

## Full Paper

## Antipruritic Effect of Ginsenoside Rb1 and Compound K in Scratching Behavior Mouse Models

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Received March 7, 2005; Accepted July 20, 2005

**Abstract.** The antipruritic and vascular permeability-inhibitory effects of ginsenoside Rb1, a main component of ginseng frequently used as a traditional medicine in Asian countries, and its metabolite compound K by intestinal microflora were investigated in scratching behavior animal models induced by compound 48/80, substance P, and histamine. Ginsenoside Rb1 and compound K orally administered 1 and 6 h before the treatment of compound 48/80 showed antipruritic effect. These ginsenosides administered at a dose of 50 mg/kg 6 h before the treatment of compound 48/80 inhibited scratching behaviors by 51% and 64%, respectively, compared with that of the control. These ginsenosides also inhibited the vascular permeability of skin. Compound K intraperitoneally administered 1 h before the treatment of compound 48/80 potently inhibited the scratching behaviors induced by compound 48/80. However, intraperitoneally administered ginsenoside Rb1 did not inhibit scratching behaviors. Compound K inhibited compound 48/80-, substance P-, and histamine-induced scratching behaviors, with 50% inhibitory doses of 4.2, 5.9, and 3.8 mg/kg, respectively, and vascular permeability, with 50% inhibitory doses of 5.8, 6.8, and 4.1 mg/kg, respectively. These results suggest that ginsenoside Rb1 and its metabolite compound K by intestinal microflora can improve scratching behaviors.

**Keywords:** compound K, scratching behavior, compound 48/80, histamine, substance P

## Introduction

Pruritus (itch) is an unpleasant cutaneous sensation, which provokes the desire to scratch, can be local or widespread, and can be associated with atopic dermatitis, urticaria, or systemic disorders (cholestasis, uraemia). Many endogenous chemical agents, like amines, proteases, growth factors, neuropeptides, opioids, eicosanoids, and cytokines, can act as pruritogens (1–3). Scratching can cause skin lesions and contribute to severe psychological disturbances (4), and therefore, inhibition of this response is consistently beneficial for improving the quality of life. To evaluate the effect of itching-inhibitory agents, compound 48/80, substance P, or histamine-induced scratching behavior mouse models were used (5–7). However, there is no specific remedy available for this common symptom.

Ginsenosides are major components of ginseng (the root of *Panax ginseng* C.A. MEYER, Araliaceae), which

is frequently used as a crude substance taken orally as a traditional medicine in Asian countries (8). These ginsenosides have been reported to show various biological activities, including antiallergic (9, 10) and anti-tumor activities (11, 12). These pharmacological actions originate from compound K, which are biotransformed from ginsenosides Rb1, Rb2, and Rc by human intestinal microflora (10–12).

During the screening program to discover antipruritic compounds from natural products, ginseng was found to show inhibitory activity on scratching behavior animal models induced by compound 48/80. Therefore, ginsenoside Rb1, which is a main component of ginseng, and its metabolite compound K by intestinal microflora was isolated and their antipruritic effects were investigated on scratching behavior animal models induced by compound 48/80, substance P, and histamine.

## Materials and Methods

## Materials

Azelastine, compound 48/80, histamine, substance

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P, and Evans blue were purchased from Sigma Chem. Co. (St. Louis, MO, USA). The ginsenoside Rb1 and compound K (Fig. 1) were isolated using the previously published methods (11, 13).

### Animals

The male BALB/c mice (18–22 g) were supplied from Charles River Orient Experimental Animal Breeding Center (Seoul, Korea). All animals were housed in wire cages at 20–22°C, a relative humidity of  $50 \pm 10\%$  humidity, a frequency of air ventilation of 15–20 times/h, and 12-h illumination (07:00–19:00; intensity, 150–300 Lux), fed standard laboratory chow (Charles River Orient Experimental Animal Breeding Center) 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).

