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Antiasthmic Effect of Fermented *Artemisia princeps* in Asthmic Mice Induced by Ovalbumin

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Abstract *Artemisia princeps* Pampanini (AP) was fermented with *Bifidobacterium infantis* K-525 and its antiasthmic effect investigated. AP and fermented AP (FAP) reduced the IgE level in the blood of ovalbumin-induced asthmic mice. Moreover, FAP reduced the IgE, proinflammatory cytokine IL-6, and IL-4 levels in the trachea, as well as in the lung of the experimental asthmic mice, whereas AP only reduced the IgE and IL-6 levels in the lungs. Nonetheless, AP and FAP both inhibited the mRNA expression of IL-6 and TNF-α in IgE-induced RBL-2H3 cells. The *in vivo* antiasthmic effect of FAP was more potent than that of AP. Therefore, these findings suggest that the enhanced antiasthmic effect of AP after bifidus fermentation was possibly due to the regulation of the proinflammatory cytokine biosynthesis of IL-6 and TNF-α.

Keywords: *Artemisia princeps*, mugwort, asthma, IgE, fermentation

Allergic asthma is a chronic and complex inflammatory disease of the lungs characterized by reversible obstruction of the airway, hyperresponsiveness, infiltration of inflammatory cells into lung tissue, mucus overproduction, and overexpression of Th2-mediated cytokines, including IL-4 and TNF-α, in the airways of allergic asthmatics [21]. The etiology of asthma reactivity is based on IgE-mediated pharmacological processes of a variety of cell populations, such as mast cells and basophils [20]. Antihistamines, steroids, and immunosuppressants have all been used against allergic diseases [14, 15, 19]; however, the improvement is still limited. Therefore, herbal medicine has been proposed as an alternative remedy, and its effectiveness is receiving increasing attention [1].

Artemisia princeps Pampanini (AP) and *Artemisia asiatic* Nakai (Family Asteracease) are widely used in traditional medicine, and their pharmacologically active constituent is eupatilin, which has inhibitory effects on gastric ulcers, ulcerative colitis, and naproxen-induced and ethanol-induced gastric damage, as shown in experimental animals [5, 10, 11]. It also induces apoptosis in human promyelocytic leukemia cells and cell-cycle arrest in ras-transformed human mammary epithelial cells, plus inhibits tyrosine kinase in activated guinea pig lung mast cells [6, 14, 16]. Recently, Kim *et al.* [8] reported that eupatilin isolated from *Artemisia asiatica* ameliorated the airway inflammation in allergic asthmatic mice, whereas previous study by the present authors [18] found that *Artemisia princeps* (AP) inhibited an *in vivo* passive cutaneous anaphylaxis (PCA) reaction. Moreover, when AP was fermented with *Bifidobacterium* sp., its PCA reaction-inhibitory and scratching behavior-inhibitory effects were increased [18]. However, the antiasthmic effect of AP and fermented AP (FAP) has not yet been studied.

Accordingly, in the present study, AP was fermented with *Bifidobacterium* K-525 and its antiasthmic activity examined in ovalbumin (OVA)-induced mice.

The AP, which had been cultured in Ganghwado and brewed for 2 years, was extracted with 80% ethanol, and then concentrated *in vacuo*. The extract (5 g) was suspended in 1 l of water and incubated for 24 h at 37°C with *Bifidobacterium infantis* K-525, as previously reported [5, 6, 17]. Thereafter, the same volume of ethanol was added to the fermented mixture, which was then centrifuged, concentrated *in vacuo*, and used as the FAP agent.

Male BALB/c mice (20–25 g) were supplied from the Orient Experimental Animal Breeding Center (Seoul, Korea).

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All the 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 given water *ad libitum*. All the 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).

The BALB/c mice were sensitized by an intraperitoneal injection of a mixture of 1 ml of 0.005% OVA and 1 ml of an aluminum hydroxide gel (alum, Rehydragel; Reheis, Berkeley Heights, NJ, U.S.A.). The nonsensitized 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 by an ultrasonic nebulizer (Ultra-Neb 99; DeVilbiss, Somerset, PA, U.S.A.). The aerosol was circulated through a large acrylic cylindrical chamber, into which the mice were placed for 1 h per day for 5 days, and then the mice were sacrificed on the 6th day. The levels of IgE in the blood and IL-4 and IL-6 in the trachea and lungs were measured using an enzyme-linked immunosorbent assay (ELISA). The blood, lungs, and trachea prepared from the asthmic mice were homogenated in a 50 mM Hepes buffer. The supernatant (50 μl) was transferred into 96-well ELISA plates, and the IgE, IL-4, and IL-6 concentrations were determined using commercial ELISA kits (Pierce Endogen, Inc., Rockford, IL, U.S.A.) [9].

