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Inhibitory Effect of Astragaloside I and IV on Passive Cutaneous Anaphylaxis Reaction and Scratching Behaviors in Mice

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Abstract – To evaluate the antiallergic effect of the dried root of *Astragalus membranaceus* Bunge (AM) (Leguminosae), which inhibited the mouse passive cutaneous anaphylaxis (PCA) reaction in a preliminary experiment, its main constituents, astragalosides I and IV, were isolated and their antiallergic effects were investigated. Astragalosides I and IV inhibited the PCA reaction induced by the IgE-antigen complex, and the scratching behaviors induced by compound 48/80. These constituents reduced the protein expressions of TNF-α and IL-4 in IgE-induced RBL-2H3 cells. These findings suggest that astragalosides I and IV as well as AM can improve IgE-induced anaphylaxis and scratching behaviors.
Keywords *Astragalus membranaceus*, astragaloside, allergy, anaphylaxis, itching

Introduction

Mast cells and basophils are well-known critical participants in various biological processes of allergic diseases (Stevens and Austen, 1989). 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 (Mitre and Nutman, 2006). 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 (Wuthrich, 1989). 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 (Sakuma *et al.*, 2001; Schafer-Korting *et al.*, 1996; Simons, 1992), but improving these diseases is very difficult. Therefore, herbal medicines have been advanced for allergic diseases, and their effectiveness has received increasing attention (Bielory, 2004).

Astragalus membranaceus Bunge (Leguminosae) is a traditional Chinese medicinal herb that is well-known for its vital-energy tonifying, skin reinforcing, diuretic, and tissue generative actions (Bensky and Gamble, 1993; Wu and Chen, 2004). The dried root of *A. membranaceus* contains isoflavones, saponins and polysaccharides (Li *et al.*, 2007; Ma *et al.*, 2002). Among these constituents, astragaloside which is a representative constituent of *A. membranaceus*, has been reported to exhibit protective effect for myocardinal ischemia, nociceptive, herpes virus and coxsackie virus, and diabetic nephropathy (Wu and Chen, 2004; Yin *et al.*, 2004). However, its antiallergic effect has not been studied.

Therefore, the antiallergic activities, anti-passive cutaneous anaphylaxis and anti-scratching behavior reactions, of astragaloside I and IV, which is isolated from *A. membranaceus* inhibiting passive cutaneous anaphylaxis reaction in mice, were investigated.

Experimental

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, USA). Astragalosides I and IV (Fig. 1) were isolated from *A. membranaceus* according to the previous reports (Kitagawa *et al.*, 1983).

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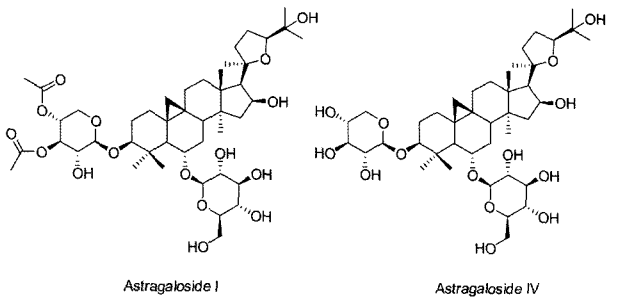

Fig. 1. Structure of main constituents, astragalosides I and IV, isolated from the dried root of *Astragalus membranaceus*.

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 (Orient Experimental Animal Breeding Center, Seoul, Korea) and allowed water *ad libitum*. All procedures relating to the animals and their care were conducted in accordance with 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.* (2003). 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-HSA, *via* the tail vein. The test agents were orally or intraperitoneally administered 1 h prior to the DNP-HSA injection. Thirty min after the DNP-HSA injection, the mice were sacrificed, their dorsal skins removed and the pigmented area measured. After extraction with 1 mL of 1 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.* (1998). 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 unmanned 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.

Enzyme-Linked Immunosorbent Assay (ELISA) of IL-4 and TNF-α in RBL-2H3 Cells Stimulated by IgE-antigen Complex – RBL-2H3 cells (5×10^5 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 and TNF-α concentrations then determined using commercial ELISA Kits (Pierce Biotechnology, Inc., Rockford, IL, USA) (Matsuda *et al.*, 2004).

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, the ethanol extract of *A. membranaceus* was found to inhibit the mouse PCA reaction induced by IgE-antigen complex at doses of 100 and 250 mg/kg by 34 and 48%, respectively. Therefore, main constituents, astragalosides I and IV, from *A. membranaceus* were isolated, and their PCA reaction-inhibitory effect in mouse were measured (Table 1). The PCA reaction was induced by an injection of IgE and antigen, with astragalosides administered orally 1 h prior to the challenge with antigen. The IgE-antigen complex potently induced the PCA reaction. These astragalosides showed potent inhibition against the PCA reaction. Astragalosides also inhibited the scratching behavior induced by compound 48/80 (Table 2). Of astragalosides, astragaloside IV more potently inhibited these allergic reactions. Astragaloside IV, at doses of 10 and 50 mg/kg, reduced PCA reaction by 44 and 53% and inhibited the scratching behavior frequency by 39 and 54%, respectively. Nevertheless, their inhibitory effects were weak, compared with those of dexamethasone.

Table 1. Inhibitory effect of astragalosides I and IV on the passive cutaneous anaphylaxis and scratching behavior reactions in mice

Agent	Dose (mg/kg)	PCA reaction[a]
Astragaloside I	10	12 ± 1[c]
	50	44 ± 6[e]
Astragaloside IV	10	22 ± 5[d]
	50	53 ± 13[e]
Azelastine	10	79 ± 5[f]

[a)] Data means the inhibition (%) which were calculated from 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.
[c,d,e,f)] Items with the same letter in each column were not significantly different.
Inhibition values indicate the mean ± S.D. (n = 6).