### Behavioral experiments

Before the experiment, the BALB/c mice were put into acrylic cages ( $22 \times 22 \times 24$  cm) for about 10 min for acclimation. The behavioral experiments were performed according to the method of Sugimoto et al. (14). The rostral part of the skin on the back of mice was clipped, and 50  $\mu\text{g}/50 \mu\text{l}$  of compound 48/80, 300  $\mu\text{g}/50 \mu\text{l}$  of histamine or 300  $\mu\text{g}/50 \mu\text{l}$  of substance P for each mouse was intradermally injected with the use a 29-gauge needle. The scratching agents were dissolved in saline and then used. Control mice received a saline injection in the place of the scratching agent. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage; and for

the observation of scratching, their behaviors were recorded using an 8-mm video camera (SV-K80; Samsung, Seoul, Korea) under unmanned conditions. Scratching of the injected site by the hind paws was counted and compared with that of other sites, such as the ears. Each mouse was used for only one experiment. The mice generally showed several scratches for 1 s, and a series of these behaviors was counted as one incident of scratching for 60 min. Ginsenoside Rb1 and compound K were orally or intraperitoneally administered 1 h or 6 h before the scratching agents.

### Measurement of vascular permeability

The increase in vascular permeability caused by scratching agents was assessed as reported previously (14). After the intradermal injection of 50  $\mu\text{g}/50 \mu\text{l}$  of compound 48/80, 300  $\mu\text{g}/50 \mu\text{l}$  of histamine, or 300  $\mu\text{g}/50 \mu\text{l}$  of substance P into the rostral part of the back of each mouse, 0.2 ml of 1% saline solution of Evans blue was injected intravenously. Ginsenoside Rb1 and compound K were orally or intraperitoneally administered 1 or 6 h before the scratching agents. Mice were sacrificed 60 min later by cervical dislocation and the scratching agent-injected site excised. The skin specimen was dissolved in 1 ml of 1 M KOH solution by overnight incubation, and 4 ml of a mixture of 0.2 M phosphoric acid solution-acetone (5:13) was added. After vigorous shaking, the precipitates were filtered off and the amount of dye was measured colorimetrically at 620 nm.

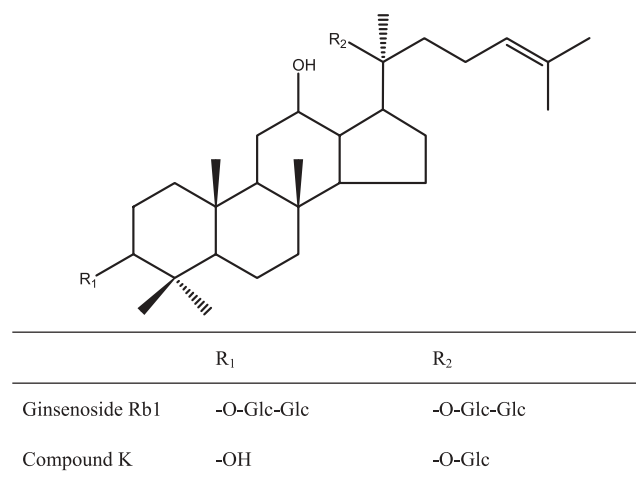
### Statistics

All the data were expressed as the mean  $\pm$  S.D., and statistical significance was analyzed by one way ANOVA followed by the Student-Newman-Keuls test.

## Results

### Effect of orally administered ginsenoside Rb1 and compound K on scratching behavior induced by compound 48/80

Ginsenoside Rb1, which is a main component of ginseng, and its metabolite compound K by intestinal microflora were isolated and their antipruritic effects were investigated on the compound 48/80-induced scratching behavior animal model (Fig. 2). When compound 48/80 was injected into the rostral part of the back skin of BALB/c mice, scratching behaviors were significantly induced. When ginsenoside Rb1 was orally administered 1 h before the treatment of the scratching agent compound 48/80, it did not inhibit the scratching behaviors. However, when ginsenoside Rb1 was orally administered 6 h before the treatment of compound



**Fig. 1.** Structure of the ginsenoside Rb1 isolated from ginseng and its metabolite compound K.

48/80, scratching behaviors were potently inhibited. Orally administered ginsenoside Rb1 at a dose of 50 mg/kg inhibited the scratching behaviors by 51%. When compound K was orally administered 1 or 6 h before the treatment of compound 48/80, it potently inhibited the scratching frequency. Compound K (50 mg/kg) inhibited the scratching behaviors by 64%. Ginsenoside Rb1 administered 6 h and compound K administered 1 and 6 h before the treatment of compound 48/80 also decreased the vascular permeability of skin induced by compound 48/80.

