J. Microbiol. Biotechnol. (2006), **16**(11), 1791–1798

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Metabolism of Ginsenoside Rg5, a Main Constituent Isolated from Red Ginseng, by Human Intestinal Microflora and Their Antiallergic Effect

SHIN, YONG-WOOK[1], EUN-AH BAE[2], MYUNG JOO HAN[2], AND DONG-HYUN KIM[1]*

[1]College of Pharmacy and [2]Department of Food and Nutrition, Kyung Hee University, Seoul 130-701, Korea

Received: June 21, 2006
Accepted: July 28, 2006

Abstract When ginsenoside Rg5, a main component isolated from red ginseng, was incubated with three human fecal microflora for 24 h, all specimens showed hydrolyzing activity: all specimens produced ginsenoside Rh3 as a main metabolite, but a minor metabolite 3β,12β-dihydroxydammar-21(22),24-diene (DD) was observed in two specimens. To evaluate the antiallergic effect of ginsenoside Rg5 and its metabolites, the inhibitory effect of ginsenoside Rg5 and its metabolite ginsenoside Rh3 against RBL-2H3 cell degranulation, mouse passive cutaneous anaphylaxis (PCA) reaction induced by the IgE-antigen complex, and mouse ear skin dermatitis induced by 12-O-tetradecanoilphorbol-13-acetate (TPA) were measured. Ginsenosides Rg5 and Rh3 potently inhibited degranulation of RBL-2H3 cells. These ginsenosides also inhibited mRNA expression of proinflammatory cytokines IL-6 and TNF-α in RBL-2H3 cells stimulated by IgE-antigen. Orally and intraperitoneally administered ginsenoside Rg3 and orally administered ginsenoside Rg5 to mice potently inhibited the PCA reaction induced by IgE-antigen complex. However, intraperitoneally administered ginsenoside Rg5 nearly did not inhibit the PCA reaction. These ginsenosides not only suppressed the swelling of mouse ears induced by TPA, but also inhibited mRNA expression of cyclooxygenase-2, TNF-α, and IL-4 and activation of transcription factor NF-κB. These inhibitions of ginsenoside Rh3 were more potent than those of ginsenoside Rg5. These findings suggest that ginsenoside Rg5 may be metabolized *in vivo* to ginsenoside Rh3 by human intestinal microflora, and ginsenoside Rh3 may improve antiallergic diseases, such as rhinitis and dermatitis.

Key words: Red ginseng, ginsenoside Rg5, ginsenoside Rh3, metabolism, intestinal microflora, allergy

Ginseng (the root of *Panax ginseng* C.A. Meyer, family Araliaceae) is frequently taken as a traditional medicine in Asian countries [31]. The major components of ginseng are ginsenosides, which contain an aglycone with a dammarane skeleton. The main components of raw ginseng are ginsenosides Rb1, Rb2, Rc, and Rf [35]. When the ginseng is steamed and dried, it is called red ginseng or heat-processed ginseng [17, 18]. Its main constituents are ginsenosides Rg3, Rg5, and Rk1, transformed from protopanaxadiol ginsenosides by steaming to prepare red ginseng or heat-processed ginseng. These ginsenosides have been reported to exhibit various biological activities [5, 15], including antiinflammatory action [25, 37], antiallergic [8, 24] and antitumor effects [20, 36]. The pharmacological actions of these ginsenosides have been explained by their biotransformation by human intestinal bacteria [1, 8, 36]. For example, ginsenoside Rg3 is transformed to ginsenoside Rh2 by human intestinal microflora [2]. The biotransformed ginsenoside Rh2 exhibited more potent antiallergic effects, such as antipassive cutaneous anaphylaxis, and antiinflammatory effects, such as biosynthesis of prostaglandin, than ginsenoside Rg3. However, studies on the metabolism of ginsenoside Rg5 and its pharmacological effects have not been thoroughly studied, although ginsenoside Rg5 is a main component with ginsenoside Rg3 in red ginseng.

In the present study, we isolated ginsenoside Rg5 from red ginseng, incubated it with human intestinal (fecal) microflora, isolated its metabolite ginsenoside Rh3, and investigated their antiallergic effect in the mouse passive cutaneous anaphylaxis (PCA) reaction induced by IgE and in mouse ear dermatitis induced by 12-O-tetradecanoilphorbol-13-acetate (TPA).

Materials and Methods

Materials

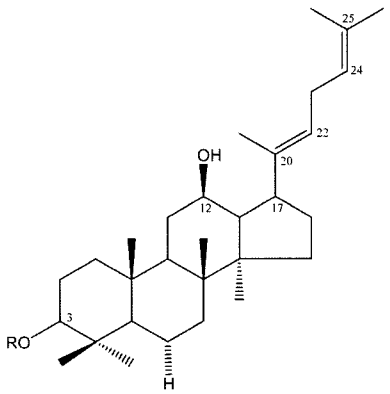
TPA, Dulbecco's modified Eagle medium (DMEM), p-nitrophenyl-*N*-acetyl-β-D-glucosaminide, anti-dinitrophenol (DNP)-IgE, DNP-human serum albumin (HSA), Evans blue,

*Corresponding author
Phone: 82-2-; Fax: 82-2-957-5030;
E-mail: dhkim@khu.ac.kr

disodium cromoglycate (DSCG), azelastine, and betamethasone were purchased from Sigma Co. (St. Louis, MO, U.S.A.). Antibody for p65 or p50 subunits of NF-κB was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, U.S.A.). Protein assay kit was purchased from Bio-Rad Laboratories Inc. (Hercules, CA, U.S.A.). TRI reagent was purchased from Molecular Research Center Inc. (Cincinnati, OH, U.S.A.).

