Antiallergic Activity of Ginsenoside Rh2

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The antiallergic activities of ginsenosides, which were isolated from acid-treated ginseng (*Panax ginseng, Araliaceae*), and their metabolites by human intestinal bacteria were measured. Ginsenoside Rh2, which is a main metabolite, had the most potent inhibitory activity on β-hexosaminidase release from RBL-2H3 cells and in the passive cutaneous anaphylaxis reaction. The inhibitory activity of ginsenoside Rh2 was more potent than that of disodium cromoglycate, a commercial antiallergic drug. This compound showed membrane stabilizing action upon differential scanning calorimetry and inhibited nitric oxide (NO) and prostaglandin E2 (PGE2) in lipopolysaccharide-stimulated RAW cells. However, this ginsenoside Rh2 did not inhibit the activation of hyaluronidase and did not scavenge active oxygen. These results suggest that ginsenoside Rh2 can exhibit antiallergic activity originating from cell membrane-stabilizing activity and antiinflammatory activity by the inhibition of NO and PGE2 production.

KEYWORDS ginsenoside Rg3; ginsenoside Rh2; antiallergic activity; *Panax ginseng*; intestinal bacteria

Ginseng (the root of *Panax ginseng* C.A. MEYER, family Araliaceae) is frequently used as a crude substance taken orally in Asian countries as a traditional medicine. The major components of ginseng are ginsenosides, which contain an aglycone with a dammarane skeleton.2-4 These ginsenosides have been reported to show various biological activities including antiinflammatory activity5 and antitumor effects (inhibition of tumor-induced angiogenesis and the prevention of tumor invasion and metastasis).5-8 The pharmacological actions of these ginsenosides have been explained by the biotransformation of ginsenoside Rb1, Rb2, and Rc from ginseng by human intestinal bacteria.6-10 For example, protopanaxadiol ginsenosides are transformed to 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (compound K) by human intestinal bacteria. This transformed compound K induces an antimitotic or antinecrotic effect by blocking tumor invasion or preventing chromosomal aberration and tumorigenesis.7,11 Ginsenoside Rg3, which is a main component of Ginseng Radix Rubra (red ginseng),12 is metabolized to ginsenoside Rh2 by human intestinal bacteria.10 This transformed ginsenoside Rg3 also shows antitumor activity.13,14

In addition, Sugiyama et al. reported that ginsenoside Rg3 suppresses histamine release from mast cells due to stimulation with compound 48/80 in vitro,15 and Ro et al. reported that ginsenoside Rb1 and Rc also partly inhibited the release of histamine and leukotrienes during the activation of guinea pig lung mast cells in vitro.16 However, the antiallergic effects of ginsenoside Rh2 have not been studied. We therefore isolated ginsenoside Rg3 from red ginseng, transformed it to ginsenoside Rh2, by human intestinal bacteria, isolated ginsenoside Rh2 and measured antiallergic activities.

MATERIALS AND METHODS

**Materials** Hyaluronidase from bovine testis, p-nitrophenyl-N-acetyl-β-D-glucosaminide, Freund’s complete adjuvant, anti-dinitrophenol (DNP)-IgE, DNP-human serum albumin (HSA), Evans blue dye, disodium cromoglycate (DSCG), hyaluronic acid potassium salts, α-phthalaldehyde, trichloroacetic acid, N2-monomethyl-L-arginine (L-NMMA), dexamethasone, azelastine, allopurinol, caffeine, acid, and histamine·2HCl were purchased from Sigma Chemical Co. (U.S.A.). Griess reagent was purchased from Promega Co. (U.S.A.). The prostaglandin E2 (PGE2) EIA kit was purchased from Cayman Chemical.

Ginsenoside Rb1, ginsenoside Rg3, and Δ20-ginsenoside Rg3 were isolated from acid-treated ginseng (AG), and ginsenoside Rh2 and Δ20-ginsenoside Rh2 were from fermented AG according to our previous method.10 RAW 264.7 cells (murine macrophages) and RBL 2H3 cells (rat basophils) were obtained from the Korean Cell Line Bank.

