



Anti-allergic Effects of White Rose Petal Extract and Anti-atopic Properties of its Hexane Fraction

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Rosa rugosa is a species of rose native to eastern Asia. The root of *R. rugosa* has been used to treat diabetes mellitus, pain and chronic inflammatory disease, and a *R. rugosa* petal extract has a strong anti-oxidant effect. In the present study, we examined if solvent fractions from white rose petal extract (WRPE) had any anti-allergic or anti-atopic effects not previously reported. WRPE and butanol and hexane fractions effectively reduced systemic anaphylactic reactions and anti-dinitrophenyl (DNP) IgE-mediated passive cutaneous anaphylaxis in mice, with the greatest inhibition observed for the hexane fraction. In addition, a significant reduction of scratching behavior by mice after histamine injection suggested this fraction's potential anti-allergic effect. At the cell level, the hexane fraction markedly inhibited β -hexosaminidase release from RBL-2H3 mast cells and suppressed the expressions of mRNA interferon- γ and interleukin-4 cytokines produced by T helper cells (type 1 and 2). These results strongly support that the hexane fraction may have an effect on atopic dermatitis, as these 2 cell types play central roles in the pathogenesis of atopic dermatitis. In conclusion, these results suggest that either the hexane fraction or one of its components may be beneficial for the treatment of allergic diseases, including atopic dermatitis.

Key words: Allergy, Atopic dermatitis, Anaphylaxis, β -Hexosaminidase, Interferon- γ , Interleukin-4

INTRODUCTION

Allergy, also often referred to as atopy, is a disorder of the immune system. Allergic reactions occur after exposures to environmental substances known as allergens. Mechanistically, allergy is 1 of 4 types of hypersensitivity. Known as type I hypersensitivity, allergy is characterized by the extensive activation of mast cells and basophils by immunoglobulin E (IgE), which is an important mediator of allergic reactions. Unlike urticaria and angioedema that are IgE-mediated allergic skin disorders, atopic dermatitis includes

both cell-mediated and IgE-mediated mechanisms during its pathogenesis (Incovaia et al., 2008).

In the early stages, a type I hypersensitivity reaction against allergen induces responses in type 2 helper T cells (Th2 cells), which belong to a subset of T cells that produce Th2 cytokines, such as interleukin-4 (IL-4). This is followed by interactions with B cells, whose role is the production of antibodies, including IgE. Subsequently, immediate hypersensitivity (anaphylaxis) is mediated by histamine release in response to allergen cross-linking of IgE that is bound to Fc ϵ RI receptors on mast cells (Galli et al., 2008). In atopic dermatitis, Th2 cells show increased production of IL-4. This stimulates plasma cells to increase IgE production, as well as promoting further Th2 cell development (Back et al., 1995). Thus, the most promising anti-allergic or anti-atopic candidates should both

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inhibit the degranulation of mast cells and suppress the T helper cells involved in the disease pathogenesis, including Th1 (late phase) and Th2 cells (early phase) (Wollenberg et al., 2008).

R. rugosa is a species of rose that is native to large parts of eastern Asia. It is found in northeastern China, Japan, Korea and southeastern Siberia where it grows along coastal regions, often on sand dunes. Its major uses have been as an ingredient in tea, a source of rose oil and for producing jam from its fruits (Kamijo et al., 2008). In addition, the roots of *R. rugosa* have been used in traditional medicine to treat diabetes mellitus, pain and chronic inflammatory diseases in Korea (Kyohaksa, 2003). Recently, it was reported that petals of *R. rugosa* have selective antibacterial activities, especially against intestinal bacteria (Kamijo et al., 2008), and have an anti-oxidant activity (Cho et al., 2003a, 2003b). The petals are also known to contain many hydrolysable tannins, which may have strong anti-oxidant and anti-inflammatory properties (Hatano et al., 1990). Thus, these pharmacological properties of *R. rugosa* extracts may provide for the development of new medicinal sources for skin allergic diseases, including atopic dermatitis.

