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

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Abstract: 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-antigen 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 Rb1, Rb2, Rc and Rf, have been isolated. However, RG contains genuine saponins, ginsenosides Rg3 and Rh2. Ginsenosides Rg3 and Rh2 were produced from protopanaxadiol ginsenosides by steaming to prepare RG3). 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 Rg3 suppressed histamine release from mast cells caused due to stimulation with compound 48/80 (condensation product of N-methyl-p-methoxyphenethylamine with formaldehyde) in vitro10).

We also reported the antiallergic and anti-inflammatory effect of RG and ginsenoside Rh211,12), antiallergic and passive cutaneous anaphylaxis reaction (PCA)-inhibitory effects of compound K13) and antiallergic effect of ginsenoside Rh2.6) However, antiallergic effects such as PCA reaction scratching behavior reactions, and its mechanism of RG and its ingredients saponin 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 methods15,16).

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,
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 (22x22x24 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% CO₂. They were then washed with 500 ml of Siraganian buffer (pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM MgCl₂, 25 mM PIPES, 40 mM NaOH) and incubated in 160 µl of Siraganian buffer containing 5.6 mM glucose, 1 mM CaCl₂ and 0.1% BSA for additional 10 min at 37°C. The cells were exposed to 40 µl of test agents for 20 min, treated with 20 µl 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 Na₂CO₃/NaHCO₃. 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 extracted 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...