

*Full Paper***Red Ginseng Inhibits Scratching Behavior Associated With Atopic Dermatitis in Experimental Animal Models**Keiichi Samukawa¹, Yasukatsu Izumi^{1,*}, Masayuki Shiota¹, Takafumi Nakao¹, Mayuko Osada-Oka¹, Katsuyuki Miura², and Hiroshi Iwao¹¹Department of Pharmacology, ²Department of Applied Pharmacology and Therapeutics, Osaka City University Medical School, Osaka 545-8585, Japan

Received October 5, 2011; Accepted January 23, 2012

Abstract. Pruritus is a severe symptom that is difficult to treat in atopic dermatitis patients. Red ginseng (RG), a natural medicine, has various biological activities such as anti-inflammatory effects. In this study, we examined the efficacy of RG extract (RGE) and its mechanism on experimental atopic dermatitis in mice. The effects of RGE on vascular permeability and itching were first evaluated. Histamine-induced permeability and itching were significantly inhibited by embrocation with RGE as well as diphenhydramine, an antihistamine drug. Next, we assessed the therapeutic effect of topical RGE in a mouse model of atopic dermatitis. Dermatitis was induced by repeated application of 2,4-dinitrofluorobenzene (DNFB) acetone solution to the mouse ear. The effects of tacrolimus (a calcineurin blocker), dexamethasone (a corticosteroid), and RGE on dermatitis and associated scratching behavior were compared. Repeated DNFB application caused frequent scratching behaviors and ear swelling. Topical treatment with tacrolimus, dexamethasone, and RGE for 8 days before the final challenge with DNFB significantly inhibited ear swelling. Tacrolimus and RGE significantly inhibited scratching behavior, whereas dexamethasone failed to do so. DNFB-induced nerve growth factor expression and nerve fiber extension were significantly attenuated by tacrolimus and RGE, but not by dexamethasone. RGE may have the potential for treatment of atopic dermatitis.

Keywords: atopic dermatitis, hapten, pruritus, red ginseng, substance P

Introduction

Atopic dermatitis is a chronic and relapsing inflammatory skin disease accompanied by severe itching and effective therapeutic strategies have not been established yet (1, 2). Various ointments, including corticosteroids and calcineurin inhibitors, have been used for inflammatory eczema, and antihistamines and antiallergic drugs have been used for pruritus (2). Chronic and severe pruritus reduces the quality of life in patients, and scratching damages the skin barrier and worsens inflammation of the skin, resulting in more itching (1). Therefore, the regulation of itching and scratching is one of the most important aims for the treatment of atopic dermatitis.

Although antihistamines are frequently used to sup-

press pruritus in atopic dermatitis, there is little evidence to demonstrate their effectiveness (3). Topical treatment with corticosteroids is very effective for the treatment of atopic dermatitis because these agents have anti-inflammatory activity. However, the prolonged or irrelevant use of steroids often causes a variety of adverse effects, for example, skin atrophy, striae, and telangiectasia (2, 4, 5). Tacrolimus has been introduced as an ointment for treatment of atopic dermatitis and treatment with tacrolimus ointment improved the clinical score in an assessment of pruritus (6 – 8). In addition, it could be applied safely for lesions in the face and neck and its anti-itch property has been suggested (9 – 11). However, treatment with tacrolimus ointment causes several local side effects such as erythema, pruritus, and irritation. Preclinical animal studies with topical calcineurin inhibitors have demonstrated an increased risk of cutaneous malignancy (12). Therefore, the development of a safer drug is warranted.

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Published online in J-STAGE on March 2, 2012 (in advance)
doi: 10.1254/jphs.11182FP

Several natural medicines have been topically tested as potential therapeutics for atopic dermatitis (13 – 15). Red ginseng (RG, *Ginseng Radix Rubra*) has been used as a traditional medicine, especially in Asian countries. The major components of RG are ginsenosides (16), which have been reported to exhibit various biological activities, including anti-inflammatory and antitumor effects (17, 18). In addition, antiallergic properties of ginsenosides have recently been reported (19 – 21).

In this study, we examined the effects of RG on histamine-induced vascular permeability in rats and on experimental dermatitis in mice caused by repeated application of 2,4-dinitrofluorobenzene (DNFB) solution, which induced eczematous changes of the ear and caused a persistent increase in scratching behavior (22). Tacrolimus and RG significantly reduced DNFB-induced increase in scratching behavior and ear swelling. The effect may be mediated, in part, by inhibition of nerve fiber extension and nerve growth factor (NGF) expression and promotion of the degradation of substance P.

Materials and Methods

Animals

All procedures involving animals were performed in compliance with the Osaka City University animal care guideline. The study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). Male Wistar rats, 5 – 6 weeks of age, and ICR mice and BALB/c mice, 8 – 10 weeks of age (Japan SLC, Inc., Hamamatsu) were used in the present study.