Isolation of Ginsenoside Rg5 and its Metabolites by Human Intestinal Microflora

Fresh human feces (5 g) were suspended in 100 ml of anaerobic dilution medium [4, 26], centrifuged at 500 ×*g* for 10 min, and the resulting supernatant was centrifuged at 10,000 ×*g* for 30 min and washed twice with the same medium. The resulting precipitate (intestinal microflora fraction) was suspended in 200 ml of the same medium containing 150 mg of ginsenoside Rg5 (purity, >90%), isolated according to a previous method [14], anaerobically incubated for 24 h at 37°C, and extracted with EtOAc. This EtOAc fraction was chromatographed on a silica gel column using $CHCl_3$-MeOH (20:1) to isolate ginsenoside Rh3 (35 mg; purity, >90%) as a main metabolite and 3β,12β-dihydroxydammar-21(22),24-diene as a minor (2 mg, purity, >80%) product. The purity of isolated ginsenosides (Fig. 1) was assayed by HPLC [Hitachi HPLC system: column, Lichrosorb NH_2 (25×0.4 cm, 5 mm, Merck Co.); elution solvent, mixtures of solvent A (acetonitrile/water/isopropanol=80:5:15) and solvent B (acetonitrile/water/isopropanol=80:20:15); gradient profile of solvent A to solvent B, from 70:30 to 0:100 for 0–20 min and from 0:100 for 20–40 min; Detector, ELSD 800 (Alltech Associates Inc., Deerfield, IL, U.S.A.)].



	R
Ginsenoside Rg5	Glc-Glc-
Ginsenoside Rh3	Glc-
3,12-Dihydroxydammar-21(22),24-diene	H

Fig. 1. Structure of ginsenosides Rg5 and Rh3.

Ginsenoside Rg5: white powder, mp 186-188, FAB-MS *m/z*=768 $(M^{+1})^+$.

Ginsenoside Rh3 [13]: white powder, mp 158–161, FAB-MS *m/z*=605 $(M^{+1})^+$.

3β,12β-Dihydroxydammar-21(22),24-diene (DD) [23]: white powder, FAB-MS *m/z*=442 $(M^{+1})^+$

Assay of Metabolic Activity of Ginsenoside Rg5 by Fecal Microflora and Isolated Bacteria from Human Feces

The reaction mixture containing 5 ml of 1 mM ginsenoside Rg5, 2.5 ml of the above fecal microflora (or intestinal bacterial) suspension (0.5 g wet weight/ml), and 2.5 ml of the anaerobic dilution medium [4] was anaerobically incubated for 24 h at 37°C. The reaction mixture (0.5 ml) was periodically taken out, adjusted to pH 2 with 0.1 N HCl, extracted with EtOAc, evaporated, and assayed by TLC [TLC plates, silica gel $60F_{254}$ (Merck Co., U.S.A.); developing solvent, $CHCl_3$ - MeOH-H_2O (65:35:10 v/v, lower phase)]. The plates were stained by spraying with MeOH-H_2SO_4 (95:5 v/v), followed by heating. The stained TLC plates were then analyzed by a TLC scanner (Shimadzu model CS-9301PC, Tokyo, Japan). Intestinal bacteria were isolated as previously reported [4].

Activity Assay of β-Hexosaminidase Released from RBL-2H3 Cells Stimulated by IgE with DNA-HSA

The inhibitory activity of test ginsenosides against the release of β-hexosaminidase from RBL-2H3 cells was evaluated according to Choo *et al.* [8].

Assay of Cytokine mRNA Expressions in RBL-2H3 Cells Stimulated by IgE with DNA-HSA by Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The test agents were added, after RBL-2H3 cells pretreated with IgE had been stimulated with DNP-HSA for 1 h and then incubated for 20 min in the CO_2 incubator and then DNP-HSA-treated for 40 min. RT-PCR analysis for RBL-2H3 cells treated with test agents and/or IgE with DNP-HSA was performed by the modified method of Matsuda *et al.* [19].

Animals

The male SD rats (180–220 g) and female ICR mice (20–25 g) were supplied from Orient Experimental Animal Breeding Center (Seoul, Korea). All animals were housed in wire cages at 20–22°C and 50±10% humidity, fed standard laboratory chow (Orient Experimental Animal Breeding Center, Seoul, Korea), 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).

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

Histamine released from rat peritoneal exudate cells induced by compound 48/80 was assayed using the method of Choo *et al.* [8].

PCA Reaction

An IgE-dependent cutaneous reaction was measured according to the method of Choo *et al.* [8]. Male ICR mice (25–30 g) were injected intradermally with 50 μl of anti-DNP IgE (10 μg) 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. Test agents were orally or intraperitoneally 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).