However, there have been no studies of white rose petal extract (WRPE) or its solvent fractions for their anti-allergic or anti-atopic properties. Therefore, in the present study, we examined if solvent fractions from WRPE (butanol and hexane fractions) had any anti-allergic profiles, and could possibly be applicable to the treatment of atopic dermatitis.

MATERIALS AND METHODS

Plant materials and chemicals

White rose petals were freshly collected at Jincheon, Chungbuk in 2006, dried and ground in a rotor speed mill (Laval Lab Inc., QC, Canada). Pulverized petals were sterilized using a 70% ethanol spray, followed by drying at 80°C for 24 h. The dried flowers were stored at 4°C before use. Solvent fractions, including water, butanol and hexane, were prepared and supplied by Chungju National University (Chungju, Korea) by using a conventional methods (Hannan et al., 2006) and kept lyophilized until use. Chemicals, compound 48/80, anti-dinitrophenyl (DNP) IgE, DNP-bovine serum albumin (BSA), phorbol 12-myristate 13-acetate (PMA) and histamine were purchased from Sigma (St. Louis, MO, U.S.A.). Anti-CD3, a T cell activator, was obtained from eBioscience (San Diego, CA, U.S.A.). Stock solutions were prepared by dissolving in either saline (oral treatment) or 10% DMSO (cell treatment).

Animals

Seven-week-old male ICR mice (28-30 g) were purchased from KOATECH (Pyeongtaek, Korea) and housed at the Laboratory Animal Research Center of Chungbuk National University. The animals were maintained at a constant temperature of $22 \pm 1^\circ\text{C}$, relative humidity of $55 \pm 10\%$ and 12-h light/dark cycle. They were fed standard rodent chow and purified water. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised in 1996), and the protocol was approved by the Institutional Animal Care and Use Committee of the Laboratory Animal Research Center, Chungbuk National University, Korea.

Cell culture

BALB/c mouse spleens were aseptically removed after sacrifice and single primary splenocytes were prepared by mechanical dissociation in cold phosphate buffered saline (PBS) at pH 7.2. Erythrocytes were depleted using a red blood cell lysis buffer (eBioscience) containing ammonium chloride, which lyses red blood cells with minimal effect on lymphocytes. Splenocytes were cultured in RPMI complete medium with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, U.S.A.). The medium also contained an anti-CD3 monoclonal antibody (mAb) (eBioscience) and varying concentrations of lyophilized fractions. Splenocytes were cultured at 5×10^6 cells/mL in 96-well microtiter plates or at 1×10^7 cells/mL in 24-well plates and maintained at 37°C in a humidified atmosphere containing 5% CO₂. RBL-2H3 mast cells were grown in RPMI 1640 medium (Hyclone) supplemented with 10% FBS, 4 mM L-glutamate (Invitrogen, Carlsbad, CA, U.S.A.), 100 U/mL penicillin and 100 µg/mL streptomycin (Invitrogen). Cultures were maintained under 5% CO₂ at 37°C in a tissue culture flask. For all experiments, the cells were grown to greater than 90% confluence and subjected to no more than 20 cell passages.

Compound 48/80-induced systemic anaphylaxis

Compound 48/80-induced systemic reactions were induced as previously described (Li et al., 2005). In brief, male ICR mice were intraperitoneally injected with a mast cell degranulator, compound 48/80 [8 mg/kg body weight (BW)]. WRPE extract and the solvent fractions (3.2 mg to 100 mg/kg BW) were dissolved in saline and intraperitoneally injected 30 min before the injection of compound 48/80 (n=6/group). Mortality was monitored for 1 h after induction of anaphylactic shock.