Drugs and antigens

RG produced from 6-year-old ginseng was obtained

from Ohki Pharmaceutical Co., Ltd. (Tokyo) which was provided from Korea Ginseng Corp. (Seoul, Republic of Korea). Five-hundred grams of red ginseng was crushed and refluxed for 2 h twice in 5 L of 70% methanol. The filtrate was evaporated to dryness under reduced pressure to give a brownish extract. This extract was chromatographed over a Lichroprep Rp-18 column with a Water-Methanol system to obtain the saponin fraction (RG extract, RGE). By using a high-performance liquid chromatography method, as previously reported (23), the content of ginsenosides in RGE used in this study was determined (Fig. 1 and Table 1).

Histamine dihydrochloride and diphenhydramine hydrochloride, which are antihistaminic agents, were purchased from Wako Chemicals (Osaka). RGE and diphenhydramine were dissolved in a hydrophilic ointment (Maruishi Pharmaceutical Co., Osaka). Histamine was dissolved in Tyrode's solution before use. Aluminum hydroxide hydrate (alum; LSL Co., Tokyo) and 2,4-dinitrophenylated ascaris extract (DNP-Ascaris, LSL Co.) were used as inducers of immunoglobulin E (IgE). DNFB (Nacalai Tesque, Kyoto) was used as an inducer of a cutaneous reaction. Tacrolimus and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Experimental protocol

The first series of experiments was performed to determine the role of RGE on histamine-induced vascular permeability. Under anesthesia induced by injection of sodium pentobarbital (50 mg/kg, intraperitoneally), the hair on the back of rats was carefully cut. After 30 min (stabilization of the skin), diphenhydramine (1%) or RGE (1% or 0.01%) was applied. Hydrophilic ointment alone was used as the control. After 1 h, Evans blue (50

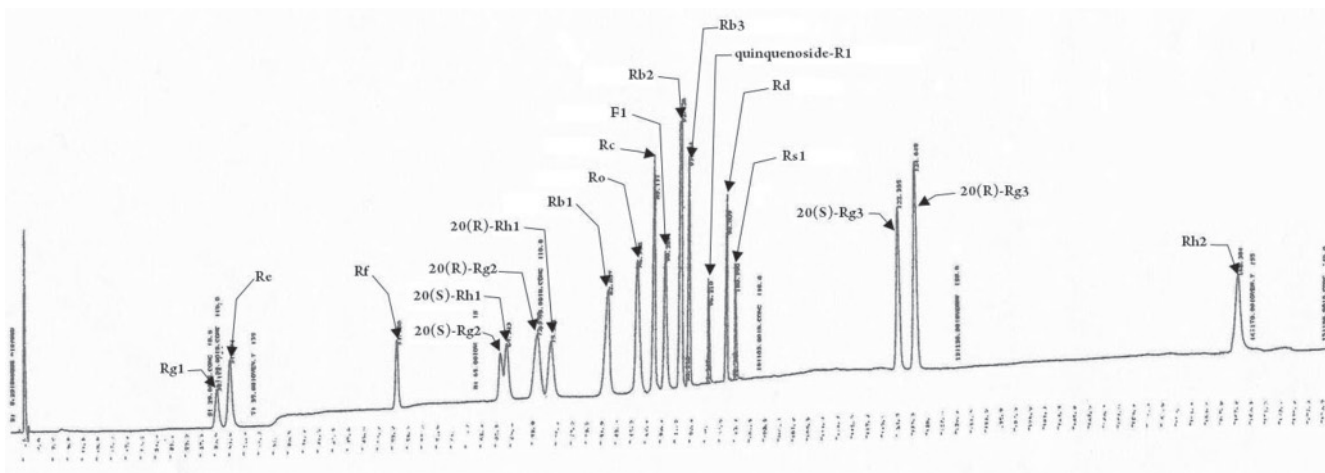


Fig. 1. High-performance liquid chromatography pattern of ginsenosides in red ginseng extract used in this study.

Table 1. The content of ginsenosides in red ginseng extract used in this study

Ginsenoside	Rate of content (%)
Ro	0.160
Rb1	0.598
Rb2	0.243
Rb3	0.051
Rc	0.243
Rd	0.068
Re	0.239
Rf	0.116
Rg1	0.296
20(S)-Rg2	0.232
20(R)-Rg2	0.007
20(S)-Rg3	0.020
20(R)-Rg3	0.015
20(S)-Rh1	0.015
20(R)-Rh1	0.019
Rs1	0.041
Quinquenoside-R1	0.041

mg/kg) was intravenously injected and at once, 100 μ L of histamine (100 μ g/mL) dissolved in Tyrode's solution or Tyrode's solution alone was injected intracutaneously into the back of the rats. After 30 min, the skin around the histamine injection site (circle of 10 mm in diameter) was removed and the leakage for 30 min was calculated. The value was corrected for the dye concentration in each rat serum. Additionally, the behavior experiment was performed according to the previous method (24). In short, 300 μ g (50 μ L) of histamine for each ICR mouse was intracutaneously injected. Control mice received a saline injection. Mouse scratching behavior was captured on videotape for 1 h after the intracutaneous injection and the number of scratching was counted. Diphenhydramine or RGE (1% or 0.01%) was applied to on the back of mice 1 h before histamine injection.

