J. Ginseng Res.
Vol. 31, No. 3, 137-141 (2007)

Inhibitory Effects of Red Ginseng on Passive Cutaneous Anaphylaxis and Scratching Behavior Reactions in Mice

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(Received July 2, 2007; Accepted August 31, 2007)

Abtract : To evaluate the antiatopic effect of Korea Red Ginseng (RG, steamed root of *Panax ginseng* C.A. Meyer, Family Araliaceae), its inhibitory effect on passive cutaneous anaphylaxis reaction and itching in mice was measured. RG and its ingredient saponin fraction (SF) potently inhibited passive cutaneous anaphylaxis (PCA) reaction and scratching behaviors. RG at a dose of 100 mg/kg and SF at a dose of 50 mg/kg significantly inhibited the scratching frequency by 32% and 38%, respectively. RG and SF also inhibited the degranulation and protein expression of tumor necrosis factor (TNF)-α and interleukin (IL)-4 of RBL-2H3 cells induced by IgE-antign complex. However, polysaccharide fraction of RG did not inhibit it. Based on these findings, RG can improve allergic skin disorders atopic dermatitis and contact dermatitis by the regulation of TNF-α, and IL-4 produced by mast cells and basophils and their membrane stabilization.

Key words : Red ginseng, scratching behaviors, passive cutaneous anaphylaxis, atopic dermatitis, allergic activity.

INTRODUCTION

Red ginseng (RG, the steamed root of *Panax ginseng* C.A. Meyer, family Araliaceae) is frequently used as a traditional medicine taken orally in Korea, China, Japan and Asian countries. The major components of ginseng are ginsenosides and polysaccharides.[1,2] Many kinds of saponins, such as ginsenosides Rb_1, Rb_2, Rc and Rf, have been isolated. However, RG contains genuine saponins, ginsenosides Rg_3 and Rh_2[3,4]. Ginsenosides Rg_3 and Rh_2 were produced from protopanaxadiol ginsenosides by steaming to prepare RG[5]. These ginsenosides have been reported to show various biological activities including anti-inflammatory activity, antiallergic, endothelium-independent aorta relaxation and anti-tumor effects.[6–9] Particularly, Sugiyama *et al*. reported that ginsenoside Rg_3 suppressed histamine release from mast cells caused due to stimulation with compound 48/80 (condensation product of N-methyl-p-methoxyphenethylamine with formaldehyde) *in vitro*[10]

We also reported the antiallergic and anti-inflammatory effect of RG and ginsenoside Rh_1[11,12], antiallergic and passive cutaneous anaphylaxis reaction (PCA)-inhibitory effects of compound K[13] and antiallergic effect of ginsenoside Rh_2[6]. However, antiatopic effects such as PCA reaction scratching behavior reactions, and its mechanism of RG and its ingredients saponins fraction (SF) and polysaccharide fraction (PF) have not been thoroughly studied.

Therefore, the present study is to investigate the inhibitory effect of RG, SF and PF on passive cutaneous anaphylaxis reaction and itching in mice.

MATERIALS AND METHODS

Materials

p-Nitrophenyl-N-acetyl-β-D-glucosaminide, Freund's complete adjuvant, anti-dinitrophenol (DNP)-IgE, DNP-human serum albumin (HSA), Evans blue, trichloroacetic acid, betamethasone, and azelastine were purchased from Sigma Chemical Co. (U.S.A). RG water extract was donated from KT&G (Korea). SF and PF were isolated according to the previous reported methods[15,16].

Animals

The male ICR mice (20-25 g) were supplied from the Orient Co., Ltd, a branch of Charles River Laboratories (Seoul, Korea). All animals were housed in wire cages,

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maintained at 20-22°C and 50±10% humidity, fed standard laboratory chow (the Orient Co., Ltd), 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) and the Animal Care and Use Guidelines of Kyung Hee University, Korea.

Passive Cutaneous Anaphylaxis (PCA) Reaction

An IgE-dependent cutaneous reaction was measured according to the previous method of Choo *et al.*[13] The male ICR mice (25-30 g) 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 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).

