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Effect of Fermented Lactic Acid Bacteria on Antiallergic Effect of *Artemisia princeps* Pampanini

SHIN, YONG-WOOK[1], EUN-AH BAE[1], BOMI LEE[1], SUNG-WON MIN[1], NAM-IN BAEK[2], SU-NO RYU[3], HAE-GON CHUNG[4], AND DONG-HYUN KIM[1]*

[1]*College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea*
[2]*Graduate School of Biotechnology and PMRC, Kyung Hee University, Suwon 449-701, Korea*
[3]*Department of Agricultural Science, Korea National Open University, Seoul 110-791, Korea*
[4]*Ganghwa Agricultural R&D Center, Kyunggi-Do, Korea*

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Abstract *Artemisia princeps* Pampanini, which is named as *Sajabalssuk* (SJ-1) in Korea, was fermented with lactic acid bacteria (LAB), and their antiallergic activities were investigated. When SJ-1 was fermented with some LAB isolated from human feces, the inhibition of NO production in RAW264.7 cells and antioxidant activities of SJ-1 were not affected. However, the inhibitory activity of SJ-1 against degranulation of RBL-2H3 cells induced by IgE was increased by LAB fermentation. Among the LAB tested, *Bifidobacterium infantis* K-525 provided the most potent inhibitory effect of SJ-1 against degranulation of RBL-2H3 cells. SJ-1 extract fermented with *B. infantis* K-525 (F-SJ-1) potently inhibited the mouse passive cutaneous anaphylaxis reaction induced by IgE with antigen, skin dermatitis induced by 12-*O*-tetradecanoylphorbol-13-acetate, and scratching behaviors induced by compound 48/80. These inhibitory activities of F-SJ-1 were more potent than those of SJ-1. These findings suggest that the inhibition of SJ-1 extract against IgE-induced allergic diseases, such as rhinitis and asthma, can be enhanced by LAB fermentation.

Key words: *Artemisia princeps* Pampanini, *Sajabalssuk*, antiallergic effect, passive cutaneous anaphylaxis reaction, lactic acid bacteria

Artemisia princeps Pampanini (Family Asteraceae), which includes eupatilin, acacetin, and eudesmane as main components [14], has long been used for the treatment of inflammation, diarrhea, gastric ulcer, and many circulatory disorders [9]. The *Artemisia princeps* Pampanini cultivated in Ganghwado, named as *Sajabalssuk* (SJ-1) in Korea, contains a high content of eupatilin, compared with that cultivated at other places, such as China [9]. Shin *et al.* [17] reported that, although SJ-1 inhibits NO production in RAW264.7 cells stimulated by lipopolysaccharide (LPS), it hardly inhibits allergic reactions such as the passive cutaneous anaphylaxis (PCA) reaction, scratching behaviors, and skin dermatitis.

In addition, most herbal medicines contain bioactive secondary metabolites that are modified, such as sugars [7, 8, 10–12]. To express or increase their pharmacological activities, the secondary metabolites may be transformed. Therefore, we screened whether lactic acid bacteria (LAB) could express or increase the pharmacological activity of herbal medicines such as *Ginseng radix* and *Puerariae flos* [1, 2, 4], since studies on the fermentation of herbal medicines by intestinal microflora are of great importance to the development of their bioactive agents.

To prepare an extract of SJ-1 fermented with LAB, SJ-1 cultured in Ganghwado and brewed for 2 years was extracted with 80% ethanol, and then concentrated under vacuum. The extract (0.5 g) was suspended in 100 ml of water and incubated for 24 h at 37°C with each lactic acid bacterium (0.2 g as dry weight). The same volume of ethanol was added in the fermented mixture, centrifuged, and then concentrated under vacuum. The extract was used as a fermented SJ-1 agent.

The inhibitory activity of test agents against the release of β-hexosaminidase from RBL-2H3 cells was evaluated according to Choo *et al.* [4]. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging (antioxidant) activity of test agents was measured according to the method of Xiong *et al.* [22]. Assay of LPS-induced NO production in RAW264.7 cells was performed according to the methold of Choo *et al.* [4].

