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Inhibitory Effect of Protopanxatriol Ginsenosides in an Oxazolone-induced Mouse Psoriatic Model

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Abstract : When the inhibitory effect of ginsenoside (G) Re isolated from ginseng and its metabolites G-Rg1, G-F1, G-Rh1 and protopanaxatriol in mouse ear skin psoriasis stimulated by oxazolone was investigated, G-Re and its metabolites suppressed mouse ear swelling stimulated by oxazolone. Among these agents tested, G-Rh1 most potently suppressed ear swelling as well as mRNA expression of COX-2 and proinflammatory cytokines IL-1β, TNF-α and interferon-γ. These findings suggest that G-Rh1 may improve chronic dermatitis and psoriasis.

Key words : ginseng, ginsenoside Re, ginsenoside Rh1, contact dermatitis.

INTRODUCTION

Ginseng (the root of *Panax ginseng* C.A. Meyer, Araliaceae), whose major components of ginseng are protopanxadiol (PD) and protopanaxatriol (PT) ginsenosides, which contain an aglycone with a dammarane skeleton,[1)] is frequently taken orally as a traditional medicine in Asian countries. These ginsenosides have been reported to exhibit various biological activities, including anti-inflammatory,[2)] and antiallergic[3,4)] and antitumor effects.[5,6)] The diversified pharmacological actions of these ginsenosides have been supported by their biotransformation by human intestinal microflora.[7,8)] PPT ginsenosides are mainly transformed to 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (compound K, C-K) or ginsenoside Rh2 (G-Rh2) by human intestinal bacteria.[8-10)] These transformed PD ginsenosides, C-K and G-Rh2, exhibit antiallergic effects, such as anti-passive cutaneous anaphylaxis, and anti-inflammatory effects.[3,11)] PPT ginsenosides are mainly transformed to G-Rh1, G-F1 or PPT via G-Rg1 by intestinal microflora (Fig. 1).[4,12,13)] These transformed G-Rh1 also exhibit antiallergic effects, such as anti-passive cutaneous anaphylaxis, and anti-inflammatory effect.[3,13)] However, the antipsoriatic effects of G-Rg1, G-F1 and PT except G-Rh1 have not been studied.

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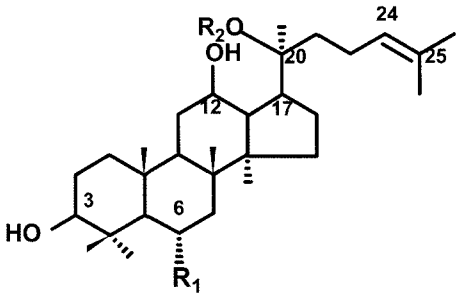
In the present study, we evaluated the antipsoriatic effect of G-Re and its metabolites in mouse ear dermatitis models induced by oxazolone.

MATERIALS AND METHODS

Materials

The oxazolone, betamethasone and RNase-free DNase were purchased from Sigma Co. (St Louis, MO, U.S.A). The TRI reagent was purchased from Molecular Research



Ginsenoside	R1	R2
Ginsenoside-Re	Rha-Glc-O-	Glc-
Ginsenoside Rg1	Glc-O-	Glc-
Ginsenoside F1	H	Glc-
Ginsenoside-Rh1	Glc-O-	H
Protopanaxatriol	H	H

Fig. 1. Structures of ginsenoside Re (G-Re) and its metabolites

Center Inc. (Cincinnati, Ohio, U.S.A.). The isolation and purity of G-Re (purity >90%), G-Rg1 (purity, >95%), G-Rh1 (purity, >95%) and G-F1 (>90%) and PT (>90%) were performed according to the previous methods.[12,13]

Animals

The female 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, with 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 of the 'Principles of Laboratory Animals Care' (NIH publication no. 85-23, revised 1985).

Oxazolone-induced dermatitis

Oxazolone-induced dermatitis was measured according to the method of Fujii *et al.*[14] Each group contained 6 female ICR mice (20-25 g). Mice were sensitized by application of 100 μl of 1.5% oxazolone, in ethanol, to the abdomen. A total of 20 μl of 1% oxazolone, in a mixture of acetone and olive oil (4:1), was then applied to both sides of the mouse ear, every 3 days, starting from 7 days after sensitization. The ear thickness was measured using a Digimatic Micrometer 72 h after each application of the oxazolone; test agents [0.02% (4 μg/ear) or 0.05% (10 μg/ear)] were applied in a total volume of 20 μl to both sides of the ear 30 min before and 3 h after each application of oxazolone

