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Shini-San Inhibits Mast Cell-Dependent Immediate-Type Allergic Reactions

H.M.Kim[1], Y.H.Lee[1], H.J.Chae[2], H.R.Kim[2], S.H.Baek[3], K.S.Lim[4] and C.Y.Hwang[4]

[1]Department of Oriental Pharmacy, College of Pharmacy,
[2]Department of Dental Pharmacology, School of Dentistry,
[3]Division of Chemistry, Technical and Biological Science, College of Natural Sciences, and
[4]College of Oriental Medicine, Center of Oriental Medicinal Science,
Wonkwang University, Iksan, Chonbuk, 570-749, South Korea

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Abstract: Shini-San has been used for treatment of allergic disease in Korea. However, its effect in experimental models remains unknown. The mast cell plays a pivotal role in initiating allergic response by secreting intracytoplasmic granular mediators such as histamine. The present report describes an inhibitory effect of Shini-San on mast cell-mediated immediate-type allergic reactions. Topical application of compound 48/80 can induce an ear swelling response in normal ($WBB6F_1$-+/+) mice but not in congenic mast cell-deficient $WBB6F_1$-W/W^v mice. Shini-San inhibited concentration-dependent mast cell-dependent ear swelling response induced by compound 48/80 in normal mice. Shini-San inhibited concentration-dependent passive cutaneous anaphylaxis induced by anti-dinitrophenyl (DNP) immunoglobulin E (IgE) in rats by topical application. Shini-San also inhibited in concentration-dependent fashion the histamine release from the rat peritoneal mast cells by compound 48/80 or anti-DNP IgE. Moreover, Shini-San had a significant inhibitory effect on compound 48/80-induced systemic anaphylactic reaction. These results indicate that Shini-San inhibits immediate-type allergic reactions by inhibition of mast cell degranulation *in vivo* and *in vitro*.

Shini-San, a traditional Oriental medicine, has been used for treatment of various allergic diseases in South Korea. However, it is still unclear how Shini-San prevents allergic diseases. It is a "wind-cold" discutient and nasal decongestant and is principally used in the treatment of nasal congestion with headache, sinusitis, and allergic rhinitis (Chang, 1986).

It is now well established that mast cells trigger immediate-type allergic reactions in response to allergens by releasing chemical mediators (Church and Caulfield, 1993). Degranulation of mast cells is caused by non-immunologic secretagogues like substance P (Hua *et al.*, 1996), compound 48/80 (Shin *et al.*, 1997), extracellular ATP (Sudo *et al.*, 1996) and so on, which result in rapid and marked histamine release (Pearce, 1989). Histamine induced anaphylactic responses such as vasodilation, increased vascular permeability

and contraction of smooth muscles. Thus, mast cells play some important roles in diverse immunological and pathological processes (Wershil and Galli, 1994). Compound 48/80 is best known as a potent inducer of degranulation and of the release, from mast cells, of histamine and other chemical mediators that are responsible for anaphylactic reactions (Allansmith *et al.*, 1989). The secretory response of mast cells can also be induced by aggregation of their cell surface-specific receptors for IgE by the corresponding antigen (Segal *et al.*, 1977; Metzger *et al.*, 1986; Alber *et al.*, 1991). The anti-IgE antibody has been established to induce the passive cutaneous anaphylaxis (PCA) reaction as a typical model for the immediate type allergic reactions. The rat skin is a useful site for studying PCA (Saito and Nomura, 1989). In this paper, we show that Shini-San inhibits both compound 48/80-induced ear swelling response and anti-dinitrophenyl (DNP) IgE-induced PCA. We also investigated the influence of Shini-San on compound 48/80 or anti-DNP IgE-induced histamine release and compound 48/80-induced systemic anaphylactic reaction.

Materials and Methods

Reagents

Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), bovine serum albumin (BSA), and metrizamide were purchased from Sigma Chemical Co. (St. Louis, MO). Tissue culture flasks and plates were obtained from Nunc (Naperville, IL).