Assay of scratching behavior frequency

The scratching behavioral experiment in male mice was performed according to the method of Sugimoto *et al*.[16] Briefly, the mice were placed in acrylic cages (22×22×24 cm) and allowed to acclimatize for about 10 min. The rostral part of the skin on the back of the mice was clipped, and 300 μg/50 μl of histamine in ICR mice then intradermally injected into each mouse. Immediately after the intradermal injection, the mice (one animal/cage) were placed back in the same cage, and the scratching behavior was recorded using an 8-mm video camera (SV-K80, Samsung, Seoul, Korea). The scratching frequency of the injected site with the hind paws was counted for 60 min. The test agents were orally administered 1 h before the scratching agent.

Assay of inhibitory activity against β-hexosaminidase release of RBL-2H3 cells

The inhibitory activity of test agents against the release of β-hexosaminidase from RBL-2H3 cells was evaluated according to Choo *et al*.[13]. RBL-2H3 cells were grown in Dulbecco's modified Eagle Medium supplemented with 10% fetal bovine serum and L-glutamine. Before the experiment, cells were dispensed into 24-well plates at a concentration of 5×10^5 cells per well, and using a medium containing 0.5 μg/ml of mouse monoclonal IgE, the cells were sensitized by incubation overnight at 37°C in 5% CO_2. They were then washed with 500 ml of Siraganian buffer (pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM PIPES, 40 mM NaOH) and incubated in 160 μl of Siraganian buffer containing 5.6 mM glucose, 1 mM $CaCl_2$ and 0.1% BSA for additional 10 min at 37°C. The cells were exposed to 40 μ*l* of test agents for 20 min, treated with 20 μ*l* of antigen (DNP-HSA, 1 μg/ml) for 10 min at 37°C to activate cells and to evoke allergic reactions. The reaction was stopped by cooling in an ice bath for 10 min. The reaction mixture was centrifuged at 2000 rpm for 10 min and 25 μl aliquots of the supernatant were transferred to 96 well plates and incubated with 25 μl of substrate (1 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide) for 1 h at 37°C. The reaction was stopped by adding 200 μl of 0.1 M Na_2CO_3/ $NaHCO_3$. Absorbance was measured by using an ELISA reader at 405 nm.

Reverse transcription – polymerase chain reaction (RT-PCR)

For isolation of mRNA from RBL-2H cells, the cultured cells were immediately placed in liquid nitrogen and pulverized in mortar. mRNA was extract from the pulverized tissue by using TRI reagent (Cincinnati, Ohio, USA) according to the manufacturer's instructions. The respective primer sets were prepared according to the method of Shin *et al*.[17]. The RT-PCR was performed with AccPower® RT/ PCR Premix (Bioneer, Seoul, Korea). Optimization of cycle number was performed to ensure that production accumulation was in the linear range. Amplified products were separated by electrophoresis on 1.5% agarose gel containing ethidium bromide. The gels were photographed under UV light. The GAPDH gene was used as an internal control. The signal intensity of each RT-PCR product was estimated by Shimazu 9301-PC scanner (Tokyo, Japan).

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.

RESULTS

Inhibition of RG on PCA Reaction

PCA reaction in mice was induced by the intradermal

Table 1. Inhibitory effects of red ginseng and its ingredients on passive cutaneous anaphylaxis reaction in mice.

Agent	Dose (mg/kg)	Inhibition[a)] (%)
RG	100	32.0±6.6[c]
	200	40.9±7.4[c]
SF	25	31.5±4.5[c]
	50	41.3±1.9[c]
PF	25	14.2±2.4[c]
	50	12.7±1.3[c]
Azelastine	10	72.1±5.3[d]

[a]The amounts of extravasated Evan blue from the dorsal skin (1 ×1cm) of the control stimulated with the IgE-antigen complex and vehicle-treated groups were 24±4 and 9±2 µg, respectively. Each experiment consisted of six observations. All inhibitory values indicate mean ± S.D.
[b,c,d]Those with the same letter are not significantly different in each column (P<0.05).