Male and female ICR mice (20–22 g) and male BALB/c mice (18–22 g) were purchased from the Charles River Orient Experimental Animal Breeding Center (Seoul, Korea). All

*Corresponding author
Phone: 82-2-961-0374; Fax: 82-2-957-5030;
E-mail: dhkim@khu.ac.kr

animals were housed in wire cages at 20–22°C, with relative humidity of 50±10% humidity, frequency of air ventilation of 15–20 times/h, and 12 h illumination (07:00–19:00; intensity, 150–300 Lux). They were fed standard laboratory chow (Charles River Orient Experimental Animal Breeding Center, Seoul, Korea) and allowed water *ad libitum*. All procedures related to the animals and their care conformed to the international guidelines *Principles of Laboratory Animals Care* (NIH publication no. 85-23, revised 1985).

An IgE-dependent cutaneous anaphylaxis reaction was measured according to the method of Choo *et al*. [4]. Briefly, male ICR mice were injected intradermally with 10 μg of anti-DNP-HSA IgE (Sigma Co., U.S.A.) into 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 was injected with 200 μl of 3% Evans blue phosphate-buffered saline containing 200 μg of DNP-HSA, *via* the tail vein. The test agents were orally or intraperitoneally administered 1 h prior to DNP-HSA injection. Thirty min after DNP-HSA injection, the mice were sacrificed and their dorsal skins removed for measurement of the pigment area. After extraction with 1 ml of 1.0 N KOH and 4 ml of a mixture of acetone and 0.6 N phosphoric acid (13:5), the amount of dye was determined colorimetrically (absorbance at 620 nm).

A TPA-induced dermatitis was measured according to the method of Reynolds *et al*. [13]. Each group contained 6 male ICR mice (20–25 g). TPA (3 μg/20 μl acetone, Sigma Co., U.S.A.) was applied to the inner and outer ear surfaces of the mouse every day for 3 days to induce skin dermatitis. Test agents, dissolved in an oil-based vehicle, were topically applied to the same site at 1 and 12 h after TPA treatment. The control group received TPA and the vehicle. On the third day, the test agents were treated 1 h after the TPA treatment. The thicknesses of both ears of each mouse were measured using a Digimatic Micrometer (Mitsutoyo Co., Tokyo, Japan) 3 h after final treatment of the test compounds.

The behavioral experiments were performed according to the method of Sugimoto *et al*. [20]. Before the experiment, BALB/c male mice were put into acrylic cages (22×22×24 cm) for about 10 min for acclimation. The rostral part of the skin on the back of the mouse was clipped, and 50 μg/50 μl of compound 48/80 for each mouse was intradermally injected with the use of a 29-gauge needle. The compound 48/80 was dissolved in saline and then used. Control mice received a saline injection in place of the scratching agent. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage to observe for scratching; their behaviors were recorded using an 8-mm video camera (SV-K80, Samsung, Seoul, Korea) under unmanned conditions. Scratching of the injected site by the hind paws was counted and compared with that of other sites, such as the ears. Each mouse was used for only one experiment. Mice generally showed several scratches for 1 s, and a series of these behaviors was counted as one incident of scratching during 60 min. The test agents were orally administered 1 h before the scratching agent.

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

To evaluate the effect of fermented LAB on the antiallergic activity of SJ-1, the inhibitory activity of SJ-1 fermented with and without LAB, which were isolated from human intestinal microflora, on LPS-induced NO production in RAW264.7 cells was measured (Table 1). The SJ-1 fermented with and without LAB all potently inhibited NO production with IC_{50} values of 8–15 μg/ml. The inhibitory activity of SJ-1 was not significantly different from that of fermented SJ-1. However, when SJ-1 was fermented by LAB, its antioxidant activity was significantly increased. When SJ-1 was fermented with *B. infantis* K-525, the antioxidant activity of SJ-1 was most strongly increased.

To evaluate the inhibitory effect of SJ-1 fermented with LAB against degranulation of mast cells and basophils, their inhibitory effect in the β-hexosaminidase release (degranulation) of RBL-2H3 cells induced by IgE was also investigated (Table 1). SJ-1 and LAB-fermented SJ-1 all inhibited the degranulation of RBL-2H3 cells. Among them, SJ-1 fermented with *B. infantis* K-525 most potently inhibited the degranultion of RBL-2H3 cells.

