

***Stachys riederi* Inhibits Mast Cell-Mediated Acute and Chronic Allergic Reactions**

Tae-Yong Shin*

College of Pharmacy, Woosuk University, Jeonju, Jeonbuk, South Korea

ABSTRACT

The effect of aqueous extract of *Stachys riederi* var. *japonica* Miq. (Labiatae) (SRAE) on the mast cell-mediated allergic and inflammatory reactions were investigated. SRAE inhibited systemic allergic reaction induced by compound 48/80. SRAE dose-dependently inhibited passive cutaneous anaphylaxis (PCA) activated by anti-dinitrophenyl (DNP) IgE. SRAE also dose-dependently inhibited the histamine release from rat peritoneal mast cells (RPMC) activated by compound 48/80 or anti-DNP IgE. Moreover, SRAE inhibited the secretion of tumor necrosis factor- α (TNF- α) and interleukin (IL)-6 in phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187-stimulated human mast cells (HMC-1 cells). These results provide evidences that SRAE may be beneficial in the treatment of acute and chronic allergic diseases.

Key Words: *Stachys riederi*; Mast cells; Allergic reaction; Tumor necrosis factor- α ; Interleukin-6.

*Correspondence: Tae-Yong Shin, College of Pharmacy, Woosuk University, Jeonju, Jeonbuk 565-701, South Korea; Fax: (82-63) 290-1597; E-mail: tyshin@woosuk.ac.kr.

INTRODUCTION

In general, immediate hypersensitivity, which involves urticaria, allergic rhinitis and asthma, is mediated by various chemical mediators released from mast cells. The mast cell has long been thought to play a crucial role in the development of many physiological changes during immediate allergic responses.^[1] 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.^[2] Mast cell degranulation can be elicited by a number of positively charged substances, collectively known as the basic secretagogues of mast cells.^[3] The most potent secretagogues include the synthetic compound 48/80 and polymers of basic amino acids.^[4] An appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylaxis.^[5] The secretory response of mast cells can also be induced by aggregation of their cell surface-specific receptors for IgE by the corresponding antigen.^[6–8] It has been established that the anti-IgE antibody induced passive cutaneous anaphylaxis (PCA) as a typical model for mast cell-dependent immediate type allergic reaction.^[9] Although mast cells also store small amounts of cytokines in their granules,^[10] these cells dramatically increase their production of tumor necrosis factor- α (TNF- α), interleukin (IL)-6 and other cytokines after stimulated with phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187. The finding that activated mast cells are also a source of several cytokines suggests an additional role of mast cells in late-phase reactions and other persistent inflammatory processes.^[11] Both TNF- α and IL-6 activate inflammatory cells and stimulate them to synthesize other cytokines. The *Stachys riederi* var. *japonica* Miq. (Labiatae), well known as Seok-Jam-Pul in Korea, has been used for centuries as traditional medicine. This plant is used for management of hemorrhage, cough and skin diseases.^[12] This paper deals with an evaluation of the effect of SRAE on the compound 48/80-induced systemic allergic reaction, anti-DNP IgE antibody-induced PCA, and histamine release from RPMC. The effect of SRAE on phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated TNF- α and IL-6 secretion in human mast cells (HMC-1 cells) was also investigated.

MATERIALS AND METHODS

Reagents

Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), α -minimal essential medium (α -MEM), o-phthaldialdehyde, phorbol 12-myristate 13-acetate, calcium ionophore A23187 and metrizamide were purchased from Sigma Chemical Co. (St Louis, MO). Recombinant TNF- α and IL-6 were obtained from R&D Systems Inc. (USA).

Animals

ICR mice and Sprague–Dawley rats were purchased from Dae-Han Biolink (Chungbuk, South 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 the temperature of $22 \pm 2^{\circ}\text{C}$ and relative humidity of $55 \pm 5\%$ throughout the study.