TPA-Induced Dermatitis

TPA-induced dermatitis was measured according to the previous method of Park *et al.* [22]. Each group contained 6 ICR mice (20–25 g). TPA (3 μg/20 μl acetone) was applied to the inner and outer surfaces of the mouse ear every day for 3 days to induce dermatitis. Test agents dissolved in an oil-based vehicle were topically applied to the same ear (1 and 10 μg/20 μl) at 1 and 12 h after TPA treatment. Normal group received vehicle alone. Control group received TPA and vehicle. On the third day, test agents were treated 1 h after TPA treatment. The thickness of mice ears was measured using a Digimatic Micrometer (Mitsutoyo Co., Tokyo, Japan), 3 h after final treatment with test agents.

Ear tissue extract for RT-PCR analysis was performed according to the modified method of Shin *et al.* [32].

Statistical Analysis

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

RESULTS

Metabolism of Ginsenoside Rg5 by Human Intestinal Microflora

To investigate the metabolites of ginsenoside Rg5 produced by human intestinal microflora, ginsenoside Rg5 was anaerobically incubated with a human fecal microflora for 24 h, and the metabolites were then extracted with EtOAc, purified by silica-gel column chromatography, and identified. Two metabolites, one major and one minor, were observed from the TLC. From instrumental analyses, the main metabolite was identified to be ginsenoside Rh3, and the minor was DD.

When ginsenoside Rg5-hydrolyzing activity in three samples of human feces was preliminarily assayed, all specimens showed hydrolyzing activity: all specimens produced ginsenoside Rh3 as a main metabolite, but the minor metabolite DD was observed in two specimens (data not shown). The average activities transforming ginsenoside Rg5 to ginsenoside Rh3 and DD were 0.42± 0.28 and 0.02±0.01 nmol/h/mg wet weight of fecal bacteria, respectively (Fig. 2A). When ginsenoside Rg5 was incubated with the most potent ginsenoside Rg5-hydrolyzing fecal

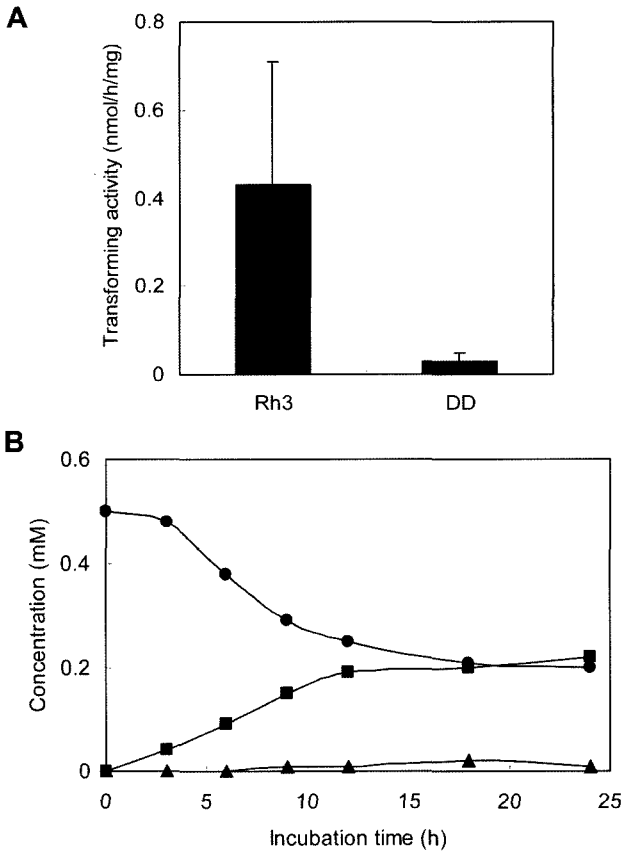


Fig. 2. Metabolism of ginsenoside Rg5 by human intestinal microflora.

A. The activity transforming ginsenoside Rg5 to ginsenoside Rh3 and DD. Human fecal suspension was prepared and its activity was assayed according to Materials and Methods. Transforming activity of ginsenoside Rg5 to ginsenoside Rh3 or 3β,12β-dihydroxydammar-21(22),24-diene (DD) was calculated from total transformed ginsenoside Rh3 and DD. Rh3, the activity transforming ginsenoside Rg5 to ginsenoside Rh3; DD, the activity transforming ginsenoside Rg5 to DD. **B.** Time course of ginsenoside Rg5. The metabolites were assayed according to Materials and Methods. ●, ginsenoside Rg5; ■, ginsenoside Rh3; ▲, DD.

Table 1. Transforming activity of ginsenoside Rg5 by intestinal bacteria isolated from human feces.

	Transforming activity[a] (nmol/h/mg)	
	Ginsenoside Rh3	DD
Bacteroides stercoris HJ-15	0.51	0.02
Bacteroides fragilis JY-6	0.25	0.01
Eubacterium A-44	0.22	-
Lactobacillus acidophilus 1-2	-[b]	-
Streptococcus faecium S-9	0.01	-
Bifidobacterium longum H-1	0.38	-
Bifidobacterium breve K-110	0.02	-

[a]Isolated bacteria were cultured in GAM broth and collected according to Materials and Methods. Transforming activity of ginsenoside Rg5 to ginsenoside Rh3 or 3β,12β-dihydroxydammar-21(22),24-diene (DD) was calculated from total transformed ginsenoside Rh3 and DD according to Materials and Methods.
[b]Not detectable.