The second series of experiments was performed to compare the effects of dexamethasone, tacrolimus, and RGE in a mouse model of atopic dermatitis. Dermatitis and scratching behavior were induced by repeated treatment of each surface of both ears with DNFB solution in BALB/c mice, as previously described (22, 25). Briefly, 2 weeks after sensitization with DNP-Ascaris and alum (day 15), a total volume of 100 μ L of DNFB dissolved in acetone at a concentration of 0.15% was topically applied every 48 h to both sides of each ear lobe, 5 times in total (days 15, 17, 19, 21, and 23). Two weeks after the fifth

application (day 37), the mice received a similar final challenge. RGE (1% or 0.01%), tacrolimus (0.1%), and dexamethasone (0.05%) were dissolved in ethanol and given topically to mice for 8 days before the final DNFB challenge. Control and vehicle mice were treated with ethanol.

At 4 h after the DNFB challenge, the ears were immediately frozen in liquid nitrogen and stored at -80°C until use (i.e., measurements of mRNA expression). At 24 h after DNFB challenge, the ears were fixed overnight in 10% neutral formalin and embedded in paraffin for histopathological analysis.

Evaluation of cutaneous reactions and scratching behavior

Cutaneous reactions were evaluated by ear swelling induced by the final challenge with DNFB. Ear thickness was measured using a dial thickness gauge (Mitutoyo Co., Ltd., Kawasaki) and expressed as the increase in ear thickness compared to the pre-challenge value. Mouse scratching behavior was captured on videotape for 2 h after the final challenge with DNFB, and the number of scratching was counted.

RNA preparation and analysis

Total RNA from mouse ears was isolated using ISOGEN (Nippon Gene Co., Toyama). To elucidate the gene expression levels, we subjected the RNA samples to quantitative real time-polymerase chain reaction (qRT-PCR, ABI Prism 7700; Applied Biosystems, Carlsbad, CA, USA), as previously reported (26). One-step qRT-PCR reactions were performed using 100 ng of total RNA per reaction. The primers and probes used for qRT-PCR are shown in Table 2. For analysis, transcript levels were normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Histopathological observation

Excised skin specimens were fixed in 10% neutral formalin and embedded in paraffin. Sections, 5- μ m-thick, were prepared and stained with hematoxylin and eosin. Nerve fibers were observed immunohistochemically using an anti-protein gene product 9.5 (PGP 9.5) antibody (Ultraclone Ltd., Isle of Wight, UK). Stained nerve fibers visualized by an enzyme reaction in the epidermis were quantitatively evaluated by using image-analyzing software (Micro Analyzer; Nihon Poladigital, Tokyo) and indicated as the number of positive cells.

Statistical analyses

All data were presented as means \pm S.E.M. Comparisons among groups were made using one-way analysis of variance (ANOVA) followed by the Bonferroni test us-

Table 2. Sequences of the quantitative real time–polymerase chain reaction (qRT-PCR) probes and primers used in this study

Symbol	Forward primer	Probe	Reverse primer
Gapd	ACTGGCATGGCCTTCCG	TTCCTACCCCCAATGTGTVVGTCGT	CAGGCGGCACGTCAGATC
Ngf	TGCCAAGGACGCAGCTTTC	CATTACGCTATGCACCTCACTGCGGC	CAGTGATCAGAGTGTAGAACAACATG
Mme	TGTCCTGGAGGTTTCATAATGGATC	AGCAGCCTCAGCCGAACTACAAGGA	CGTAGTTAGCACACCGTCTCC
ppt1	TGCCGGTGCTTACTCCAAAC	CTGTGCTTGACACCAGGCGTTCTTGC	CGGTACACACTCTCCTTGATAGG

