48/80-induced or IgE-mediated anaphylactic responses by blocking histamine release from RPMC.

8. MESB attenuates compound 48/80-induced calcium uptake into RPMC

It is well established that an increase of calcium uptake of RPMC contributes to the release of histamine (Akagi et al. 1994). Therefore, we measured the calcium uptake. Treatment with MESB alone showed no change in calcium uptake. However, calcium uptake was greatly increased by the stimulation of RPMC with compound 48/80 (data not shown). The compound 48/80-induced calcium uptake was inhibited in a concentration-dependent manner of MESB (Fig. 7). Moreover, DSCG (a reference drug) significantly reduced compound 48/80-induced calcium uptake into RPMC at 0.1～1 mg/mL. Our observations propose that MESB may inhibit histamine release through blocking calcium uptake into RPMC.

9. MESB increases intracellular cAMP level in RPMC

The cAMP pathway is critical to the regulation of mast cell activation. An increase of cAMP is known to precede the inhibition of histamine release from mast cells activated by compound 48/80 (Kaliner and Austen 1974). To investigate the mechanism of MESB on the reduction of histamine release from RPMC stimulated by compound 48/80,

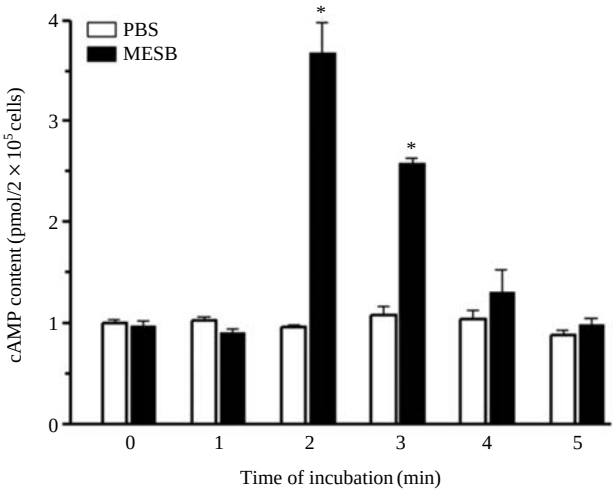

Fig. 8. Effect of the methanol extract of *Scutellaria baicalensis* (MESB) on the cAMP levels of rat peritoneal mast cells (RPMC). RPMC were treated with MESB (1 mg/mL) for indicated time at 37°C, and then cAMP levels were measured. Each bar represents the mean ± SEM of five independent experiments. *$p < 0.05$, significantly different from the value at 0 min.

we assessed the intracellular cAMP level. The level of cAMP was not changed in unstimulated RPMC. When RPMC were incubated with MESB at 1 mg/mL, the cAMP level increased at 2 ~ 3 min and decreased to basal level from 4 min (Fig. 8). From these results, it is suggested that MESB inhibits histamine release by increasing the cAMP level in RPMC.

Discussion

Scutellaria baicalensis has been shown to have a broad spectrum of biological activities, including anti-inflammatory and anti-allergic activities based on its long history in clinical application (Zhang et al. 2003). This study confirms that MESB has anti-anaphylactic properties. MESB significantly inhibits compound 48/80-induced systemic anaphylaxis-like reaction, plasma histamine release and ear swelling response. Pretreatment with MESB also suppresses degranulation of RPMC activated by compound 48/80. From a morphologic point of view, the cell sizes appear to be somewhat larger than the control, but there is no significant difference in size between the two groups. Furthermore, MESB attenuates compound 48/80-induced histamine release from RPMC. It is well-recognized that compound 48/80 can induce the mast cell-dependent, non-specific anaphylactic reaction. The mechanism of anaphylaxis-like response triggered by compound 48/80 is considered to be due to the massive release of vasoactive amines such as histamine from mast cells and basophils (Nishikawa and Kitani 2008). As mentioned above, histamine is a typical preformed mediator that causes various pathophysiologic events in acute allergic reactions (Lim 2003). Thus, it is inferred that MESB inhibits mast cell-mediated anaphylaxis-like reactions by reducing histamine release from RPMC.

PSA and PCA represent models of acute allergic reactions in which mast cells appear to be essential (Neel et al. 2004). MESB profoundly suppresses anti-DNP IgE-mediated PSA and PCA. In view of the inhibitory effects of MESB on PSA and PCA *in vivo*, we examined its effect on RPMC triggered by IgE. In agreement with these *in vivo* effects, MESB dose-dependently inhibited antigen-induced histamine release from RPMC. The mechanism of the protection against anti-DNP IgE, while not clear at present, may be suggested only in some particular conditions. Future work will be required to elucidate the protective mechanism of MESB.

