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Anti-allergic Effects of *Shensuyin*

Lee Seung-Eon, Shin Jo-Young, Lee Si-Hyeong*

Department of Internal Medicine, College of Oriental Medicine, Wonkwang University
Professional Graduate School of Oriental Medicine, Wonkwang University*

蔘蘇飮의 항알레르기 效果

이승언, 신조영, 이시형*

원광대학교 한의과대학 폐계내과학교실, 원광대학교 한의학전문대학원*

모든 알레르기 반응의 중심축이 되는 비만세포는 주로 피부, 위장관 및 호흡기관의 점막에 분포하고 있다. 활성화된 비만세포는 즉각형 알레르기 반응을 일으키는 여러 인자들을 방출시키게 된다. 方藥合編에 따르면 蔘蘇飮은 알레르기 鼻炎, 發熱, 風寒, 頭痛, 기침에 效能이 있는 處方이다. 본 硏究는 蔘蘇飮의 肥滿細胞 의존성 아나필락시 반응(anaphylactic reaction)에 대한 藥理 效果를 조사하기 위한 것이다. 蔘蘇飮은 compound 48/80으로 유발되는 전신성 아나필락시 쇼크(systemic anaphylactic shock)와 耳介 浮腫 反應(ear swelling response)을 농도 의존적으로 억제하였다. 蔘蘇飮을 0.1, 1 ㎎/㎖로 전처리하였을 때, 흰쥐 복강 肥滿細胞(rat peritoneal mast cells, RPMCs)에서 compound 48/80에 의해 유발되는 히스타민 분비는 감소하는 것으로 나타났다. 또한 蔘蘇飮은 anti-dinitrophenyl IgE에 의해 활성화 된 수동 피부 아나필락시(passive cutaneous anaphylaxis, PCA)를 농도 의존적으로 抑制하였다. 결론적으로 蔘蘇飮은 肥滿細胞 의존성 즉각형 알레르기 反應을 抑制하여, 항 아나필락시 활성(anti-anaphylactic activity)을 가지는 것으로 보여 진다.

Key Words:

Ⅰ. Introduction

Shensuyin (SSY), a Korean traditional prescription, has been used for the treatment of headache, coughs and much phlegm by cold wind, especially allergic rhinitis (hay fever). This prescription is composed of twelve oriental medicinal herbs, Ginseng Radix, Perillae Folium, Peucedani Radix, Pinelliae Tuber, Puerariae Radix, Hoelen, Aurantii nobilis Pericarpium, Platycodi Radix, Ponciri Fructus, Glycyrrhizae Radix, Zingiberis Rhizoma, and Zizyphi inermis Fructus. In recent studies, SSY have anti-allergic effects through inhibiting on ear swelling formation in the contact dermatitis response induced by picryl chloride[1]. However it is not known how SSY prevents mast cell-mediated anaphylactic reactions in experimental model.

The immediate-type allergic reaction is involved in many allergic disorder as urticaria, allergic rhinitis, asthma and sinusitis. These reactions are mediated by various chemical mediators released from mast cells[2]. Mast cells, a central component of all allergic disease, are distributed in the skin and the mucosa of gastrointestinal or respiratory tracts. Histamine is one of the well characterized

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· 교신저자 : 이시형, 전북 익산시 신용동 344-2 원광대학교 부속 익산한방병원 6내과
(Tel. 063-850-2106 Fax.
E-mail : beginstar@dreamwiz.com)

and potent vasoactive mediator implicated in the acute phase of immediate-type hypersensitivity reactions among the substances released on degranulation of mast cells caused by non-immunologic secretagogues like substance P, compound 48/80, ATP[3-5]. In systemic immediate hypersensitivity, the decrease in vascular tone and leakage of plasma caused by released mediators leads to a fall in blood pressure and shock, anaphylactic shock, which is fatal. Compound 48/80, well-known histamine releaser, induces ear swelling in skin anaphylactic reaction model[6]. Ear swelling response is a traditional predictation one of dermal sensitization in humans using mice[7].