Preparation of SRAE

The plants of *Stachys riederi* were collected in Waju, Jeonbuk, South Korea, on the 25th of August, 2000. A voucher specimen (number WSP-00-17) was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The plant sample was extracted with purified water at 70°C for 5 h. The extract was filtered through Whatman No. 1 filter paper and the filtrate was lyophilized. The yield of dried extract from starting crude materials was about 6.2%. 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 Allergic Reaction

Compound 48/80-induced systemic anaphylactic reaction was examined as previously described.^[13] Mice were given an intraperitoneal injection of 0.008 g/kg body weight (BW) of the mast cell degranulator compound 48/80. SRAE was administered by intraperitoneal injection from 0.005 to 1 g/kg BW 1 h before the injection of compound 48/80 ($n = 10/\text{group}$). In time dependent experiment, SRAE (1 g/kg) was injected intraperitoneally at 5 min, 10 min, 20 min, 30 min and 40 min after compound 48/80 injection ($n = 10/\text{group}$). Mortality was monitored for 1 h after induction of anaphylactic shock.

PCA Reaction

An IgE-dependent cutaneous reaction was generated by sensitizing skin with an intradermal injection of anti-DNP IgE followed 48 h later with an injection of DNP-HSA into the tail vein. The mice were injected intradermally with 0.5 μg of anti-DNP IgE into each of 2 dorsal skin sites that had been shaved 48 h earlier. Each mouse, 48 h later, received an injection of 100 μg of DNP-HSA in PBS containing 4% Evans blue (1:4) via tail vein. SRAE (0.001 to 1 g/kg BW) was orally administered 1 h before the challenge. Then thirty min after the challenge, the mice were killed 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 (5:13) based on the method of Katayama et al.^[14] The absorbent intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan).

Assay of Histamine Release

The histamine content was measured by the o-phthalaldehyde spectrofluorometric procedure of Shore et al.^[15] The fluorescent intensity was measured at 438 nm (excitation at 353nm) in a spectrofluorometer (Shimadzu, RF-5301 PC, Japan).

Preparation of RPMC

RPMC were isolated as previously described.^[16] 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 NaHCO₃, 2.7 mM KCl, 0.3 mM NaH₂PO₄ and 0.1% gelatin), into the peritoneal cavity and the abdomen was gently massaged for about 90 s. 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 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.^[17] 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 $400 \times g$. 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.

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 SRAE (0.001 to 1 mg/mL) 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 16 h. The cells were preincubated with the SRAE (0.001 to 1 mg/mL) at 37°C for 10 min prior to the challenge with DNP-HAS (1 µg/mL). The cells were separated from the released histamine by centrifugation at $400 \times g$ for 5 min at 4°C. Residual histamine in cells was released by disrupting the cells with perchloric acid and centrifugation at $400 \times g$ for 5 min at 4°C.

Assay of TNF- α and IL-6 Secretion

TNF- α and IL-6 secretion was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as described previously.^[18] HMC-1 cells were cultured with α -MEM plus 10% FBS and resuspended in Tyrode buffer A. The cells were sensitized with PMA (20 nM) plus A23187 (1 µM) for 6–9 h in the absence or presence of SRAE. The ELISA was sensitive to TNF- α and IL-6 concentrations in the medium above 0.01 ng/mL. The ELISA was performed by coating 96-well plates with 6.25 ng/well of murine monoclonal antibody with specificity for murine TNF- α and IL-6 respectively. Before use and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween-20 and twice with PBS alone. All reagents used in this assay and the coated wells were incubated for 1 h at room temperature. For the standard curve, rTNF- α and rIL-6 were added to serum previously determined to be negative to endogenous TNF- α and IL-6. After exposure to the medium, the assay plates were exposed sequentially to biotinylated anti-human TNF- α and IL-6, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablets substrates. Optical density readings were

Table 1. Effect of SRAE on compound 48/80-induced systemic allergic reaction.