Anti-dinitrophenyl (DNP) IgE-mediated passive cutaneous anaphylaxis (PCA) reaction

An IgE-dependent skin reaction was generated in ICR mice by sensitizing an area of shaved dorsal skin with an intradermal injection of 0.2 µg anti-DNP mouse IgE antibody in 20 µL phosphate buffered saline (PBS). DNP-ovalbumin (1 mg/kg) containing 0.5% Evan's blue was intravenously injected into the tail vein 48 h later to evaluate vascular permeability. WRPE and the solvent fractions were intraperitoneally (20 mg/kg or 100 mg/kg) or orally (300 mg/kg) administered 1 h before antigen injection. Thirty minutes after the Evan's blue injection or antigen challenge, mice were sacrificed by terminal anaesthesia and dorsal skin was removed for the measurement of the pigmented area. The amount of extravasated Evan's blue dye was determined by incubation of biopsy specimens in 1 mL formamide at 55°C for 24 h and the color intensity was measured at 620 nm using a spectrophotometer, UV mini-1240 (Shimadzu, Kyoto, Japan). The amount of dye was calculated from an Evan's blue standard curve (Kim et al., 2005; Kwon et al., 2008).

Histamine-induced cutaneous reaction and scratching behavior

The dorsal skin of ICR mice was clipped and 20 µL of histamine dihydrochloride (0.2 µg/site) was injected intradermally. At the same time, 0.25 mL of 0.5% Evan's blue saline solution was injected intravenously, and the amount of extravasated dye was measured for 30 min after the histamine injection (Inagaki et al., 1999). To evaluate mouse scratching behavior, 20 µL of histamine (50 µg/site) was injected intradermally into the rostral part of the back skin. At the same time, the mouse was placed in an observation cage and scratching behavior was monitored for 30 min. Because mice scratch very quickly and several times for about 1 s using the hind paws, a series of scratching was counted as 1 instance (Sugimoto et al., 1998; Inagaki et al., 2001). The hexane fraction (32, 100 or 320 mg/kg) was orally administered 30 min before histamine injection.

β-Hexosaminidase secretion assay

Mast cell degranulation was determined by measuring the release of a granule marker, β-hexosaminidase. RBL-2H3 cells were grown on 48-well plates (2.5×10^5 cells/mL) and experiments were carried out 3 h after plating. Cells were then treated overnight with 1 µg/mL IgE (Sigma). To remove excess IgE before stimulation, cells were washed 4 times with extracellular buffer (125 mM NaCl, 5 mM KCl, 1.5 mM CaCl₂, 1.5 mM MgCl₂, 20 mM HEPES, pH 7.3). The cells were then stimulated with 400 ng/mL DNP-BSA (Sigma) suspended in 500 µL extracellular buffer with 0.1% BSA, and incubated at 37°C for 1 h. Following incubation, 50 µL of the supernatant was incubated with 200 µL of 1 mM *p*-nitrophenyl *N*-acetyl-beta-*D*-glucosamine (Sigma) in 0.05 M citrate buffer (pH 4.5) for 3 h at 37°C. The enzyme reaction was terminated by the addition of 500 µL of 0.05 M sodium carbonate buffer (pH 10.0) and the OD of each reaction was read at 405 nm (SupraMax340, Molecular Devices, Sunnyvale, CA, U.S.A.). The effects of various treatments on β-hexosaminidase release were described as % of IgE control absorbance value.

Quantitative real-time PCR

To analyze cytokine mRNAs, anti-CD3-treated mouse splenocytes were cultured in 10% FBS RPMI1640 medium for 24 h with hexane fraction or cyclosporine A. Total RNA extracts from mouse splenocytes stimulated by anti-CD3 mAb were prepared by the Trizol method (Invitrogen). cDNA was synthesized from RNA by the reverse transcription of 1 µg of total RNA using the Improm-II reverse transcription system (Promega, Madison, WI, U.S.A.) and oligo dT primers for a total volume of 20 µL. PCR amplifications were performed using the primers described in Table I (Bioneer, Deajeon, Korea). Quantitative real-time PCR reactions were run on a Rotor-Gene 6000 (Corbett Research, Sydney, Australia) using SYBR Green PCR Master Mix (Qiagen, CA, U.S.A.) in 20 µL reaction mixtures. Each real-time PCR master mix contained 10 µL 2X enzyme mastermix, 7.0 µL RNase free water, 1 µL of each primer (10 pM each) (Table I)

