

Ginsenoside Rh1 Possesses Antiallergic and Anti-Inflammatory Activities

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Key Words

Ginsenoside Rh1 · *Panax ginseng* · Antiallergic activity · Inflammation · iNOS · COX-2

Abstract

Background: Ginseng (the root of *Panax ginseng* C.A. Meyer, Araliaceae) has been reported to possess various biological activities, including anti-inflammatory and antitumor actions. In this study, we investigated the anti-allergic activity of ginsenosides isolated from ginseng.

Method: We isolated ginsenosides by silica gel column chromatography and examined their in vitro and in vivo antiallergic effect on rat peritoneal mast cells and on IgE-induced passive cutaneous anaphylaxis (PCA) in mice. The in vitro anti-inflammatory activity of ginsenoside Rh1 (Rh1) in RAW264.7 cells was investigated. **Results:** Rh1 potently inhibited histamine release from rat peritoneal mast cells and the IgE-mediated PCA reaction in mice. The inhibitory activity of Rh1 (87% inhibition at 25 mg/kg) on the PCA reaction was found to be more potent than that of disodium cromoglycate (31% inhibition at 25 mg/kg); Rh1 was also found to have a membrane-stabilizing action as revealed by differential scanning calorimetry. It also inhibited inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression in RAW 264.7 cells, and the activation of the transcription factor, NF- κ B, in nuclear fractions. **Conclu-**

sion: The antiallergic action of Rh1 may originate from its cell membrane-stabilizing and anti-inflammatory activities, and can improve the inflammation caused by allergies.

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Introduction

Ginseng (the root of *Panax ginseng* C.A. Meyer, Araliaceae) is taken orally as a traditional medicine in Asian countries. The major components of ginseng are ginsenosides, which contain an aglycone with a dammarane skeleton [1, 2]. These ginsenosides have been reported to exhibit various biological activities, including anti-inflammatory (adjuvant) [3] and antitumor effects (inhibition of tumor-induced angiogenesis and the prevention of tumor invasion and metastasis) [4, 5]. The pharmacological actions of these ginsenosides have been explained by their biotransformation by human intestinal bacteria [6–8]. For example, protopanaxadiol ginsenosides are transformed to 20-O- β -D-glucopyranosyl-20(S)-protopanaxadiol (compound K) by human intestinal bacteria. This transformed compound induces an antimetastatic or anticarcinogenic effect by blocking tumor invasion or preventing chromosomal aberration and tumorigenesis [5]. However, few studies on antiallergic effects of ginsenosides have been reported.

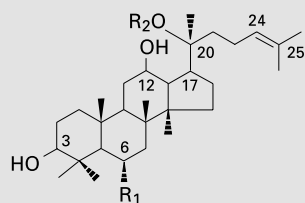
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Ginsenosides	R ₁	R ₂
Protopanaxatriol	OH	H
Rh1	Glc-O-	H
Rg1	Glc-O-	Glc-
Re	Rha-Glc-O-	Glc-

Fig. 1. Structure of protopanaxatriol ginseng saponins.

Therefore the antiallergic and anti-inflammatory activity of protopanaxatriol ginseng saponins isolated from ginseng were measured.

Materials and Methods

Materials

The hyaluronidase from bovine testis, compound 48/80, egg albumin, *p*-nitrophenyl-N-acetyl- β -D-glucosaminide, anti-dinitrophenyl (DNP)-IgE, DNP-human serum albumin (HSA), Evans blue, disodium cromoglycate (DSCG), hyaluronic acid potassium salts, *o*-phthalaldehyde, trichloroacetic acid, histamine $\cdot$ 2HCl, N^G-monomethyl-L-arginine (*L*-NMMA), lipopolysaccharide (LPS) and indomethacin were all purchased from Sigma Chemical Co. (USA). Griess reagent was purchased from Promega Co. (USA). Anti-rabbit inducible nitric oxide synthase (iNOS), anti-goat cyclooxygenase-2 (COX-2), anti-rabbit nuclear factor- κ B (NF- κ B) and anti-mouse β -actin antibodies were purchased from Santa Cruz Biotechnology (USA). The prostaglandin E₂ (PGE₂) EIA kit and COX (ovine) Inhibitor Screening Assay kits were purchased from Cayman Chemical. RAW 264.7 cells were obtained from the Korean Cell Line Bank. Ginsenoside Re (Re), ginsenoside Rg1 (Rg1) and ginsenoside Rh1 (Rh1), and protopanaxatriol were isolated from ginseng (fig. 1), according to a previously published method [9].