The secretory responses of mast cells can be induced by aggregation of their cell surface-specific receptors for immunoglobulin E (IgE) by the corresponding antigen[8-10]. It has been established that the anti-IgE antibody induces passive cutaneous anaphylaxis (PCA) as a typical *in vivo* model for immediate-type hypersensitivity reactions in anaphylactic reactions. Rats skins are useful sites for studying PCA[11].

The purpose of this study is to evaluate the effects of SSY on the allergic reaction. In the present study, the author examine the effects of SSY on the compound 48/80-induced systemic anaphylactic shock and ear swelling response. The author also investigate histamine release from rat peritoneal mast cells (RPMCs) and anti-dinitrophenyl (DNP) IgE antibody-induced PCA. In addition the author does alcian blue/nuclear fast red staining to confirm the inhibitory effects of SSY on the compound 48/40-induced degranulation of RPMCs.

II. Materials and Methods

1. Materials

Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), metrizamide, *o*-phthaldialdehyde (OPA), and evans blue were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The α-minimal essential medium was purchased from Flow Laboratories (Irvine, UK). Fetal bovine serum(FBS) was purchased from Life Sciences(Grand Island, NY, USA).

2. Animals

The original stock of male ICR mice and male Sprague-Dawley rats were purchased from the Dae-Han Experimental Animal Center (Daejon, Korea), and the animals were maintained at the College of Pharmacy, Wonkwang University. The rats were housed in a laminar air-flow room maintained at a temperature of 22±1℃ and relative humidity of 55±10% throughout the study. No animal was used more than once. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985).

3. Preparation of SSY

The SSY was obtained from Wonkwang University Oriental Medicine Hospital(Iksan, South Korea). A voucher specimen (number 1-01-60) was deposited at the Herbarium of Wonkwang University. An extract of SSY was prepared by decocting the dried prescription of herbs with boiling distilled water. The duration of decoction was about 3 hs. The decoction was filtered, lyophilized and kept at 4°C. The samples were dissolved in saline and then filtered through 0.45-

Table 1. The composition of SSY

Name of crude materials	Weight(g)
Ginseng Radix	4
Perillae Folium	4
Peucedani Radix	4
Pinelliae Tuber	4
Puerariae Radix	4
Hoelen	4
Aurantii nobilis Pericarpium	3
Platycodi Radix	3
Ponciri Fructus	3
Glycyrrhizae Radix	3
Zingiberis Rhizoma	3
Zizyphi inermis Fructus	3
Total amount	42

㎛ syringe filter(Table 1).

4. Compound 48/80-induced systemic anaphylactic reaction

Mice were given an intraperitoneal injection of the mast cell degranulator compound 48/80 (8 ㎎/㎏). SSY was dissolved in saline and administered orally 1 h before the injection of compound 48/80. Mortality was monitored for 30 min after induction of anaphylactic reaction.

5. Ear swelling response

Compound 48/80 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. Ear thickness 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 40 min after compound 48/80 or vehicle injection. SSY was administered orally 1 h before the compound 48/80-injection (100 ㎍/site). The values obtained would appear to represent the effect of compound 48/80 rather than the effect of the vehicle injection (physical swelling), since the ear swelling response evoked by physiologic saline returned to almost baseline thickness within 40 min.

6. Preparation of RPMCs

RPMCs were isolated as previously described[12]. In brief, rats were anesthetized by ether, and injected with 20 ㎖ of Tyrode buffer B (NaCl, glucose, $NaHCO_3$, KCL, NaH_2PO_4) containing 0.1% gelatin (Sigma) into the peritoneal cavity; the abdomen was gently massaged for about 90 sec. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells was aspirated by Pasteur pipette. Then 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.[13]. In brief, peritoneal cells suspended in 1 ㎖ of Tyrode buffer B were layered onto 2 ㎖ of 0.225 g/㎖ metrizamide (density 1.120 g/㎖; Sigma) 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 ㎖ of 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) containing calcium. 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.