Animals

WB-+/+ and C57BL/6-+/+ mice were raised in our laboratory, and (WB x C57BL/6) F_1-+/+ and W/W^v mice (hereafter called WBB6F_1-+/+ and -W/W^v mice) were purchased from the Japan SLC (Hamamatsu, Japan). WBB6F_1- W/W^v mice are genetically deficient in mast cells (Kitamura *et al.*, 1978). Mice were used at 2 to 4 months of age. The original stock of Wistar rats and C57BL/6 mice were purchased from Dae-Han Experimental Animal Center (Eumsung, Chungbuk, South Korea), and the animals were kept at the College of Pharmacy, Wonkwang University. The animals were housed five to ten per cage in a laminar air flow room maintained under a temperature of 22 ± 1°C and relative humidity of 55 ±10% throughout the study.

Preparation of Extracts

The plant materials were obtained from College of Oriental Medicine, Wonkwang University (Korea). The sample was extracted with distilled water at 60°C for 3 hrs. The extract was filtered through a 0.45 mm filter, lyophilized, and kept at 4°C. The ingredients of 42 g Shini-San include 4 g of Flos Magnoliae, 4 g of Radix Asari, 4 g of Radix Ligustici tenuissimae, 4 g of Rhizoma Cimicifugae, 4 g of Rhizoma Cnidii, 4 g of Lignum akebiae, 4 g of Radix Ledebouriellae, 4 g of Radix Osterici koreani, 4 g of Radix Glycyrrhizae, and 4 g of Radix Angelicae dahuricae. The ingredients correspond to parts of the following plants: *Magnolia denudata* Desr. (Magnoliaceae), *Asarum sieboldii* Miq. (Aristolochiaceae),

Ligusticum tenuissimum Kitag. (Umbelliferae), *Cimicifuga heracleifolia* Kom. (Ranunculaceae), *Cnidium officinale* Makino (Umbelliferae), *Akebia quinata* Decne. (Lardizablaceae), *Ledebouriella divaricata* (Turcz.) Ueke (Umbelliferae), *Ostericum koreanum* (Max.) Kitagawa. (Umbelliferae), *Glycyrrhiza uralensis* Fisch. (Leguminosae), *Angelica dahurica* (Fisch.) Benth. et Hooker (Umbelliferae), *Xanthium strumarium* L. (Compositae). 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 and 0.1 % BSA) before use.

Ear Swelling Response

Compound 48/80 (20 mg/ml) was freshly dissolved in saline and injected intradermally into the dorsal aspect of a mouse ear using a microsyringe with a 28 gauge hypodermic needle. Thickness of ears was measured with a digimatic micrometer (Mitutoyo, Japan) under mild anesthesia. Ear swelling response represented an increment in thickness above baseline control values. Ear swelling response was determined 30 min after compound 48/80 or vehicle injection. The value seemed to represent the effect of the compound 48/80 itself but not an amount of the vehicle injected (physical swelling) because within 30 minutes, the ear swelling response evoked by physiologic saline nearly returned to the baseline thickness.

PCA

An IgE-dependent skin reaction was generated by sensitizing the skin with an intradermal injection of anti-DNP IgE followed 48 hrs after with an injection of DNP-HSA into the rat's tail vein. The anti-DNP IgE and DNP-HSA were diluted in PBS. The rats were injected intradermally with 0.5 mg (50 μl) of anti-DNP IgE into each of four dorsal skin sites that had been shaved 48 hrs earlier. The sites were outlined with a water-insoluble red marker. Forty-eight hours later, each rat received an injection of 2 mg of DNP-HSA in PBS containing 4% Evans blue (1 : 4) through the tail vein. Shini-San was administered topically or intradermally 4 hrs before the challenge. Shini-San was diluted in 70% ethanol. Thirty minutes after the challenge, the rats were sacrified, 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 1.0 N KOH and 9 ml of mixture of acetone and phosphoric acid (5 : 13) in accord with the method of Katayama *et al.* (1978). The absorbent intensity of the extraction was measured at 620 nm by using a spectrophotometer, and the amount of dye was calculated with the Evans blue measuring line.