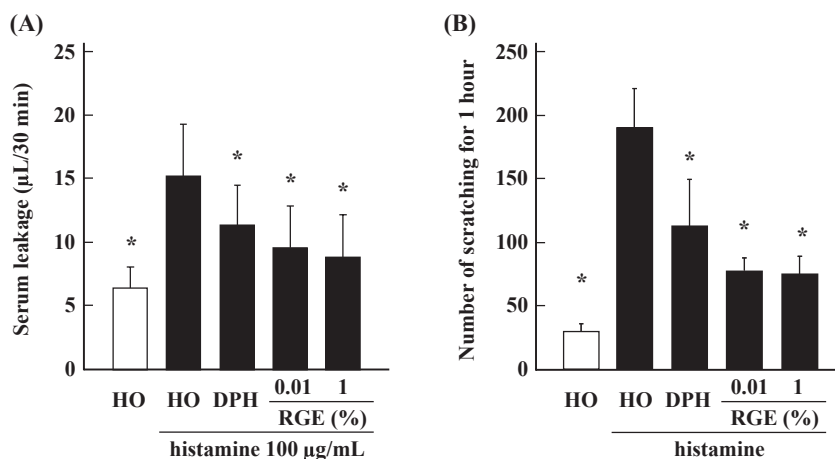


Fig. 2. Effect of red ginseng extract (RGE) on the amount of serum leakage per 30 min (A) and scratching behavior (B) induced by histamine. HO indicates hydrophilic ointment alone; DPH indicates hydrophilic ointment, including diphenhydramine (1%); RGE indicates hydrophilic ointment, including Korean red ginseng. Values represent the mean \pm S.E.M. [$n = 5 - 8$ (A) and $5 - 7$ (B)]. * $P < 0.05$ vs. HO with histamine.

ing StatView (SAS Institute, Inc., Cary, NC, USA). Differences were considered to be statistically significant at a value of $P < 0.05$.

Results

Effect of RGE on histamine-induced vascular permeability and scratching behavior

Intracutaneous injection of histamine remarkably increased vascular permeability. Preapplication of RGE (at both 1% and 0.01%) as well as diphenhydramine significantly inhibited vascular permeability (Fig. 2A). There was no significant difference among the groups treated with 1% and 0.01% of RGE or diphenhydramine.

Intracutaneous injection of histamine also induced marked scratching behavior of ICR mice (Fig. 2B). Topical treatment with not only diphenhydramine but also RGE inhibited the histamine-induced scratching behavior.

Characterization of mouse ear dermatitis

Immunized mice were repeatedly treated with DNFB (5 times) and challenged once again 2 weeks later. Changes in histopathological features and ear swelling are shown in Fig. 3. Ear thickness of mice without DNFB application had not changed during the experiments. Repeated challenge performed every other day caused

potent inflammation with significant scab formation. Thickening of the epidermis and dermis and strong accumulation of inflammatory cells were observed. During the 2-week period between the fifth and final challenge, the scab dropped off and the inflammation decreased. However, the final challenge induced remarkable inflammation and increased ear swelling.

Effects of dexamethasone, tacrolimus, and RGE on dermatitis

Effects of topical application of tacrolimus, dexamethasone, and RGE on dermatitis were examined. Drug application was performed daily for 8 days from 7 days after the fifth DNFB challenge (day 30) until the day of the final challenge (day 37). Histopathological images at 24 h after the final challenge are shown in Fig. 4A. In DNFB-challenged mice, significant thickening of the epidermis and dermis and remarkable accumulation of inflammatory cells were observed. Dexamethasone completely inhibited ear swelling at both 6 and 24 h after the final DNFB challenge (Fig. 4B). Inhibition by tacrolimus at 6 h was comparable to that of dexamethasone, but was less at 24 h. RGE significantly inhibited ear swelling at 6 h after the final challenge, but did only slightly inhibit it at 24 h.

The numbers of scratching behaviors observed in 2 h after the final challenge are shown in Fig. 5. Frequent