7. Histamine assay

Purified RPMCs were resuspended in Tyrode buffer A containing calcium for the treatment with compound 48/80. RPMC suspensions (2×10^5 cells/㎖) were pre-incubated for 10 min at 37℃ before the addition of compound 48/80 for stabilization. The cells were pre-incubated with the SSY for 20 min, and then incubated for 15 min with compound 48/80 (6 g/㎖). The reaction was stopped by cooling the tubes in ice. The cells were separated from the released histamine by centrifugation at 400 × g for 5 min at 4℃. Residual histamine in the cells was released by disrupting the cells with perchloric acid and centrifugation at 400 × g for 5 min at 4℃. The histamine content was measured by the OPA spectrofluorometric procedure of Shore et al.[14]. The fluorescent intensity was measured at 440 ㎚ (excitation at 360 ㎚) in spectrofluorometer.

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

$$\% \text{ inhibition} = (A - B)\ 100 / A$$

where A is histamine release without SSY and B is histamine release with SSY.

8. Alcian blue-nuclear fast red (NFR) staining

In order to compare the status of mast cells before or after the addition of SSY would make it clear whether SSY affects the degranulation process or not, alcian blue-NFR staining was performed. Mast cells were centrifuged with cytospin at 28 g for 5 min and then fixed with Carnoy's solution. Cells were stained with 1% alcian blue and NFR. They were then rinsed in distilled water and gradually dehydrated in a series of 80%, 90%, 95% and 100% alcohol. The slides were cleared in xylene and mounted with mounting medium.

9. PCA reaction

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 mice tail vein. The DNP-HSA was diluted in phosphate-buffered saline (PBS). The mice were injected intradermally with 100 ng of anti-DNP IgE into each of three dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Forty-eight hours later, each mouse received an injection of 200 ㎕ of the 1:1 mixture of 1 ㎎/㎖ DNP-HSA in PBS and 4% Evans blue via the tail vein. One hour before this injection, SSY was administered orally. The mice were sacrificed 40 min after the intravenous challenge. The dorsal skin of the mouse was removed for measurement of the pigment area. The amount of dye was then determined colorimetrically after extraction with 0.5 ㎖ of 1.0 mol/l KOH and 4.5 ㎖ of a mixture of acetone and phosphoric acid (with the ratio of 5:13), based on the method of

Katayama et al.[15]. The absorbent intensity of the extraction was measured at 620 ㎚ in a spectrofluorometer, and the amount of dye was calculated with the Evans blue measuring-line.

10. Statistical analysis

The results were expressed as mean ± S.E.M. for the number of experiments. Statistical significance was compared between each treated group and control by the Student's *t*-test. Results with $P < 0.05$ were considered statistically significant.

III. Results

1. Effects of SSY on compound 48/80-induced systemic anaphylaxis

To assess the contribution of SSY in anaphylactic reactions, the author first used the *in vivo* model of systemic anaphylactic reaction. Compound 48/80 (8 ㎎/㎏) was used as a systemic fatal anaphylaxis inducer. After the injection of compound 48/80, the mice were monitored for 30 min, after which the mortality rate was determined. As shown in Table 2, an oral administration of saline as a control induced a fatal reaction in 100% of each group. When the SSY was treated at concentrations ranging from 0.01 to 1 g/㎏ for 1 h, the mortality with compound 48/80 was reduced dose-dependently(Table 2).

2. Effects of SSY on ear swelling response

The previous study showed that compound 48/80 significantly induced an ear swelling response at concentration of 50-200 ㎍ per site[16]. Therefore the author chose a concentration of 100 g/site for compound 48/80 induced optimal ear-swelling response in this experiment. As shown in Table 3, when mice were pre-treated with SSY for 1 h, the ear swelling response derived from compound 48/80 was reduced in dose-dependent manner ($P<0.05$) (Table 3).

