

Vitex rotundifolia L. prevented airway eosinophilic inflammation and airway remodeling in an ovalbumin-induced asthma mouse model

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Received 15 November 2011; accepted 27 August 2012

Abstract

Vitex rotundifolia L. (VR) has long been used in China and Korea in traditional medicine. This study was conducted to evaluate the ability of *Vitex rotundifolia* L. to prevent airway inflammation and remodeling in an ovalbumin (OVA)-induced murine asthma model. The total cell number and number of inflammatory cells in the bronchoalveolar lavage (BAL) fluid were counted. The levels of cytokines in the BAL fluid and serum IgE levels were measured using an ELISA. For histological analysis, hematoxylin and eosin staining, periodic acid-Schiff staining and immunohistochemistry were evaluated. The release of total cells into the BAL fluid was significantly inhibited in OVA-induced asthmatic mice treated with VR extract. In addition, eosinophilia and lymphocytosis were reduced significantly in mice that received VR extract. Furthermore, levels of the T_H2 cytokines IL-4 and IL-5 and pro-inflammatory cytokine TNF- α in the BAL fluid and total IgE in serum were markedly suppressed by VR extract. OVA-specific IgE in the serum and IL-13 in the BAL fluid were decreased, but not significantly. The allergic effects of VR extract were accompanied by a reduction in airway hyperresponsiveness. Additionally, morphologic findings demonstrated that VR extract substantially inhibited OVA-induced eosinophilia, goblet cell hyperplasia and smooth muscle mass production. This finding suggests that VR extract may have pharmacological effects that would be useful for the treatment of asthma via the inhibition of the T_H2 response and airway remodeling.

Keywords: airway hyperresponsiveness, eosinophilia, goblet cell hyperplasia, smooth muscle actin, T_H2 response

Introduction

Asthma is an inflammatory disease characterized by the presence of increased numbers of leucocytes, especially eosinophils and lymphocytes, in the bronchial tissues. The clinical symptoms of asthma are wheezing, coughing, shortness of breath and chest discomfort (1). The initial stages of asthma are a result of airway inflammation in which leucocytes, especially eosinophils, play pathogenic roles (2). During an asthma attack, eosinophils selectively migrate into the airways, where they infiltrate and cause inflammation. The severity of asthma is determined by the degree of airway narrowing or airway hyperresponsiveness (AHR) induced by activated lymphocytes in the airway. The severity of asthma is also associated with

airway remodeling, which is characterized by goblet cell hyperplasia, pulmonary fibrosis and increased airway smooth muscle mass (3). Both the pro-inflammatory cytokine TNF- α and the T_H2 cytokine interleukin IL-13 are well-known remodeling-associated cytokines. Asthma inflammation is also induced by cytokines released from T_H2 lymphocytes, which can act via positive feedback mechanisms to promote the production of more inflammatory mediators, including other cytokines (4). Among T_H2 cytokines, IL-4, IL-5 and IL-13 are directly linked with the immunopathogenesis of asthma (5). This is because these cytokines both regulate the main processes involved in IgE production and differentiate and activate eosinophils (4, 6).

Many natural products used in oriental medicine have been found to be effective agents for the treatment of asthma (7, 8). The results of our previous studies suggested that Moutan Cortex Radicis reduced eotaxin secretion (9) and that Bambusae Caulis in Taeniam reduced airway inflammation (10). However, the biomolecular activity of many natural products has yet to be confirmed by experimental testing (11). Additionally, despite their remarkable curative ethnopharmacological abilities, most natural products have not been widely used in western societies because their mode of action at the molecular level is not well understood. One such product, the dried fruit of *Vitex rotundifolia* L. (*Vitex trifolia* L. var. *simplicifolia* Cham.; VR), has long been used in China and Korea as a traditional medicine for treatment of various allergic diseases and upper respiratory infections (12). It contains the flavonoids such as artemetin, quercetagenin, 5,3'-dihydroxy-6,7,4'-trimethoxyflavanone and casticin, of which casticin is the primary active compound, and several studies have investigated the chemical constituents of the extracts of this fruit and the molecular mechanisms responsible for its effects (13–15).

In this study, we investigated the ability of VR extract to prevent airway eosinophilic inflammation and airway remodeling activity using an ovalbumin (OVA)-induced murine asthma model. Furthermore, we determined that VR substantially attenuates airway inflammation through the suppression of the T_H2 response, and that this activity resulted in the abrogation of airway remodeling.

Methods

Preparation of the VR extract

VR-extract granules were purchased from Sun Ten Pharmaceutical (Taipei, Taiwan). The granules were weighed precisely to 100mg and then dissolved in 10ml of distilled water by stirring overnight at room temperature. Next, the solution was centrifuged for 10 min at 3000 r.p.m. (Eppendorf, Hamburg, Germany), after which the supernatant was sterilized by passing the solution through a 0.22 μ m syringe filter. The stock of the VR extract was then diluted several times and used in the experiments. The stock (KHOP00210) was stored at 4°C until use.