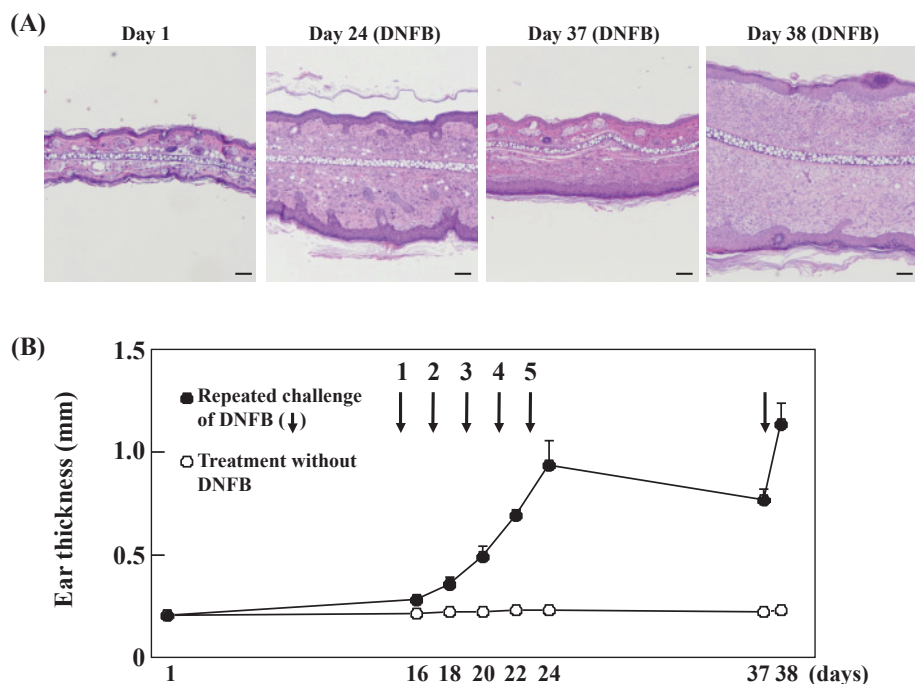


Fig. 3. Changes in histopathological features and ear swelling of the mouse ear repeatedly treated with 2,4-dinitrofluorobenzene (DNFB). A) Representative histopathological images of DNFB-challenged skin lesions stained by hematoxylin and eosin, original magnification: $\times 100$ (bar = $100\ \mu\text{m}$). B) Time course of the changes in ear thickness. Arrow indicates the point of DNFB challenge. Values represent the mean \pm S.E.M. ($n = 8 - 12$).

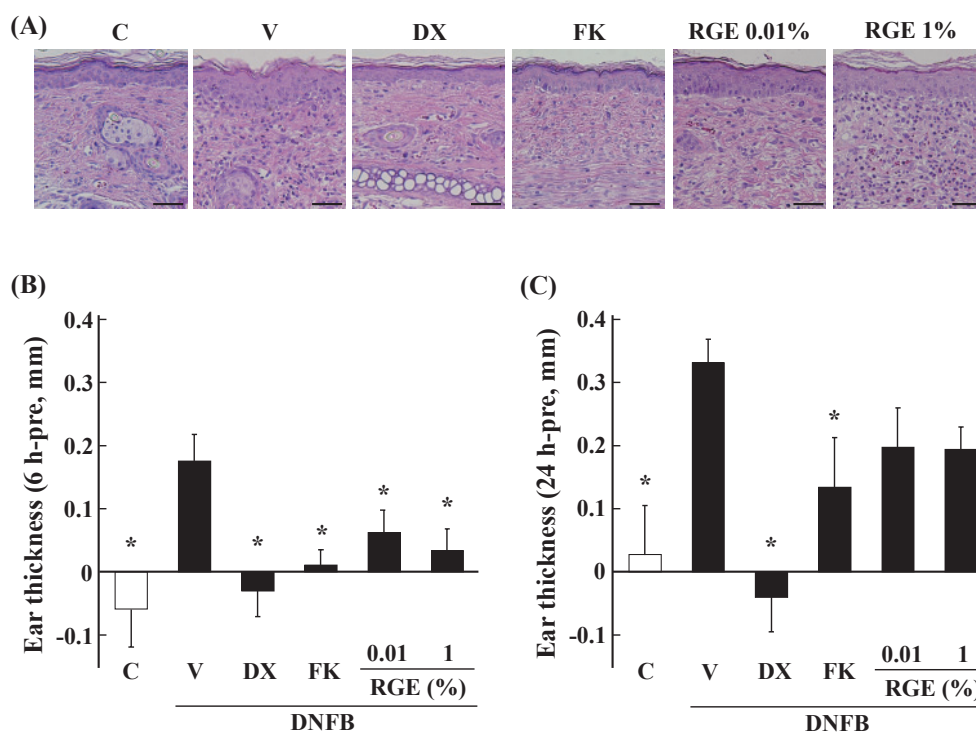


Fig. 4. Effects of dexamethasone, tacrolimus, and Korean red ginseng extract (RGE) on the histopathological features and ear swelling caused by the final challenge with 2,4-dinitrofluorobenzene (DNFB). A) Representative histopathological images of skin lesions stained by hematoxylin and eosin, original magnification: $\times 200$ (bar = $100\ \mu\text{m}$). Skin lesions were excised 24 h after the final challenge with DNFB. C indicates non-challenge with DNFB; V indicates no medication; DX indicates dexamethasone; FK indicates tacrolimus. Values represent the mean \pm S.E.M. ($n = 6 - 8$). * $P < 0.05$ vs V.

scratching behavior was induced by challenge with DNFB. Treatment with tacrolimus significantly inhibited scratching behavior. RGE also decreased the incidence dose-dependently, although its effect was less potent than that of tacrolimus. On the other hand, dexametha-

sone failed to inhibit scratching behavior.

Effects of dexamethasone, tacrolimus, and RGE on nerve fiber extension and nerve growth factor expression