Table 2. Inhibitory effects of red ginseng and its ingredients on the scratching behaviors induced by histamine in mice

Agent	Dose (mg/kg)	Inhibition[a)] (%)
RG	100	32 ± 4.5[d,e]
	200	53 ± 5.6[f]
SF	25	30 ± 5.2[d,e]
	50	38 ± 5.9[e]
PF	25	-[b)]
	50	5 ± 0.4[c]
Azelastine	10	63 ± 12.7[f]

All agents were administered p.o. prior to compound histamine. Each experiment consisted of six observations. All inhibitory values indicate mean ± S.D.
[a]Scratching behavior frequency numbers of normal control, which was treated with saline alone, and control group, which was treated with histamine and saline, for 1 h were 85 ± 5 and 3 ± 2, respectively.
[b]Not determined.
[c,d,e,f]Those with the same letter are not significantly different in each column (P<0.05).

injection of anti-DNP-HSA. And then RG water extract was orally administered 60 min prior to challenge with DNP-HSA antigen and their inhibitory potency of PCA reaction was measured (Table 1). RG extract potently inhibited PCA reaction, and at doses of 100 and 200 mg/kg inhibited PCA reaction by 32 and 40.9%, respectively. Therefore, we isolated the representative ingredients, SF and PF, from RG and measured their inhibitory effect against PCA reaction. The saponin fraction potently inhibited the PCA reaction, but the PF did not exhibit the PCA reaction-inhibitory effect.

Table 3. Inhibitory effects of RG and its ingredients on the β-hexosaminidase release from RBL 2H3 cells induced by IgE-antigen complex.

Agent	IC_{50} (mg/ml)
RG	> 0.2 (32)
SF	0.19
PF	> 0.2 (12)
Azelastine	0.02

RBL-2H3 cells, which were grown in DMEM supplemented with 10% fetal bovine serum and L-glutamine, were dispensed into 24 well plates, at a concentration of 5×10^5 cells per well, and sensitized using 0.5 µg/ml of mouse monoclonal IgE. They were then washed with 500 µl of siraganian buffer, exposed to test agents for 20 min, and treated with 20 µl of antigen (DNP-HSA, 1 µg/ml) for 10 min at 37°C. The release of β-hexosaminidase from RBL-2H3 cells was measured according to the method of Choo *et al.*[18)] The values in parenthesis indicate degranuation-inhibitory percent at a dose of 0.2 mg/ml.

Inhibitory activity of RG and its ingredients on histamine-induced scratching behaviors in mice

To evaluate the antiatopic effect of RG, we measured the inhibitory effects of RG and its ingredients on histamine-induced scratching behaviors in mice. RG and its SF potently inhibited scratching behaviors. However, PF did not inhibit it. RG at a dose of 100 mg/kg and SF at a dose of 50 mg/kg significantly inhibited the scratching frequency by 32% and 38%, respectively. RG and SF also reduced the vascular permeability of skin induced by compound 48/80 (Data not shown). Their inhibitory activities against vascular permeability were proportion to their inhibitions against scratching behaviors.

Inhibitory activity of RG and its ingredients against degranulation of RBL-2H3 cells

To evaluate the inhibitory mechanism of RG against PCA and scratching behavior reactions, their inhibitory effect in β-hexosaminidase release (degranulation) of RBL-2H3 cells induced by IgE-complex was investigated (Table 1). RG and SF all inhibited the degranulation of RBL-2H3 cells. Among them, SF potently inhibited it. However, PF did not inhibit it.

Inhibition of RG and its ingredients on cytokine production of RBL-2H3 cells

The inhibitory effects of RG and its ingredients against protein expression of TNF-α and IL-4 were also measured in RBL-2H3 cells stimulated by IgE-antigen complex by the analysis of RT-PCR analysis (Fig. 1). RG and its ingredients inhibited the mRNA expressions of TNF-α

