Inhibitory Effect of Schizandrin on Passive Cutaneous Anaphylaxis Reaction and Scratching Behaviors in Mice

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To evaluate the antiallergic effect of the fruit of Schizandra chinensis Bail (Family Magnoliaceae), which inhibited the mouse passive cutaneous anaphylaxis (PCA) reaction in a preliminary experiment, its main constituent, schizandrin, was isolated and its antiallergic effect investigated. Schizandrin inhibited the PCA reaction induced by the IgE–antigen complex, the scratching behaviors induced by compound 48/80 and the serum IgE production induced by ovalbumin. Schizandrin also inhibited the in vitro degranulation of compound 48/80-induced rat peritoneal mast cells and IgE-induced RBL 2H3 cells. Schizandrin reduced the protein expressions of TNF-α and IL-4 in IgE-induced RBL 2H3 cells. These findings suggest that schizandrin can improve IgE-induced anaphylaxis and scratching behaviors.

Key words allergy; schizandrin; anaphylaxis; IgE

Mast cells and basophils are well-known critical participants in various biological processes of allergic diseases.1—3) These cells express surface membrane receptors, with high affinity and specificity for IgE. The interaction of antigen-bound IgE in surface membrane receptors causes the release of histamine, prostaglandins, leukotrienes and cytokines.4,5) These cytokines activate chemotaxis and phagocytosis of neutrophils and macrophages. Finally, cytokine-induced reactions cause tissue inflammation. These allergic diseases are now rapidly increasing chronic health problem in most countries.6) Antiallergic agents, such as anti-histamines, steroids and immunosuppressants, have been used against allergic diseases, such as allergic rhinitis, atopic dermatitis, asthma and food allergies.7—9) but improving these diseases is very difficult. Therefore, herbal medicines have been advanced for allergy; schizandrin; anaphylaxis; IgE

Materials Dulbecco’s modified Eagles medium (DMEM), fetal bovine serum, dinitrophenol-human serum albumin (DNP-HSA), ovalbumin (OVA), p-nitrophenyl-N-acetyl-β-D-glucosaminide, cremophor EL and compound 48/80 were purchased from Sigma Co. (St. Louis, MO, U.S.A.). Schizandrin (purity, 95.3%) (Fig. 1) was isolated from the fruits of SZ, according to the previous reports.16,17)

Animals The male ICR mice (20—25 g) and male Sprague-Dawley rats were supplied by the Orient Experimental Animal Breeding Center (Seoul, Korea). All animals were housed in wire cages at 20—22 °C and 50±10% humidity, fed standard laboratory chow (Oriental Experimental Animal Breeding Center, Seoul, Korea) and allowed water ad libitum. All procedures relating to the animals and their care conformed to the international guidelines ‘Principles of Laboratory Animals Care’ (NIH publication no. 85—23, revised 1985).

Measurement of PCA Reaction An IgE-dependent cutaneous reaction was measured according to the previous method of Choo et al.18) The male ICR mice were intradermally injected, with 10 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. Forty-eight hours later each mouse received an injection of 200 µl of 3% Evans blue in PBS, containing 200 µg of DNP-HAS, via the tail vein. The test agents were orally or intraperitoneally administered 1 h prior to the DNP-HSA injection. Thirty minutes after the DNP-HSA injection, the mice were sacrificed.

MATERIALS AND METHODS

Materials Dulbecco’s modified Eagles medium (DMEM), fetal bovine serum, dinitrophenol-human serum albumin (DNP-HSA), ovalbumin (OVA), p-nitrophenyl-N-acetyl-β-D-glucosaminide, cremophor EL and compound 48/80 were purchased from Sigma Co. (St. Louis, MO, U.S.A.). Schizandrin (purity, 95.3%) (Fig. 1) was isolated from the fruits of SZ, according to the previous reports.16,17) The fruit of Schizandra chinensis Bail (SZ, Family Magnoliaceae) has frequently been used as a tonic and astringent agent in traditional Korean, Chinese and Japanese medicines. SZ, which contains lignans, such as schizandrin and gomisin A, as main constituents, has been reported to have many biological properties, including hepatoprotective, anti-inflammatory and antitumor activities.12—15) In preliminary experiments, SZ was found to inhibit the mouse passive cutaneous anaphylaxis reaction induced by the antigen–IgE complex. However, the antiallergic effect of these components remains to be thoroughly studied.