Table I. Primer sequences for real-time RT-PCR

Gene (murine)	Primer	Amino acid sequences	Product size (bp)	Accession no.
IFN-γ	Forward	5'- GTTCTGGGCTTCTCCTCCTG-3'	245	NM_008337
	Reverse	5'- CTGGCTCTGCAGGATTTTCA-3'		
IL-4	Forward	5'- ATATCCACGGATGCGACAAA-3'	252	NM_021283
	Reverse	5'- AAGCCCCGAAAGAGTCTCTGC-3'		
β-actin	Forward	5'- CTAGGCACCAGGGTGTGATG-3'	291	NM_007393
	Reverse	5'- CTACGTACATGGCTGGGGTG-3'		

and 1 μ L diluted template. PCR was performed with an initial pre-incubation step for 10 min at 95°C, followed by 45 cycles of 95°C for 15 s, annealing at 52°C for 15 s and extension at 72°C for 10 s. Melting curve analysis was used to confirm formation of the expected PCR product, and products from all assays were additionally tested by 1.2% agarose gel electrophoresis to confirm the correct lengths and patterns using a conventional reverse transcription-PCR method. An inter-run calibrator was used, and a standard curve was created for each gene to obtain PCR efficiencies. Relative sample expression levels were calculated using Rotor-Gene 6000 Series Software 1.7, and were expressed relative to β -actin and corrected for between-run variability. Results for the experimental samples were expressed as the percentages of the internal control gene.

Statistical analysis

The results are given as means $\pm$ S.D. One-way ANOVA (SPSS 12.0 version) followed by a Tukey's multiple comparisons test was used to make statistical comparisons between groups. A p -value < 0.05 was considered statistically significant.

RESULTS

Compound 48/80-induced systemic anaphylaxis

To investigate anti-allergic effects of a crude extract and solvent fractions of WRPE, we used compound 48/80 to induce systemic anaphylaxis in an *in vivo* model. After the intraperitoneal injection of compound 48/80 (8 mg/kg), mice were monitored for 1 h to determine their mortality. As shown in Table II, compound 48/80 induced fatal shock in a vehicle only group (100% mortality). However, WRPE and the solvent fractions prevented fatal shock in dose-dependent manners. Interestingly, WRPE and the hexane and butanol fractions prevented the compound 48/80-induced shock at lower doses (32 mg/kg), whereas the water fraction was not preventative. This indicated that any pharmacologically active ingredients were concentrated in these 2 fractions.

Anti-DNP IgE-mediated PCA reactions

Next, we examined which fraction effectively ameliorated responses to allergy induction. For these experiments, localized extravasation of Evan's blue dye was induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. As shown in Fig. 1, oral administration of WRPE and the solvent fractions (300 mg/kg) significantly inhibited anti-DNP IgE-mediated PCA reactions. Greater in-

Table II. Effects of white rose petal extract (WRPE) and its distilled water (WRPE-DW), butanol (WRPE-BtOH) and hexane (WRPE-hexane) fractions on systemic anaphylactic shock induced by compound 48/80

Treatment (mg/kg)	Compound 48/80 (mg/kg)	Mortality (%)
Vehicle	-	8
		10/10 (100)
WRPE	10	8
	32	8
	100	8
		6/6 (100)
WRPE-DW	10	8
	32	8
	100	8
		6/6 (100)
WRPE-BtOH	3.2	8
	10	8
	32	8
	100	8
		6/6 (100)
WRPE-hexane	3.2	8
	10	8
	32	8
	100	8
		5/6 (83.3)
		1/6 (16.7)
		0/6 (0)
		0/6 (0)