Animals

Male Hartley guinea pigs, SD rats and ICR mice were supplied from Daehan experimental animal breeding center. All animals were housed in wire cages at 20–22°C and 50 $\pm$ 10% humidity, fed standard laboratory chow (Samyang Feed Production Co.) and allowed water ad libitum. All procedures relating to animals and their care conformed to the international guidelines *Principles of Laboratory Animals Care* (NIH publication No. 85-23, revised 1985) and *Guiding Principles for the Care and Use of Laboratory Animals* of Kyung Hee University, Korea.

Passive Cutaneous Anaphylaxis Reactions

The IgE-dependent passive cutaneous anaphylaxis (PCA) reactions were measured according to the method of Katayama et al. [10]. Male ICR mice (25–30 g) were injected intradermally with 10 μ g 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 μ l of 3% Evans blue PBS containing 200 μ g of DNP-HSA via the tail vein. Ginsenosides or DSCG were administered 1 h prior to the DNP-HSA injection. Thirty minutes after the 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).

Anti-Histamine Action of Ginsenosides

The male Hartley guinea pigs (300 $\pm$ 30 g) were sacrificed by exsanguination. Ileal strips were prepared and suspended in a 10-ml magnus tube (32°C, 95% O₂ + 5% CO₂) containing Tyrode solution. Each ginsenoside was added to the preparation 30 s before treatment with histamine (1 $\times$ 10⁻⁶ M). Contraction was calculated as a percentage of the maximal response to histamine.

Assay of Histamine Release from Rat Peritoneal Exudate Cells Induced by Compound 48/80

The male Wistar rats (200 $\pm$ 20 g) were exsanguinated and injected intraperitoneally with 20 ml of physiological solution (137 mM NaCl, 2.7 mM CaCl₂, 1.0 mM MgCl₂ $\cdot$ 6H₂O, 5.6 mM glucose, 1 unit of heparin/ml and 5 mM phosphate buffer at pH 7.2). The abdominal region was gently massaged for 2 min, and the peritoneal exudate cells collected. The cell suspension was centrifuged at 300 g and 4°C for 5 min, and washed several times with physiological solution. The cells obtained from 5 animals were then pooled (5 $\times$ 10⁴/ml cells). The peritoneal exudate cell suspension (2.5 ml) was mixed with various concentrations of ginsenosides or DSCG in 0.5 ml of physiological solution each, and the mixtures preincubated at 37°C for 5 min. The controls and blanks were treated in the same manner. The reaction mixture (3.0 ml) was then mixed with 0.5 ml of compound 48/80, cooled to 4°C and centrifuged at 2,500 g and 4°C for 10 min. The histamine released into the supernatant and residue was measured according to the method of Shore et al. [11].

Nitric Oxide Analysis

Nitric oxide (NO) was determined by measuring the amount of nitrite in the cell culture supernatant using Griess reagent according to the manufacturer's protocol [12]. The RAW 264.7 cells were stimulated with LPS (1 μ g/ml) and ginsenosides for 24 h, then briefly centrifuged, and 150 μ l of the cell culture supernatant was mixed with 150 μ l of Griess reagent, and incubated for 10 min at room temperature (light protected). The absorbance was measured using an ELISA reader at 540 nm and compared with a standard calibration curve prepared from sodium nitrite [13].

Measurement of iNOS Enzyme Activity

The RAW 264.7 cells were stimulated with LPS (1 μ g/ml) for 16 h and washed twice with PBS. They were then incubated with ginsenosides or *L*-NMMA for 16 h. Cells were briefly centrifuged, and 150 μ l of the supernatant mixed with 150 μ l of Griess reagent and incubated for 10 min at room temperature (light protected). The absorbance

was measured using an ELISA reader at 540 nm, and compared with a standard calibration curve prepared from sodium nitrite [13].