**Assay of Antiallergic Activity in RBL 2H3 Cell Line**

The inhibitory activity of ginsenosides against the release of β-hexosaminidase from RBL-2H3 cells was evaluated according to the method of Choi et al.17 RBL-2H3 cells were grown in Dulbecos modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and L-glutamine and split every 3 d. Before the experiment, cells were dispersed into 24-well plates at a concentration of 5×10⁵ cells/well, using the medium containing 0.5 μg/ml of mouse monoclonal IgE and were incubated overnight at 37 °C in 5% CO2 for sensitization of cells. The cells were washed with 500 μl of siraganian buffer (pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM MgCl2, 25 mM piperazine-N,N’-bis(2-ethanesulfonic acid), 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. Then cells were exposed to 40 μl of test agents for 20 min, followed by treatment with 20 μl of antigen (DNP-HSA, 1 μg/ml) for 10 min at 37 °C to activate cells to evoke allergic reactions (degranulations). The reaction was stopped by cooling in an ice bath for 10 min. The reaction mixture was centrifuged at 800×g for 10 min and 25-μl aliquots of 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. The absorbance was measured with an ELISA reader at 405 nm.

**Nitric Oxide Analysis**

Nitric oxide (NO) was determined by measuring the amount of nitrite from cell culture supernatant using the Griess reagent according to the manu-
facturers protocol. RAW 264.7 cells were stimulated with lipopolysaccharide (LPS) (1 µg/ml) and test agents for 24 h. Cells were briefly centrifuged and 150 µl of cell culture supernatant was mixed with 150 µl of Griess reagent and incubated 10 min at room temperature (light protected). The absorbance was measured using an ELISA reader at 540 nm against a calibration curve with sodium nitrate as a standard. 

**Assay of PGE2** The RAW 264.7 cells were seeded at 5 × 10^4 cells/well in flat-bottomed 96-well plates. The test agents and LPS (1 µg/ml) were added to the culture medium and incubated at 37 °C for 20 h. The medium was collected in a microfuge tube and centrifuged at 1800 × g for 10 min. The supernatant was decanted into a new microfuge tube, and the amount of PGE2 determined using a PGE2 Enzyme-Immuno-Assay kit (Cayman Chemical). 

**Assay of Hyaluronidase Activity** Hyaluronidase activity was determined according to the method of Kakegawa et al. 

**Assay of Antioxidant Activity** 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and xanthine oxidase (XOD)-inhibitory activities of test agents isolated were measured according to the method of Xiông et al. Superoxide anion radical generation of compounds isolated was measured according to the method of Xiông et al. 

**Differential Scanning Calorimetry** Differential scanning calorimetry was performed according to a previously described method. Briefly, either 200 µl of salt buffer (SB: 154 mM NaCl, 2.7 mM KCl, 5.6 mM glucose, 5 mM HEPES, pH 7.4) alone or SB containing one of the test compounds and 5 mg of dipalmitoylphosphatidylcholine (DPPC) were sonicated for 1 min at 45°C. Twenty microliters of this dispersion was sealed in an aluminum sample pan and then scanned in a DSC-2010 (TA Instruments, U.S.A.) located at the Korea Basic Science Institute. 

**Animals** Male Hartley guinea pigs (250 g) and male ICR mice (20—22 g) were supplied by Daehan Experimental Animal Breeding Center. All animals were housed in wire cages and fed with standard laboratory chow (Samyang Feed Production Co.) and water ad libitum. 

**Passive Cutaneous Anaphylaxis Reaction** The IgE-dependent passive cutaneous anaphylaxis reaction was measured according to the previous method of Katayama et al. 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 after each mouse received an injection of 200 µl of 3% Evans blue in PBS containing 200 µg of DNP-HSA via the tail vein. The test agents were administered 1 h prior to DNP-HSA injection. Thirty minutes after DNP-HSA injection, the mice were killed and their dorsal skins were removed for measurement of the pigmented 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). 

**Antihistamine Activity** Male Hartley guinea pigs (350 ± 30 g) were killed by exsanguination and the ilea were prepared in cold Tyrode solution. The ileal strip prepared was then suspended in a 10 ml Magnus tube (32°C, 95% O2 + 5% CO2) containing the Tyrode solution. Each test agent was added to the preparation 30 s before the treatment of histamine (1 × 10^-6 M). The percent contraction was shown as a percent of the maximal response to histamine. 