SJ-1 fermented with *B. infantis* K-525 (F-SJ-1) also potently scavenged DPPH-radical. Therefore, to compare the *in vivo* antiallergic activity of SJ-1 with F-SJ-1, we measured

Table 1. Effect of lactic acid bacteria fermentation on the inhibitory effect of SJ-1 on LPS-induced NO production in RAW264.7 cells, IgE-induced β-hexosaminidase release in RBL-2H3 cells, and radical production of DPPH.

Fermented lactic acid bacteria	IC_{50} (μg/ml)		
	NO production	β-Hexosaminidase release	DPPH radical
None	8	51	60
B. magnum K-321	8	52	55
B. minimum K-506	10	43	48
B. infantis K-525	8	42	46
L. brevis II-46	15	43	47

IC_{50} is 50% inhibitory concentration.

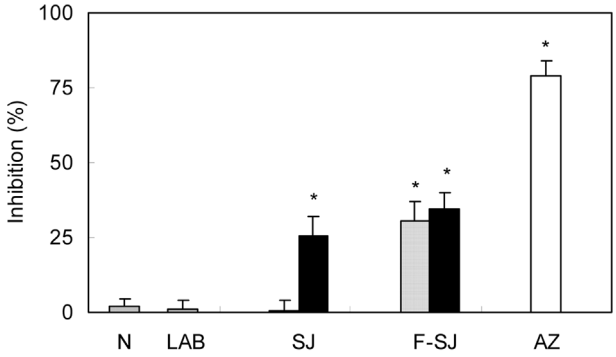

Fig. 1. Inhibitory effect of SJ-1 femented with and without *B. infantis* K-525 on IgE-induced passive cutaneous anaphylaxis in mice.

The SJ-1 was extracted with 80% ethanol and used as an SJ-1 extract (SJ). The extract was fermented with *B. infantis* K-525 for 24 h at 37°C, concentrated, and then used as fermented SJ-1 extract (F-SJ). Each agent [0 (▨), 10 (□), 20 (▩), and 50 (■) mg/kg] was orally administered 1 h prior to DNP-HSA injection. The positive agent was orally administered 10 mg/kg of azelastine (AZ). Normal group (N) was treated with vehicle alone. LAB group was treated with extract of *B. infantis* K-525 alone. All values are mean±SD (n=5). *Significantly different, compared with that of normal control.

the inhibitory effect of SJ-1 and F-SJ-1 on mouse PCA reaction induced by IgE with antigen (Fig. 1). F-SJ-1 more potently inhibited the PCA reaction than SJ-1 did, although both SJ-1 and F-SJ-1 inhibited the PCA reaction.

The anti-inflammatory effect of SJ-1 and F-SJ-1 on TPA-induced mouse ear skin dermatitis was measured (Fig. 2).

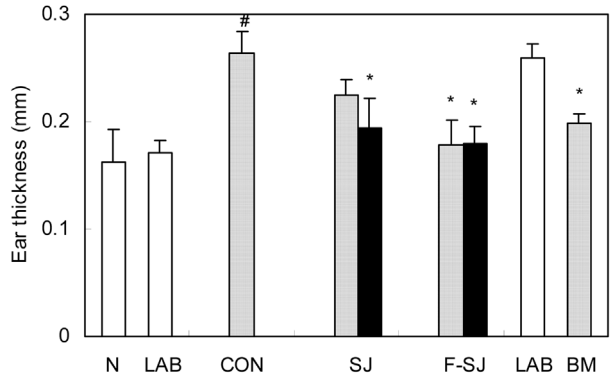

Fig. 2. Inhibitory effect of SJ-1 femented with and without *B. infantis* K-525 in TPA-induced skin dermatitis of mouse ears.