Measurement of COX Enzyme Activity

The COX activities of the purified enzymes were determined using the COX inhibitor screening assay kit (Cayman Chemical). In brief, ovine COX-1, or COX-2, were preincubated with and without various concentrations of the test sample in 0.1 M Tris-HCl (pH 8.0), containing 5 mM EDTA, 2 mM phenol and 1 μ M heme, at 37 °C for 2 min. The reactions were initiated by adding arachidonic acid to a final concentration of 100 μ M, and incubated at 37 °C for 2 min. One twentieth the volume of 1 M HCl was added to the reaction mixtures to stop enzyme catalysis, followed by one tenth the volume of saturated stannous chloride (50 mg/ml). The reaction mixtures were incubated for 5 min at room temperature, and then the amount of PGE₂ formed during the reaction was measured by an enzyme immunoassay.

Assay of PGE₂

The RAW 264.7 cells were seeded at 5×10^4 cells per well in flat-bottomed 96-well plates. The ginsenosides, or indomethacin, and LPS (1 μ g/ml) were added to the culture medium, and incubated at 37 °C for 20 h. The medium was collected in a microcentrifuge tube and centrifuged at 2,800 g for 10 min. The supernatant was decanted into a new microcentrifuge tube, and the amount of PGE₂ determined using a PGE₂ Enzyme-Immuno-Assay kit (Cayman Chemical).

Western Blot Analysis of iNOS, COX-2 and NF- κ B

Western blot analyses of the iNOS, COX-2 and NF- κ B were performed according to the method of Ishihara et al. [13]. The RAW 264.7 cells were plated in 60-mm culture dishes (3×10^6 cells), the ginsenosides, or L-NMMA, and LPS (1 μ g/ml) added to the culture medium, and the cells incubated at 37 °C for 6–20 h. The cells were lysed on ice for 15 min in a hypotonic buffer, containing 10 mM Tris (pH 8.0), 1.5 mM MgCl₂, 1 mM DTT, 0.1% NP-40, 5 μ g/ml pepstatin A and 5 μ g/ml aprotinin, and centrifuged at 12,000 g and 4 °C for 15 min. The supernatant was used as the cytosol fraction for the Western blot assays of iNOS and COX-2 protein expression. The pelleted nuclear fractions for the Western blot assays of NF- κ B protein expression were resuspended in the extraction buffer, containing 10 mM Tris (pH 8.0), 50 mM KCl, 300 mM NaCl, 1 mM DTT, 5 μ g/ml pepstatin A and 5 μ g/ml aprotinin, and then lysed on ice for 30 min. The lysed nuclear fraction was centrifuged at 12,000 g and 4 °C for 30 min. The cell lysates (40 μ g) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions, and transferred to polyvinylidene difluoride membranes (Millipore, UK) at 30 V for 2 h. The membranes were blocked with 2% skim milk in PBS, containing 0.05% Tween 20, and incubated for 2 h at room temperature, and for a further 2 h with anti-iNOS, anti-COX-2, anti-NF- κ B antibodies (Santa Cruz) as primary antibodies. After removing the primary antibodies, the membranes were washed three times with PBS containing 0.05% Tween 20 and then incubated with a 1: 2,000 dilution of the secondary antibody conjugated with horseradish peroxidase for 1 h. The membranes were washed three times, and the reaction products visualized using an enhanced chemiluminescence Western blot system (Pierce).

Assay of Hyaluronidase Activity

Hyaluronidase activity was determined according to the method of Kagekawa et al. [14].