Therefore, to evaluate the antiallergic activity of SZ, a main constituent, schizandrin, was isolated from the fruit of SZ and its in vivo inhibitory activity against scratching behaviors and passive cutaneous anaphylaxis (PCA) investigated.

MATERIALS AND METHODS

Materials Dulbecco’s modified Eagles medium (DMEM), fetal bovine serum, dinitrophenol-human serum albumin (DNP-HSA), ovalbumin (OVA), p-nitrophenyl-N-
their dorsal skins removed and the pigmented area measured. After extraction with 1 ml 1.0 M KOH and 4 ml of a mixture of acetone and 0.2 M phosphoric acid (13:5), the amount of dye was determined colorimetrically at 620 nm.

Scratching Behavioral Experiments Male BALB/c mice were placed in acrylic cages (22×22×24 cm) for about 10 min to become acclimatized. The behavioral experiments were performed according to the method of Sugimoto et al.21 The rostral part of the skin on the back of the mice was clipped, and 50 µg/50 µl of compound 48/80 (dissolved in saline) intradermally injected into each mouse. Control mice received a saline injection in the place of the compound 48/80. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage and the scratching behaviors recorded using an 8-mm video camera (SV-K80, Samsung, Seoul, Korea) under unmannned conditions. Scratching of the injected site with the hind paws was counted and compared with that of other sites, such as the ears. Each mouse was used for only one experiment. The mice generally showed several scratches per second, and a series of these behaviors was counted as one incident of scratching over a 60 min period. The test agents were orally administered 1 h before the scratching agent.

Assay of Serum IgE in Mouse Asthmic Mice Induced by OVA The BALB/c mice were sensitized using an intraperitoneal injection of 1 ml of a mixture of 0.005% OVA and 1ml aluminum hydroxide gel (alum, Rehydragel; Reheis, Berkeley Heights, NJ, U.S.A.). The non-sensitized mice received an intraperitoneal injection of alum alone. On the 10th day, the mice were given an intraperitoneal booster injection of the same antigen or alum. On the 17th day after sensitization, the mice were challenged with aerosolized 5% OVA, which was generated using an ultrasonic nebulizer (UltraNeb 99; DeVilbiss, Somerset, PA, U.S.A.). The aerosol was circulated through a large acrylic cylindrical chamber, with mice then placed in the chamber for 1 h per day. The OVA aerosol challenge was repeated for 5 d. Test agents [50 mg/kg (schizandrin) or 10 mg/kg (betamethasone)] were orally administered 1 h prior to the 5 d OVA aerosol challenge. The mice were sacrificed, and the level of IgE in the blood measured according to the previously reported method.20

Enzyme-Linked Immunosorbent Assay (ELISA) of IL-4 ad TNF-α in RBL-2H3 Cells Stimulated by IgE–Antigen Complex RBL-2H3 cells (5×10^6 cells), previously cultured in DMEM, were treated with 0.5 µg/ml of mouse monoclonal IgE to sensitize the cells. The cells (1.8 ml) were exposed to 0.2 ml of the test agents (dissolved in 0.5% dimethyl sulfoxide) for 4 h, followed by treatment with 0.2 ml DNP-HSA (1 µg/ml) for 40 min at 37°C. The supernatant (50 µl) was transferred to 96-well ELISA plates, and the IL-4 ad TNF-α concentrations then determined using commercial ELISA Kits (Pierce Biotechnology, Inc., Rockford, IL, U.S.A.).

Assay of Degranulation of RBL-2H3 Cells and Rat Peritoneal Mast Cells Stimulated by IgE–Antigen Complex The inhibitory activity of test agents against the release of β-hexosaminidase from RBL-2H3 cells and histamine of rat peritoneal mast cells was evaluated according to Choo et al.18 Statistical Analysis All data were expressed as the mean ± standard deviation, with statistical significance analyzed using one-way ANOVA followed by a Student–Newman–Keuls test.

RESULTS AND DISCUSSION

During the screening program to evaluate the antiallergic activity of herbal medicines, SZ was found to inhibit the mouse PCA reaction induced by IgE–antigen complex. Therefore, a main constituent, schizandrin, from SZ was isolated, and its PCA reaction-inhibitory effect in mouse measured (Table 1). The PCA reaction was induced by an injection of IgE and antigen, with schizandrin administered orally 1 h prior to the challenge with antigen. The IgE–antigen complex potently induced the PCA reaction. Schizandrin showed potent inhibition against the PCA reaction. Schizandrin also inhibited the scratching behavior induced by compound 48/80. Schizandrin, at doses of 10 and 50 mg/kg, reduced the scratching behavior frequency by 7 and 45%, respectively. The effect of schizandrin on the serum IgE production in asthmic mice induced by OVA was also measured. Schizandrin was found to inhibit the serum IgE concentration by 30%. Nevertheless, its inhibitory effect was weak compared with that of betamethasone.