cAMP pathway is supposed to be critical to the activation of mast cells. It has been reported that agents that induce the elevation of intracellular cAMP level can attenuate the stimulated release of mediators from mast cells (Tasaka et al. 1986, Weston and Peachell 1998). Moreover, several studies have shown an algorithm between cAMP and calcium uptake in RPMC. In general, increased cAMP inhibits superoxide anion generation *via* cAMP-dependent protein phosphorylation in RPMC stimulated by compound 48/80 (Fukuishi et al. 1997). Decreased superoxide anion as well as cAMP impedes inositol 1,4,5-triphosphate or guanosine triphosphate-induced calcium release from the endoplasmic reticulum (Yoshii et al. 1988, Akagi et al. 1994). Accordingly, calcium-filling state in the endoplasmic reticulum blocks a calcium influx into RPMC, which leads to the reduction of the free intracellular calcium content (Hoth and Penner 1993). Consequently, decreased intracellular calcium prevents histamine release from RPMC (Yoshii et al. 1988, Akagi et al. 1994). Interestingly, treatment of MESB transiently increases cAMP level beyond the basal level. Although the mechanism of MESB-induced cAMP production has not been elucidated, MESB may activate adenylate cyclase directly or indirectly, otherwise inhibit cAMP phosphodiesterase. In addition, MESB pre-

vents compound 48/80-induced calcium uptake of RPMC in a dose-dependent fashion. According to this observation, the inhibitory mechanism of MESB on histamine release from compound 48/80-treated RPMC may be due to the increase of intracellular cAMP. Subsequently, we speculate that increased cAMP inhibits calcium uptake into RPMC *via* a cascade of intracellular events described above and then the decrease of free intracellular calcium content hinders the histamine release from RPMC.

This study has a few limitations, however, probably stemming from the functional compartments in RPMC for cAMP on histamine release (Alfonso et al. 2000). It has been described earlier that histamine release from RPMC is not inhibited at all by the increase of cAMP, such as the increase of cAMP through β stimulation by isoproterenol (Marquardt and Wasserman 1982). Therefore, the possibility that the increase of cAMP by MESB may not inhibit histamine release from RPMC cannot be excluded. Alternatively, it can also be assumed that the modulatory effect of cAMP on histamine release depends more on the cross-talk of the activated signal transducting pathway than on the final level of cAMP (Alfonso et al. 2000). In this sense, further studies are needed to elucidate the detail relationship between cAMP and histamine release in RPMC.

In summary, the present results demonstrate that MESB attenuates both compound 48/80-induced anaphylaxis-like and IgE-mediated anaphylactic reactions in *in vivo* and *in vitro* murine models. Generally, extraction and isolation is known to be the first important steps for separation, characterization, and quantification of flavonoids from plant materials. Flavonoids are often most soluble in organic solvents less polar than water. Thus, aqueous methanol is a popular choice of solvent. As previously described, *Scutellaria baicalensis* is extracted with 70～80% methanol, and subsequently fractionated with diethyl ether and n-butanol through a silica gel column chromatography, followed by collection of several fractions including wogonin, wogonoside, ganhuangenin and 3,5,7,2′,6′-pentahydroxyflavone (PHF) (Lim 2003). On the basis of this, we used the whole methanol extract of *Scutellaria baicalensis*, which is likely to contain the above-mentioned flavonoids. However, the active components that are responsible for the biological effect are not clear at this time. Previous reports have shown the anti-allergic activity of the extracts of *Scutellaria baicalensis* (Lim 2002, 2003). Among the various components, wogonin, wogonoside, ganhuangenin and PHF markedly inhibit histamine release from peritoneal exudate cells stimulated with calcium ionophore or compound 48/80. This earlier observation agrees with our result that the histamine release from compound 48/80-treated RPMC is reduced in a dose-dependent manner of MESB. Thus, these findings suggest that flavonoids might be active components attributed to the anti-anaphylactic activity of *Scutellaria baicalensis*. The effort to identify active components from MESB is ongoing in our laboratory.

In conclusions, *Scutellaria baicalensis* may have beneficial effects in the prevention or treatment of mast cell-mediated allergic diseases.

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황금은 비만세포를 매개로 하는 아나필락틱 반응을 억제한다.

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간추림 : 비만세포는 즉시형 과민반응과 알레르기성 질환의 발현에 중요한 역할을 한다. 황금은 동양의학에서 전통적으로 널리 사용된 약초로, 약리작용에는 항암, 항바이러스, 항박테리아, 항염증 효과 등이 있다. 그러나, 황금이 비만세포를 매개로 한 아나필락틱 반응에 미치는 영향을 다룬 연구가 거의 없는 실정이다.

본 연구에서 우리는 황금의 메탄올 추출물이 compound 48/80 혹은 anti-DNP IgE에 의한 쥐 모델 아나필락시에 미치는 영향을 관찰하였다. 이러한 결과를 보다 확실히 입증하고자, 비만세포의 히스타민 유리에 초점을 맞춰서 황금이 이들 세포에 대해 억제효과를 보이는지 확인하였다.

황금은 compound 48/80에 의한 전신성 아나필락시양 반응과 그에 따른 혈장 내 히스타민 유리 그리고 귀의 부종반응을 억제하였다. 황금은 또한 anti-DNP IgE에 의해 수동적으로 유도된 전신 및 피부 아나필락시 반응을 억제하였다. 시험관 내 실험에서, 황금은 compound 48/80에 의해 유도된 비만세포의 탈과립을 억제하였으며, compound 48/80 혹은 anti-DNP IgE로 활성화된 비만세포로부터의 히스타민 유리를 감소시켰다. 이외에도 compound 48/80에 의해 유도된 세포 내 칼슘 유입이 황금에 의해 농도의존적으로 억제되었다. 아울러 황금은 일시적으로 비만세포 내 cAMP의 양을 증가시켰다.

이상의 결과들은 황금이 효과적인 항 아나필락틱 활성을 가지고 있음을 제시한다.

찾아보기 낱말 : 비만세포, 아나필락틱 반응, 황금, 히스타민, 칼슘, cAMP

[†]공동 제1저자로 동등한 역할을 수행하였음.

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