

Compound K, a Metabolite of Ginsenoside Rb1, Inhibits Passive Cutaneous Anaphylaxis Reaction in Mice

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Abstract : To understand the anti-allergic mechanism of compound K, which is a metabolite of ginsenoside Rb1, a main constituent of the root of *Panax ginseng* C.A. Meyer (family Araliaceae), its inhibitory effect against IgE-antigen complex (IAC)-induced passive cutaneous anaphylaxis (PCA) reaction in mice and mRNA and protein expressions of allergic cytokines in IAC-stimulated RBL-2H3 cells were investigated. Orally administered ginsenoside Rb1 more potently inhibited PCA reaction when administered at 5 h prior to the IAC treatment than when administered at 1 h before. However, compound K orally administered 1 h before IAC treatment showed a more potent anti-PCA reaction effect than when treated at 5 h before. Orally administered ginsenoside Rb1 more potently inhibited PCA reaction induced by IAC in mice than intraperitoneally treated one, apart from orally administered its metabolite, compound K, which was more potent than the orally administered one. The compound K, a metabolite of ginsenoside Rb1, inhibited mRNA and protein expressions of IL-4 and TNF- α and the activation of their transcription factor NF- κ B and MAPK in IAC-stimulated RBL-2H3 cells. These findings suggest that orally administered ginsenoside Rb1 may be dependent on its metabolism by intestinal microflora in the intestine and the compound K may improve allergic diseases by the inhibition of IL-4 and TNF- α expression.

Key words : allergy, ginsenoside Rb1, compound K, anaphylaxis, IgE

INTRODUCTION

Mast cells and basophils are well-known critical participants in various biological processes of allergic diseases.¹⁻³⁾ These cells express surface membrane receptors, with high affinity and specificity for IgE. The interaction of antigen-bound IgE in surface membrane receptors causes the release of histamine, prostaglandins, leukotrienes and cytokines.^{4,5)} These cytokines activate chemotaxis and phagocytosis of neutrophils and macrophages. Finally, cytokine-induced reactions cause tissue inflammation. These allergic diseases are now rapidly increasing chronic health problem in most countries.⁶⁾ Anti-allergic agents, such as anti-histamines, steroids and immunosuppressants, have been used against allergic diseases, such as allergic rhinitis, atopic dermatitis, asthma and food allergies,⁷⁻⁹⁾ but improving these diseases is very difficult. Therefore, herbal medicines have been advanced for allergic diseases, and their effectiveness has received increasing attention.^{10,11)}

Ginseng (the root of *Panax ginseng* C.A. Meyer, family Araliaceae) is frequently used as a crude substance, and is taken orally in Asian countries as a traditional medicine. The major components of interest in ginseng are ginsenosides, which contain an aglycone with a dammarane skeleton.^{12,13)} These ginsenosides have been previously reported to show various biological activities, which include anti-inflammatory,¹⁴⁾ and anti-tumor activities (i.e., the inhibition of tumor-induced angiogenesis and the prevention of tumor invasion and metastasis).^{15,16)} The pharmacological actions of these ginsenosides have been explained on the basis of the biotransformation of ginsenosides by human intestinal bacteria.¹⁷⁻²²⁾ For example, the protopanaxadiol ginsenosides are transformed to 20-O- β -D-glucopyranosyl-20 (S)-protopanaxadiol (compound K) by human intestinal bacteria. Compound K shows anti-metastatic and/or anti-carcinogenic effects by blocking tumor invasion or by preventing chromosomal aberration and tumorigenesis.^{6,22)} In our previous reports, compound K showed anti-allergic effects against anaphylaxis, dermatitis and scratching behavior.²³⁾ Nevertheless, its anti-allergic mechanism of compound K has not been thoroughly studied.

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Therefore, to understand the anti-allergic mechanism of compound K, we transformed ginsenoside Rb1 by *Bifidobacterium* H-1, a human intestinal bacterium, and isolated its metabolite, compound K, and investigated its anti-allergic effects *in vitro* and *in vivo*.

