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Sanguisorba officinalis Inhibits Immediate-type Allergic Reactions

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Abstract – The effect of aqueous extract of *Sanguisorba officinalis* L. (Rosaceae) root (SOAE) on the immediate-type allergic reactions by anal therapy was investigated. SOAE (0.01 to 1 g/kg) dose-dependently inhibited systemic anaphylaxis induced by compound 48/80 in mice. When SOAE was pretreated at the same concentrations with systemic anaphylaxis, the plasma histamine levels were reduced in a dose-dependent manner. SOAE (0.1 and 1 g/kg) also significantly inhibited local anaphylaxis activated by anti-DNP IgE. SOAE (0.001 to 1 mg/ml) dose-dependently inhibited the histamine release from rat peritoneal mast cells (RPMC) activated by compound 48/80 or anti-DNP IgE. The level of cyclic AMP (cAMP) in RPMC, when SOAE (1 mg/ml) was added, transiently and significantly increased compared with that of basal cells. These results provide evidence that anal therapy of SOAE may be beneficial in the treatment of allergic diseases.
Key words – *Sanguisorba officinalis*, anal therapy, anaphylaxis, compound 48/80, anti-DNP IgE, histamine, cyclic AMP

Introduction

The dried root of *Sanguisorba officinalis* L. (Rosaceae), well known as Ji - Yu in Korea, has been used for centuries as traditional medicine. It has been known to be effective in many kinds allergic skin disease such as urticaria, eczema, and allergic dermatitis as a traditional oriental medicine (Chang and But, 1987; Zu, 1998). The mast cells have been thought to play a major role in the development of many physiologic changes during allergic responses (Kim and Lee, 1999). Mast cell activation both by IgE-dependent and IgE-independent stimuli, bring about the process of degranulation that results in the fusion of the cytoplasmic membranes with the plasma membrane. This is accompanied by both the fast external release of granule-associated stored mediators (histamine, chemotactic factors, neutral proteases, proteoglycans, cytokines, etc) as well as by the generation and release of newly generated mediators, such as products of arachidonic acid metabolism (Metcalfe *et al.*, 1981) and, at later times, by the production and release of an array of cytokines (Church *et al.*, 1997). 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). Mast cell degranulation can be elicited by a number of positively charged substances, collectively known as the basic secretagogues of mast cells (Lagunoff *et al.*, 1983). Compound 48/80 and polymers of basic amino acids, such as substance P, are some of the most potent secretagogues of mast cells (Ennis *et al.*, 1980). Compared with the natural process, a high concentration of compound 48/80 induces almost a 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 (Allansmith *et al.*, 1989; Shin *et al.*, 1999). The secretory response of mast cells can also be induced by aggregation of their cell surface-specific receptors for immunoglobulin E (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 model for the immediate hypersensitivity. Anal therapy is a kind of drug delivery system through the anus, which is utilized in the patients to whom oral administration is impossible. The drug absorbed in the rectum can avoid first-pass effect in the liver and circulate the whole-body directly. The absorption rate in the rectum is faster than that in the gastrointestinal tract. The absorption rate and total amount through the rectum have little difference with those of a venous administration. Thus, anal therapy can be expected to have good efficacy by the increased absorption rate and the strong medical action (Won *et al.*, 2001; Yi *et al.*, 2001). It was previously reported that *Sanguisorba officinalis*

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inhibited the allergic reaction by intraperitoneal and oral-administration (Shin *et al.*, 2002). We administered medicine to the experimental animal through the anus and investigated the inhibitory effect against allergic reaction.

This paper deals with an evaluation of the effect of SOAE on compound 48/80-induced systemic anaphylaxis and anti-dinitrophenyl (DNP) IgE antibody-induced PCA by anal therapy and histamine release from RPMC. We also investigated intracellular cAMP content to clarify the mechanism by which the SOAE inhibited histamine release from RPMC.

Materials and Methods

Reagents – Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), α-minimal essential medium (α-MEM), ortho-phthaldialdehyde and metrizamide were purchased from Sigma Chemical Co. (St Louis, MO). cAMP was purchased from Amersham Pharmacia Biotec (UK).

Animals – The original stock of ICR mice and SD rats were purchased from Dae-Han Experimental Animal Center (Daejeon, Korea), and the animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed five to ten per cage in a laminar air flow room maintained under a temperature of 22±2°C and relative humidity of 55±5% throughout the study.