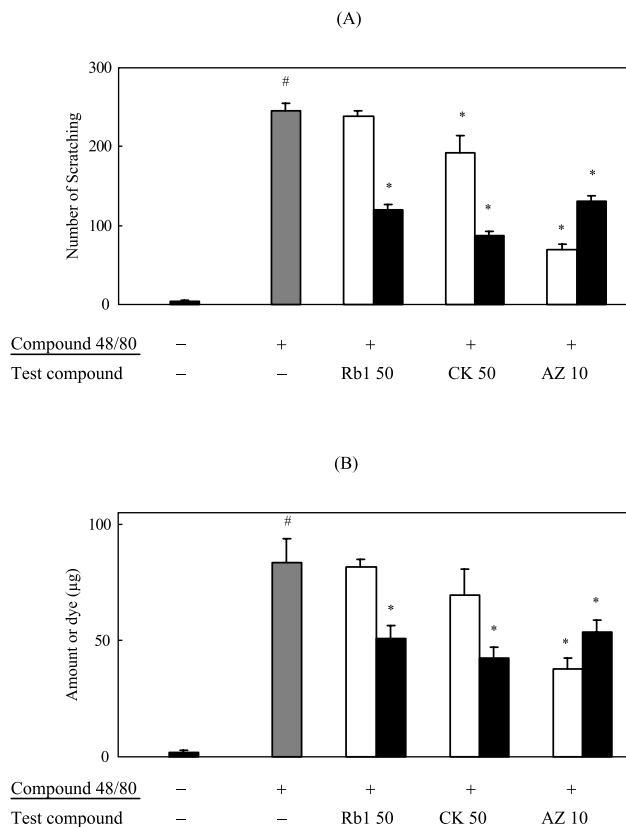
*Effect of intraperitoneally administered ginsenosides on scratching behavior induced by compound 48/80, substance P, or histamine*

The scratching-inhibitory effect of intraperitoneally administered ginsenoside Rb1 and compound K was

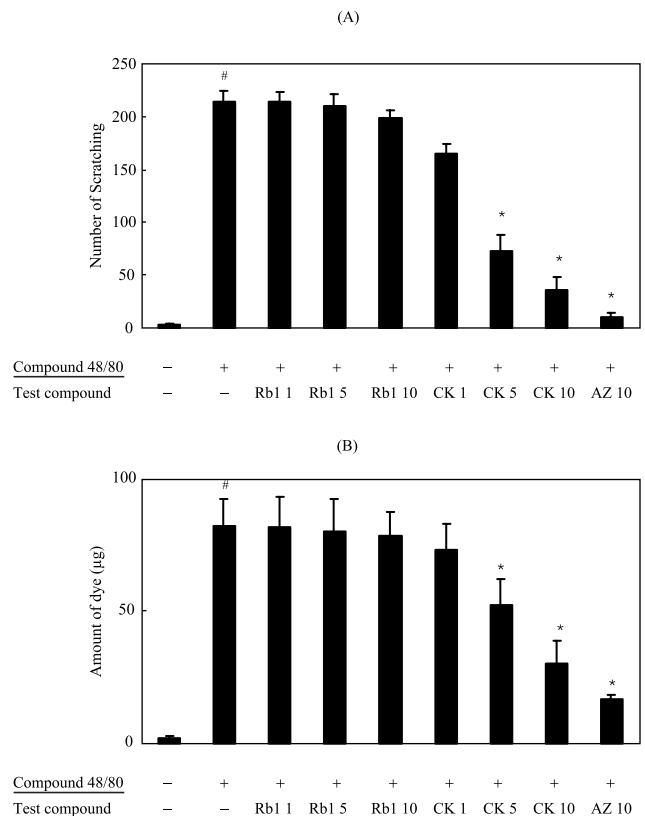
evaluated (Fig. 3). Intraperitoneally administered ginsenoside Rb1 did not exhibit the inhibitory effect against scratching behaviors. However, compound K potently inhibited the scratching frequency. Compound K also decreased the vascular permeability of skin induced by compound 48/80. Compound K potently inhibited the scratching behaviors and vascular permeability, with 50% inhibitory doses of 4.2 and 5.8 mg/kg, respectively. Its inhibitory potency was comparable with that of a commercially available azelastine.