To evaluate the distribution of nerve fibers in the epi-

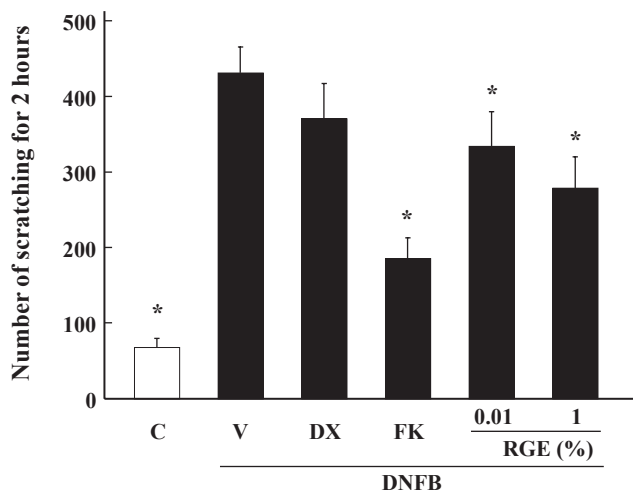


Fig. 5. Effects of dexamethasone, tacrolimus, and Korean red ginseng extract (RGE) on scratching behavior. Scratching behavior induced after the final challenge was evaluated for 2 h and is indicated as number of incidences in 2 h. Abbreviations are the same as in the legend of Fig. 4. Values represent the mean \pm S.E.M. ($n = 12 - 14$). * $P < 0.05$ vs V.

dermis of the ear, immunohistochemical analyses were performed with an antibody to PGP 9.5. Representative examples of PGP 9.5-immunostained sections at 24 h after the final DNFB challenge are shown in Fig. 6A. The results of the quantitative evaluation of stained nerve fibers in the epidermis are shown in Fig. 6B. The area of PGP 9.5 immunoreactivity in the epidermis was clearly enlarged after DNFB challenge compared to that without DNFB challenge. The increase was significantly repressed with tacrolimus and RGE. However, dexamethasone treatment did not result in a change in the size of the PGP 9.5-immunoreactive area.

To investigate the effect of dexamethasone, tacrolimus, and RGE on NGF, mRNA expression of NGF was evaluated by qRT-PCR. NGF mRNA expression was significantly increased at 4 h after DNFB challenge (Fig. 6C). The increased expression of NGF was significantly attenuated with tacrolimus or 1% RGE, while dexamethasone did not change it.

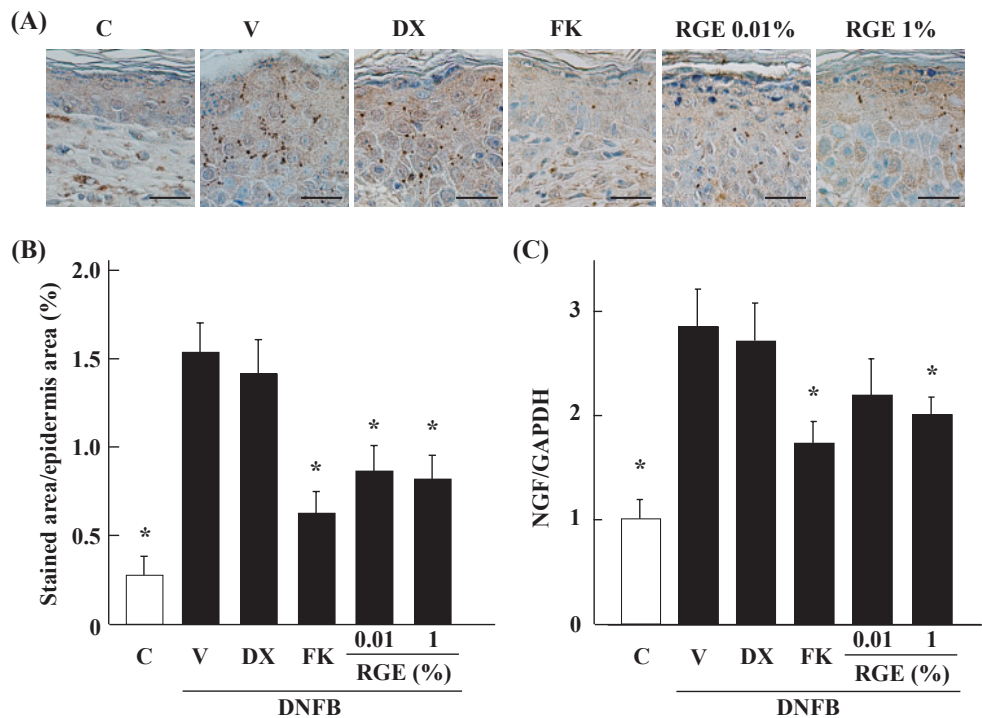


Fig. 6. Effects of dexamethasone, tacrolimus, and Korean red ginseng extract (RGE) on nerve fiber extension and gene expression of nerve growth factor (NGF). Representative immunohistochemical images of the skin lesion by staining for protein gene product (PGP) 9.5 (A) and quantification of the area of PGP 9.5-immunoreactive nerve fibers in the epidermis (B). Ear specimens were excised 24 h after the final challenge with 2,4-dinitrofluorobenzene (DNFB) (bar = 50 μ m). Abbreviations are the same as in the legend of Fig. 4. Values represent the mean \pm S.E.M. ($n = 6 - 8$). * $P < 0.05$ V. C) Gene expression of NGF. The bar graph shows each mRNA value, corrected for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA value. Ear samples were excised 4 h after the final challenge with DNFB. Mean values in the control group are represented as 1. Abbreviations are the same as in the legend of Fig. 4. Values represent the mean \pm S.E.M. ($n = 6$). * $P < 0.05$ vs. V.