Experimental animals

Pathogen-free, 6-week-old male BALB/c mice were purchased from Charles River Technology Inc. (South Korea) and maintained in a pathogen-free laboratory at Kyung Hee University. The mice were kept in an air-conditioned room and acclimated for at least 1 week prior to use. The mice had free access to food and water throughout the experiment. All experimental procedures complied with the requirements of the Animal Care and Ethics Committee of Kyung Hee University. Twenty-four mice were divided into four groups of six.

Sensitization and inhalational exposure

Animal studies were conducted using specific pathogen-free, 6-week-old male BALB/c mice (Charles River Technology Inc.) that were sensitized by intra-peritoneal (i.p.) injection of 100 μ g of chicken OVA and 20mg aluminum hydroxide (Sigma-Aldrich, MO, USA) on days 0 and 14. The OVA-challenged mice were exposed to 1.5mg of OVA intra-nasally on days 15, 16 and 17. The positive control group of asthma-induced mice was then treated with 10mg kg⁻¹ of dexamethasone (Dexa, Sigma-Aldrich), while the other group was treated with 100mg kg⁻¹ of VR extract between days 15 and 17 via orogastric gavage. Two groups of mice were treated with Dexa and VR extract via an orogastric gavage route 1 h before the OVA challenge. The negative control group was sensitized and challenged with PBS. Mice were sacrificed on day 18 (Fig. 1).

Collection of bronchoalveolar lavage fluid and serum

Bronchoalveolar lavage (BAL) fluid was collected by lung lavage using 1 ml of PBS administered via the trachea. After three lavages, the BAL fluid was centrifuged for 10 min at 1300 r.p.m. The cell concentrations were then determined using a hemacytometer, and differential cell counts were conducted using slides prepared by cytocentrifugation and Diff-Quick staining. Approximately 500 cells were counted. The supernatant was stored at -80°C until subsequent cytokine measurement. Immediately prior to the first and final antigen challenges, blood was collected and sera were obtained by centrifugation and stored at -80°C until subsequent determination of total IgE and OVA-specific IgE.

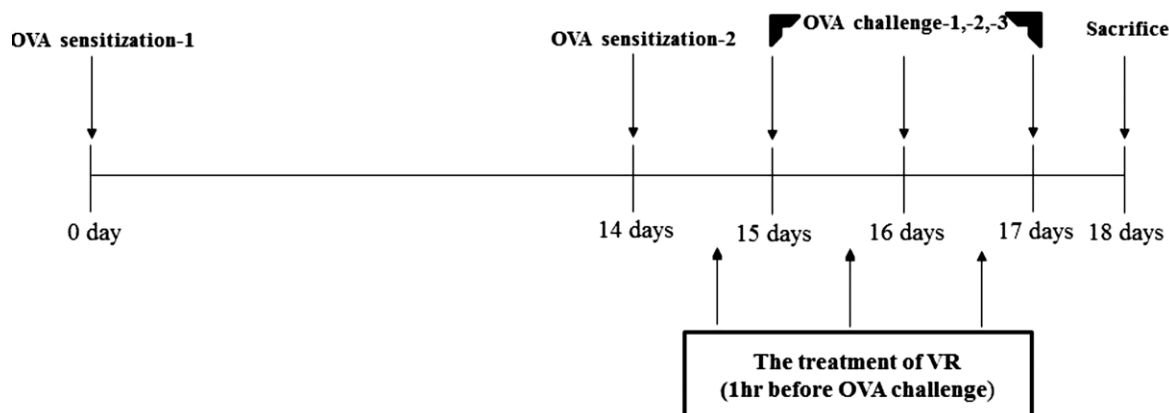


Fig. 1. Treatment protocol of asthma model.

Measurement of levels of cytokines in BAL fluid and total IgE and OVA-specific IgE in the serum

The concentrations of IL-4, IL-5, IL-13, TNF- α , total IgE and OVA-specific IgE were measured by a sandwich ELISA using a Mouse CytoSet Kit (Invitrogen, CA, USA), OptEIA Set mouse IgE microtiter plate (BD Biosciences, CA, USA) and Mouse OVA-specific IgE ELISA assay kit (DS Pharma Biomedical Co., Ltd, Osaka, Japan). All assays were conducted in triplicate. The optical density was measured at 450nm in a microplate reader.