To understand the antiallergic mechanism of schizandrin, its degranulation-inhibitory activities against rat peritoneal mast cells and RBL-2H3 cells were measured (Fig. 2). Schizandrin inhibited the degranulation of rat peritoneal mast cells induced by compound 48/80 and RBL-2H3 cells induced by the IgE–antigen complex; its IC₅₀ values were 97 and 95 µM, respectively, and those of azelastine were 32 and 26 µM, respectively. When the inhibitory effect of schizandrin on the protein expressions of proinflammatory cytokines, TNF-α and IL-4, in RBL-2H3 cells induced by IgE–antigen complex was measured using an ELISA assay, a concentration of 50 µM was found to inhibit the expressions of TNF-α and IL-4 by 42 and 37%, respectively (Fig. 3).

Azelastine, a representative antiallergic drug,22 is an H1-receptor antagonist, which decreases the mediator release.

### Table 1. Effect of Schizandrin on the Mouse Passive Cutaneous Anaphylaxis (PCA) Reaction Induced by IgE, the Scratching Behaviors Induced by Compound 48/80 and the Asthma Induced by Ovalbumin

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (mg/kg)</th>
<th>PCA reaction (%)</th>
<th>Scratching behaviors (%)</th>
<th>IgE production (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizandrin</td>
<td>10</td>
<td>61±11e</td>
<td>7±5e</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>72±9f</td>
<td>45±9f</td>
<td>30±6e</td>
</tr>
<tr>
<td>Azelastine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>82±15f</td>
<td>76±6f</td>
<td>—</td>
</tr>
<tr>
<td>Betamethasone</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>78±8f</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>—</td>
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<td>—</td>
</tr>
</tbody>
</table>

a) The amounts of extravasated Evan blue from the dorsal skin (1×1 cm) of the control stimulated with the IgE–antigen complex and vehicle-treated groups were 25±3 and 11±2 µg, respectively. b) Scratching behavior number frequency of the normal control, which was treated with saline alone, and the control group, which was treated with compound 48/80 and saline, for 1 h were 239±25 and 3±1, respectively. c) The mice were sensitized by an intraperitoneal injection of 5 mg ovalbumin (OVA) mixed with alum, given an intraperitoneal booster injection of the same antigen and then challenged with aerosolized 5% OVA. Test agents were orally administered 1 h prior to the 5 day OVA aerosol challenge. The mice were sacrificed, and the level of IgE in the blood measured using an ELISA kit. The amounts of blood IgE in the control stimulated with ovalbumin and vehicle-treated groups were 71±5.1 and 10.5±2.1 ng/ml, respectively. d) Not determined. e–g) Items with the same letter in each column were not significantly different. Inhibition values indicate the mean±S.D. (n=6).
drin against scratching behaviors may be different from that of pruritogen. Therefore, the inhibitory mode of schizan-
drins may not be due to the inhibition of mast cell degranulation and cy-
tokine expressions of TNF-\(\alpha\) and IL-4, in RBL-2H3 cells as well as the mRNA expressions (data not shown). The previous investigations have reported that derivatives of schizandrin, such as gomisin A and schizandrin A, isolated from SZ, potently inhibited NF-\(\kappa\)B activation and exhibited anti-inflammatory and PAF-antagonic effects.\(^2\,\text{17,25}\) These results suggest that schizandrin may inhibit NF-\(\kappa\)B activation in RBL-2H3 cells.

Based on these findings, the inhibitory effect of schizandrin against the PCA reaction and scratching behaviors may be due to the inhibition of mast cell degranulation and cytokine expressions of TNF-\(\alpha\) and IL-4. Nevertheless, the anti-PCA reaction and anti-scratching behavior mechanisms of schizandrin can not be sufficiently established. Therefore, its inhibitory mechanisms remain to be solved. Finally, these findings suggest that schizandrin may improve the IgE-induced anaphylaxis and scratching behaviors.

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REFERENCES