The ear tissues for reverse transcriptase-polymerarse chain reaction (RT-PCR) analysis were excised 6 h after the last application of oxazolone, frozen in liquid nitrogen and homogenized by a mortar and pestle that had been prechilled in liquid nitrogen. Total RNA was extracted using TRI reagent according to the manufacturer's instructions, and treated with RNase-free DNase. The concentration of the RNA content was determined by measuring the absorbance of RNA at 260 and 280 nm, and the RNA samples were stored at -70°C until RT-PCR analysis. The RT-PCR was performed with AccPower® RT/PCR Premix (Bioneer, Seoul, Korea) according to the previously reported method of Shin *et al.*[11]

Histopathological study

The ears of the mice were excised 72 h after the last application of oxazolone, fixed in 10%-buffered formalin solution, embedded in paraffin using standard methods, cut into 5-μm sections, stained with hematoxylin-eosin and then assessed under light microscopy.

Statistical analysis

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

RESULTS AND DISCUSSION

Psoriasis is a chronic and inflammatory skin disorder. Psoriasis patients have been shown to have an interferon (IFN)-γ producing Th1 bias in lesion skin and peripheral blood, although cylcooxygenase (COX)-2 is also induced, which together are thought to develop cytokine networks of Th1 cells.[15-17] Fujii *et al.* developed the skin dermatitis of mouse ears stimulated by oxazolone as an experimental psoriastic model.[14] Steroids, betamethasone, and the immunosuppressants, FK-506, for Th1 cells, have been used clinically for psoriasis.[18-20] However, it also exhibited side effects, such as severe nephrotoxicity and neurotoxicity. Therefore, new agents for clinical uses should be developed from Chinese traditional medicines.

We evaluated the effects of G-Re and its metabolites by

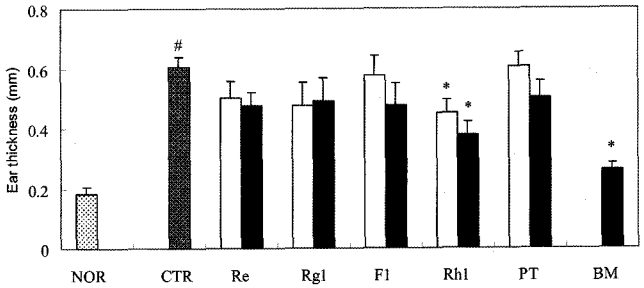


Fig. 2. The effects of ginsenoside Re (G-Re) and its metabolites on the oxazolone-induced ear thickness of mice. Values represent the mean ± S.D. for five mice. Mouse ears were excised 72 h after the last application of oxazolone and stained with hematoxylin-eosin. Negative control mice received 1.5% oxazolone to the abdomen, but not applied it to the ear (NOR). Oxazolone (1%) was applied to both sides of the ear every 3 days, starting from 7 days after sensitization, and vehicle (ethanol) (CTR), G-Re (Re), G-Rg1 (Rg1), G-F1 (F1), G-Rh1 (Rh1), protopanxatriol (PT) or 0.05% betamethasone (BM) were applied to the ear 30 min before and 3 h after each application of oxazolone. Open and closed bars indicate 0.02% and 0.05% agents, respectively. #Significantly different from the normal control group ($P<0.05$). *Significantly different from the control group ($P<0.05$).