Table 2. Inhibitory effect of astragalosides I and IV on scratching behaviors in mice

Agent	Dose (mg/kg)	Inhibition[a]
Astragaloside I	10	27 ± 4[c]
	50	39 ± 8[c,d]
Astragaloside IV	10	38 ± 9[c,d]
	50	54 ± 12[d]
Azelastine	10	75 ± 8[e]

[a)] 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 243 ± 21 and 3 ± 1, respectively.
[c,d,e)] Items with the same letter in each column were not significantly different.
Inhibition values indicate the mean ± S.D. (n = 6).

To understand the antiallergic mechanism of these astragalosides, their inhibitory effects in the protein expressions of proinflammatory cytokine TNF-α and IgE-switching cytokine IL-4 in RBL-2H3 cells induced by IgE-antigen complex were measured using an ELISA assay (Fig. 2). Astragalosides I and IV at a concentration of 50 μM were found to inhibit the expressions of TNF-α by 25 and 27%, and IL-4 by 20 and 28%, respectively.

Mast cells produce histamine, as well as proinflammatory cytokines, especially TNF-α, IL-4 and IL-6 (Zhao *et al.*, 2005). These cytokines have a critical biological role in the allergic reaction. Mast cells are a principal source of TNF-α in human dermis, and degradation of mast cells in the dermal endothelium is abrogated by the inhibition of the action of TNF-α (Kempuraj *et al.*, 2003). IL-4 produced from mast cells induces IgE production in B cells and is associated with anaphylaxis and pruritic reactions (Mitre and Nutman, 2006). The inhibition of proinflammatory cytokine expression from mast cells

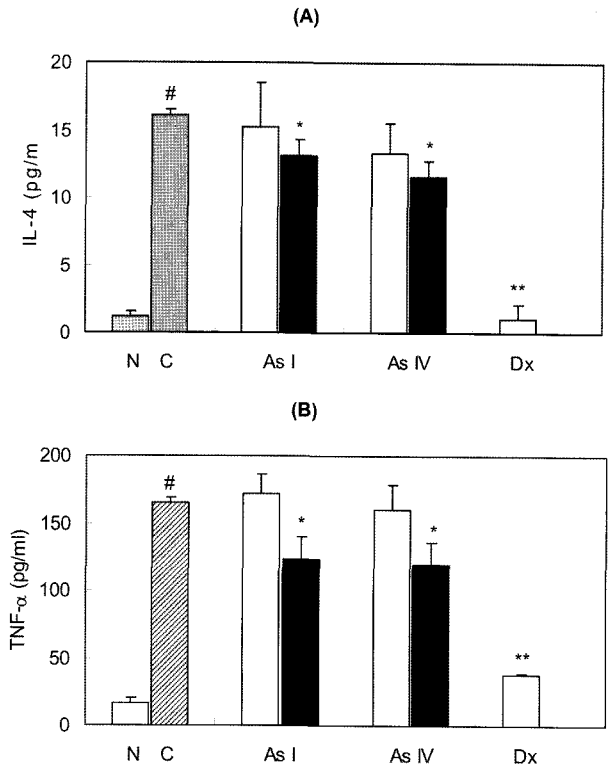

Fig. 2. Effect of astragalosides I and IV on the protein expressions of IL-4 (A) and TNF-α (B) in RBL-2H3 cells induced by the IgE-antigen complex. RBL-2H3 cells (5×10^5 cells) were treated with 0.5 μg/mL of mouse monoclonal IgE, exposed to 20 μM (white bar) and 100 μM (black bar) of agents (N, normal control group; C, control group; As I, astragaloside I; As IV, astragaloside IV; Dx, dextramethasone) for 4 h, followed by treatment with 0.2 ml of dinitrophenol-human serum albumin (DNP-HSA, 1 μg/mL) for 40 min at 37°C, and ELISA for TNF-α and IL-4 were then performed. The normal control was treated with vehicle alone instead of the agents and IgE-antigen, and control group was treated only without the agent. Values represent the mean ± S.D. for duplicate experiments. #Significantly different from the normal control group ($^{\#}P < 0.05$). *Significantly different from the control group ($^{*}P < 0.05$).

must be one of the key indicators of reduced allergic symptoms. In the preliminary study, *A. membranaceus* inhibited the PCA reaction in mice. Therefore, main constituents astragalosides I and IV isolated from *A. membranaceus* were found to inhibit mouse PCA and scratching behavior reactions. From our *in vitro* study, these astragalosides were also found to inhibit the protein expressions of some cytokines, TNF-α and IL-4, in RBL-2H3 cells.

Generally, glycosides of natural products are not easily absorbed from intestine to the blood for their hydrophilic characterization. Astragaloside I is cycloastragenol glycoside conjugated with diacetylated D-xylose and D-glucose while astragaloside IV is cycloastragenol glycoside

conjugated with D-xylose and D-glucose. Hence, astragaloside I is more hydrophilic component than astragaloside IV. Therefore, if astragaloside I is orally administered, its absorption from the intestine to the blood is difficult, compared to astragaloside IV. Thus, to absorb these glycosides from intestine, these compounds must be metabolized to hydrophobic metabolites (Kim, 2002; Kobashi and Akao, 1997). Therefore, astragaloside IV may show more potent inhibition than astragaloside I. These results suggest that the inhibitory effect of *A. membranaceus* and its main constituents astragalosides I and IV against the PCA reaction and scratching behaviors may be due to the inhibition of cytokine expressions of TNF-α and IL-4.

Finally, these findings suggest that *A. membranaceus* and its main constituents I and IV may improve the IgE-induced anaphylaxis and scratching behaviors.

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