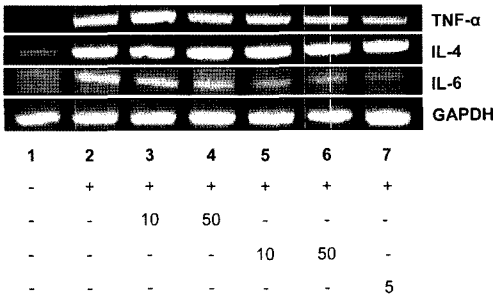


Fig. 3. Effect of ginsenosides Rg5 and Rh3 on cytokine mRNA expression levels of RBL-2H3 cells induced by IgE with DNP-HSA. RBL-2H3 cells pretreated with IgE were stimulated with DNP-HSA for 1 h and treated with ginsenosides Rg5 or Rh3 for 20 min at the CO_2 incubator.
Bovine serum was added and then incubated for 40 min. RT-PCR analysis for RBL-2H3 cells treated with tested agents and/or IgE with DNP-HSA was performed. G-Rg5, ginsenoside Rg5; G-Rh3, ginsenoside Rh3; Dexa, dexamethasone.

specimen for 24 h and periodically assayed for metabolites (Fig. 2B), ginsenoside Rg5 began to be mainly transformed to ginsenoside Rh3, and subsequently produced DD after 9 h incubation. A major metabolite was ginsenoside Rh3, with DD as a minor product, after 24 h incubation.

Among the isolated intestinal bacteria, *Bacteroides stercoris* HJ-15 and *Bifidobacterium longum* H-1 most potently transformed ginsenoside Rg5 to ginsenoside Rh3 (Table 1).

Inhibition of Ginsenosides Rg5 and Rh3 on Degranulation and Cytokine Production of RBL-2H3 Cells

In the preliminary experiment, red ginseng inhibited PCA reaction *in vivo*. Therefore, to evaluate *in vitro* whether its main and genuine ginsenoside Rg5 and its metabolite Rh3 possess antiallergic activity, their inhibitory effects on β-hexosaminidase release from RBL-2H3 cells induced by IgE with antigen were examined (Table 2). Ginsenosides Rg5 and Rh3 potently inhibited β-hexosaminidase release of RBL-2H3 cells induced by IgE with antigen, with IC_{50} values of 0.09 and 0.04 mM, respectively. These ginsenosides also potently inhibited histamine release of peritoneal mast cells induced by compound 48/80, with IC_{50} values of 0.12 and 0.02 mM, respectively. The degranulation inhibitory activities of ginsenoside Rh3 against rat peritoneal mast and RBL-2H3 cells was more potent than those of ginsenoside Rg5.

Whether ginsenosides Rg5 and Rh3 inhibited mRNA expressions of cytokines IL-4, IL-6, and TNF-α in RBL-2H3 cells stimulated by IgE with DNP-HSA was investigated by using RT-PCR analysis (Fig. 3). IgE with antigen significantly induced mRNA expression of these cytokines. However, ginsenosides Rg5 and Rh3 with IgE antigen inhibited mRNA expression of IL-6 and TNF-α. The ginsenosides Rg5 and Rh3 at a dose of 0.05 mM inhibited mRNA expression of TNF-α and IL-6 by 17% and 34%, and 19% and 43%, respectively.

Table 2. Inhibitory effect of ginsenosides on the release of β-hexosaminidase from RBL-2H3 cells induced by IgE with DNP-HSA and histamine from rat peritoneal mast cells by compound 48/80.

Agent	IC_{50} (mM)	
	RBL-2H3 cells	Peritoneal mast cells
Red ginseng[a]	>0.2	0.05
Ginsenoside Rg5	0.09	0.12
Ginsenoside Rh3	0.04	0.02
Azelastine	0.02	0.02
DSCG	0.50	0.57

Histamine released by compound 48/80 and β-hexosaminidase activity released from RBL-2H3 cells induced by IgE with DNP-HSA were 82.7±4.4% and 75.0±8.8%, respectively.
[a]Final concentration unit of red ginseng BuOH extract was mg/ml.
All values are mean±SD (n=3).

Inhibition of Ginsenosides Rg5 and Rh3 on PCA Reaction

To evaluate the *in vivo* antiallergic activity of ginsenosides Rg5 and Rh3, the PCA reaction in mice was induced by the intradermal injection of IgE, where these ginsenosides were administered orally or intraperitoneally 60 min prior to challenge with DNP-HSA antigen, and the inhibitory potency of the PCA reaction was measured (Table 3). Orally administered ginsenosides Rg5 and Rh3 potently inhibited the PCA reaction. Intraperitoneally administered ginsenoside Rh3 also significantly inhibited the PCA reaction. However, intraperitoneally administered ginsenoside Rg5 showed weak inhibition against the PCA reaction. The inhibitory potency of intraperitoneally administered ginsenosides was stronger than that of orally administered ones.

Table 3. Inhibitory effect of ginsenosides against PCA reaction.