The SJ-1 was extracted with 80% ethanol and used as an SJ-1 extract (SJ). The extract was fermented with *B. infantis* K-525 for 24 h at 37°C, concentrated, and then used as fermented SJ-1 extract (F-SJ). N, treated vehicle alone; CON, treated TPA alone; dotted bar in SJ, treated TPA and 0.005% SJ-1; black bar in SJ, treated TPA and 0.01% SJ-1; dotted bar in F-SJ, treated TPA and 0.005% F-SJ-1; black bar in F-SJ, treated TPA and 0.01% F-SJ-1; white bar in LAB, treated with extract of *B. infantis* K-525 alone; dotted bar in LAB, TPA and LAB; BM, treated TPA and 0.01% betamethasone. All values are mean±SD (n=5). #Significantly different, compared with that of normal control. *Significantly different, compared with that of control.

Both SJ-1 and F-SJ-1 potently inhibited skin dermatitis. F-SJ-1 topically applied at a dose of 0.005% inhibited the PCA reaction by 89%. The inhibitory potency of F-SJ-1 was higher than that of SJ-1, and was comparable to that of betamethasone.

SJ-1 and F-SJ-1 also inhibited compound 48/80-induced scratching behaviors in mice (Fig. 3). F-SJ-1 significantly inhibited the scratching frequency by 51%. These extracts at a dose of 50 mg/kg also decreased the vascular permeability of skin induced by compound 48/80 (data not shown). The inhibitory activities of these extracts against vascular permeability were proportion to their inhibitory effect on scratching behaviors.

Allergic reactions, including rhinitis, asthma, and anaphylaxis, produce many inflammatory mediators and cause scratching, inflammation, pain, and increased vascular permeability [19, 21]. Antihistamines, NSAIDs, steroids and immunosuppressants are representative agents against these allergic diseases [5, 15, 18, 20]. However, repeated application of these agents causes side effects [16]. Therefore, herbal medicines have been used for allergic diseases, and its effectiveness has received increasing attention [3]. SJ-1 cultivated in Ganghwado contains a high content of euphatilin compared with those produced by other places [14], and weakly inhibits allergic reactions. In the present study, when SJ-1 was fermented with LAB, the inhibitory effect of SJ-1 and LAB-fermented SJ-1 against LPS-induced NO

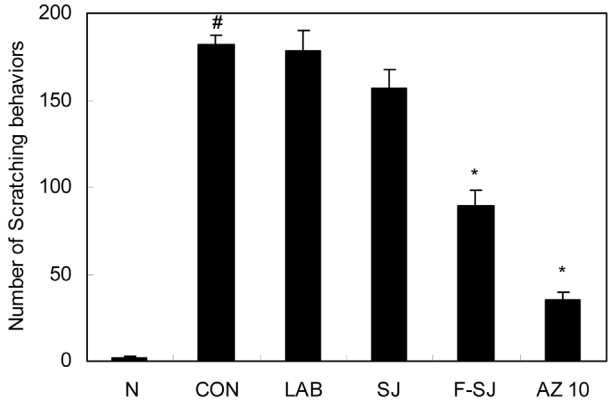

Fig. 3. Inhibitory effect of SJ-1 femented with and without *B. infantis* K-525 on compound 48/80-induced scratching behaviors in mice.

The SJ-1 was extracted with 80% ethanol and used as an SJ-1 extract (SJ). The extract was fermented with *B. infantis* K-525 for 24 h at 37°C, concentrated, and then used as fermented SJ-1 extract (F-SJ). Each extract (50 mg/kg) was orally administered. The positive agent was orally administered 10 mg/kg of azelastine (AZ). The scratching agent compound 48/80 (50 μg/50 μl) for each mouse was intradermally injected 1 h after the administration of test agents. Normal group was treated with vehicle (saline) alone (N). Control group (CON) was with compound 48/80 and vehicle, and LAB group was with compound 48/80 and extract of *B. infantis* K-525. All values are mean±SD (n=5). #Significantly different, compared with that of normal control. *Significantly different, compared with that of control.

production in RAW264.7 cells was not significantly different. However, SJ-1 fermented with LAB (F-SJ-1), particularly *B. infantis* K-525, showed potent inhibition against degranulation of RBL-2H3 cells, and it also inhibited the PCA reaction induced by IgE with antigen, skin dermatitis induced by TPA, and scratching behaviors induced by compound 48/80. These results suggest that the antiallergic activity of SJ-1 can be increased by LAB fermentation. Finally, we believe that F-SJ-1 can improve IgE-induced allergic diseases such as rhinitis and asthma.

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