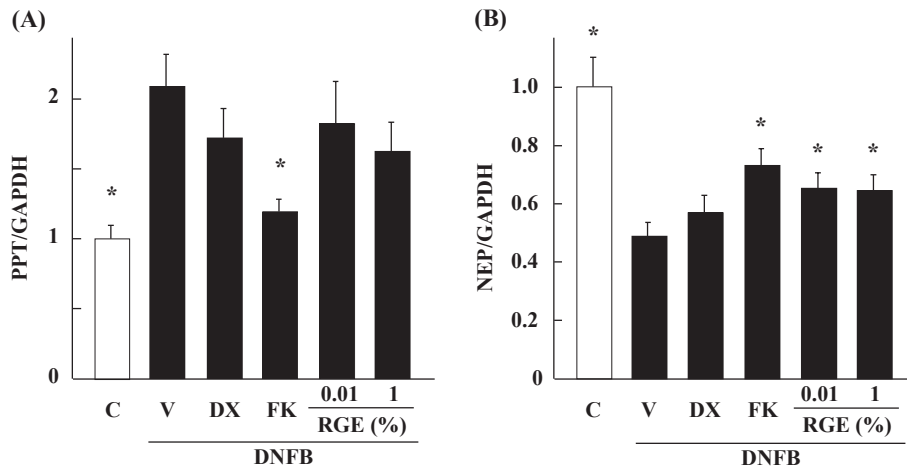


Fig. 7. Effects of dexamethasone, tacrolimus, and Korean red ginseng on gene expressions of preprotachykinin (PPT) (A) and neutral endopeptidase (NEP) (B). The bar graph shows each mRNA value, corrected for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA value. Ear samples were excised 4 h after the final challenge with 2,4-dinitrofluorobenzene. Mean values in the control group are represented as 1. Abbreviations are the same as in the legend of Fig. 4. Values represent the mean \pm S.E.M. ($n = 6$). * $P < 0.05$ vs V.

Effects of dexamethasone, tacrolimus, and RGE on the metabolic pathway of substance P

We investigated the effect of dexamethasone, tacrolimus, and RGE on the metabolic pathway of substance P because it is a neuropeptide that is released from sensory nerve fibers to cause or expand inflammation (27, 28). Preprotachykinin (PPT) is converted into substance P, which is metabolized by neutral endopeptidase (NEP). Therefore, mRNA expressions of PPT and NEP were evaluated by real time qRT-PCR (Fig. 7). DNFB challenge significantly increased the expression of PPT. The increased expression of PPT was significantly attenuated with tacrolimus. Dexamethasone or RGE tended to reduce the expression of PPT. DNFB challenge decreased the expression of NEP. The expression of NEP returned to normal levels by tacrolimus or RGE treatment for 8 days before the final DNFB challenge. In contrast, treatment with dexamethasone failed to cause similar changes.

Discussion

We demonstrated in the present study that topical RGE attenuates histamine-induced vascular permeability. Furthermore, RGE improved atopic dermatitis in the mouse ear, induced by repeated challenge with DNFB. RGE attenuated DNFB-induced nerve fiber extension, NGF expression, and the metabolic pathway of substance P.

Corticosteroids and tacrolimus have been used as remission-maintenance therapy in topical preparations for patients with atopic dermatitis. One of the most important aims in the treatment of atopic dermatitis is the regulation of itching and scratching. There are several studies that compared the effect of tacrolimus with that of dexamethasone, a mild corticosteroid, in experimental models of

atopic dermatitis (22, 29, 30). In an interesting experimental model, dermatitis was induced by repeated application of a hapten, DNFB, to the mouse ear, resulting in frequent scratching behaviors and ear swelling (22, 25). Topically applied tacrolimus exhibits potent inhibitory actions with regard to scratching behavior in spite of its relatively weak anti-inflammatory activity, whereas dexamethasone exhibits potent anti-inflammatory activity without affecting the scratching behavior. Tacrolimus inhibits nerve fiber extension into the epidermis, suggesting that the tacrolimus-induced inhibition contributes to reduced scratching behavior.

RG is a traditional medicine frequently used as a crude substance in Asian countries where it is taken orally. The major components of raw ginseng are ginsenosides, which contain an aglycone with a dammarane skeleton (16). Ginsenosides have been reported to exhibit various biological activities, including anti-inflammatory and antitumor effects (17, 18). In addition, the antiallergic properties of ginsenosides have recently been noted. For example, Tachikawa et al. reported that ginsenoside Rg3 suppresses histamine release from mast cells after stimulation with compound 48/80 in vitro (31). Ro et al. reported that ginsenosides Rb1 and Rc, in part, inhibit the release of histamine and leukotrienes during activation of guinea-pig lung mast cells in vitro (32). The antiallergic properties of RG have strongly suggested their possible use as potential antiatopic agents.