Agent	Dose (mg/kg)	Inhibition (%)	
		p.o.	i.p.
Saline	-	0	0
Red ginseng BuOH extract	100	32±4.8[b]	-[f]
Ginsenoside Rg5	10	58±4.8[c]	31±7.7[b]
	25	67±5.8[c,d,e]	42±5.9[b]
Ginsenoside Rh3	10	62±6.2[c,d]	65±7.9[c]
	25	75±6.8[d,e]	73±10.5[c]
DSCG	25	15±3.9[a]	-
Azelastine	10	88±8.8[e]	95±1.2[d]

All agents were administered p.o. or i.p. prior to antigenin challenge.
Values are expressed as means±SD (n=5).
[a,b,c]Items with the same letter are not significantly different in each column.
[d]Not determined.

Inhibition of Ginsenosides Rg5 and Rh3 on TPA-Induced Mouse Ear Dermatitis

To evaluate the inhibitory effect of ginsenosides Rg5 and Rh3 against contact dermatitis, their effect in TPA-induced mouse ear dermatitis was measured (Fig. 4). When TPA was applied to the mouse ear, erythema (reddening of the skin) and edema were observed. Ear thickness measured as an index of skin inflammation increased at 48 h to 209%. Betamethasone used as a positive agent at a dose of 10 mg/mouse ear potently suppressed ear swelling with a suppressive rate of 78%. Ginsenosides Rg5 and Rh3 at a dose of 10 mg/mouse ear also potently suppressed ear swelling and their suppressive rates were 57% and 77% on the third day, respectively. For histopathological analysis, we excised the ear at the third day and stained it with hematoxylin-eosin. These ginsenosides improved the swelling induced by the application with TPA (data not shown). Ginsenoside Rh3 showed more potent inhibition than ginsenoside Rg5.

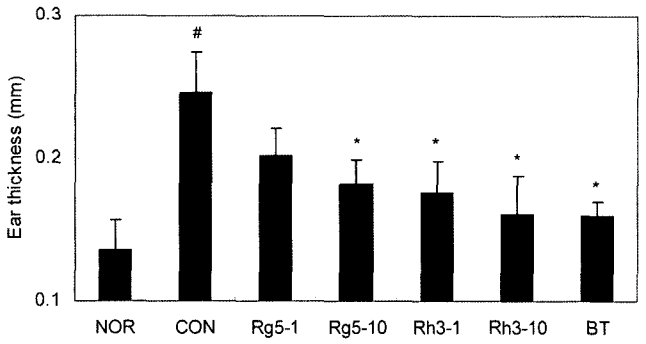


Fig. 4. Effects of ginsenosides Rg5 and Rh3 on the thickness of mice ears induced by TPA.
NOR, vehicle alone (normal); CON, TPA alone treated control; Rg5-1, 1 μg/20 μl ginsenoside Rg5; Rg5-10, 10 μg/20 μl ginsenoside Rg5; Rh3-1, 1 μg/20 μl ginsenoside Rh1; Rh3-10, 10 mg/20 ml ginsenoside Rh1; BT, 10 μg/20 μl betamethasone. Values represent means±S.D. for six mice. #Significantly different from the normal control group (#P<0.05). *Significantly different from the control group (*P<0.05; **P<0.001).

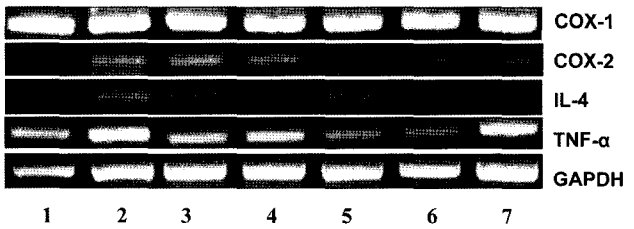


Fig. 5. Effect of ginsenosides Rg5 and Rh3 on mRNA expressions of COXs and cytokines in TPA-induced mouse ear dermatitis.
1, Vehicle alone-treated normal group; 2, oxazolone alone-treated control group; 3, 1 μg ginsenoside Rg5 with oxazolone; 4, 10 μg ginsenoside Rg5 with oxazolone; 5, 1 μg ginsenoside Rh3 with oxazolone; 6, 10 μg ginsenoside Rh3 with oxazolone; 7, 10 μg betamethasone with oxazolone.

Effect of Ginsenosides Rg5 and Rh3 on Expression of COXs and Cytokines Induced by TPA

The effect of ginsenosides Rg5 and Rh3 in mRNA expression levels of COX-1, COX-2, and cytokines of mouse ear dermatitis induced by TPA was investigated by using RT-PCR analysis (Fig. 5). TPA significantly induced COX-2 mRNA expression, but did not induce that of COX-1. The application of these ginsenosides in TPA-stimulated mice did not affect COX-1 mRNA expression, but potently inhibited mRNA levels of COX-2. TPA also significantly induced mRNA expression of TNF-α and IL-4. Ginsenosides Rg5 and Rh3 also inhibited the mRNA expression of TNF-α induced by TPA. The ginsenosides also inhibited mRNA expression of IL-4. The ginsenoside Rh3 more potently inhibited these mRNA expression levels than ginsenoside Rg5. When the effect of these agents on a transcription factor NF-κB, which modulates inflammatory reactions [14], was examined to further dissect the antidermatitic mechanism of ginsenosides Rg5 and Rh3, electrophoretic mobility shift assay showed that ginsenosides Rg5 and Rh3 significantly repressed the NF-κB DNA-binding activity in TPA-induced mouse ear dermatitis (data not shown).