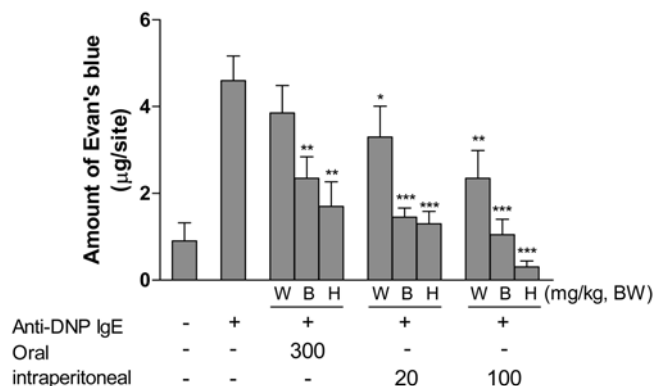


Fig. 1. Effects of WRPE and butanol and hexane fractions on PCA reactions. Mice were treated by oral (300 mg/kg, BW) or intraperitoneal (20, 100 mg/kg, BW) route 1 h prior to challenge with antigen. Evan's blue dye was extracted as described in Materials and Methods and measured by spectrophotometer. *, **, ***Statistically significant at $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. control, respectively ($n = 5$). W, WRPE; B, butanol fraction; H, hexane fraction; BW, body weight.

hibitions of PCA reactions were found after intraperitoneal injections of the butanol and hexane fractions in dose-dependent manners (Fig. 1). Interestingly, the hexane fraction strongly inhibited Evan's blue leakage at 100 mg/kg (3 times higher than the butanol fraction). Thus, we used the hexane fraction, which may contain non-polar compounds that are not derived from fatty acids and function as hormones, vitamins,

photopigments and other specialized roles, for additional studies.

Histamine-induced cutaneous reaction and scratching behavior

The hexane fraction, which showed the most potent inhibitions for both systemic and local anaphylaxis reactions, was examined for its effects on cutaneous reactions and scratching behaviors induced by an intradermal injection of histamine. As shown in Fig. 2A, 32, 100 and 320 mg/kg of the hexane fraction markedly inhibited the histamine-induced vascular permeability increase in a dose-dependent manner, 21.4%, 57.4% and 66.5%, respectively (Fig. 2A). In addition, as shown in Fig. 2B, following the injection of histamine (0.5 μ g/site), 32 and 100 mg/kg of the hexane fraction significantly reduced the numbers of scratching behaviors to 59.8 and 58.5, respectively, compared with 83.2 in the control group. In particular, the most significant reduction was observed at 320 mg/kg, which may be closely associated with the

inhibition of enhanced vascular permeability shown in Fig. 2A.

β -Hexosaminidase release by RBL-2H3 cells

To confirm an anti-atopic effect of the hexane fraction and to predict an Fc ϵ RI receptor-mediated signaling in conjunction with calcium-dependent signals, release of β -hexosaminidase, an indicator of mast cell degranulation, was measured in the supernatants of RBL-2H3 cell cultures after treatment with DNP-BSA in the presence of varying doses of the hexane fraction (0.1 to 50 μ g/mL). As shown in Fig. 3, a high dose of the hexane fraction (50 μ g/mL) effectively inhibited the release of β -hexosaminidase by RBL-2H3 cells to the extent of a positive control, cyclosporine A. This inhibitory effect indicates that components in the hexane fraction may have anti-allergy-specific properties and led us to speculate that the hexane fraction might also have anti-atopic effects, which are caused by both cell- and IgE-mediated mechanisms.

Th1/Th2 cytokines from mouse splenocytes

A Th1 (IFN- γ) and a Th2 (IL-4) cytokine were examined at the gene level to determine if the hexane fraction inhibited these T cell receptor-mediated cytokines' expressions, which are both critically required for atopic dermatitis. The expressions of IFN- γ and IL-4 mRNAs were examined in the presence of varying concentrations of the hexane fraction (1 to 100 μ g/mL). Cyclosporine A was included for comparison. mRNA levels for the selected Th1/Th2 cytokines were as-