3. Effects of SSY on histamine release from RPMCs

The author next examined the effects of SSY on compound 48/80 induced histamine release from RPMCs. As shown in Fig. 1, inhibitory effects of SSY on compound 48/80 induced histamine release was significant 55.4% and 72.5% at concentrations of 0.1 and 1 ㎎/㎖. However SSY has a little effect on the inhibition of histamine release at low concentration (0.01 ㎎/㎖) (Fig. 1).

Table 2. Effects of SSY on compound 48/80-induced systemic anaphylactic reaction in mice

SSY dose (g/㎏)[a]	Compound 48/80 (8 ㎎/㎏)[b]	Mortality (%)[c]
None (saline)	+	100.0
0.01	+	50.0
0.1	+	33.3
1	+	16.7
1	-	0.0

[a] The groups of mice were orally pretreated with 200 ㎕ of saline or SSY was given at various doses 1 h before the compound 48/80 injection.
[b] The compound 48/80 solution was intraperitoneally given to the groups of mice (n=10).
[c] Mortality (%) is presented as the 'Number of dead mice x 100/Total number of experimental mice'.

Table 3. Effects of SSY on compound 48/80-induced ear-swelling response in mice[a]

SSY dose (g/kg)[b]	Thickness (㎜)[c]	Inhibition (%)
None	0.293±0.002	-
0.01	0.247±0.010*	15.60
0.1	0.199±0.007*	32.12
1	0.135±0.009*	53.76

[a] Twenty ㎕ of compound 48/80 (100 ㎍/site) were applied intradermally.
[b] The mice were orally administered with the indicated concentration of SSY for 1 h prior to the compound 48/80 application (n=10).
[c] Each datum represents the mean ± S.E.M.
*$P<0.05$; significantly different from the saline value.

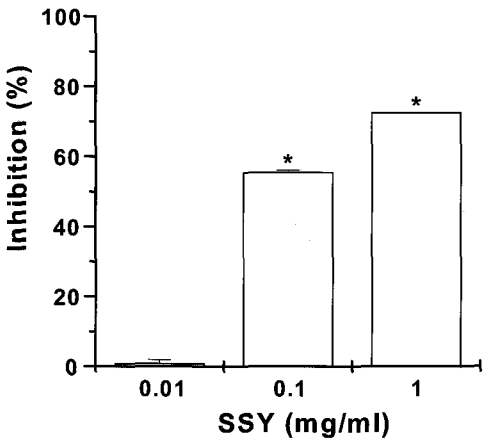

Fig. 1. Effects of SSY on compound 48/80-induced histamine release from RPMCs.

RPMCs (2×10^5 cells) were pre-incubated with various concentrations of SSY at 37℃ for 10 min prior to incubation with compound 48/80. *$P<0.05$; significantly different from the saline value.

4. Effects of SSY on degranulation of mast cells

The photographs of alcian blue-NFR stained-RPMC are shown in Fig. 2. Compound 48/80-stimulated RPMC in the absence SSY was extensively degranulated compared with SSY-treated cell, which is correlated with an inhibition of histamine release. The results show that SSY inhibits the compound 48/80-induced degranulation from mast cells(Fig. 2).