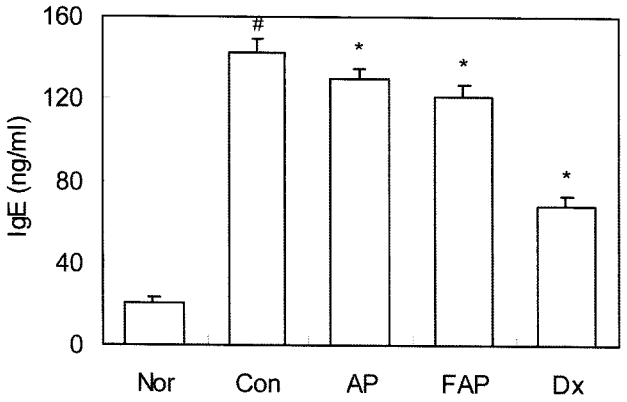

Fig. 1. Effect of AP and FAP on IgE level in blood of asthmic mice induced by OVA.
The BALB/c mice were sensitized with an intraperitoneal injection of OVA mixed with alum and given a booster injection. The mice were then challenged with aerosolized 5% OVA for 5 days after sensitization. For the mice in the normal group (Nor), the OVA was not treated. The test agents [Con, saline alone; AP, *Artemisia princeps* extract (50 m/kg); FAP, Fermented *Artemisia princeps* extract (50 mg/kg); and Dx, dexamethasone (10 mg/kg)] were orally administered 1 h before the OVA aerosol challenge. On the next day, the mice were sacrificed and the level of IgE in the blood measured using an ELISA kit (BD Biosciences, San Diago, CA, U.S.A.). The IgE values indicate mean±SD (n=5). # The control group is significantly different ($p<0.05$) compared with the normal group. * Significantly different ($p<0.05$), compared with the control group.

A reverse transcription-polymerase chain reaction (RT-PCR) analysis of RBL-2H3 cells treated with the test agents and/or IgE with DNP-HSA was performed using the modified method of Shin *et al.* [17].

All the data were expressed as the mean±standard deviation, and statistical significance was analyzed by a one-way ANOVA, followed by a Student-Newman-Keuls test.

When screening for antiallergic agents from natural products, we previously found that AP inhibited the PCA reaction induced by an IgE-antigen complex and the scratching behavior induced by compound 48/80 in mice, and these inhibitory effects of AP were increased by bifidus fermentation. In addition, Kim *et al.* [8] previously reported that the main constituent, eupatilin, isolated from *Artemisia asiatica* hd an antiasthmic effect. However, the antiasthmic effect of AP and its fermented form has not yet been studied. Therefore, AP was fermented, as previously reported [18], and the IgE level in the blood of OVA-induced asthmic mice investigated (Fig. 1). Whereas the OVA immunization increased the blood IgE level in the mice, AP treatment decreased the induced blood IgE level, and FAP treatment inhibited the blood IgE level increment more potently than AP. FAP also decreased the IgE levels in the lungs and trachea (Fig. 2), whereas AP only reduced the IgE level in the lungs. Furthermore, FAP significantly reduced the proinflammatory cytokine IL-6 level in the trachea and lungs, as determined by an ELISA assay, whereas the AP only inhibited the IL-6 expression in the lungs. Finally, in contrast to AP, FAP reduced the IgE-switching cytokine IL-4 level in the trachea and lungs, although its inhibitory effect was not significant in the trachea.

To understand the antiasthmic mechanism of AP and FAP, their degranulation-inhibitory activities against RBL-2H3 cells were measured, where AP and FAP both inhibited the degranulation of RBL-2H3 cells induced by the IgE-antigen complex, as previously reported [18] (data not shown). When their inhibitory effects on the mRNA expression of the proinflammatory cytokines TNF-α and IL-6 in RBL-2H3 cells induced by the IgE-antigen complex were measured using an RT-PCR assay, AP and FAP inhibited both IL-6 and TNF-α expressions (Fig. 3), where FAP inhibited the proinflammatory cytokine expression more potently than AP. However, neither AP nor FAP inhibited the mRNA expression of IgE-switching cytokine IL-4.

Mast cells and basophils are well known as critical participants in various biologic processes of allergic diseases [2, 21]. These cells express surface membrane receptors with a high affinity and specificity for IgE. The interaction of antigen-bound IgE in surface membrane receptors releases histamine, prostaglandins, leukotrienes, and cytokines [2, 11]. These cytokines activate the chemotaxis