MATERIALS AND METHODS

1. Materials

Dulbecco's modified Eagle medium (DMEM) and radio-immunoprecipitation assay (RIPA) lysis buffer were purchased from Sigma Co. (St Louis, MO, USA). A protease inhibitor cocktail was from Roche Applied Science (Mannheim, Germany). ELISA kits were obtained from Pierce Biotechnology, Inc., (Rockford, IL, USA). Antibodies for NF- κ B (pp65 and p65) and p38 MAP kinase (pp38 and p38) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Enhanced chemiluminescence (ECL) immunoblot system was obtained from Pierce Co. (Rockford, IL, U.S.A.).

The ginsenoside Rb1 (purity, >92%) and compound K (purity, >95%) (Fig. 1) were isolated from the root of *Panax ginseng* C.A. Meyer, according to the previous reports.^{24,25)}

2. Animals

The male ICR mice (20-25 g) were supplied by the 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 the animals and their care conformed to the international guidelines 'Principles of Laboratory Animals Care' (NIH

publication no. 85-23, revised 1985).

3. Passive cutaneous anaphylaxis (PCA) reaction

An IgE-dependent cutaneous reaction was measured according to the previous method of Choo *et al.*²⁶⁾ The male ICR mice were intradermally injected, 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 in PBS, containing 200 μ g of DNP-HSA, *via* the tail vein. The test agents were orally or intraperitoneally administered 1 h or 5 h prior to the DNP-HSA injection. Thirty min after the DNP-HSA injection, the mice were sacrificed, their dorsal skins removed and the pigmented area measured. After extraction with 1 mL of 1.0 M KOH and 4 mL of a mixture of acetone and 0.2 M phosphoric acid (13:5), the amount of dye was determined colorimetrically at 620 nm.

4. Enzyme-linked immunosorbent assay (ELISA) and immunoblot

RBL-2H3 cells were performed by the method of Choo *et al.*²⁶⁾ Briefly, the previously cultured RBL-2H3 cells or HMC cells (5×10^5 cells) were treated with 0.5 μ g/mL of mouse monoclonal IgE for sensitization of cells. The cells (1.8 mL) were exposed to 0.2 mL of test agents for 20 min (for RT-PCR), for 1 h (for immunoblot) or 4 h [for enzyme-linked immunoassay (ELISA)], followed by the treatment with 0.2 mL of DNP-HSA (1 μ g/mL) for 40 min at 37°C. Total RNA was extracted by using RNeasy[®] Minikit, and then RT-PCR for IL-4, TNF- α and GAPDH were performed. The immunoblot for NF- κ B (pp65 and p65) was performed to the method of Lee *et al.*²⁷⁾ The supernatant (50 μ L) was transferred into 96-well (ELISA) plates and then IL-4 and TNF- α concentrations were determined using commercial ELISA kits (Pierce Biotechnology, Rockford, IL, USA).

5. Statistical analysis

All data were expressed as the mean \pm standard deviation, with statistical significance analyzed using one-way ANOVA followed by a Student-Newman-Keuls test.

RESULTS AND DISCUSSION

To evaluate the anti-allergic activity of compound K, ginsenoside Rb1 was transformed to compound K by *Bifidobacterium* H-1, its metabolite compound K isolated and

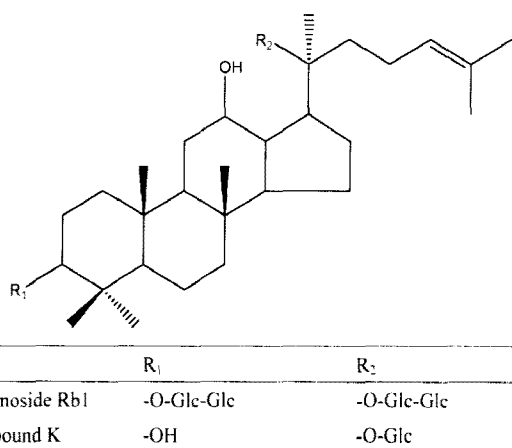


Fig. 1. Structure of ginsenoside Rb1 and its metabolite compound K.