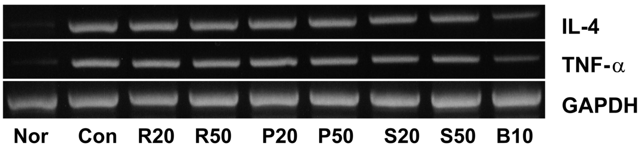

Fig. 1. The effects of RG and its ingredients on the mRNA expression of TNF-α and IL-4 in RBL-2H3 cells induced by the IgE-antigen complex. RBL-2H3 cells (2.5×10^6 cells) were treated with 0.5 μg/mL of mouse monoclonal IgE for 1 h, and then exposed to 0.2 mL of the test agents [N, normal; C, vehicle alone; R20, 20 μg/ml of red ginseng (RG); R50, 50 μg/ml of RG; P20, 50 μg/ml of polysaccharide fraction (PF); P50, 50 μg/ml of PF; S20, 20 μM/ml of saponin fraction (SF); S50, 50 μM/ml of SF; B10, 10 μM betamethasone) for 20 min, followed by treatment with 0.2 mL dinitrophenol-human serum albumin (DNP-HSA, 1 μg/mL) for 1 h at 37°C, and then RT-PCR for TNF-α and IL-4 were performed. Normal (Nor) was treated with vehicle alone instead of agents and IgE-antigen complex.

and IL-4. Of them, SF most potently inhibited the mRNA expressions of TNF-α and IL-4.

DISCUSSION

Allergic diseases such as atopic dermatitis, asthma, allergic rhinitis, and food allergy afflict up to 20% of the human population in most countries[18]. The etiology of allergy reactivity is based on IgE-medicated pharmacological processes of a variety of cell populations such as mast cell and basophils[19]. Degradation of mast cells and basophils with antigen-crosslinked IgE releases histamine, prostaglandins, leukotrins and cytokines affecting lymphocytes, macrophages, eosinophils and neutrophils. Finally cytokine-induced reaction causes tissue damages such as atopic dermatitis. Therefore, antiatopic dermatitis with antiPCA reaction and/or antiscratching behavior actions may be beneficial agents for allergic diseases.

RG and its SF potently inhibited PCA reaction induced by IgE-antigen complex and histamine-induced scratching behaviors. SF inhibited the release of β-hexosaminidase from RBL-2H3 cells. However, the inhibitory effect of SF was weaker than that of the representative constituents, ginsenoside Rh_2, of RG previously reported. This result suggests that RG almost did not contain the active ginsenoside Rh_2. Nevertheless, if RG or its SF is orally administered, its ginsenosides may be metabolized to the ginsenoside Rh_2 by intestinal microflora and express the anti-PCA reaction like the previous reports. RG did not exhibit the significant antihistamine effect against guinea pig ileum[11], although Tachikawa *et al*. reported that ginsenoside Rg_3 weakly inhibited histamine-induced ileum contractions of guinea-pig[10]. The previous studies reported that ginsenoside Rh_1, compound K and ginsenoside Rh_2 showed more potent membrane stabilizing effect than those of disodium cromoglycate[6,7,13]. These results suggest that the inhibitory action of these ginsenosides on the release of β-hexosaminidase may be due to protection of the cytolytic response by antigen-IgE and these ginsenosides after all showed the most potent inhibitory activity on PCA reaction.

RG and its SF also potently inhibited the scratching behaviors (itching) induced by histamine. Iitching, which provokes the desire to scratch, can be local or widespread and associated with atopic dermatitis, uriticaria or systemic disorders (cholestasis, uraemia). Many endogenous chemical agents, like amines, proteases, growth factors, neuropeptides, opioids, ecosanoids and cytokines, can act as pruritogens[19-21]. Scratching can cause skin lesions and contribute to severe psychological disturbances[22], and therefore, inhibition of this response is consistently beneficial for improving the quality of life.

RG, particularly SF, significantly inhibited mRNA expression of proinflammatory TNF-α and IgE-inducing IL-4 in RBL-2H3 cells induced by IgE-antigen complex. These findings suggest that RG can improve allergic skin disorders atopic dermatitis and contact dermatitis by the regulation of TNF-α, and IL-4 produced by mast cells and basophils and their membrane stabilization. Therefore, we believe that RG can show extensive antiatopic and allergic effects and its SF can be a candidate for the therapeutic agent for allergy.

ACKNOWLEGEMENT

This work was supported by the grant from the Korean Society of Ginseng (2006).

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