**RESULTS**

**Effects of Ginsenoside Rg3 and Rh2 on β-Hexosaminidase Release from RBL-2H3 Cells** To investigate whether the ginsenosides isolated from acid-treated ginseng, of which the components are similar to those of red ginseng, possess antiallergic activity, we examined the inhibitory effects of ginsenoside Rg3, A^20^-ginsenoside Rg3, and their metabolites by human intestinal bacteria on β-hexosaminidase release from RBL-2H3 cells induced by IgE (Table 1). Among the compounds tested, ginsenoside Rh2 had the strongest inhibitory activity on the β-hexosaminidase release induced by IgE and was stronger than DSCG. However, ginsenoside Rb1, ginsenoside Rg3 and A^20^-ginsenoside Rg3 exhibited little inhibitory activity. However, their metabolites, ginsenoside Rh2 and A^20^-ginsenoside Rh2 showed potent inhibitory activity. β-Hexosaminidase release induced by antigen (DNP-HSA) was 45.2% for the cell content of 2 × 10^4 cells/well. Spontaneous β-hexosaminidase release was 3.2% of the cell contents. Ginsenoside Rh2 showed the strongest inhibitory activity, with an IC_{50} value of 0.1 mM. 

**Effects of Ginseng and Ginsenosides on PCA Reaction** To determine the inhibitory effect of ginsenoside Rg3, A^20^-ginsenoside Rg3, and their metabolites on the PCA reaction in mice, these compounds were administered orally or intraperitoneally 60 min prior to challenge with antigen (Table 2). Ginsenosides inhibited the PCA reaction in mice. Among the compounds tested, ginsenoside Rh2 administered intraperitoneally showed the strongest inhibitory activity and significantly inhibited PCA at a dose of 25 mg/kg with in-
hibitory activity of 88±8.2%. Ginsenoside Rh2 inhibited the PCA reaction even if it was administered orally, and exhibited effects stronger than those of DSCG.

**Hyaluronidase-Inhibitory, Active Oxygen-Scavenging, and Membrane-Stabilizing Activities** When the inhibitory effects of ginsenoside Rg3, Δ20-ginsenoside Rg3, and their metabolites on the activation of hyaluronidase were investigated, they did not inhibit the activation of hyaluronidase. Even when the active oxygen-scavenging activity of these compounds was examined, they did not show any activity (Table 3).

In addition, when the murine macrophage cell line RAW 264.7 was stimulated with LPS for 24 h, the nitrite content in the media significantly increased. However, when the cells were treated with 1.0 to 100 μM of ginsenosides, the nitrite content was significantly reduced in a dose-dependent manner. The ginsenoside Rh2 was the most potent: its IC50 value was 32 μM, while that of L-NMMA was most potent at 37 μM. The ginsenoside Rh2 potently inhibited PGE2 production in LPS-stimulated RAW264.7 cells.

We measured the phase transition temperature for the liposomes prepared by DPPC with and without ginsenoside Rh2 (Fig. 1). Heat absorption of the liposomes prepared with DPPC alone occurred at 41.26 °C (phase transition temperature). However, when the liposomes were prepared by adding ginsenoside Rh2, the transition was initiated at lower temperatures and the peak appearance became weaker and broader in a dose-dependent manner. A similar effect was observed with the addition of DSCG, a commercial agent, but was weak compared with that of ginsenoside Rh2.

**DISCUSSION**

Allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, and food allergy afflict up to 20% of the human population in most countries.23) The etiology of allergen reactivity is based on IgE-mediated pharmacologic processes in a variety of cell populations such as mast cells and basophils.24) Degranulation of mast cells and basophils with antigen cross-linked IgE releases histamine, prostaglandins, leukotrienes, and cytokines affecting lymphocytes, macrophages, eosinophils, and neutrophils. Finally cytokine-induced reaction causes tissue injury. Therefore antiallergic agents with antiinflammatory actions may be beneficial drugs for allergic diseases.