Preparation of Rat Peritoneal Mast Cells (RPMC)

RPMC were isolated as previously described (Shin *et al.*, 1997). 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) containing 0.1% gelatin (Sigma Chemical Co.), into the peritoneal cavity, and the abdomen was gently massaged for about 90 sec.

The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells was aspirated by a Pasteur pipette. Thereafter, the peritoneal cells were sedimented at 150 x g for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from major components of rat peritoneal cells, that is, macrophages and small lymphocytes, according to the method described by Yurt *et al.* (1977). In brief, peritoneal cells suspended in 1 ml Tyrode buffer B were layered on 2 ml of 22.5% w/v metrizamide (density, 1.120 g/ml, Sigma Chemical Co.) and centrifuged at room temperature for 15 min at 400 x 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 cells were viable, as judged by Trypan blue uptake.

Inhibition of Histamine Release

Mast cell suspensions (1 x 10^6 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 Shini-San preparations, and then incubated (10 min) with the compound 48/80. Mast cell suspensions (1 x 10^6 cells/ml) were also sensitized with anti-DNP IgE (10 μg/ml) for 6 h. The cells were preincubated with the Shini-San at 37°C for 10 min prior to challenge with DNP-HSA (1 μg/ml). The cells were separated from the released histamine by centrifugation at 400 x g for 5 min at 4°C. Residual histamine in cells was released by disrupting the cells with perchloric acid and centrifuging at 400 x g for 5 min at 4°C. Histamine content was measured by the o-phthalaldehyde spectroflurometric procedure of Shore *et al.* (1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer.

Assay of Histamine Release

The inhibition percentage of histamine release was calculated by to the following equation:

$$\% \text{ Inhibition} = \frac{\text{Histamine release without Shini-San - Histamine release with Shini-San}}{\text{Histamine release without Shini-San}} \times 100$$

Compound 48/80-induced Systemic Anaphylactic Reaction

Mice were given an intraperitoneal injection of 8 mg/kg of the mast cell degranulator compound 48/80. Shini-San was administered orally before the injection of compound 48/80. Mortality was monitored for 1 hr after induction of anaphylactic shock. After the mortality test, blood was obtained from the heart of each mouse.

Statistical Analysis

Data were expressed as mean ± S.E. for the number of experiments. Student's t-test was

used to compare between groups. $P < 0.05$ was considered statistically significant.

Results

Effect of Shini-San on Compound 48/80-induced Ear Swelling Response

In initial experiments, we confirmed that topical application of compound 48/80 can induce an ear swelling response in normal ($WBB6F_1$-+/+) mice but not in the congenic mast cell-deficient $WBB6F_1$-W/W^v mice (Figure 1). Compound 48/80 significantly induced an ear swelling response at concentrations of 50-200 μg/site. We chose a concentration of 100 μg/site for compound 48/80 for optimal ear swelling response in subsequent experiments. As shown in Figure 2, when mice were pretreated with Shini-San for 30 min, the ear swelling response with compound 48/80 was reduced in concentration-dependent fashion. The inhibition was significant at doses of 100 and 1000 μg/site.

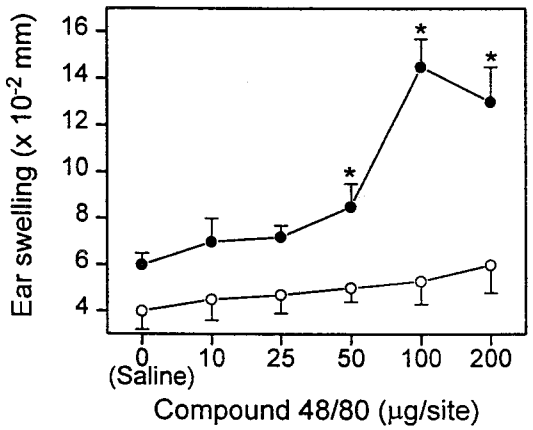

Figure 1. Induction of ear swelling by topical application of compound 48/80 or vehicle (Saline) alone in the ear skin of $WBB6F_1$-+/+ and -W/W^v mice. Ten microliters of various concentrations of compound 48/80 were applied topically to both ears of mice. Ear swelling was measured 30 min after application of compound 48/80. (●), $WBB6F_1$-+/+ mice; (O), $WBB6F_1$-W/W^v mice. Each datum represents the mean ± S.E. of 3 independent experiments. *$P < 0.05$; significantly different from the saline value.