Preparation of SOAE – The *Sanguisorba officinalis* was purchased from the oriental drug store, Bohwa Dang (Jeonju, Korea). A voucher specimen (number WSP-00-13) was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The plant sample was extracted with distilled water at 70°C for 5 h (two times). The extract was filtered through Whatman No. 1 filter paper and the filtrate was lyophilized, and kept at –4°C. The yield of dried extract from starting crude materials was about 11.3%. 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 mM $MgCl_2$, 5.6 mM glucose, 0.1% bovine serum albumin) before use.

Compound 48/80-induced systemic anaphylaxis – Compound 48/80-induced systemic anaphylactic reaction was examined as previously described (Yi *et al.*, 2001). Mice were given an intraperitoneal injection of 0.008 g/kg body weigh (BW) of the mast cell degranulator, compound 48/80. The dried SOAE was dissolved in saline and administered anally ranging from 0.005 to 1 g/kg 1 h before the injection of compound 48/80 (n=10/group). In time dependent experiment, SOAE (1 g/kg BW) was administered anally at 0 min, 5 min, 10min, 20 min and 30 min after compound 48/80 injection (n=10/group). Mortality was monitored for 1 h after induction of anaphylactic shock. After the mortality test, blood was obtained from the heart of each mouse.

PCA reaction – 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 tail vein. The anti-DNP IgE and DNP-HSA were diluted in PBS. The mice were injected intradermally with 0.5 μg of anti-DNP IgE into each of two dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Each mouse, 48 h later, received an injection of 1 mg of DNP-HSA in PBS containing 4% Evans blue (1:4) *via* the tail vein. SOAE (0.001 to 1 g/kg BW) was anally administered 1 h before the challenge. Then 30 min after the challenge, the mice were sacrificed and the dorsal skin was removed for measurement of pigment area. The amount of dye was then determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of mixture of acetone and phosphoric acid (13:5) based on the method of Katayama *et al.* (1978). The absorbent intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan) and the amount of dye was calculated with the Evans blue measuring-line.

Preparation of plasma and histamine determination – The blood was centrifuged at 400×g for 10 min. The plasma was withdrawn and histamine content was measured by the ortho-phthaldialdehyde spectrofluorometric procedure of Shore *et al.* (1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer (Shimadzu, RF-5301 PC, Japan).

Preparation of RPMC – RPMC were isolated as previously described (Kanemoto *et al.*, 1993). In brief, rats were anesthetized by ether and injected 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 and 0.1% gelatin) into the peritoneal cavity and the abdomen was gently massaged for about 90 seconds. 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.* (1977). In brief, peritoneal cells suspended in 1 ml of Tyrode buffer B were layered on 2 ml of metrizamide (22.5 w/v%) 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 Tyrode buffer A. 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 – Purified RPMC were resuspended in Tyrode buffer A for the treatment of compound 48/80. RPMC suspensions (2×10^5 cells/ml) were preincubated for 10 min at 37°C before the addition of compound 48/80 (5 μg/ml). The cells were preincubated with the SOAE preparations, and then incubated (10 min) with the compound 48/80. RPMC suspensions (2×10^5 cells/ml) were also sensitized with anti-DNP IgE (10 μg/ml) for 6 h. The cells were preincubated with the SOAE at 37°C for 10 min prior to the challenge with DNP-HSA (1 μg/ml). The cells were separated from the released histamine by centrifugation at 400×g for 5 min at 4°C. Residual histamine in cells was released by disrupting the cells with perchloric acid and centrifugation at 400×g for 5 min at 4°C.

Measurement of cAMP level – The cAMP level was measured according to the method of Peachell *et al.* (1988). In brief, purified mast cells were resuspended in prewarmed (37°C) Tyrode buffer A. Typically, an aliquot of cells (2×10^5 cells/ml) were added to an equivalent volume (50 μl) of prewarmed buffer containing the drug in an Eppendorf tube. The reaction was allowed to proceed for discrete time intervals, terminated by the addition of ice-cold acidified ethanol (0.9 ml of 86% ethanol/1 M HCl, 99:1) with brief vigorous vortexing and then snap frozen in liquid nitrogen. The sample was later thawed and vortexed, then the debris was sedimented in a centrifuge (400×g at 4, for 5 min), and an aliquot (0.9 ml) of the supernatant was removed and evaporated to dryness under reduced pressure. The dried sample was reconstituted in assay buffer (150-200 μl) and stored frozen. The cAMP level was determined by enzyme immunoassay, using a commercial kit (Amersham Pharmacia Biotech).

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.