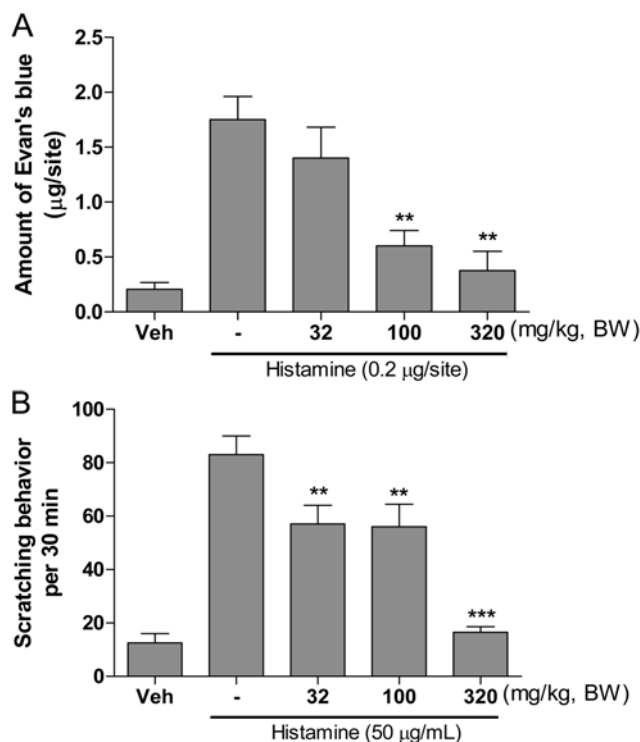


Fig. 2. Effects of hexane fraction on histamine-induced cutaneous reaction and scratching behavior. **A** The hexane fraction was orally administered (32, 100, 320 mg/kg, BW) 30 min before histamine injection. Evan's blue dye was extracted as described in Materials and Methods and measured by spectrophotometer. **B** Scratching behavior was measured for 30 min after the intradermal injection of histamine to the back skin of mice. **Statistically significant at $p < 0.01$ vs. control ($n = 5$). Veh, vehicle.

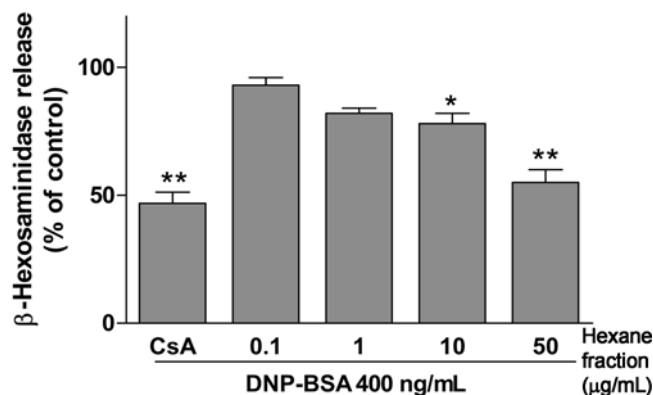


Fig. 3. β -hexosaminidase release from RBL-2H3 mast cells. RBL-2H3 cells (2.5×10^5 /mL) were sensitized with 1 μ g/mL of DNP-specific IgE overnight and pre-treated with varying doses of the hexane fraction (0.1-50 μ g/mL) for 1 h. Cells were stimulated with 400 ng/mL of DNP-BSA for 1 h. β -hexosaminidase release from RBL-2H3 cells was measured using a microplate reader at 405 nm. Cyclosporine A (0.1 μ M) was used as a positive control. Results are expressed as means $\pm$ S.D. from 3 separate experiments. * $p < 0.05$, ** $p < 0.01$ vs. vehicle-treated control (DNP-BSA alone).