Kim et al. have recently reported that topical RG and its ginsenosides suppress 2-chloro-1,3,5-trinitrobenzene-induced atopic dermatitis-like skin lesions in NC/Nga mice, via partial suppression of the local T helper 2 (Th2) cell response and tumor necrosis factor (TNF)- α mRNA expression (19). RG may be considered as a potential topical therapeutic agent in the management of atopic dermatitis. In their study, RG was used in advance of the

onset of the disease. Furthermore, the effect of RG on scratching behavior is still unclear. In the present study, repeated stimulations and final stimulation were separated by 14 days in order to observe the secondary response, and the effects of topical dexamethasone, tacrolimus, and RG were compared. Topical application of RG for 8 days before the final challenge with DNFB significantly inhibited scratching behavior after the final challenge. Furthermore, RG also significantly inhibited ear swelling at 6 h after the final challenge. The inhibitory activity of RG with regard to ear swelling seems to last for at least 6 h after challenge with DNFB. On the other hand, tacrolimus inhibited ear swelling for 24 h after DNFB challenge, suggesting that topical application of RG may be necessary twice or three times a day. In contrast, dexamethasone did not affect the scratching behavior. These results confirmed those of previous reports (22, 30). Tacrolimus and RG inhibited DNFB-induced nerve fiber extension, but dexamethasone did not affect it, suggesting that the increase in intraepidermal nerve fibers, as well as skin barrier dysfunction, play a crucial role in the increased itching sensation.

In patients with atopic dermatitis, plasma NGF levels are well correlated with disease severity (33). It has also been reported that NGF is abundantly released from human keratinocytes *in vitro* (34) and highly expressed in the epidermis of NC/Nga mice (35, 36). Furthermore, in NC/Nga mice, treatment with an anti-NGF antibody abolished nerve fiber extension as well as skin lesions (35). In this study, tacrolimus and RG (1%) inhibited DNFB-induced NGF mRNA expression. In consideration of the previous report (35), NGF seems to be an important regulator of nerve fiber extension.

Substance P is a neuropeptide that is released from sensory nerve fibers to cause or expand inflammation (27, 28). Released substance P stimulates mast cells to cause mediator release. The mast cell-derived vasoactive mediator histamine potentiates inflammation and induces itching. Thus, substance P potentiates inflammation and itching. Furthermore, substance P is reported to be a putative mediator for itching and induces scratching behavior in mice directly as well as indirectly through mast cell activation (37, 38). On the other hand, it is known that topical tacrolimus exhibits burning or pruritus as a frequent adverse effect in the application area, which is resolved within the first week of treatment (39). Ständer et al. demonstrated that topical application of tacrolimus is followed by an initial release of substance P and calcitonin gene-related peptide from primary afferent nerve fibers in murine skin (40). These data suggest that released substance P is responsible for inflammation and itching. A previous report showed that tacrolimus treatment depletes the substance P content in the dermis and

that the depletion of substance P contributes, at least in part, to inhibition of scratching behavior by tacrolimus (22). Substance P is an evolutionarily conserved protein and the major peptide derived from PPT (41). NEP uses substance P as its substrate (42). The proinflammatory properties of substance P and cytokines have been linked to the activities of various endopeptidases (42, 43). Furthermore, NEP^{-/-} mice show augmented allergic contact dermatitis (44). In the present study, DNFB-induced decrease in NEP expression levels was reversed by tacrolimus and RG. Tacrolimus also inhibited the DNFB-induced PPT expression. Dexamethasone did not affect the expressions of PPT and NEP. The results suggest that RG may inhibit scratching behavior by promoting the degradation of substance P.

Taken together with the previous reports that ginsenosides Rh2, Rg3, Rb1, Rc, and Ro have anti-inflammatory effect (17, 19–21, 31, 32, 45), our observations suggest that the beneficial effects of RGE depend on partially one or more of these ginsenosides. Actually, these are included in RGE used in this study (Fig. 1). However, further study is needed to verify our assumption.

In conclusion, we demonstrated that topical treatment with RG inhibits histamine-induced vascular permeability and contributes to the inhibition of DNFB-induced scratching behavior by decreasing, at least in part, nerve fiber extension and NGF expression and promoting the degradation of substance P.

Acknowledgments

The authors thank Akina Omura, Ayako Soga, Yuta Nakanishi, Ryohei Mineo, Eri Oki, Hikaru Kitahara, Akie Kimura, Natsuki Tanaka, Shigeki Fukushima, Akie Ikushima, Takahiro Kawai, Ayumi Fujimoto, Masaki Yamagata, Yuka Ueda, Yuki Kobayashi, Junko Yamada, Natsuki Hamazawa, and Chiori Asahi for their technical assistance.

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