Result

SOAE inhibits compound 48/80-induced systemic anaphylaxis – To determine the effect of SOAE by anal therapy in systemic anaphylaxis, we used compound 48/80 (0.008 g/kg) as a fatal anphylaxis inducer. After the intraperitoneal 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 anal administration of 200 μl saline as a control induced a fatal shock in 100% of mice. When the SOAE was anally administered at a concentrations ranging from 0.005 to 1 g/kg BW for 1 h, the mortality with compound 48/80 was reduced dose-dependently. Of special note, SOAE inhibited compound 48/80-inducced mortality 100% with the dose of 1 g/kg. In addition, the mortality of mice administered anally with SOAE (1 g/kg) 0 min, 5 min, 10 min, 20 min and 30 min after compound 48/80 injection increased time-dependently (Table 2).

Table 1. Effect of SOAE on compound 48/80-induced systemic anaphylaxis

SOAE treatment (g/kg BW)	Compound 48/80 (0.008 g/kg BW)	Mortality (%)
None (saline)	+	100
0.005	+	100
0.01	+	90
0.05	+	50
0.1	+	30
0.5	+	20
1	+	0
1	–	0

Groups of mice (n=10/group) were anally pretreated with 200 μl saline or SOAE. SOAE was given at various doses 1 h before the compound 48/80 injection. The compound 48/80 solution was intraperitoneally given to the group of mice. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead mice×100/total number of experimental mice.

Table 2. Time-dependent effect of SOAE on compound 48/80-induced systemic anaphylaxis

SOAE treatment (g/kg, BW)	Time (min)	Compound 48/80 (0.008 g/kg BW)	Mortality (%)
None (saline)	0	+	100
1	0	+	0
	5	+	0
	10	+	0
	20	+	20
	30	+	100

Groups of mice (n=10/group) were anally pretreated with 200 μl saline or SOAE. SOAE (1 g/kg) was given at 0 min, 5 min, 10 min, 20 min and 30 min after the compound 48/80 injection. The compound 48/80 solution was intraperitoneally given to the group of mice. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead mice×100/total number of experimental mice.

SOAE inhibits compound 48/80-induced plasma histamine release – The ability of SOAE to influence compound 48/80-induced plasma histamine release was investigated. SOAE was given from 0.01 to 1 g/kg BW 1 h before (n=10/group) compound 48/80 injection. SOAE dose-dependently inhibited compound 48/80-induced plasma histamine release. The inhibition rate of histamine by SOAE was significant at doses of 0.1 to 1 g/kg (Table 3).

SOAE inhibits anti-DNP IgE-induced PCA – Local

Table 3. Effect of SOAE on compound 48/80-induced plasma histamine release

SOAE treatment (g/kg, BW)	Compound 48/80 (0.008 g/kg BW)	Amount of histamine (μg/ml)
None (saline)	+	0.201±0.018
0.01	+	0.193±0.026
0.05	+	0.184±0.020
0.1	+	0.139±0.016*
0.5	+	0.079±0.018*
1	+	0.062±0.009*

Groups of mice (n=10/group) were anally pretreated with 200 μl saline or SOAE. SOAE was given at various doses 1 h before the compound 48/80 injection. Each datum represents the mean±SEM of three independent experiments. *p<0.05; significantly different from the saline value.

Table 4. Effect of SOAE on the 48 h PCA.

SOAE treatment (g/kg BW)	Anti-DNP IgE plus DNP-HSA	Amount of dye (μg/site)
None (saline)	+	6.703±0.741
0.001	+	6.217±0.534
0.01	+	5.892±0.499
0.1	+	3.174±0.372*
1	+	1.745±0.211*

SOAE was administered anally 1 h prior to the challenge with antigen. Each datum represents the mean±SEM of three independent experiments. *p<0.05; significantly different from the saline value.

extravasation is induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Anti-DNP IgE was injected into the right dorsal skin sites. As a control, the left dorsal skin site of these mice was injected with saline alone. After 48 h, all animals were injected intravenously with DNP-HSA plus Evans blue dye. SOAE was administered anally 1 h prior to the challenge with antigen. As shown in Table 4, SOAE (0.1 and 1 g/kg) by anal therapy showed a marked inhibition in PCA reaction.

SOAE inhibits compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC – The inhibitory effect of SOAE on compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC are shown in Table 5 and Table 6. SOAE dose-dependently inhibited compound 48/80-induced or anti-DNP IgE-mediated histamine release at concentrations of 0.001 to 1 mg/ml. Especially, SOAE significantly inhibited the compound 48/80-induced or anti-DNP IgE-mediated histamine release at the concentrations of 0.1 and 1 mg/ml.