SRAE treatment (g/kg BW)	Compound 48/80 (0.008 g/kg BW)	Mortality (%)
None (saline)	+	100
0.005	+	100
0.01	+	80
0.05	+	50
0.1	+	40
0.5	+	20
1	+	10
1	—	0

Groups of mice ($n = 10/\text{group}$) were intraperitoneally pretreated with 200 μl saline or SRAE. SRAE 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 $\times 100/\text{total number of experimental mice}$.

made within 10 min of the addition of the substrate on a Titertek Multiscan (Flow Laboratories) with a 405 nm filter.

Statistical Analysis

The results obtained were expressed as mean $\pm$ S.E. The Student's t-test was used to make a statistical comparison between the groups. Results with $p < 0.05$ were considered statistically significant.

Table 2. Time-dependent effect of SRAE on compound 48/80-induced systemic allergic reaction.

SRAE treatment (g/kg BW)	Time (min)	Compound 48/80 (0.008 g/kg BW)	Mortality (%)
None (saline)		+	100
1	5	+	10
	10	+	30
	20	+	60
	30	+	80
	40	+	100

Groups of mice ($n = 10/\text{group}$) were intraperitoneally pretreated with 200 μl saline or SRAE. SRAE (1 g/kg) was given at 5 min, 10 min, 20 min, 30 min and 40 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 $\times 100/\text{total number of experimental mice}$.

Table 3. Effect of SRAE on the 48 h PCA.

SRAE treatment (g/kg BW)	Anti-DNP IgE plus DNP-HSA	Amount of dye ($\mu\text{g}/\text{site}$)
None (saline)	+	5.481 ± 0.587
0.001	+	4.659 ± 0.501
0.01	+	4.385 ± 0.414
0.1	+	$2.741 \pm 0.283^*$
1	+	$1.700 \pm 0.189^*$

SRAE was administered orally 1 h prior to the challenge with antigen. Each datum represents the mean $\pm$ S.E. of three independent experiments.

* $p < 0.05$; significantly different from the saline value.

RESULT

SRAE Inhibits Compound 48/80-Induced Systemic Allergic Reaction

To assess the contribution of SRAE in allergic reaction, an in vivo model of systemic allergic reaction was used. Compound 48/80 (0.008 g/kg BW) was used as a systemic fatal allergic reaction inducer. As shown in Table 1, an intraperitoneal injection of 200 μL saline as control induced a fatal shock in 100% of each group. When mice were pretreated with SRAE at concentration ranging from 0.005 to 1 g/kg BW for 1 h, the mortality with compound 48/80 was reduced dose-dependently. In addition, the mortality of mice injected intraperitoneally with SRAE (1 g/kg BW) 5 min, 10 min, 20 min, 30 min and 40 min after compound 48/80 injection increased time-dependently (Table 2).

SRAE Inhibits PCA

PCA reaction is one of the most important in vivo models of anaphylaxis in local allergic reaction.^[19] As described in the experimental procedure, local extravasation

Table 4. Effect of SRAE on compound 48/80-induced histamine release from RPMC.

SRAE treatment (mg/mL)	Compound 48/80 (5 $\mu\text{g}/\text{mL}$)	Amount of histamine ($\mu\text{g}/\text{mL}$)
None (saline)	+	0.138 ± 0.014
0.001	+	0.124 ± 0.010
0.01	+	0.103 ± 0.018
0.1	+	$0.065 \pm 0.013^*$
1	+	$0.021 \pm 0.006^*$

The cells (2×10^5 cells/mL) were preincubated with SRAE at 37°C for 10 min prior to incubation with compound 48/80. Each datum represents the mean $\pm$ S.E. of three independent experiments.

* $p < 0.05$; significantly different from the saline value.

Table 5. Effect of SRAE on IgE-mediated histamine release from RPMC.

SRAE treatment (mg/mL)	Anti-DNP IgE plus DNP-HSA	Amount of histamine (μg/mL)
None (saline)	+	0.163 ± 0.011
0.001	+	0.158 ± 0.021
0.01	+	0.136 ± 0.016
0.1	+	0.080 ± 0.012*
1	+	0.036 ± 0.002*

The cells (2×10^5 cells/mL) were preincubated with SRAE at 37°C for 10 min prior to challenge with DNP-HSA. Each datum represents the mean ± S.E. of three independent experiments.

