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Antiallergic effect of ginseng fermented with *Ganoderma lucidum*

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Abstract : Ginseng (the root of *Panax ginseng* CA Meyer, family Araliacease), which is used in Korea, China and Japan as a herbal medicine, was fermented with *Ganoderma lucidum* (GL) and their antiallergic effects were investigated. Of GLs used for fermentation, KCTC 6283 potently produced ginsenoside Rh2, followed by KFRI M101. KCTC 6532, and ginsenoside Rd, followed by KFRI M101. Oral administration of these fermented ginseng extracts inhibited allergic reactions, passive cutaneous anaphylaxis reaction induced by IgE and scratching behaviors induced by compound 48/80. Of them, the ginseng extract fermented by KCTC 6532 and KFRI M101 potently inhibited allergic reactions compared to that fermented by KCTC 6283. These findings suggest that the fermentation of ginseng with GL can increase its antiallergic activity and the increment of its antiallergic effect may be due to the biotransformation of ginseng saponins to ginsenosides Rd and Rh2.

Key words : ginseng, *Ganoderma lucidum*, antiallergic effect, fermentation.

INTRODUCTION

Ginseng (the root of *Panax ginseng* C.A. Meyer, Family Araliaceae) is frequently used as a crude substance, and taken orally in Asian countries as a traditional medicine. The major components of interest in ginseng are ginsenosides, which contain an aglycone with a dammarane skeleton[1,2]. These ginsenosides have been previously reported to show various biological activities, which include anti-inflammatory[3] and anti-tumor activities (i.e., the inhibition of tumor-induced angiogenesis and the prevention of tumor invasion and metastasis)[4,5]. The pharmacological actions of these ginsenosides have been explained on the basis of the biotransformation of ginsenosides by human intestinal microflora[7-10]. For example, the protopanaxadiol ginsenosides are transformed to 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (compound K) by human intestinal bacteria. The transformed compound K induces antimetastatic and/or anticarcinogenic effects by blocking tumor invasion or by preventing chromosomal aberration and tumorigenesis[5,11]. To increase the pharmacological effect of ginseng, the ginsengs fermented by lactic acid bacteria have been developed. However, the studies on the ginsengs fermented by mushrooms, particularly, *Ganoderma lucidum* Karst. (GL, family Polyporaceae), which has been frequently used in traditional Chinese medicine for hepatitis, tumor and immunological disorders[12,13], have not been performed.

Therefore, we fermented ginseng with GLs, and investigated their antiallergic effects.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagles medium (DMEM), fetal bovine serum, dinitrophenol-human serum albumin (DNP-HSA), ovalbumin (OVA), cremophor EL and compound 48/80 were purchased from Sigma Co. (St. Louis, MO, USA).

The mycelia of *G. lucidum* KCTC 6283, KCTC 6532 (from the Biological Resource Center, Daejeon, Chungnam), and KFRI M101 (Korea Food Research Institute, Sungnam, Gyunggi, Korea) were used.

Fermentation of ginseng by GL

Raw ginseng (4 year-old the root of *Panax ginseng* C.A. Meyer, Ansung Ginseng Cooperative Association, Korea)

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were finely washed with distilled water, chopped and dried at 60°C by hot-air dryer. The dried ginseng flakes (100 g) were mixed well with 210 ml of distilled water, and autoclaved in 121°C for 15 min. A seed mycelia disc (20 mm) of *G. lucidum* was inoculated in the autoclaved ginseng media and then incubated in growth chamber for 35 days. The sterilized ginsengs with or without fermentation were then extracted with 100 ml of distilled water twice. The water extracts were extracted with ethyl acetate, and then concentrated in vacuo. These were used as non-fermented and fermented agents.

Extraction of saponin from fermented ginseng and determination of its ginsenoside content

The ginseng samples with and without fermentation were homogenized and freeze-dried. The dried powder (2 g) was extracted with 30 ml of 80% methanol in boiling water for 1 h and filtered through filter paper (Whatman No. 1). Its insoluble residue was extracted with the same solvent. The filtrate was concentrated by a rotary evaporator at 60°C, readjusted with 10 ml of distilled water, and extracted with 10 ml of diethyl ether twice. The aqueous layer was extracted with 10 ml of water-saturated butanol for 4 times. The combined butanol layer was concentrated by a rotary evaporator. The concentrate was dissolved in methanol and used as a saponin fraction.