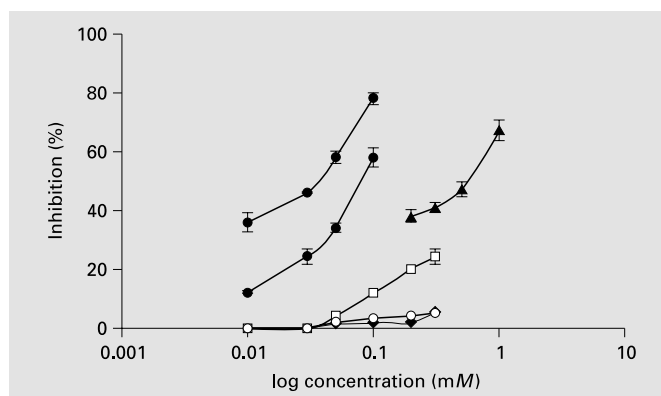


Fig. 2. Inhibitory effect of ginsenosides on the histamine released from rat peritoneal mast cells by compound 48/80. $\blacklozenge$ = Re; $\square$ = Rg1; $\bullet$ = Rh1; $\circ$ = protopanaxatriol; $\blacktriangle$ = DSCG; $\blacksquare$ = azelastine. Histamine release (%) induced by compound 48/80 was $82.7 \pm 4.4\%$. All values are means $\pm$ SD from three experiments. Inhibition (%) = (histamine release induced by compound 48/80 – histamine release induced by compound 48/80 and agent)/(histamine release (%) induced by compound 48/80) $\times$ 100.

Assay of Antioxidant Activity

1,1-Diphenyl-2-picrylhydrazyl radical scavenging, superoxide anion radical generation and xanthine oxidase-inhibitory activities of test compounds were measured according to the method of Xiong et al. [15].

Differential Scanning Calorimetry

The differential scanning calorimetry assay was performed according to the method of Tasaka et al. [16]. Either 200 μ l of salt buffer (154 mM NaCl, 2.7 mM KCl, 5.6 mM glucose and 5 mM HEPES, pH 7.4) alone, or salt buffer containing one of the ginsenosides (or DSCG) and 5 mg of dipalmitoylphosphatidylcholine (DPPC) was sonicated for 1 min above 45 °C. Twenty microliters of this dispersion was sealed in an aluminum sample pan, and a calorimetric scan was performed on a DSC-2010 apparatus (TA Instruments, USA), at the Korea Basic Science Institute.

Statistical Analysis

All the data from the in vivo experiments were expressed as the mean $\pm$ standard deviation; the statistical significance was determined by Student's t test.

Results

Inhibitory Effect of Ginsenosides on Histamine Release from Rat Peritoneal Mast Cells

To investigate whether ginsenosides possess antiallergic activity, we examined their inhibitory effects on histamine released from rat peritoneal exudate cells following addition of compound 48/80 (fig. 2). Among the sub-

Table 1. Inhibitory effect of ginsenosides and DSCG on PCA reaction

Group	Dose mg/kg	Inhibition, %	
		p.o.	i.p.
Re	25	— ^a	15 ± 3.8 ^b
Rg1	25	—	37 ± 6.7 ^c
Rh1	25	79 ± 2.6 ^b	87 ± 5.1 ^c
Protopanaxatriol	25	—	25 ± 4.5 ^{b, c}
Azelastine	25	—	89 ± 4.9 ^e
DSCG	25	—	31 ± 3.9 ^b
	100	37 ± 0.2 ^c	45 ± 2.6 ^c

All agents were administered per os (p.o.) or intraperitoneally (i.p.) prior to challenge with antigen. Values are expressed as means ± SD (n = 5).

^a Not determined.

^{b–e} Those with the same letter are not significantly different in each column (p < 0.05).

Table 2. Inhibitory effects and oxygen-scavenging activities of ginsenosides on hyaluronidase activity

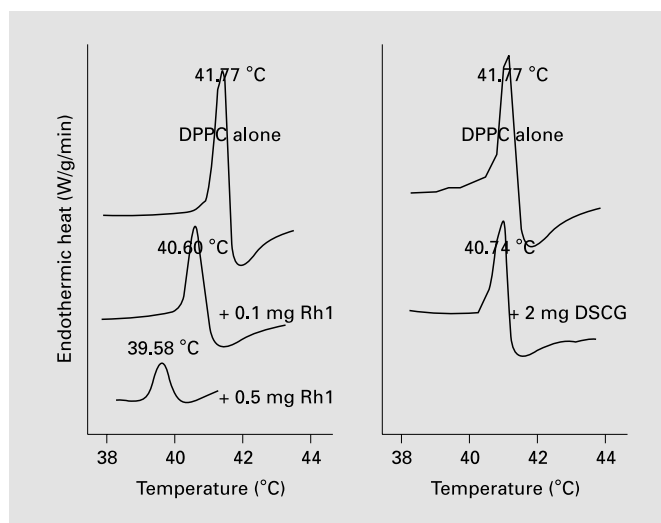
Agent	IC ₅₀ , mM			
	hyaluron- idase	DPPH	superoxide anion	xanthine oxidase
Re	>0.2	>0.2	0.14	>0.2
Rg1	>0.2	>0.2	>0.2	>0.2
Rh1	>0.2	>0.2	>0.2	>0.2
20(S)-Protopanaxatriol	>0.2	>0.2	>0.2	>0.2
Caffeic acid	— ^a	0.004	0.014	—
Allopurinol	—	—	—	0.04