*p < 0.05; significantly different from the saline value.

was induced by skin injection of anti-DNP IgE followed by a antigenic challenge. Oral administration of SRAE (0.1 and 1 g/kg) showed a significant inhibition rate in PCA reaction (Table 3).

SRAE Inhibits Histamine Release from RPMC

The inhibitory effect of SRAE on compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC are shown in Tables 4 and 5. SRAE dose-dependently inhibited compound 48/80-induced or anti-DNP IgE-mediated histamine release at concentrations of 0.001 to 1 mg/mL. In particular, SRAE significantly inhibited the compound 48/80-induced or anti-DNP IgE-mediated histamine release at the concentrations of 0.1 and 1 mg/mL.

SRAE Inhibits TNF-α and IL-6 Secretion from HMC-1 Cells

To assess the effect of SRAE in PMA plus A23187-stimulated TNF-α and IL-6 secretion, HMC-1 cells were pretreated with SRAE for 30 min prior to PMA plus

Table 6. Effect of SRAE on PMA plus A23187-stimulated TNF-α and IL-6 secretion from HMC-1 cells.

Treatment	Concentration (mg/mL)	Content (ng/mL)	
		TNF-α	IL-6
None (saline)	—	0.353 ± 0.115	0.039 ± 0.008
PMA + A 23187	—	2.974 ± 0.255	1.081 ± 0.036
PMA + A23187 + SRAE	0.1	2.574 ± 0.226	0.377 ± 0.171*
	1	1.635 ± 0.141*	0.436 ± 0.042*

PMA plus A23187-stimulated HMC-1 cells (3×10^5) were incubated for 6 h in the absence or presence of SRAE. TNF-α and IL-6 secreted into the medium are presented as the mean ± S.E. of three independent experiments.

*p < 0.05; significantly different from the saline value.

A23187-stimulation. As shown in Table 6, SRAE inhibited the secretion of TNF- α and IL-6 in PMA plus A23187-stimulated HMC-1 cells.

DISCUSSION

The present study showed that SRAE pretreatment significantly affected compound 48/80-induced non-specific systemic allergic reaction and anti-DNP IgE-induced specific local allergic reaction. SRAE also inhibited compound 48/80 or anti-DNP IgE-mediated histamine release from RPMC. These results indicate that anaphylactic degranulation of mast cells is inhibited by SRAE. The secretory property of compound 48/80 is not due to a receptor-mediated process but, like mastoparan, results from a direct activation of G-proteins.^[20,21] The evidence indicates that the protein is Gi-like and that the activation is inhibited by benzalkonium chloride.^[22] Tasaka et al.^[23] reported that compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. This result indicates that the membrane permeability increase may be an essential trigger for the release of the mediator from mast cells. In this sense, anti-allergic agents having a membrane-stabilizing action may be desirable. SRAE might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80. 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.^[24,25] PCA is one of the most important in vivo models of anaphylaxis in local allergic reaction. The SRAE administered mice are protected from IgE-mediated PCA reaction. The results obtained prove that SRAE inhibited the IgE-mediated anaphylactic reaction in vivo in a murine model. It is believed that cytokines, including TNF- α and IL-6 play a major role in triggering and sustaining the allergic inflammatory response. SRAE inhibited the secretion of TNF- α and IL-6 in PMA plus A23187-stimulated HMC-1 cells. Many signals for anaphylactic reaction, including protein kinase C and Ca²⁺, are also involved in production in mast cells distinct from that for degranulation. Further work should address the possibility that SRAE may also be active in the inhibition of human mast cell degranulation and in the treatment of human allergic disorders.

In conclusion, the results obtained in the present study provide evidence that SRAE importantly contributes to the prevention or treatment of mast cell-mediated acute and chronic allergic diseases.

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