When substance P was used as a scratching agent, it induced the scratching behaviors and increased vascular permeability (Fig. 4). Compound K inhibited scratching behaviors and vascular permeability, with 50% inhibitory doses of 5.9 and 6.8 mg/kg, respectively.

When histamine was used as an inducer of scratching behavior, histamine not only induced the scratching



**Fig. 2.** Inhibitory effect of orally administered ginsenoside Rb1, compound K, and azelastine on compound 48/80-induced scratching behavior (A) and vascular permeability (B) in BALB/c mice. Mice were treated without (Control) or with oral administration of 50 mg/kg ginsenoside Rb1 (Rb1 50), 50 mg/kg compound K (CK 50), or 10 mg/kg (AZ 10) 1 (open columns) or 6 h (closed columns) before the intradermal injection of 50  $\mu$ g/50  $\mu$ l (per mouse) compound 48/80 into the shaved back skin of mice. Normal: control mice treated with saline, instead of compound 48/80. Mean  $\pm$  S.D. (n = 6). <sup>#</sup> $P$  < 0.05, compared with normal control; \* $P$  < 0.05, compared with compound 48/80 alone.



**Fig. 3.** Inhibitory effect of intraperitoneally administered ginsenoside Rb1, compound K, and azelastine on compound 48/80-induced scratching behavior (A) and vascular permeability (B) in BALB/c mice. Mice were treated without (Control) or with intraperitoneal administration of 1 mg/kg (Rb1 1), 5 mg/kg (Rb1 5) and 50 mg/kg of ginsenoside Rb1 (Rb1 50), 1 mg/kg (CK 1), 5 mg/kg (CK 5) and 50 mg/kg of compound K (CK 50), or 10 mg/kg of azelastine (AZ 10) 1 h before the intradermal injection of 50  $\mu$ g/50  $\mu$ l (per mouse) compound 48/80 into the shaved back skin of mice. Normal: control mice treated with saline, instead of compound 48/80. Mean  $\pm$  S.D. (n = 6). <sup>#</sup> $P$  < 0.05, compared with normal control; \* $P$  < 0.05, compared with compound 48/80 alone.