^a Not determined.

stances tested, Rh1 had the strongest inhibitory activity on the histamine released due to compound 48/80, with an IC₅₀ value of 0.037 mM, which is a stronger inhibitory potency than that of commercially available DSCG. However, Re, Rg1 and Rf, and protopanaxatriol exhibited little inhibitory activity.

Inhibition of PCA Reaction by Ginsenosides

To determine the inhibitory effect of ginsenosides on PCA reactions in mice, these ginsenosides were administered, either orally or intraperitoneally, 60 min prior to challenge with the antigen (table 1). The ginsenosides

**Fig. 3.** Calorimetric scans of the liposomes made of DPPC containing Rh1 and DSCG (heating rate was 5 °C/min).

inhibited the PCA reaction in mice. Among the substances tested, Rh1, administered intraperitoneally, showed the strongest inhibitory activity (87 ± 5.1%), significantly inhibiting the PCA at a dose of 25 mg/kg. Rh1 potentially inhibited the PCA reaction, even when administered orally.

Hyaluronidase-Inhibitory, Active-Oxygen-Scavenging and Membrane-Stabilizing Activities of Ginsenosides

To understand the mechanism of the inhibitory action of Rh1 on the PCA reaction, its active-oxygen-scavenging- and hyaluronidase-inhibitory activities were measured (table 2). The ginsenosides did not inhibit the activation of hyaluronidase, or scavenge active oxygen (table 2).

To measure the membrane-stabilizing activity of the ginsenosides, the phase transition temperature of the for the liposomes prepared from DPPC, both with and without Rh1 was measured as well (fig. 3). The heat absorption of the liposomes, prepared without Rh1 or DSCG, took place at 41.77°C (phase transition temperature). However, when Rh1 was added to the liposomes, 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 weaker than that of Rh1. However, Re had no effect on the phase transition temperature (data not shown).

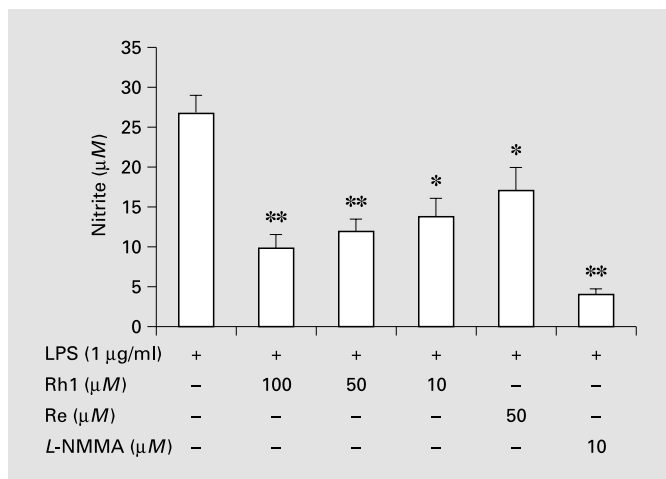


Fig. 4. Effects of Rh1 and Re on NO synthesis of LPS-stimulated RAW 264.7 cells. All values are means $\pm$ SD (n = 3). * Significantly different from LPS-stimulated group (p < 0.05). ** Significantly different from LPS-stimulated group (p < 0.01).

Effect of Rh1 on NO and PGE₂ Production by the Murine Macrophage Cell Line

To investigate the anti-inflammatory effects of Rh1 and Re, their ability to modulate NO synthesis in LPS-stimulated cultures of murine macrophages of RAW 264.7 cells was examined (fig. 4). Stimulation of the RAW 264.7 cells with LPS was found to induce the production of the NO. Rh1 inhibited the production of NO by the RAW 264.7 cells. However, cell viability, as determined by the MTT assay, was unaltered by the ginsenosides at the concentrations used.