DSCG and azelastine are representative antiallergic drugs.25,26) DSCG is an inhibitor of hyaluronidase as well as a membrane stabilizer. DSCG is mainly known to inhibit the release of chemical mediators from mast cells induced by the anigen-IgE antibody reaction, but weakly inhibits the chemical mediator from RBL-2H3 cells. Azelastine is an H1-receptor antagonist, but also decreases mediator release from mast cells and basophils.26,27) We also confirmed that DSCG weakly inhibits the release of β-hexosaminidase from RBL-2H3 cells during the present investigation. Ginsenoside Rh1 and Rg3 did not inhibit the release of β-hexosaminidase by RBL 2H3 cells induced by IgE. However, ginsenoside Rh2, which is a metabolite of ginsenoside Rg3, potently inhibits the release of β-hexosaminidase by human intestinal bacteria10) from RBL-2H3 cells. Ginsenoside Rh2 and the other ginsenosides tested neither inhibited the activation of hyaluronidase, nor scavenged the superoxide anion, although some antiallergic agents were inhibitors of hyaluronidase and scavengers of active oxygen species.

In previous reports, compound 48/80 and antigen-IgE increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane.26,27) One of the

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### Table 3. Inhibitory Effects of Ginsenosides on Hyaluronidase and NO Production by RAW 264.7 Cells and Their Active Oxygen-Scavenging Activities

<table>
<thead>
<tr>
<th>Agent</th>
<th>Hyaluronidase</th>
<th>DPPH</th>
<th>Superoxide anion</th>
<th>XOD</th>
<th>NO production</th>
<th>PGE2 production</th>
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<tr>
<td>Ginsenoside Rb1</td>
<td>&gt;0.0</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
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<td>&gt;0.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
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<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>0.082</td>
<td>&gt;0.05</td>
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<tr>
<td>Δ20-Ginsenoside Rg3</td>
<td>&gt;0.0</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ginsenoside Rh2</td>
<td>&gt;0.0</td>
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<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>0.032</td>
<td>0.008</td>
</tr>
<tr>
<td>Δ20-Ginsenoside Rh2</td>
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<td>&gt;0.2</td>
<td>&gt;0.2</td>
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<td>0.003</td>
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</table>

a) Not determined.

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**Fig. 1. Calorimetric Scans of the Liposomes Prepared with DPPC Containing Ginsenoside Rh2 and DSCG (Heating Rate 5 °C/min)**

DPPC, dipalmitoylphosphatidylcholine; DSCG, disodium cromoglycate.
mechanisms of these antiallergic compounds might be to act on the lipid bilayer membrane affecting the prevention of the perturbation. Therefore we measured the phase transition temperature of the liposomes prepared using ginsenoside Rh2. The membrane-stabilizing effects of ginsenoside Rh2 were stronger than those of DSCG, a commercial agent. These results suggest that the inhibitory action of ginsenoside Rh2 against the release of β-hexosaminidase may be due to protection of the cytolytic response by antigen-IgE. Ginsenoside Rh2 also exhibited the most potent inhibitory activity against the PCA reaction. However, the other ginsenosides weakly inhibited it. In addition, when we measured the antagonistic activity of ginsenoside Rh2 against histamine, which is released from mast cells in the pathologic process of type I allergy action, using guinea pig ileum, ginsenoside Rh2 did not cause a significant antihistamine action, although Tachikawa et al. reported that ginsenoside Rg3 weakly inhibited histamine-induced ileum contractions of guinea pig.20) These results suggest that the antiallergic action of ginsenoside Rh2 originates from the potent cell membrane-stabilizing activity.

Cytokine-activated macrophages synthesize various mediators that modulate the inflammatory response. NO and prostaglandins are two pleiotropic mediators produced at inflammatory sites.29) Stimuli such as cytokines and/or bacterial LPS induce iNOS and COX-2 protein expression. Despite its beneficial role in host defense, sustained NO and PGE2 production can be deleterious to the host, and has been implicated in the pathogenesis of various inflammatory diseases.10) Ginsenoside Rh2 significantly inhibited NO and PGE2 productions. These results suggest that ginsenoside Rh2 may show antiinflammatory activity.

When ginsenoside Rg3 was incubated with human intestinal microflora, it was easily metabolized to ginsenoside Rh2,10) which should be responsible for the inhibition of the PCA reaction. Therefore we believe that ginsenoside Rg3 in red ginseng is prodrugs that has extensive antiallergic and antiinflammatory properties and that ginsenoside Rh2 is a candidate therapeutic agent for the treatment of allergy.

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REFERENCES