The content of ginsenosides in the saponin fraction was analyzed by JASCO HPLC system (Jasco system, Japan) consisted of Inteligent UV/VIS detector UV-975, Inteligent HPLC pump PU-980, and column oven CO-965 [column, μ-Bondapak C18 (3.9 × 300 mm, Waters, Irland), mobile phase, distilled water-acetonitrile gradient system [0-30 min, 19% actonitrile; 30-31 min, 19-30% acetonitrile (linear gradient); 31-65 min 30% acetonitrile]; flow rate, 1.5 ml/min; detection, wavelength, 203 nm].

Animals

Male and female ICR mice (20 - 22 g) and male BALB/c mice (18 - 22 g) were supplied from Charles River Orient Experimental Animal Breeding Center (Seoul, Korea). All animals were housed in wire cages at 20-22°C, a relative humidity of 50 ± 10% humidity, a frequency of air ventilation of 15-20 times/h, and 12 h illumination (07:00 -19:00; intensity, 150-300 Lux), fed standard laboratory chow (Charles River Orient 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).

Assay of Passive Cutaneous Anaphylaxis (PCA) Reaction

An IgE-dependent cutaneous reaction was measured according to the previous method of Choo *et al*.[14] The male ICR mice were injected intradermally 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 PBS containing 200 μg of DNP-HSA *via* the tail vein. The test agents were orally administered 1 h prior to DNP-HSA injection. Thirty min after DNP-HSA injection, the mice were sacrificed and their dorsal skins were 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 (the absorbance at 620 nm).

Scratching Behavioral Experiment

The behavioral experiments were performed according to the method of Choo *et al*.[14] The male BALB/c mice were put into acrylic cages (22×22×24cm) for about 10 min for acclimation. The rostral part of the skin on the back of mice was clipped, and 50 μg/50 μl of compound 48/80 for each mouse was intradermally injected with the use a 29 gauge needle. The scratching agent was dissolved in saline and then used. Control mice received a saline injection in the place of the scratching agent. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage and, for the observation of scratching; their behaviors 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. The mice generally showed several scratches for 1 s, and a series of these behaviors was counted as one incident of scratching for 60 min. The test agents were orally administered 1 h before the scratching agent.

Statistics

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

Table 1. The content of ginsenosides in the ginsengs fermented by *G. lucidum* mycelia on HPLC.

	Content (%)							
	Rb2+Rb3	Rc	Rd	Rg3	Rh2	Re	Rg1	Rg2+Rh1
S. ginseng[a)]	3.02	4.12	1.76	0.79	-[b)]	2.50	2.85	0.65
KCTC 6283	1.97	1.29	1.31	0.28	0.05	0.69	1.71	0.65
KCTC 6532	0.56	0.40	7.91	0.73	0.02	1.08	1.29	0.35
KFRI M101	2.52	1.51	6.68	0.67	0.04	2.16	2.56	0.62

[a)]Sterilized ginseng in autoclave.
[b)]Not detected.

Table 2. Inhibitory effect of the ginseng fermented with and without GL on IgE-induced passive cutaneous anaphylaxis in mice.

Agent	Dose (mg/kg)	Inhibition (%)
Medium alone	100	$12\pm6^{a,b}$
Sterilized ginseng flake	100	$20\pm7^{b,c}$
Ginseng fermented by KCTC 6283	20	7 ± 5^{a}
	100	$25\pm9^{b,c}$
Ginseng fermented by KCTC 6532	20	10 ± 7^{a}
	100	$31\pm7^{c,d}$
Ginseng fermented by KFRI M101	20	$12\pm6^{a,b}$
	100	43 ± 5^{d}
Azelastine	10	76 ± 6^{e}

The ginsengs with and without autoclave sterilization were extracted with distilled water and used as ginseng and sterilized ginseng flake extracts. The extract was fermented with *Ganoderma lucidum* (GL) for 35 days at 25°C, concentrated and used as GL-fermented ginseng extract. The positive agent was orally administered 10 mg/kg of azelastine. Each extract (20 mg/kg and 100 mg/kg) was orally administered 1 h prior to DNP-HSA injection. Normal group was treated with vehicle alone. All values are means ± S.D. (n=5). [a,,b,c,d,e]Items with the same letter are not significantly different.