To determine whether the reduction of the nitrite accumulation caused by Rh1 resulted from the direct inhibition of iNOS enzyme activity, Rh1 was examined to see if it could also suppress the production of NO by the RAW 264.7 cells in which iNOS protein expression had already been induced (fig. 5). When the RAW 264.7 cells were pretreated with LPS, iNOS enzyme activity was significantly induced. However, Rh1 with LPS inhibited iNOS enzyme activity at high concentrations compared with that of iNOS protein expression.

Stimulation of the RAW 264.7 cells with LPS increased the synthesis of PGE₂, another inflammatory mediator produced by activated macrophages. As shown in figure 6, Rh1 suppressed the production of PGE₂. Therefore, the effect of the ginsenoside on COX enzyme activity was also investigated in vitro (data not shown). However, Rh1 did not inhibit the enzyme activity of either COX-1 or COX-2.

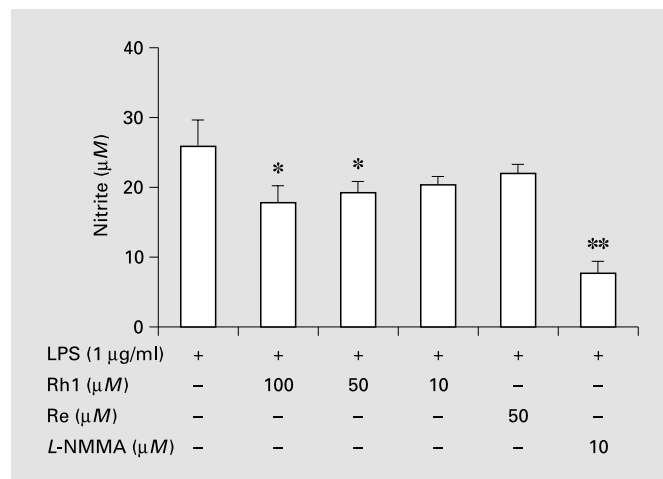


Fig. 5. Effects of Rh1 and Re on NOS activity of RAW 264.7 cells pretreated with LPS. All values are means $\pm$ SD (n = 3). * Significantly different from LPS-stimulated group (p < 0.05). ** Significantly different from LPS-stimulated group (p < 0.01).

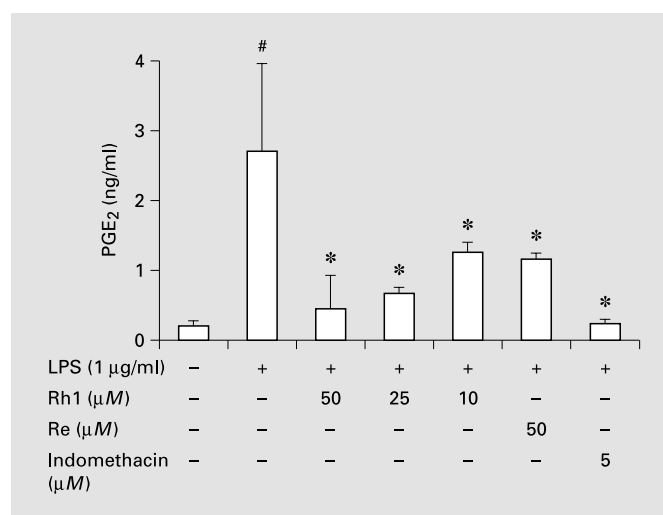
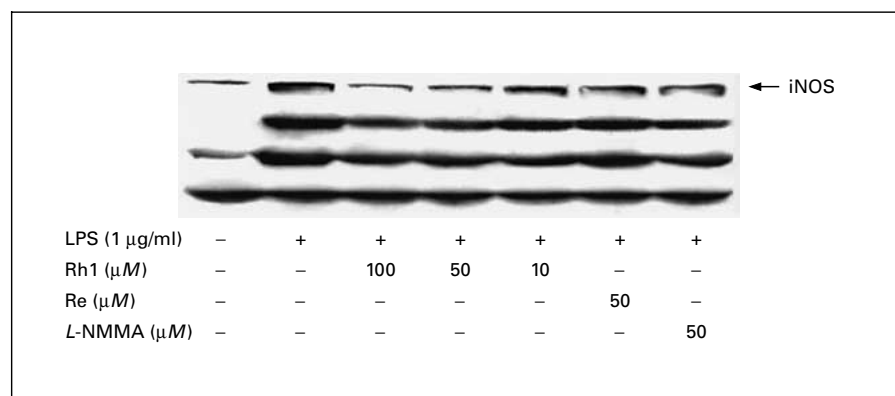