DISCUSSION

Mast cells and basophils are well-known as a critical participant in the various biologic processes including allergic diseases [7, 27, 34, 38]. These cells express on their surface membrane receptors with high affinity and specificity for IgE. The interaction of multivalent antigens with surface-bound IgE initiates a series of biochemical events that culminates in the release of histamine and the production of cytokines, which store within their numerous cytoplasmic granules as biologically potent inflammatory mediators, and causes allergic diseases, such as anaphylaxis and rhinitis. Anti-histamines, steroids, and immunosuppressants have been using against allergic diseases [29, 30, 33]. However, improving these diseases is too difficult. Therefore, traditional herbal medicines have been advanced

for allergic diseases, and its effectiveness has received increasing attention [6, 39].

Many researchers have focused on ginsenosides as antiallergic components in ginseng. The antiallergic actions of these ginsenosides have been explained by their biotransformation by human intestinal bacteria [1, 2]. For example, when ginsenoside Rg3 were transformed to ginsenoside Rh2 by human intestinal microflora, the biotransformed ginsenoside Rh2 exhibited the potent antiallergic effects [2]. Ginsenoside Rg5 is a main component with ginsenoside Rg3 in red ginseng. Nevertheless, the antiallergic effect of ginsenoside Rg5 has not been studied.

In the present study, we isolated ginsenoside Rg5 from red ginseng and its main metabolite ginsenoside Rg3 produced by the incubation with intestinal microflora, and evaluated the antiallergic effects. First, ginsenoside Rg5 weakly inhibited the degranulation of RBL-2H3 cells and rat peritoneal mast cells. However, ginsenoside Rh3, which is a metabolite of ginsenoside Rg5 by human intestinal bacteria, potently inhibited it, as potently as azelastine. Neither of these ginsenosides inhibited the activation of hyaluronidase, nor scavenged the superoxide anion (data not shown), although some antiallergic agents have been found to be inhibitors of hyaluronidase and scavengers of active oxygen species [9, 11]. The ginsenosides Rg5 and Rh3 did not exhibit antihistamine action (data not shown). In previous reports, ginsenosides such as compound K and ginsenoside Rh2 prevented perturbation of the lipid membrane caused by compound 48/80 [8, 24]. Therefore, these reports suggest that the inhibitory action of these ginsenosides such as ginsenoside Rh3 on the degranulation of mast cells may be due to its ability to protect cells from the cytolytic response induced by IgE with antigen or compound 48/80. Furthermore, these ginsenosides inhibited cytokine biosynthesis of IL-6 and TNF-α in RBL-2H3 cells induced by IgE with DNP-HSA. These results suggest that ginsenosides may regulate the degranulation and cytokine biosynthesis of mast cells and basophils, which causes allergic diseases.

We also evaluated their antiinflammatory effects on a TPA-induced mouse ear dermatitis model. The treatment of TPA was accompanied by substained swelling and redness, as previously reported [22]. COX-2, which is a marker of acute inflammatory disease, was also induced. Ginsenosides Rg5 and Rh3 significantly reduced sustained swelling (thickness) of mice ears induced by TPA. The ginsenosides Rg5 and Rh3 not only inhibited the mRNA expression of COX-2, but also the activation of NF-κB transcription factor, which is an upstream modulator of COX-2 gene expression in the mouse ear [10, 12]. Furthermore, the ginsenoside Rh3 significantly inhibited TNF-α and IL-4 mRNA expression. These results suggest that ginsenosides Rg5 and Rh3 can improve inflammatory skin disorders, such as contact dermatitis or psoriasis, by the regulation of COX-2 and TNF-α produced by macrophage cells and IL-4 produced by Th2 cells. The inhibitory effect of ginsenoside Rh3 was more potent than that of ginsenoside Rg5. Nevertheless, the effect of ginsenoside Rg5 may be similar to that of ginsenoside Rh3, because ginsenoside Rg5 was metabolized to ginsenoside Rh3 by human intestinal microflora.

Orally administered ginsenosides Rg5 and Rh3 and intraperitoneally administered ginsenoside Rh3 inhibited mouse PCA reaction induced by IgE. However, intraperitoneally administered ginsenoside Rg5 exhibited weak inhibition against the PCA reaction. In addition, if ginseng is administered orally, its protopanaxadiol ginsenoside Rg5 can be easily metabolized to ginsenoside Rh3. However, ginsenoside Rg5 was not metabolized to ginsenoside Rh3 in rat liver homogenate (data not shown). These results suggest that ginsenoside Rg5 may not be metabolized to ginsenoside Rh3 in tissues such as liver, when it was intraperitoneally administered. Therefore, the *in vitro* biological effect of ginsenoside Rg5 is different to the *in vivo* administered one. Based on these findings, we believe that the inhibitory effect of orally administered ginsenoside Rg5 may be due to the ginsenoside Rh3 metabolized by intestinal microflora and the metabolized ginsenoside Rh3 can improve IgE-induced allergic diseases such as rhinitis and asthma.

Acknowledgment

This work was supported by a grant from Kyung Hee University, KHU-2003-1101 (M. J. Han).