5. Effects of SSY on PCA

Another way to evaluate anaphylactic reactions *in vivo* models is to induce PCA[17]. Local injection of anti-DNP IgE followed by an intravenous antigenic challenge was performed. Anti-DNP IgE was injected into dorsal skin sites. After 48 h, all animals were injected intravenously with DNP-HSA containing Evans blue dye. The cutaneous anaphylactic reaction was best visualized by the

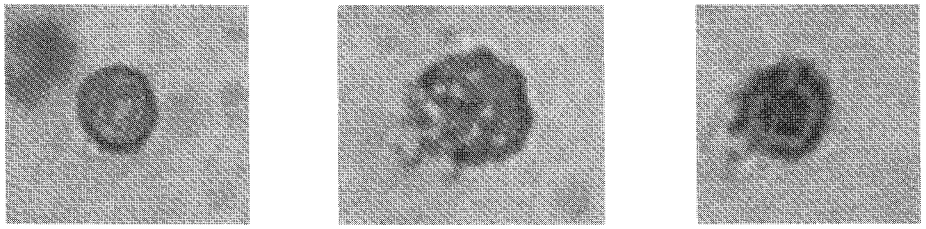

Fig. 2. The photographs of alcian blue-NFR stained mast cells.

Isolated RPMC was pre-incubated at 37 ℃ for 10 min (A). Compound 48/80 - stimulated RPMC was incubated for 10 min in the absence (B) or in the presence (C) of SSY (1 ㎎/㎖). Magnifications were × 400.

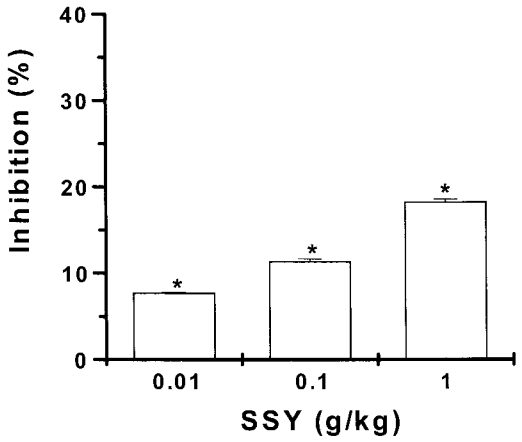

Fig. 3. Effects of SSY on 48 h PCA in mice.

SSY was administered orally 1 h prior to the challenge with antigen (DNP-HSA). Each datum represents the mean ± S.E.M. $^{*}P<0.05$; significantly different from the saline value.

extravasation of dye. When SSY was orally administered to the mouse, the PCA reaction was significantly inhibited in a dose-dependent manner(Fig. 3).

Ⅳ. Discussion

In the present study SSY pre-treatment profoundly affected compound 48/80-induced systemic anaphylatic reaction, ear swelling response, degranulation, and histamine release from RPMCs. SSY also inhibited anti-DNP IgE-induced PCA reaction in murine model by oral administration.

The stimulation of mast cells with compound 48/80 is conceived to initiate the activation of a signal transduction pathway to lead histamine release from mast cells. Some reports have shown

that compound 48/80 and other polybasic compounds are able to activate G proteins and compound 48/80 activates mast cell phospholipase D (PLD) via heterotrimeric GTP-binding proteins[18-21]. They identified recombinant $G_{\beta 2\gamma 2}$ subunit markedly synergized PLD activation by compound 48/80 in permeabilized RBL-2H3 cells[22]. The report that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane indicates that the membrane permeability increase may be an essential trigger for the release of mediators from mast cells[23]. Thus, it is possible to hypothesize that SSY might act on the lipid bilayer membrane affecting the prevention of the perturbation induced by compound 48/80.

The study that the treatment with compound 48/80 by differential alcian blue/safranin staining decreased the number of mast cells in skin showed a possibility that the mast cells cannot be detected by alcian blue due to the complete degranulation by compound 48/80[24].

The effective method to evaluate skin anaphylactic reactions *in vivo* models is to induce PCA[25-9]. The SSY administered mouse is protected from IgE-mediated PCA and these mechanism of the protection against anti-DNP IgE may be suggested only in some particular conditions. It is conceivable that SSY inhibits the initial phase of immediate type allergic reactions, probably through interference with the degranulation system.

In conclusion, these results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by SSY. The author believe that administration of SSY may have a clinical applicability to the allergic disorders.

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