Fig. 6. Effects of Rh1 and Re on PGE₂ synthesis of LPS-stimulated RAW 264.7 cells. All values are means $\pm$ SD (n = 3). # Significantly different from normal group (p < 0.05). * Significantly different from LPS-stimulated group (p < 0.05).

Effect of Rh1 on iNOS and COX-2 Protein Expression

Whether Rh1 could affect iNOS and COX-2 protein expressions was also examined. Stimulation of the RAW 264.7 cells with LPS resulted in accumulation of iNOS protein, as determined by Western blot analysis (fig. 7). Rh1 reduced the levels of iNOS protein in a dose-dependent manner. Densitometry scans of the respective blots

Fig. 7. Effects of Rh1 and Re on iNOS, COX-2 and NF- κ B protein expressions of LPS-stimulated RAW 264.7 cells by Western blot analysis.



showed that at Rh1 concentrations of 10, 50 and 100 μ M, the levels of the iNOS protein were reduced by 27, 70 and 84%, respectively, compared with the control cells stimulated with LPS. These amounts of iNOS protein correlated with the reduced accumulation of nitrite. *L*-NMMA at a concentration of 50 μ M, which was used as a positive control, also inhibited the accumulation of nitrite by 48% compared with LPS-stimulated control cells. However, the inhibitory activity of Re was weaker than that of Rh1.

Stimulation of the RAW 264.7 cells with LPS also induced the expression of the COX-2 protein, as determined by Western blot analysis. Rh1 reduced the levels of COX-2 protein expression in a dose-dependent manner. Rh1 at concentrations of 10, 50 and 100 μ M reduced the levels of COX-2 protein by 23, 48 and 61%, respectively, compared with the control cells stimulated with LPS. These amounts of COX-2 protein correlated with the reduced accumulation of PGE₂. Inhibition by *L*-NMMA amounted to 49% compared with LPS-stimulated control cells. However, the inhibitory activity of Re was weaker than that of Rh1. Therefore, the effect of Rh1 on the activation of the nuclear transcription factor, NF- κ B, which induced both iNOS and COX-2, was investigated. The NF- κ B in the nuclear fraction was activated by LPS. However, Rh1 inhibited the activation of NF- κ B in the RAW 264.7 cells stimulated with LPS: at Rh1 concentrations of 10, 50 and 100 μ M, the levels of NF- κ B in nuclei were reduced by 23, 48 and 61%, respectively, compared with the control cells stimulated with LPS. The amounts of NF- κ B protein correlated with the reduced accumulation of iNOS and COX-2. *L*-NMMA at a concentration of 50 μ M caused 56% inhibition compared with the control cells stimulated with LPS. However, the inhibitory activity of Re was weaker than that induced by Rh1.

Discussion

The etiology of the allergy reactivity is based on the IgE-mediated pharmacological processes of a variety of cell populations, such as mast cells and basophils [15]. Degradation of mast cells and basophils with antigen-cross-linked IgE releases histamine, prostaglandins, leukotrienes and cytokines. These cytokines activate the chemotaxis and phagocytosis of neutrophils and macrophages. Finally, the cytokine-induced reaction causes tissue inflammation. Therefore, antiallergic agents, with anti-inflammatory actions, may be beneficial in allergic diseases.