Effect of Shini-San on Anti-DNP IgE-induced PCA

Another way to test mast cell-mediated skin allergic reaction is to induce PCA (Wershil et al, 1987). As described in Experimental Procedures, local extravasation is induced by local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Anti-DNP IgE was injected in the right dorsal skin sites of rats. As a control, the left dorsal skin site of these rats was injected with saline alone. After 48 hrs, all animals were injected intravenously with DNP-HSA treated with Evans blue dye. The cutaneous allergic reaction was

best visualized by the extravasation of the dye. The topical applications of Shini-San inhibited PCA in a concentration-dependent manner (Table 1).

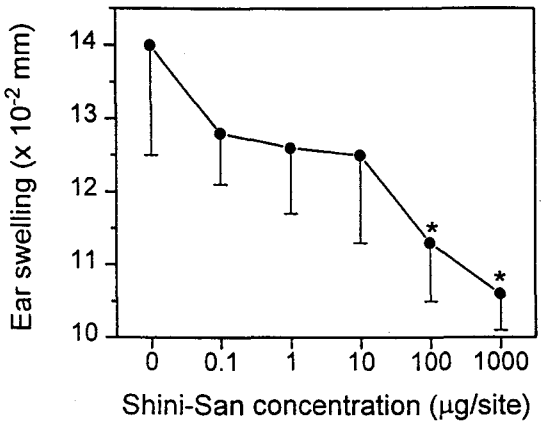

Figure 2. Effect of Shini-San on compound 48/80-induced ear swelling response in mice. Ten microliters of compound 48/80 (100 μg/site) were applied topically to the ears of mice. The skins of the ears were treated with the indicated concentrations of Shini-San for 30 min prior to the compound 48/80 application. Each datum represents the mean ± S.E. of 3 independent experiments. *P < 0.05; significantly different from the saline value.

Table 1. Effect of Topical Application of Shini-San on PCA in Mice

Treatment:	Concentration (mg/kg)	Amount of dye# (μg/site)
Saline (Control)	-	8.41 ± 1.65
Shini-San	0.1	8.05 ± 1.70
	1	7.52 ± 1.33
	10	7.13 ± 0.91
	100	6.54 ± 1.14
	1000	5.17 ± 0.83*

Each Shini-San (10 pl) was applied topically 4 h prior to the challenge with antigen.
#Each amount of dye represents the mean ± S.E. of 3 independent experiments.
*P < 0.05; significantly different from the saline value.

Effect of Shini-San on Histamine Release from RPMC

We next examined the effect of Shini-San on compound 48/80 or anti-DNP IgE-induced histamine release from PRMC. Shini-San inhibited compound 48/80 or anti-DNP IgE-induced histamine release from RPMC in a concentration-dependent manner (Figure 3). Inhibitory effect of Shini-San on histamine release was more significant in the case of anti-DNP IgE treatment.

Effect of Shini-San on Compound 48/80-induced Systemic Anaphylactic Reaction

Finally, we investigated whether Shini-San could also inhibit compound 48/80 induced-systemic anaphylactic reaction. Shini-San inhibited concentration-dependently compound 48/80-induced systemic anaphylactic reaction in mice (Table 2). Especially, Shini-San inhibited compound 48/80-induced anaphylactic reaction 60% with the dose of 1000 mg/kg. Treatment with Shini-San (1000 mg/kg) detected no physiological differences by appearance.