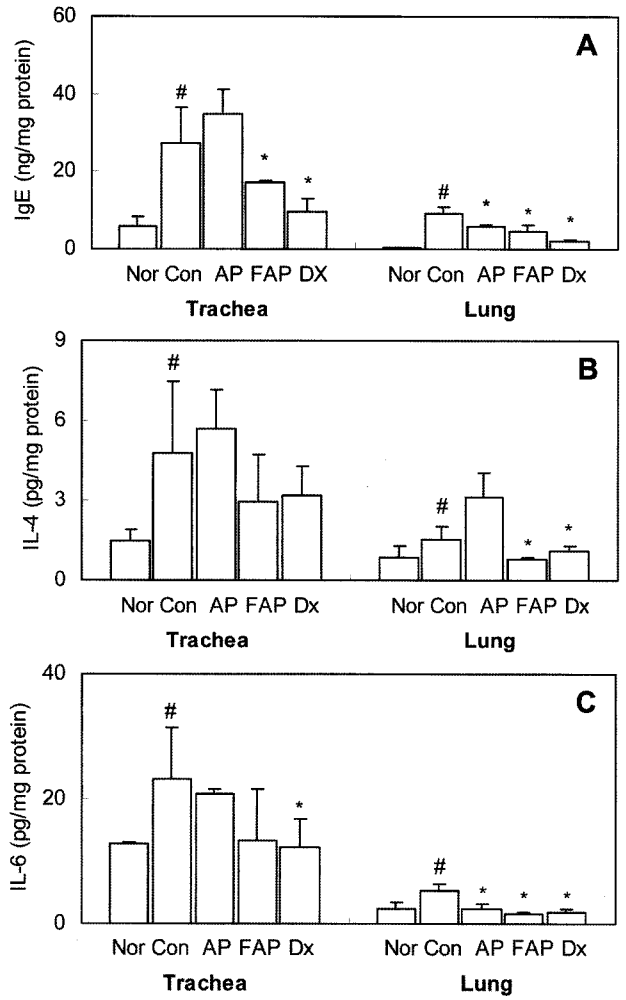

Fig. 2. Effect of AP and FAP on levels of IgE (**A**) and cytokines IL-4 (**B**) and IL-6 (**C**) in trachea and lungs of asthmic mice induced by OVA.

The sensitization of the mice was performed as described in Fig. 1. Nor, normal group (saline alone); Con, saline alone in OVA-induced mice; AP, 50 mg/kg AP; FAP, 50 mg/kg FAP; and Dx, dexamethasone (10 mg/kg). The cytokine values indicate mean±SD (n=5). # The control group is significantly different ($p<0.05$) compared with the normal group. * Significantly different ($p<0.05$), compared with the control group.

and phagocytosis of neutrophils and macrophages. Finally, cytokine-induced reactions cause tissue inflammation.

Betamethasone, a representative antiallergic medicine [15], has also been found to reduce the OVA-induced blood IgE level in mice. Yet, AP and FAP not only inhibited the blood IgE level increment in OVA-induced mice, but also reduced the expression of proinflammatory cytokine IL-6 in asthmic mice. The inhibition of proinflammatory cytokine expression from mast cells is a key indicator of reduced allergic symptoms [11, 20]. IL-4 induces IgE production in B lymphocytes. Therefore, the induction of IgE in B lymphocytes is abrogated by the inhibition of IL-4 production. Nonetheless, whereas AP did not reduce the IgE production-stimulating cytokine IL-4 level in asthmic

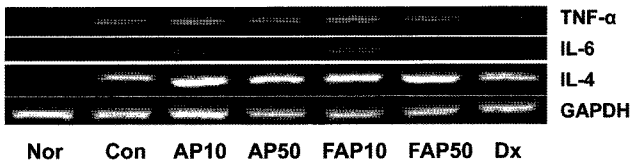

Fig. 3. Effect of AP and FAP on mRNA expression of TNF-α, IL-6, and IL-4 in RBL-2H3 cells induced by IgE-antigen complex.

The RBL-2H3 cells (5×10^5 cells) were treated with 0.5 μg/ml of mouse monoclonal IgE, exposed to 0.2 ml of the agents (Nor, normal; Con, control treated with vehicle alone; AP10, 10 μg/ml AP; AP50, 50 μg/ml AP; FAP10, 10 μg/ml FAP; FAP50, 50 μg/ml FAP; and Dx, 10 μM dexamethasone) 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 then a RT-PCR for TNF-α and IL-4 was performed. The normal group was only treated with the vehicle, instead of the agents and IgE-antigen.

mice, FAP did inhibit the IL-4 production. In a previous study, AP and FAP inhibited the PCA reaction induced by an IgE-antigen complex in mice and protected the degranulation of mast cells by the same IgE-antigen complex [18]. The inhibitory effect of FAP against asthma was more potent than that of AP. Therefore, these results suggest that the antiasthmic effects of FAP may have been due to the inhibition of cytokine biosynthesis and degranulation. Its inhibition of IL-4 production reduced the level of IgE, which induces asthma, whereas its inhibition of IL-6 production improved the inflammation induced by the degranulation of mast cells.

Consequently, based on these findings, AP enforced the antiasthmic effect of bifidus fermentation, plus FAP exhibited potential for protecting against IgE-mediated skin allergic diseases.

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