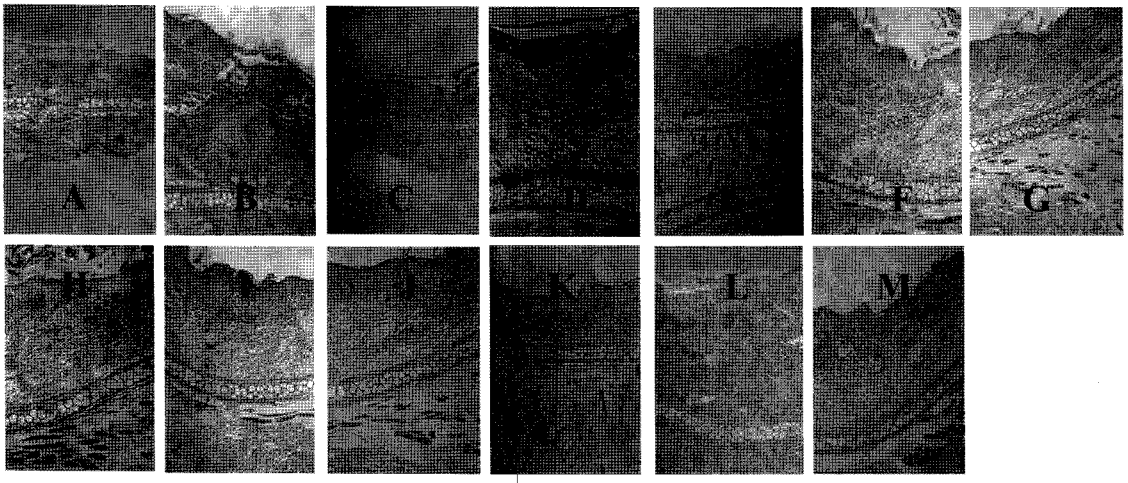


Fig. 3. Histopathological pictures of mouse ears after repeated application of oxazolone with or without ginsenoside Re (G-Re) and its metabolites. Mouse ears were excised 72 h after the last application of oxazolone and stained with hematoxylin-eosin. As a negative control, mice were only sensitized with 1.5% oxazolone to the abdomen, but with no application to the ear (A). 1 % Oxazolone was applied to both sides of the ear every 3 days, starting from 7 days after sensitization, and vehicle (ethanol) (B), 0.05% betamethasone (C), 0.02% G-Re (D), 0.05% G-Re (E), 0.02% G-Rg1 (F), 0.05% G-Rg1 (G), 0.02% G-F1 (H), 0.05% G-F1 (I), 0.02% G-Rh1 (J), 0.05% G-Rh1 (K), 0.02% PT (L), or 0.05% PT (M) was applied to the ear 30 min before and 3 h after each application of oxazolone.

using the method of Fujii *et al.*[14] (Fig. 2). The oxazolone, applied to sensitized mouse ears, caused erythema (reddening of the skin), edema and/or indurations and sometimes abrasions. The ear thickness, measured as an index of skin inflammation, increased approximately 3-fold, compared with that of normal group, which reached maximum 16 days after sensitization. Betamethasone, used as a positive agent at a concentration of 0.05%, potently suppressed the ear swelling, with a suppressive rate of 83% at 16 days. G-Re and its metabolites suppressed the ear swelling at each time-point. Among them, G-Rh1 most potently suppressed the ear swelling. The suppressive rates of G-Rh1 at the 16^{th} day at doses of 0.02% and 0.05% were 37% and 53%, respectively. For a histopathological analysis, the ears were excised on the third day and stained with hematoxylin-eosin (Fig. 3). The ears with oxazolone applied swelled so severely that the entire section could not be shown.

The effects of G-Re and its metabolites on the mRNA expression levels of COX-1, COX-2 and some cytokines of mouse ear dermatitis induced by oxazolone were investigated using RT-PCR analysis (Fig. 4). Oxazolone significantly induced COX-2 mRNA expression; however COX-1 and GAPDH mRNA were not induced. Oxazolone also induced mRNA expression of some cytokines IL-1β, TNF-α, IFN-γ and IL-4. The application of G-Re and its metabolites inhibited the cytokine mRNA expression. Among them, ginsenoside Rh1 at a concentration of 0.05% inhibited mRNA expression of COX-1, IL-1β, TNF-α and IFN-γ by 32%, 26%, 28% and 21%, respectively, but did not affect COX-1 and IL-4 mRNA expression.

Ginseng (the root of *Panax ginseng* C.A. Meyer, family Araliaceae) was found to show anti-inflammatory and antiallergic activity in TPA- and oxazolone-induced mouse ear dermatitis models.[3,4,11] These ginsenosides showed antiallergic effect when given orally not intraperitoneally to rats.[3,4] However, their metabolites, C-K and G-Rh2, showed potent antipsoriatic activity, when these ginsenosides were given intraperitoneally or orally administered. However, the antipsoriatic effect of PT ginsenosides and their metabolites have not been thoroughly studied. In the present study, we observed that the ginsenosides inhibited the sustained swelling (thickness) induced by oxazolone. Among the agents employed in this experiment, G-Rh1 most potently inhibited the swelling as well as the mRNA level of COX-2, a marker of acute inflammatory disease. These results are supported by the report of Park *et al.*, where G-Rh1 inhibited the expression levels of COX-2 by regulating the signal transduction related to the activation of NF-kB in LPS-induced RAW264.7 cells.[4] Furthermore, G-Rh1 significantly inhibited the expressions of TNF-α and IL-1β produced by macrophages, and interferon-γ produced by Th1 cells,

