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

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(Received June 1, 2009; Revised June 19, 2009; Accepted June 19, 2009)

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-κB 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.
Therefore, to understand the antiallergic 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-κB (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 μg of anti-DNP IgE, into each of two dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Forty-eight hours later each mouse received an injection of 200 μL of 3% Evans blue in PBS, containing 200 μg 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 (5x10^5 cells) were treated with 0.5 μg/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 immunosassay (ELISA)], followed by the treatment with 0.2 mL of DNP-HSA (1 μg/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-κB (pp65 and p65) was performed to the method of Lee et al.27) The supernatant (50 μL) 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 ± 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.](image-url)