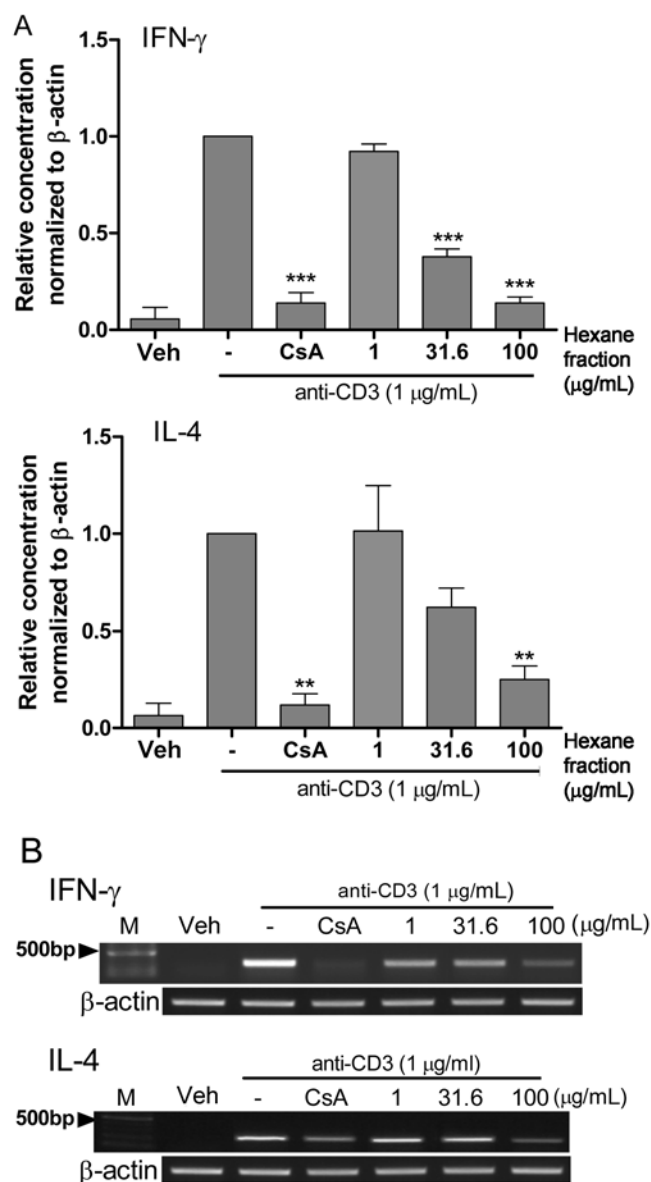


Fig. 4. Th1/Th2 cytokine mRNA expressions by splenocytes. Cells were cultured for 24 h as described in Materials and Methods, in the presence of hexane fractions (1, 31.6, and 100 μ g/mL). Cyclosporine A (0.1 μ M) was used as a positive control. Isolated mRNA was analyzed by real-time RT-PCR for IFN- γ , IL-4 and β -actin. **A** Results were internally confirmed by the comparative cycle count (Ct, cycle number threshold) against β -actin as the standard gene. **B** Conventional RT-PCR was used to confirm real-time semiquantitative RT-PCR. Anti-CD3 mAb-activated (1 μ g/mL) group was used for relative comparisons for each group. Results are mean $\pm$ S.D. (n = 3). ** p < 0.01, *** p < 0.001. Veh, vehicle.

essed using quantitative real-time PCR. As shown in Fig. 4, T cells were significantly activated by anti-CD3 mAb. However, the mRNA expression levels for IFN- γ and IL-4 were significantly inhibited after treatment

with 31.6 μ g/mL of the hexane fraction. Interestingly, 100 μ g/mL of the hexane fraction inhibited the mRNA expressions of these cytokines similar to that by cyclosporine A treatment (Fig. 4A). This result was confirmed by a conventional RT-PCR method (Fig. 4B). Thus, these results clearly demonstrate that components in the hexane fraction can effectively control T cell-derived cytokines after stimulation with anti-CD3 mAb.

DISCUSSION

Allergy is an adaptive immune response directed against non-infectious environment agents called allergens, including some non-infectious components of certain infectious organisms. Allergic skin disorders, such as urticaria, angioedema, contact dermatitis and atopic dermatitis, are clinically important. Their pathogenesis varies from local to systemic effects depending upon contacts with specific allergens that elicit allergic reactions in the skin (Incovaia et al., 2008). In allergic diseases, immediate-type hypersensitivity reactions are mediated by mast cells either in response to IgE-dependent or IgE-independent stimuli. Degranulation of mast cells results in the release of various mediators, such as histamine and pro-inflammatory cytokines.