their anti-PCA reaction effect investigated in PCA reaction mouse model (Fig. 2). The PCA reaction was induced by an injection of IgE-antigen complex (IAC), with test agents administered orally 1 h or 5 h prior to the challenge with antigen. IAC potently induced the PCA reaction. When ginsenoside Rb1 or compound K was orally administered 1 h before the treatment of IAC, compound K potently inhibited PCA reaction, although ginsenoside Rb1 showed a weak inhibition. Ginsenoside Rb1 (25 mg/kg) and compound K (25 mg/kg) orally administered 1 h before IAC treatment inhibited the PCA reaction by 33% and 86%, respectively. However, when ginsenoside Rb1 was orally administered 5 h before IAC treatment, it showed a more potent inhibition than when administered 1 h before. Ginsenoside Rb1 (25 mg/kg) orally administered 5 h before IAC treatment inhibited the PCA reaction by 54%. When compound K was orally administered 5 h before IAC treatment, it potently inhibited the PCA reaction. The compound K showed a more potent inhibition when administered 1 h before IAC treatment than when administered 5 h before. Compound K (25 mg/kg) orally administered 5 h before IAC treatment inhibited the PCA reaction by 63%.

Next, we measured PCA reaction-inhibitory effect of intraperitoneally administered ginsenoside Rb1 and com-

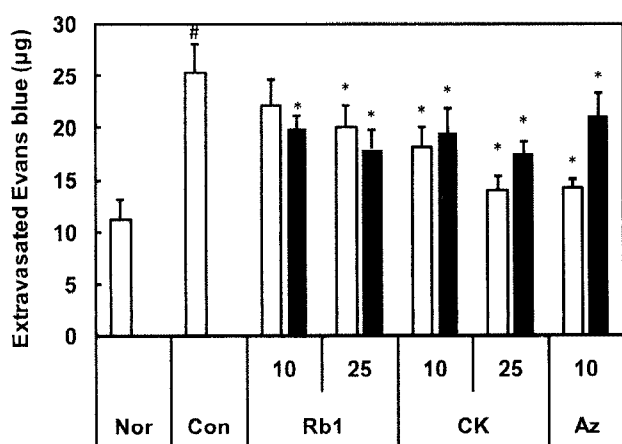


Fig. 2. Effect of orally administered ginsenoside Rb1 and compound K on passive cutaneous anaphylaxis (PCA) reaction induced by IgE-antigen complex (IAC) in mice. Test agents were orally administered 1 h or 5 h prior to antigen challenge. The mice were sacrificed. The amounts of extravasated Evan blue from the dorsal skin (1×1 cm) of the control stimulated with IAC and vehicle-treated groups were 25 ± 3 and 11 ± 2 µg, respectively. Values indicate the mean ± S.D. (n=6). #Significantly different versus normal group (P<0.05). *Significantly different versus control group (P<0.05).

ound K (Fig. 3). Intraperitoneally administered ginsenoside Rb1 weakly inhibited PCA reaction, but compound K potently inhibited it. Its inhibitory potency was comparable with that of a commercially available azelastine.

To understand the anti-PCA reaction mechanism of compound K, its inhibitory activities against IgE-switching cytokine IL-4 and proinflammatory cytokine TNF-α in IAC-induced RBL-2H3 cells were measured using an ELISA and RT-PCR assays (Fig. 4). Compound K significantly inhibited these cytokine expressions, although ginsenoside Rb1 weakly inhibited their expressions. The compound K, at concentrations of 5 and 20 µM, inhibited their expressions by 43% and 59% for TNF-α and 55% and 67% for IL-4, respectively. The compound K also inhibited the activation of NF-κB and p38 MAPK in IAC-stimulated RBL-2H3 cells.