Table 3. Inhibitory effect of the ginsengs fermented with and without GL on compound 48/80-induced scratching behaviors in mice.

	Dose (mg/kg)	Inhibition (%)
Medium alone	100	$13\pm5^{a,b}$
Sterilized ginseng flake	100	$17\pm3^{b,c}$
Ginseng fermented by KCTC 6283	20	9 ± 5^{a}
	100	$29\pm7^{c,d}$
Ginseng fermented by KCTC 6532	20	$12\pm3^{a,b}$
	100	35 ± 3^{d}
Ginseng fermented by KFRI M101	20	11 ± 4^{a}
	100	45 ± 5^{e}
Azelastine	10	47 ± 4^{e}

The ginsengs with and without autoclave sterilization were extracted with distilled water and used as ginseng and sterilized ginseng flake extracts. The extract was fermented with *Ganoderma lucidum* (GL) for 35 days at 25°C, concentrated and used as GL-fermented ginseng extract. Each extract (20 mg/kg and 100 mg/kg) was orally administered. The positive agent was orally administered 10 mg/kg of azelastine. 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 and control group was with compound 48/80 and vehicle. All values are means ± S.D. (n=5). [#]Significantly different from the normal control group ($^{\#}P<0.05$). *Significantly different from compound 48/80 alone stimulated (control) group (*$p<0.05$)

Results and Discussion

To investigate the relationship between antiallergic effect of GL-fermented ginseng and its saponin biotransformation, we fermented ginseng by GL, extracted with BuOH and then analyzed their saponin contents (Table 1). GL transformed ginseng saponins to their hydrophobic metabolites. Of them, KCTC 6532 and KFRI M101 potently produced ginsenoside Rd and KCTC 6283 and KFRI M101 potently produced ginsenoside Rh2. However, instead of the content increment of these ginsenosides, the content of hydrophilic ginsenosides Rb2 and Rc was decreased.

In addition, we investigated the antiallerigc effect of GL-fermented ginsengs in mouse passive cutaneous anaphylaxis reaction induced by IgE-antigen complex (Table 2). Fermented ginsengs potently inhibited the PCA reaction in mice, compared to that of non-fermented ginseng. Of them the ginseng fermented by KFRI M101 most potently inhibited the PCA reaction. However, these inhibitory effects were weak, compared with that of azelastine.

The inhibitory effect of the fermented ginsengs in the compound 48/80-induced scratching behavior mouse model was also investigated (Table 3). Fermented ginsengs also potently inhibited the scratching behaviors, compared to that of non-fermented ginseng. Of them, the ginseng fermented by KFRI M101 at a dose of 100 mg/kg

most potently inhibited the scratching behaviors, with a value of 45% (azelastine at a dose of 10 mg/kg inhibited it by 47%).

Allergic reactions including rhinitis, asthma and anaphylaxis produced many inflammatory mediators and caused scratching, inflammation, pain and increase of vascular permeability[15-17]. Anti-histamines, steroids and immunosuppressants did not only have potent anti-inflammatory effects, but also cause intense side reactions[16,18,19]. Therefore, herbal medicines have been advanced for allergic diseases, and its effectiveness has received increasing attention. Ginseng exhibited the anti-inflammatory and antiallerigc activities. These effects of the ginseng were increased by the fermentation of lactic acid bacteria. The pharmacological effects of many herbal medicines were also increased by intestinal bacteria[20,21]. Based on these findings, we fermented the ginseng by GLs. The GL fermentation of the ginseng more potently inhibited PCA reaction induced by IgE-antigen complex as well as scratching behaviors induced by compound 48/80 than non-fermented one. Of GLs, KFRI M101 most potently increased the antiallergic effect. KFRI M101 potently produced ginsenosides Rh2 and Rd, compared with other GLs. Based on these findings, the fermentation of ginseng with GL can increase its antiallergic effect and its increment may be due to the biotransformation of ginseng saponins to ginsenosides Rd and Rh2.

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