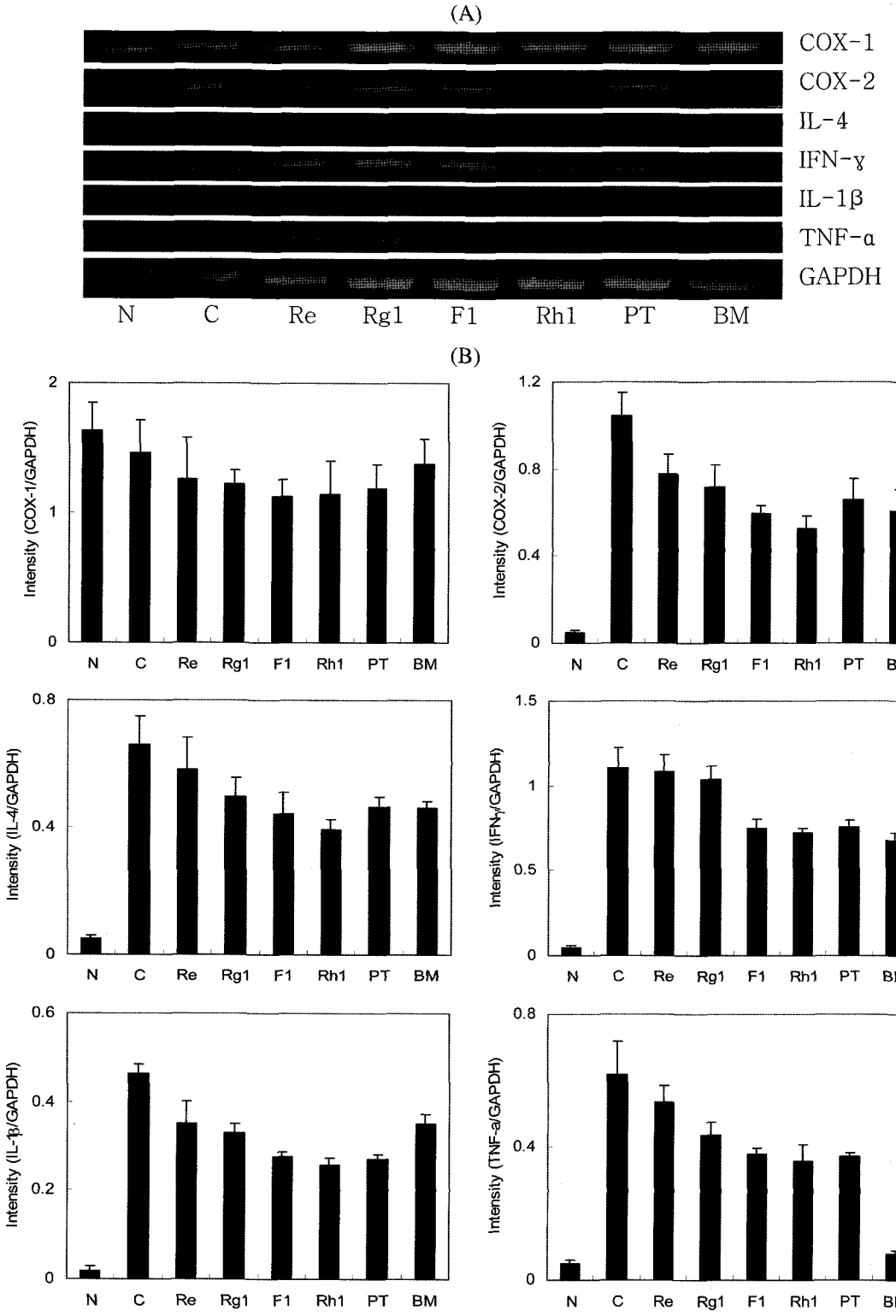


Fig. 4. The effects of ginsenoside Re (G-Re) and its metabolites on mRNA expression levels of COX-1, COX-2, IL-1β, IL-4, INF-γ, TNF-α and GAPDH in oxazolone-induced mouse ear dermatitis. (A) Assay of RT-PCR products, with constitutively expressed GAPDH mRNA used as a control. (B) Relative intensities of RT-PCR. N, Normal; C, oxazolone alone; Re, G-Re with oxazolone; Rg1, G- Rg1 with oxazolone; F1, G-F1 with oxazolone; Rh1, G-Rh1 with oxazolone; PT, protopanaxatriol with oxazolone; BM, betamethasone with oxazolone. The dose of all agents tested was 0.05%.

although weakly inhibited that of IL-4 produced by Th2 cells. These results suggest that G-Rh1 can improve acute and chronic inflammatory skin disorders, contact dermatitis or psoriasis by regulating the expression of TNF-α and IL-1β produced by macrophage cells and those of interferon-γ and IL-4 produced by Th cells.

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