REFERENCES

1. Akao, T., H. Kida, M. Kanaoka, M. Hattori, and K. Kobashi. 1988. Intestinal bacterial hydrolysis is required for the appearance of compound K in rat plasma after oral administration of ginsenoside Rb1 from *Panax ginseng*. *J. Pharm. Pharmacol.* **50:** 1155–1160.
2. Bae, E. A., M. J. Han, M. K. Choo, S. Y. Park, and D. H. Kim. 2002. Metabolism of 20(S)- and 20(R)-ginsenoside Rg3 by human intestinal bacteria and its relation to *in vitro* biological activities. *Biol. Pharm. Bull.* **25:** 58–63.
3. Bae, E. A., S. Y. Park, and D. H. Kim. 2000. Constitutive β-glucosidases hydrolyzing ginsenoside Rb1 and Rb2 from human intestinal bacteria. *Biol. Pharm. Bull.* **23:** 1481–1485.
4. Bae, E. A., M. K. Choo, E. K. Park, S. Y. Park, H. Y. Shin, and D. H. Kim. 2003. Metabolism of ginsenoside Rc by human intestinal bacteria and its related antiallergic activity. *Biol. Pharm. Bull.* **25:** 743–747.

5. Bae E.-A., J.-E. Shin, S.-H. Park, and D.-H. Kim. 2004. Inhibitory effect of ginseng polysaccharides on rotavirus infection. *J. Microbiol. Biotechnol.* **14:** 202–204.
6. Bielory, L. 2004. Complementary and alternative interventions in asthma, allergy, and immunology. *Ann. Allergy Asthma Immunol.* **93(2 Suppl 1):** S45–S54.
7. Bruhns, P., S. Fremont, and M. Daeron. 2005. Regulation of allergy by Fc receptors. *Curr. Opin. Immunol.* **17:** 662–669.
8. Choo, M. K., E. K. Park, M. J. Han, and D. H. Kim. 2003. Antiallergic activity of ginseng and its ginsenoside. *Planta Med.* **69:** 518–522.
9. Cox, J. S. G. 1967. Disodium cromglycate (FPL-670) ("Intar"): A specific inhibitor of reaginic antibody-antigen mechanism. *Nature* **216:** 1326–1329.
10. Hernandez, G. L., O. V. Volpert, M. A. Iniguez, E. Lorenzo, S. Martinez-Martinez, R. Grau, M. Fresno, and J. M. Redondo. 2001. Selective inhibition of vascular endothelial growth factor-mediated angiogenesis by cyclosporin A: Roles of the nuclear factor of activated T cells and cyclooxygenase 2. *J. Exp. Med.* **193:** 607–620.
11. Ito H., T. Miyazaki, M. Ono, and H. Sakurai. 1998. Antiallergic activities of rabdosiin and its related compounds: Chemical and biochemical evalutions. *Bioorg. Med. Chem.* **6:** 1051–1056.
12. Keum, Y. S., S. S. Han, K. S. Chun, K. K. Park, J. H. Park, S. K. Lee, and Y. J. Surh. 2003. Inhibitory effects of the ginsenoside Rg3 on phorbol ester-induced cyclooxygenase-2 expression, NF-kappaB activation and tumor promotion. *Mutat. Res.* **523–524:** 75–85.
13. Kim, D. S., N. I. Baek, J. D. Park, Y. H. Lee, S. Y. Jeong, C. B. Lee, and S. I. Kim. 1995. Preparation and structure determination of a new glycoside, (20E)-ginsenoside Rh3, and its isomer from diol-type ginseng saponins. *Yakhak Hoeji* **39:** 85–93.
14. Kim, S. I., J. H. Park, J. H. Ryu, J. D. Park, Y. H. Lee, J. H. Park, T. H. Kim, J. M. Kim, and N. I. Baek. 1996. Ginsenoside Rg5, a genuine dammarane glycoside from Korean red ginseng. *Arch. Pharm. Res.* **19:** 551–553.
15. Kim, J.-M., J.-E. Shin, M. J. Han, S.-H. Park, and D.-H. Kim. 2003. Inhibitory effect of ginseng saponins and polysaccharides on infection and vacuolation of *Helicobacter pylori*. *J. Microbiol. Biotechnol.* **13:** 706–709.
16. Kim, W. K., P. G. Jang, M. S. Woo, I. O. Han, H. Z. Paik, K. Lee, H. Lee, T. Joh, and H. S. Kim. 2004. A new anti-inflammatory agent KL-1037 represses pro-inflammatory cytokine and inducible nitric oxide synthase (iNOS) gene expression in activated microglia. *Neuropharmacology* **47:** 243–252.
17. Kitagawa, I., M. Yoshikawa, M. Yoshihara, T. Hayashi, and T. Taniyama. 1983. Chemical studies of crude drugs (1). Constituents of *Ginseng radix* rubra. *Yakugaku Zasshi* **103:** 612–622.
18. Kown, S. W., S. B. Han, I. H. Park, J. M. Kim, M. K. Park, and J. H. Park. 2001. Liquid chromatographic determination of less polar ginsenosides in processed ginseng. *J. Chromatogr. A* **921:** 335–339.
19. Mastuda, H., T. Morikawa, K. Ueda, H. Managi, and M. Yoshikawa. 2002. Structural requirement of flavonoids for inhibition of antigen-induced degranulation, TNF-α and IL-4 production from RNL-2H3 cells. *Bioorg. Med. Chem.* **10:** 2123–3128.
20. Mochizuki, M., C. Y. Yoo, K. Matsuzawa, K. Sato, I. Saiki, S. Tono-oka, K. Samukawa, and I. Azuma. 1995. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside Rb2, 20(R)- and 20(S)-ginsenoside Rg3, of Red ginseng. *Biol. Pharm. Bull.* **18:** 1197–1202.
21. Nicoloff, B. J. 1991. The cytokine network in psoriasis. *Arch. Dermatol.* **127:** 871–884.
22. Park, B. K., M. Y. Heo, H. Pak, and H. P. Kim. 2001. Inhibition of TPA-induced cyclooxygenase-2 and skin inflammation in mice by wogonin, a plant flavone from *Scutellaria radix*. *Eur. J. Pharmacol.* **425:** 153–158.
23. Park, I. H., N. Y. Kim, S. B. Han, J. M. Kim, S. W. Kwon, H. J. Kim, M. K. Park, and J. H. Park. 2002. Three new dammarane glycosides from heat processed ginseng. *Arch. Pharm. Res.* **25:** 428–432.
24. Park, E. K., M. K. Choo, E. J. Kim, M. J. Han, and D. H. Kim. 2003. Antiallergic activity of ginsenoside Rh2. *Biol. Pharm. Bull.* **26:** 1581–1584.
25. Park, E. K., M. K. Choo, M. J. Han, and D. H. Kim. 2004. Ginsenoside Rh1 possesses antiallergic and anti-inflammatory activities. *Int. Arch. Allergy Immunol.* **133:** 113–120.
26. Park, S.-Y., J.-H. Kim, and D.-H. Kim. 2005. Purification and characterization of quercitrin-hydrolyzing ct-L-rhamnosidase from *Fusobacterium* K-60, a human intestinal bacterium. *J. Microbiol. Biotechnol.* **15:** 519–524.
27. Plaut, M., J. H. Pierce, C. Whatson, J. Hanley-Hyde, R. P. Nordan, and W. E. Paul. 1989. Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophore. *Nature* **339:** 64–67.
28. Reynolds, N. J., J. J. Voorhees, and G. H. Fisher. 1998. Cyclosporin A inhibits 12-O-tetradecanoyl-phorbol-13-acetate-induced cutaneous inflammation in severe combined immunodeficient mice that lack fuctional lymphocytes. *Br. J. Dermatol.* **139:** 16–22.
29. Sakuma, S., Y. Higashi, N. Sato, T. Sasakawa, T. Sengoku, Y. Ohkubo, T. Amaya, and T. Goto. 2001. Tacrolimus suppressed the production of cytokines involved in atopic dermatitis by direct stimulation of human PBMC system. (comparison with steroids). *Int. Immunopharmacol.* **1:** 1219–1226.
30. Schafer-Korting, M., M. H. Schmid, and H. C. Korting. 1996. Topical glucocorticoids with improved risk-benefit ratio. *Drug Safety* **14:** 375–385.
31. Shibata, S., M. Fujita, H. Itokawa, O. Tanaka, and T. Ishii. 1963. Panaxadiol, a sapongenin of ginseng roots (1). *Chem. Pharm. Bull.* **11:** 759–764.
32. Shin, Y. W., E. A. Bae, S. S. Kim, Y. C. Lee, and D. H. Kim. 2005. *Int. Immunopharmacol.* **5:** 1183–1191.
33. Simons, F. E. 1992. The antiallergic effects of antihistamines (H1-receptor antagonists). *J. Allergy Clin. Immunol.* **90:** 705–715
34. Stevens, R. L. and K. F. Austen. 1989. Recent advances in the cellular and molecular biology of mast cells. *Immunol. Today* **10:** 381–386.