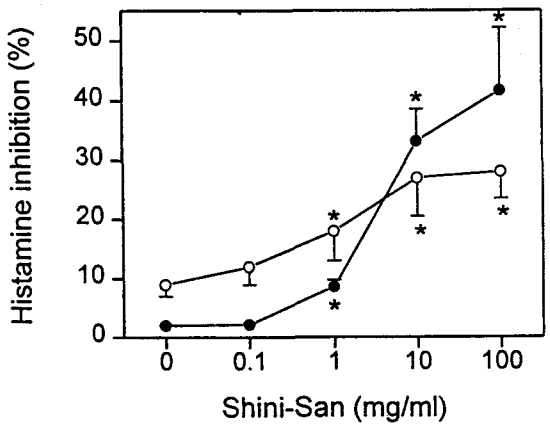

Figure 3. Effect of Shini-San on compound 48/80- or anti-DNP IgE-mediated histamine release from RPMC. RPMC (1 x 10^6 cells) were preincubated with various concentrations of Shini-San at 37°C for 10 min prior to incubation with compound 48/80 or prior to the challenge with DNP-HSA. (●), compound 48/80; (O), anti-DNP IgE. Each datum represents the mean ± S.E. of 3 independent experiments. *P < 0.05; significantly different from the saline value.

Table 2. Effect of Shini-San on Compound 48/80-induced Systemic Anaphylactic Reaction

Treatment	Concentration (mg/kg)	Compound48/80 (8 mg/kg)	Mortality (%)
Saline (Control)	-	+	100
Shini-San	0.1	+	100
	1	+	100
	10	+	94 ± 6
	100	+	77 ± 7*
	1 000	+	60*
	1 000	-	0

Mice were orally administered either 200 μl saline or Shini-San at various doses 1 h before the compound 48/80 injection (n = 10/group). Mortality (%) within 1 h following the compound 48/80 injection was represented as no. of dead mice x 100/no. of total experimental mice. Each datum represents the mean ± S.E. of 3 independent experiments.*P < 0.05; significantly different from the saline control.

Discussion

The present study showed that Shini-San potently inhibited compound 48/80-induced ear swelling response in mice and anti-DNP IgE-induced PCA reaction in rats by topical application. Shini-San also inhibited the compound 48/80 or anti-DNP IgE-induced histamine release from RPMC. In addition, although the dose of Shini-San required to inhibit compound 48/80 induced anaphylaxis was quite large, our results showed that Shini-San pretreatment profoundly affected the systemic anaphylactic reaction. There is no doubt that stimulation of mast cells with compound 48/80 initiates the activation of a 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). We showed that topical application of compound 48/80 did not induce a swelling response in ears of $WBB6F_1$-W/W^v mice that had been locally and selectively repaired of their mast cell deficiency. Therefore, we speculate that our results indicate that mast cell-mediated immediate type allergic reactions are inhibited by Shini-San. 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 membrane permeability increase may be an essential trigger for the release of mediators from mast cells. Shini-San might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80.

The Shini-San-administered rats are protected from IgE-mediated local allergic reaction. The mechanism of the protection against anti-DNP IgE, while not clear at present, may be suggested only in particular conditions. It is conceivable that Shini-San inhibits the initial phase of immediate type allergic reactions, probably through interference with the degranulation system.

Antiallergic activity has been demonstrated for a number of plant extracts (Yang *et al.*, 1997; Kang *et al.*, 1997; Kim *et al.*, 1998a; Kim *et al.*, 1998b; Kim *et al.*, 1999). Only in few cases the chemical nature of the antiallergic compounds has been fully characterized. The results obtained in this searching justify continuing with the purification of crude extracts and isolation of active compounds for improving their potential as antiallergic drugs and/or finding of new lead molecules.

In conclusion, the results obtained in the present study provide evidence that Shini-San inhibited the immediate-type allergic reactions *in vivo* and *in vitro* in a murine model. To our knowledge, this is the first report of such selective inhibition of mast cell-mediated immediate-type allergic reactions by Shini-San.

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