Clinically, histamine released from mast cells is closely related to the immediate phase of allergic inflammation, including vasodilatation, increased vascular permeability and tissue edema (Shin et al., 2005). In addition, atopic dermatitis, a systemic skin disease, plays a major role in skin barrier dysfunction and hyperreactivity after exposures to immunological stimuli, such as allergens and microbes (Leung et al., 2004). The altered skin barrier function consequently results in the passage of allergens through the skin and leads to a pivotal role played by Langerhan's cells for systemic responses, followed by the activation of Th2 cells (Novak et al., 2003).

IL-4, a Th2 cytokine, interacts with B cells that produce large amounts of IgE. IgE circulates in the blood until it binds to Fc ϵ RI receptors on the surfaces of mast cells and basophils, which are both involved in acute allergic inflammatory responses (Oettgen and Geha, 1999). Allergen-specific T cells cloned from lesions of acute atopic dermatitis skin show a Th2/Th1-like cytokine profile (Tengvall et al., 1996). However, the *in situ* expression of IL-4 in chronic atopic dermatitis lesions is accompanied by the expression of IFN- γ (Grewe et al., 1994), which suggests an additional involvement of Th1 cytokines during the chronic phase. Thus, reductions of mast cell-derived inflam-

matory allergic responses would be a central point to avoid immediate hypersensitivity, resulting in anaphylaxis. Moreover, atopic dermatitis, which is mediated by T cell and B cell-derived IgE, could also be mechanistically controlled by the suppression of cytokines from both Th1 and Th2 cells in a counter-regulatory fashion (Leung et al., 2004).

In the present study, we clearly demonstrated that WRPE and its hexane fraction significantly attenuated compound 48/80-induced systemic anaphylactic reactions and also inhibited anti-DNP IgE-mediated PCA reactions in mice. In addition, the hexane fraction from WRPE was analyzed to contain more total polyphenols, which was approximately 2-fold higher than that of butanol fraction and 2.6-fold higher than that of water fraction (data not shown), suggesting higher potencies against allergic reactions than those of butanol and water fractions. This result was confirmed by tests for histamine-induced cutaneous reactions and scratching behaviors that showed significantly reduced Evan's blue dye leakage and scratching behavior in dose-dependent manners.

At the cell level, pretreatment with the hexane fraction effectively inhibited the release of a prototypical degranulation marker, β -hexosaminidase, from RBL-2H3 mast cells. This suggests an effective preventative action against anaphylaxis that is elicited after activation of mast cells. As this result was observed for both *in vivo* and *in vitro* assessments, the hexane fraction or some component(s) in the fraction may be a good candidate for anti-allergic resources.

To verify the potential of the hexane fraction against atopic dermatitis, Th1 and Th2 cytokines, which are known as the initial triggers for the development of atopic dermatitis, were examined. The results showed that the hexane fraction effectively inhibited the expressions of a Th1 cytokine mRNA (IFN- γ), which is involved in the chronic phase of atopic dermatitis, and of a Th2 cytokine mRNA (IL-4), which is involved in the early acute phase of atopic dermatitis. The suppressions of both IFN- γ and IL-4 mRNAs may indicate that the hexane fraction mechanistically played a key role in signal pathways that are downstream of activation via the T cell receptor. Considering that both Th cells and mast cells are responsive to calcium-dependent signals, and that these 2 cell-mediated mechanisms provide clinical clues for atopic dermatitis therapy (Bornhove et al., 2002), the hexane fraction may contain potential inhibitors to the pathogenesis of atopic dermatitis through the inhibition of these 2 major cell types (Th and mast cells) that are closely associated with atopic dermatitis pathogenesis.

In summary, the results from this study provide *in*

vivo and *in vitro* evidence that a hexane fraction from WRPE effectively attenuates immediate-type allergic reactions in an experimental mouse model, and inhibits mast cell degranulation and Th1/Th2 cytokines in atopic dermatitis cell models. Therefore, we suggest that a non-polar hexane fraction of WRPE has the potential for anti-allergic and anti-atopic dermatitis effects, and that hexane fraction is believed to have advantages in pharmaceutical use for anti-atopic dermatitis.

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