Of the tested substances, Re and Rg1, and protopanaxatriol did not inhibit the release of histamine. However, Rh1, which has a glucosyl group in the 6-OH position of the protopanaxatriol skeleton, is hydrolyzed by intestinal bacteria [16], inhibited the release of histamine from rat peritoneal exudate cells and was more potent than the commercially available azelastine. However, Re and Rg1, which have one sugar group more than Rh1, and protopanaxatriol released from the glucosyl group, did not inhibit the release of histamine. None of the ginsenosides tested inhibited the activation of hyaluronidase, or scavenged superoxide anions, although some antiallergic agents were inhibitors of the hyaluronidase and scavengers of active oxygen species. In previous reports, compound 48/80 and antigen-IgE increased the permeability of the lipid bilayer membrane due to membrane perturbation [17, 18]. These antiallergic compounds might act on the lipid bilayer membrane, thus preventing the perturbation. Therefore, the phase transition temperatures of the liposomes treated with Rh1 were measured. The membrane-stabilizing effect of Rh1 was stronger than that of the commercially available DSCG. However, the other ginsenosides tested

showed no membrane-stabilizing activity. These results suggest that the inhibitory action of Rh1 on the release of β -hexosaminidase may be due to the protection of the cytolytic response of the antigen-IgE or compound 48/80 although Rh1 exhibited a weak cytotoxic effect at high concentrations ($EC_{50} = >0.3 \text{ mM}$). Rh1 also exhibited the most potent inhibitory activity on the PCA reaction. However, the other ginsenosides only caused weak inhibition. In addition, when the antagonistic activity of Rh1 on histamine released from mast cells in type I allergic reaction was measured using guinea pig ileal strips, Rh1 caused no significant antihistamine action. These results support that the antiallergic action of Rh1 may originate from a potent cell membrane-stabilizing activity.

Cytokine-activated macrophages synthesize various mediators that modulate the inflammatory response. NO and prostaglandins are two pleiotropic mediators produced by NOSs and COXs, respectively, at inflammatory sites [19]. NOSs can be classified into two major groups. Neuronal and endothelial NOSs are in general constitutively expressed, and the NO produced by such isoforms is a key regulator of homeostasis. Conversely, the iNOS plays an important role in the cytotoxic activity of activated macrophages [20]. Stimuli, such as cytokines and/or bacterial LPS, induce iNOS protein expression, which once synthesized is responsible for the prolonged, high-output production of NO. Despite its beneficial role in host defense, the sustained production of NO can be deleterious to the host, and has been implicated in the pathogenesis of various inflammatory diseases [21]. COX, which catalyzes the conversion of arachidonic acid to PGH_2 , is a rate-limiting enzyme in the biosynthesis of prostaglandins [22, 23]. Two isoforms of this enzyme have been described: the constitutive enzyme COX-1, which is present in almost all cell types, is thought to be involved in homeostatic prostanoid biosynthesis, whereas COX-2 is rapidly induced after stimulation with cytokines and/or LPS. COX-2 is predominantly expressed in the cells involved in inflammatory reactions, such as macrophages. In addition to sites of inflammation, COX-2 has also been found to be upregulated in human colorectal adenomas and adenocarcinomas [24, 25]. Rh1 inhibits the production of NO and PGE_2 , in LPS-stimulated RAW 264.7 cells, and the activity of iNOS. However, the ginsenosides did not inhibit the COX-1 and COX-2 enzyme activities in vitro. Rh1 inhibited the expression of iNOS and COX-2 proteins. When Rh1 was added to the culture system, COX-2 activity was inhibited by 35% at $25 \mu\text{M}$, compared with control (data not shown). Rh1 inhibited the activation of the NF- κ B transcription factor, which

regulates iNOS and COX-2 gene expression on the RAW 264.7 cells stimulated with LPS. However, the inhibitory activity of Re was weaker than that of Rh1. These results suggest that Rh1 may express anti-inflammatory activity via the regulation of the signal transduction related to the activation of NF- κ B.

DSCG and azelastine are representative antiallergic drugs. DSCG is an inhibitor of hyaluronidase, as well as a membrane stabilizer [18, 26]. Azelastine is an H_1 -receptor antagonist, but also decreases the release of the mediator from mast cells and basophils [18]. However, Rh1, which should be responsible for the inflammation and PCA reaction inhibitory activities, was different from these antiallergic drugs. Furthermore, when ginseng was orally administered, its protopanaxatriol ginsenosides were easily metabolized to Rh1 [16]. Therefore, we believe that the protopanaxatriol ginsenosides contained in *P. ginseng* as prodrugs have extensive antiallergic properties, and that Rh1 is a candidate therapeutic agent for allergies and inflammation.

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