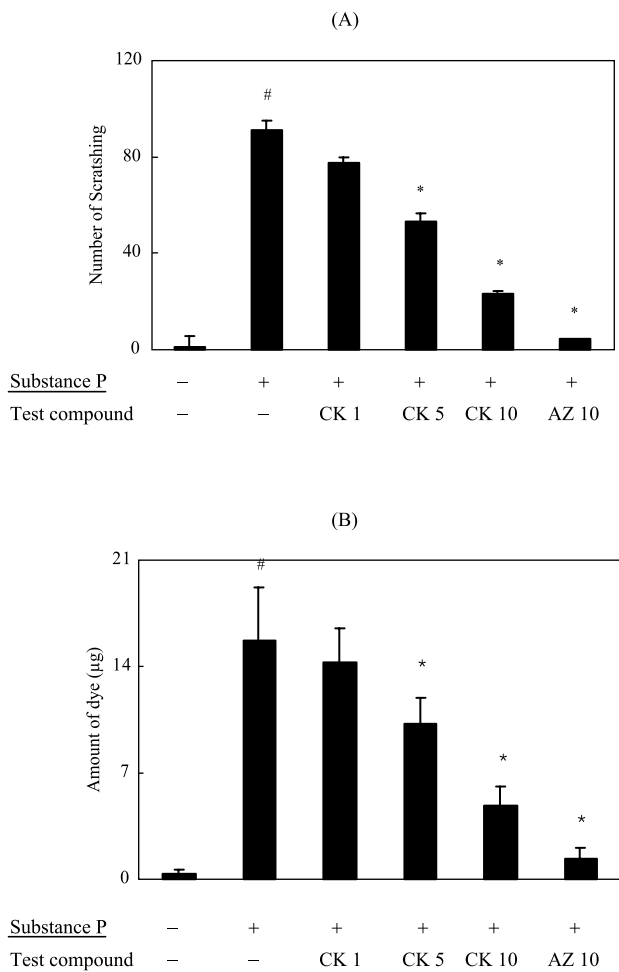
behaviors, but also increased the vascular permeability (Fig. 5). Compound K also inhibited the histamine-induced scratching behavior and vascular permeability, with 50% inhibitory doses of 3.8 and 4.1 mg/kg, respectively. Its inhibitory potency was comparable with that of the commercially available azelastine.

Discussion

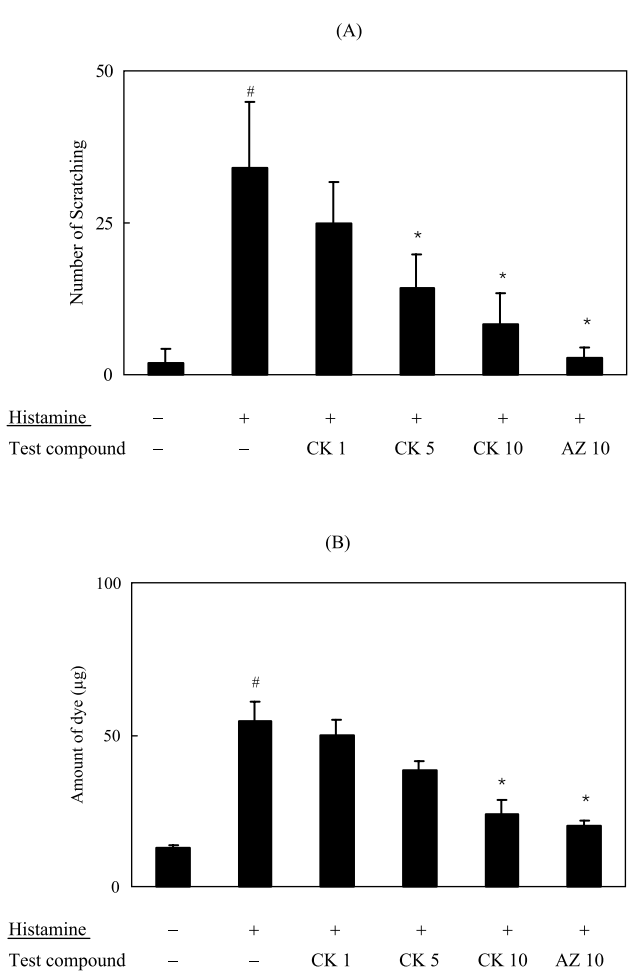
Pruritus (itch) may be associated with atopic dermatitis, urticaria, or systemic disorders (cholestasis, uraemia). The etiology of atopic dermatitis may be based on IgE-mediated pharmacological processes of a

variety of cell populations such as mast cells and basophils (15). Degranulation of mast cells and basophils, with antigen-crosslinked IgE, releases histamine, prostaglandins, leukotrienes, and cytokines (16, 17). These mediators activate chemotaxis and phagocytosis of neutrophils and macrophages, as well as induce itching (15, 18, 19).