Azelastine, a representative antiallergic drug, is an H1-receptor antagonist, which decreases the mediator release from mast cells and basophils.²⁸⁾ Quercetin, a non-steroidal anti-inflammatory flavonoid, scavenges superoxide anion and inhibits the PCA reaction.²⁹⁾ When the ginsenosides Rb1, Rb2, Rc and Rd, which is main components of ginseng, were orally administered to rats or humans, they were metabolized to compound K in the intestine and absorbed into the blood.³⁰⁻³²⁾ When ginseng was orally

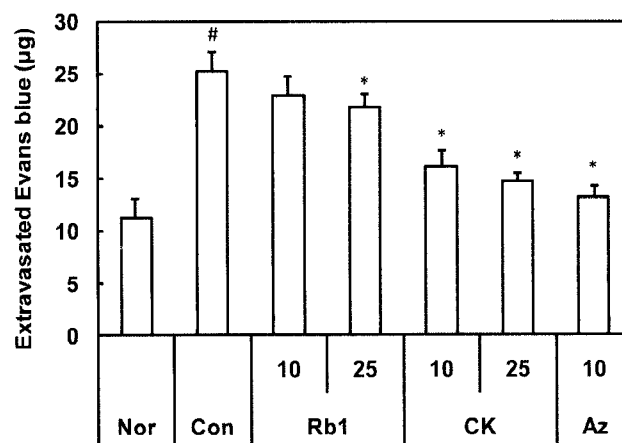


Fig. 3. Effect of intraperitoneally administered ginsenoside Rb1 and compound K on passive cutaneous anaphylaxis (PCA) reaction induced by IgE-antigen complex (IAC) in mice. Test agents were intraperitoneally administered 1 h prior to antigen challenge. The amounts of extravasated Evan blue from the dorsal skin (1×1 cm) of the control stimulated with the IAC and vehicle-treated groups were 25 ± 3 and 11 ± 2 µg, respectively. Values indicate the mean ± S.D. (n=6). #Significantly different versus normal group (P<0.05). *Significantly different versus control group (P<0.05).