SOAE increases cAMP level of RPMC – Finally, We investigated the cAMP content to clarify the mechanism by which SOAE inhibits histamine release from RPMC. When RPMC were incubated with SOAE at a concentration of 1 mg/ml, the cAMP content significantly increased. It peaked at 1 min after SOAE was added, then decreased to basal value about four min later (Fig. 1).

Table 5. Effect of SOAE on compound 48/80-induced histamine release from RPMC.

SOAE treatment (mg/ml)	Compound 48/80 (5 μg /ml)	Amount of histamine (μg/ml)
None (saline)	+	0.386±0.018
0.001	+	0.363±0.037
0.01	+	0.310±0.029
0.1	+	0.061±0.009*
1	+	0.052±0.014*

The cells (2×10^5 cells/ml) were preincubated with SOAE at 37°C for 10 min prior to incubation with compound 48/80. Each datum represents the mean±SEM of three independent experiments. *p<0.05; significantly different from the saline value.

Table 6. Effect of SOAE on IgE-mediated histamine release from RPMC.

SOAE treatment (mg/ml)	Anti-DNP IgE plus DNP-HSA	Amount of histamine (μg/ml)
None (saline)	+	0.165±0.018
0.001	+	0.157±0.022
0.01	+	0.143±0.025
0.1	+	0.093±0.016*
1	+	0.074±0.011*

The cells (2×10^5 cells/ml) were preincubated with SOAE at 37°C for 10 min prior to challenge with DNP-HSA. Each datum represents the mean±SEM of three independent experiments. *p<0.05; significantly different from the saline value.

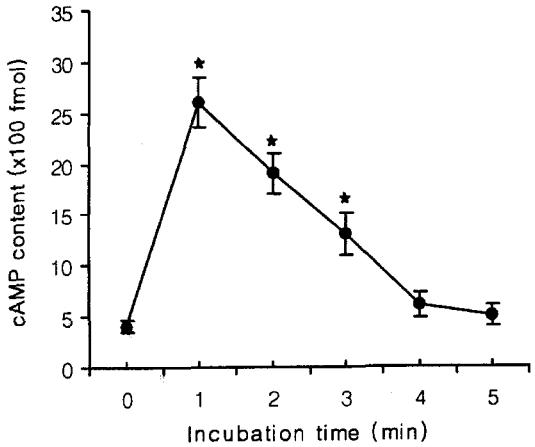

Fig. 1. Time course of increase in the cAMP level of RPMC caused by SOAE. RPMC (2×10^5 cells/ml) were pretreated with SOAE (1 mg/ml) at 37°C. Each datum represents the mean±SEM of three independent experiments. *p<0.05; significantly different from the saline value.

Discussion

The present study showed that SOAE pretreatment greatly affected compound 48/80-induced systemic anaphylaxis and anti-DNP IgE mediated PCA. SOAE inhibited the plasma

histamine levels in mice. SOAE also inhibited the compound 48/80 or anti-DNP IgE-mediated histamine release from RPMC. Therefore, we simply speculate that these results indicate that anaphylactic degranulation of mast cells is inhibited by SOAE. There is no doubt that stimulation of mast cells 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, apparently directly, to activate G-proteins (Mousli *et al.*, 1990a; Mousli *et al.*, 1990b). The evidence indicates that the protein is G inhibitory-like and that the activation is inhibited by benzalkonium chloride (Bueb *et al.*, 1990). Tasaka *et al.* (1986) reported that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. This result indicates that the permeability increase of the cell membrane may be an essential trigger for the release of the mediators from mast cells. In this sense, anti-allergic agents having a membrane-stabilizing action may be desirable. SOAE might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80. The SOAE anally administered mice were protected from local anaphylaxis, which suggests that SOAE might be useful in the treatment of allergic skin reactions. The release of histamine is known to be depressed by an increase in the intracellular cAMP content due to the activation of adenylate cyclase or inhibition of cAMP phosphodiesterase (Makino *et al.*, 1987). The intracellular cAMP content of the mast cells, when incubated with SOAE (1 mg/ml), increased about 6-fold in comparison with that of basal cells.

In conclusion, these results provide evidence that anal therapy of SOAE may be beneficial in the treatment of allergic diseases. Also, it suggests that SOAE may contain compounds with actions that inhibit mast cell-mediated allergic reactions *in vivo* and *in vitro*. Therefore, further investigation is necessary to clarify unknown anti-anaphylactic constituents that may be more active than the SOAE itself. Also, further work should address the possibility that SOAE may also be active in the inhibition of human mast cell degranulation and, therefore, in the treatment of human allergic disorders.

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