To evaluate effect of itching-inhibitory agents on scratching behavior mouse models, compound 48/80-, substance P-, and histamine were used (5 – 7). These scratching agents were intradermally injected into the rostral part of the back skin of BALB/c and ICR mice, and the scratching behaviors evaluated for 60 min. The



**Fig. 4.** Inhibitory effect of intraperitoneally administered compound K and azelastine on substance P-induced scratching behavior (A) and vascular permeability (B) in BALB/c mice. Mice were treated without (Control) or with intraperitoneal administration of 1 mg/kg (CK 1), 5 mg/kg (CK 5) and 50 mg/kg of compound K (CK 50), or 10 mg/kg of azelastine (AZ 10) 1 h before the intradermal injection of 300 μg/50 μl (per mouse) substance P into the shaved back skin of mice. Normal: control mice treated with saline, instead of substance P. Mean ± S.D. (n = 6). <sup>#</sup>*P* < 0.05, compared with normal control; <sup>\*</sup>*P* < 0.05, compared with compound 48/80 alone.



**Fig. 5.** Inhibitory effect of intraperitoneally administered compound K and azelastine on histamine-induced scratching behavior (A) and vascular permeability (B) in BALB/c mice. Mice were treated without (Control) or with intraperitoneal administration of 1 mg/kg (CK 1), 5 mg/kg (CK 5) and 50 mg/kg of compound K (CK 50), or 10 mg/kg of azelastine (AZ 10) 1 h before the intradermal injection of 300 μg/50 μl (per mouse) histamine into the shaved back skin of mice. Normal: control mice treated with saline, instead of histamine. Mean ± S.D. (n = 6). <sup>#</sup>*P* < 0.05, compared with normal control; <sup>\*</sup>*P* < 0.05, compared with compound 48/80 alone.

scratching frequencies for the 60 min period for each inducer were increased dose-dependently. These scratching agents more potently induced scratching behaviors in ICR mice than in BALB/c mice like the previously reported study (20). Particularly, compound 48/80 vigorously caused scratching behaviors in ICR mice. The correct counting of scratching behavior frequencies was too difficult. However, BALB/c mice were less sensitive to histamine. Nevertheless, in the present study, BALB/c mice were used for preparation of scratching mouse models. Histamine and substance P all caused scratching behaviors and increased vascular permeability as previously reported (5). However, the vascular permeability of substance P was weaker than that of histamine, although Kuraishi et al. reported that substance P-induced scratching behaviors and vascular permeability in BALB/c mice were similar to that of histamine (5). The difference is still to be elucidated.

When the ginsenosides Rb1, Rb2, Rc, and Rd, which are 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 (21–23). When ginseng was orally administered to humans, compound K was detected in the blood. Therefore, these results have suggested that protopanaxadiol-type ginsenosides would be metabolized to compound K in the intestine by intestinal microflora. Therefore, we studied the antipruritic effect of ginsenoside Rb1, which is a main component of ginseng, and its metabolite compound K on scratching behavior mouse models. Ginsenoside Rb1 orally administered 6 h before the treatment of scratching agent potently inhibited scratching behavior induced by compound 48/80. However, when ginsenoside Rb1 was orally administered 1 h before the treatment of scratching agent, it did not inhibit the scratching behavior. These results suggest that to express the pharmacological action of ginsenoside Rb1, it may take a time (6 h) for ginsenoside Rb1 to be metabolized to compound K by intestinal microflora. However, the intraperitoneally and orally administered compound K showed the antipruritic effect within 1 h after the treatment of compound 48/80. Compound K potently inhibited scratching behaviors induced by compound 48/80, substance P, and histamine, and it also the increased vascular permeability of the skin. However, compound K showed no histamine-antagonistic activity against rat ileum (data not shown). The result suggest that the antipruritic effect of compound K is independent of the histamine H<sub>1</sub> receptor. In addition, 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 (9). Azuma et al. reported that the membrane stabilizer tranilast, 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 (24). These results suggest that compound K may inhibit scratching behaviors by stabilizing the membrane rather than by antagonizing the histamine H<sub>1</sub> receptor, although the mechanism is still to be elucidated.

Based on these findings, protopanaxadiol ginsenosides may be metabolized to compound K by intestinal microflora, and ginseng extract including these ginsenosides can show an antipruritic effect.

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