35. Tanaka, N., O. Tanaka, and S. Shibata. 1972. Chemical studies on the oriental plant drugs. XXVIII. Saponins and sapogenins of ginseng; Stereochemistry of sapogenin of ginsenoside Rb1, Rb2 and Rc. *Chem. Pharm. Bull.* **20:** 1212–1216.
36. Wakabayashi, C., H. Hasegawa, J. Murata, and I. Saiki. 1998. *In vivo* antimetastatic action of ginseng protopanaxadiol saponins is based on their intestinal bacterial metabolites after oral administration. *Oncol. Res.* **9:** 411–417.
37. Wu, J. Y., B. H. Gardner, C. I. Murphy, J. R. Seals, C. R. Kensil, J. Recchia, G. A. Beltz, G. W. Newman, and M. J. Newman. 1992. Saponin adjuvant enhancement of antigen-specific immune responses to an experimental HIV-1 vaccine. *J. Immunol.* **148:** 1519–1525.
38. Wuthrich, B. 1989. Epidemiology of the allergic diseases: Are they really on the increase? *Int. Arch. Allergy Appl. Immunol.* **90(Suppl. 1):** 3–10.
39. Yang, S. H., C. Y. Hong, and C. L. Yu. 2001. Decreased serum IgE level, decreased IFN-gamma and IL-5 but increased IL-10 production, and suppressed cyclooxygenase 2 mRNA expression in patients with perennial allergic rhinitis after treatment with a new mixed formula of Chinese herbs. *Int. Immunopharmacol.* **1:** 1173–1182.