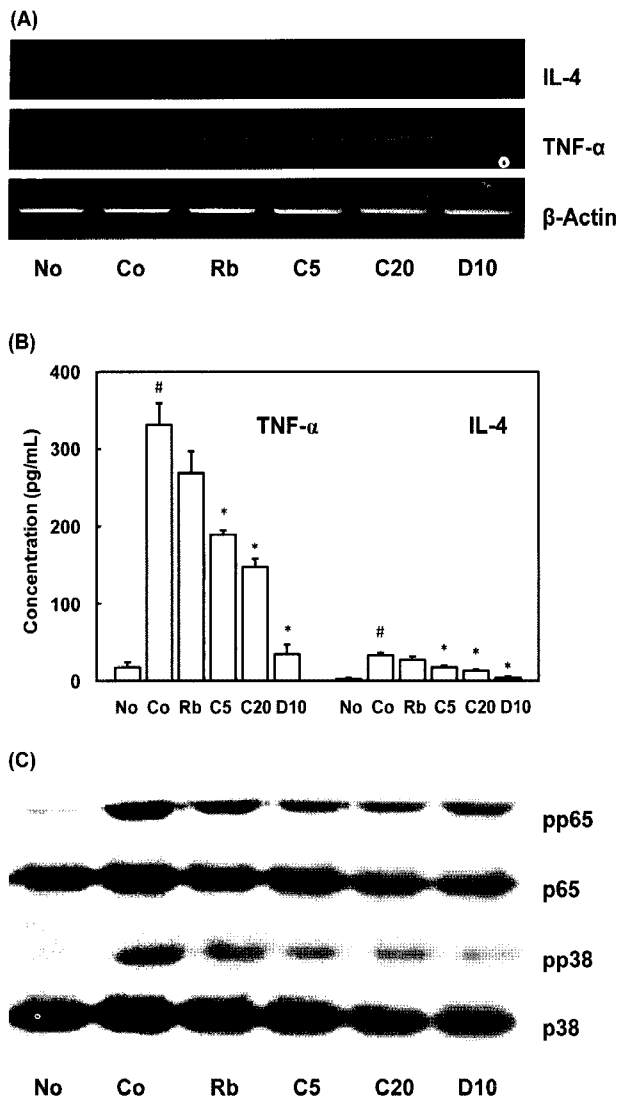


Fig. 4. Effect of ginsenoside Rb1 and compound K on mRNA and protein expression levels of some cytokine in RBL-2H3 cells induced by IgE-antigen complex. RBL-2H3 cells (5×10^5 cells) were treated with $0.5 \mu\text{g/mL}$ of mouse monoclonal IgE, exposed to 0.2 mL of agents (Co, vehicle alone; Rb20, $20 \mu\text{M}$ ginsenoside Rb1; C5, 5 or $20 \mu\text{M}$ compound K; C20, $20 \mu\text{M}$ compound K; D10, $10 \mu\text{M}$ dexamethasone) for 20 min (for RT-PCR) or 4 h (for ELISA), followed by the treatment with 0.2 mL of dinitrophenol-human serum albumin ($1 \mu\text{g/mL}$) for 40 min (mRNA expression) or 4 h (protein expression) at 37°C and then RT-PCR for IL-4 and TNF- α (A), ELISA for IL-4 and TNF- α (B) and immunoblot for NF- κ B (pp65 and p65) (C) were performed. Normal (No) was treated with vehicle alone instead of agent and IgE-antigen complex. Values represent the mean \pm S.D. for triplicate experiments. #Significantly different versus normal group ($P < 0.05$). *Significantly different versus control group ($P < 0.05$).

administered to human, compound K was detected in the blood. Therefore, these results have suggested that proto-panaxadiol-type ginsenosides should be metabolized to compound K in intestine by intestinal microflora. Therefore, we studied the anti-allergic effect of ginsenoside Rb1, which is a main component of ginseng, and its metabolite compound K on PCA mouse models. Ginsenoside Rb1 orally administered 5 h before IAC treatment potentially inhibited PCA reaction. However, when ginsenoside Rb1 was orally administered 1 h before IAC treatment, it weakly inhibited the PCA reaction. These results suggest that, to express the pharmacological action of ginsenoside Rb1, it may take a time (5 h) for ginsenoside Rb1 to be metabolized to compound K by intestinal microflora. However, intraperitoneally and orally administered compound K potentially inhibited PCA reaction within 1 h after the treatment of compound 48/80. Compound K potentially inhibited PCA reaction. Choo *et al.* reported that compound K inhibited the histamine release from RBL-2H3 cells induced by IgE as well as the passive cutaneous anaphylaxis (PCA) reaction in mice due to its potent membrane stabilizing effect.²⁶⁾ Shin and Kim reported that compound K inhibited scratching behavior caused by compound 48/80 or histamine by the inhibition of vascular permeability.²⁴⁾

Azuma *et al.* reported that a membrane stabilizer trinitilast, which inhibited the histamine release from rat peritoneal mast cells induced by antigens, as well as antigen-induced PCA reaction, possessed an inhibitory effect toward scratching behavior.³³⁾ However, we observed that compound K potentially inhibited IL-4 and TNF- α expression as well as the activation of NF- κ B and p38 (MAPK). These results suggest that compound K may inhibit these cytokine expressions by the regulation of NF- κ B and MAPK.

These results suggest that ginsenoside Rb1 may be metabolized to compound K by intestinal microflora and the metabolite compound K may improve allergic reactions, such as rhinitis and atopic dermatitis, by the inhibition of IgE-switching cytokine IL-4 and proinflammatory cytokine TNF- α and the membrane stabilizing action.

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