Preparation of lung tissues and histology

Lung tissues were removed from the mice and fixed with 4% formaldehyde overnight at 4°C. The fixed tissues were then embedded in paraffin and cut into 4- μ m sections with a microtome (Leica, Nussloch, Germany), after which the sections were placed on slide glasses, deparaffinized and stained with hematoxylin and eosin (Sigma-Aldrich) in order to examine the leucocytes that had infiltrated into the peribronchial connective tissues. In addition, the samples were stained in randomly selected sections with periodic acid-Schiff (PAS; IMEB Inc., CA, USA) to assess the goblet cell hyperplasia in the bronchi. For immunohistochemistry of the smooth muscle actin (SMA), 4- μ m sections of the lower trachea and lung tissue were treated with 0.3% H₂O₂-methanol for 30min to block the endogenous peroxidase, and then incubated at 4°C overnight with anti-SMA mouse monoclonal antibody (1:50 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The slides were incubated with the avidin-biotin peroxidase complex (ABC kit, Vector Laboratories, Burlingame, CA, USA) and the color was developed with DAB (Vector Laboratories). After immunohistochemical staining, the slides were counterstained with Harris's hematoxylin for 3min and then mounted with Canada Balsam (Showa Chemical Co. Ltd, Tokyo, Japan).

Measurement of airway hyperresponsiveness

The AHR of the mice was measured using a single-chamber, whole-body plethysmograph (Buxco Electronics, Inc., Troy, NY) according to the manufacturer's protocol. The plethysmography and enhanced pause (Penh) variable was used to estimate airway resistance. Mice were serially exposed to increasing doses of nebulized methacholine (0, 25, 50 and 100mg ml⁻¹) (Sigma-Aldrich) in PBS for 3min, and Penh values were measured for 3min following the end nebulization.

Quantitative chromatographic analysis

High performance liquid chromatography (HPLC) analysis was used for the quantification of casticin in VR extract and the serum of asthmatic mice treated with VR. The HPLC system consisted of a Nanospace SI-2/3001 pump, a 3004 column oven with a 10 μ l fixed loop and a 3002 UV detector, was purchased from Shiseido (Tokyo, Japan). Chromatographic separation was performed using a Unison UK-C18 column (150 $\times$ 2.0mm ID, 3 μ m; Imtakt, Kyoto, Japan). The mobile phase consisted of a 2% formic acid solution containing 5% acetonitrile, the flow rate was 0.2ml min⁻¹, and the separation temperature was 30°C. The UV detector was set to 350nm and was controlled by a computer running the Dschromn

program supplied by Donam Instruments (Seoul, South Korea). All solvents were filtered and degassed prior to use.

VR (0.988g) was weighed and dissolved in 20ml of methanol. The sample was then sonicated for 15min then centrifuged at 15 000 r.p.m. for 10min. Finally, the supernatant of the sample was filtered through a 0.45 μ m syringe filter (PVDF; Gelman, USA). The serum samples of mice treated with 100mg kg⁻¹ of VR extract were weighed and the sample and a 2% trichloroacetic acid solution were mixed at a rate of 1:2. The mixed solution was then sonicated for 15min, then centrifuged at 15 000 r.p.m. for 10min. The supernatant was collected and analyzed by reversed-phase HPLC. For the quantitative analysis of the serum from mice treated with VR extract, a standard casticin calibration curve was made using six different standard solutions (2.5, 5, 10, 25, 50 and 100ng ml⁻¹). The regression equation was $y = ax + b$, where y and x were the peak area and sample concentration, respectively. The calibration equation for casticin was $y = 2.63875x$ and the R^2 value was 0.9993.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance followed by a post-hoc tests when necessary (Newman-Keuls Multiple Comparison Test). All data were analyzed using GraphicPad Prism (GraphicPad Software Inc., CA, USA). Differences were considered significant at $P < 0.05$.

Results

Inhibitory effect of VR extract on leukocytes in BAL fluid

The number of total cells, eosinophils and lymphocytes in the BAL fluid from OVA-immunized and -challenged mice increased remarkably compared with normal mice that were sensitized and challenged with saline (** $P < 0.01$; Fig. 2A, B, and D). These results indicated that the allergic inflammation model of asthma was successfully established. However, oral pre-treatment with VR extract significantly inhibited the number of total cells when compared with the asthmatic controls (45.9%, ** $P < 0.01$; Fig. 2D). The differential leukocyte counts show that oral administration of VR extract significantly inhibited eosinophilia when compared with the asthmatic control (86.0%, ** $P < 0.01$; Fig. 2A). Additionally, lymphocytosis in asthmatic mice that were treated orally with VR extract was significantly reduced when compared with the asthmatic control groups (74.7%, ** $P < 0.01$; Fig. 2B). Furthermore, the VR extract-treated group showed a 15.6% reduction in macrophage numbers, but this difference was not significant (Fig. 2C). Therefore, treatment of the induced asthma model with VR extract resulted in a significant reduction in the number of inflammatory cells that was as effective as dexamethasone.

Regulation of cytokines released into BAL fluid by VR extract

To investigate the possible inhibitory mechanisms of VR extract in airway inflammation and remodeling, the levels of IL-4, IL-5, IL-13 and TNF- α were examined using ELISA. In the asthmatic control mice, IL-4, IL-5, IL-13 and TNF- α levels were higher in BAL fluid compared with samples from normal mice.

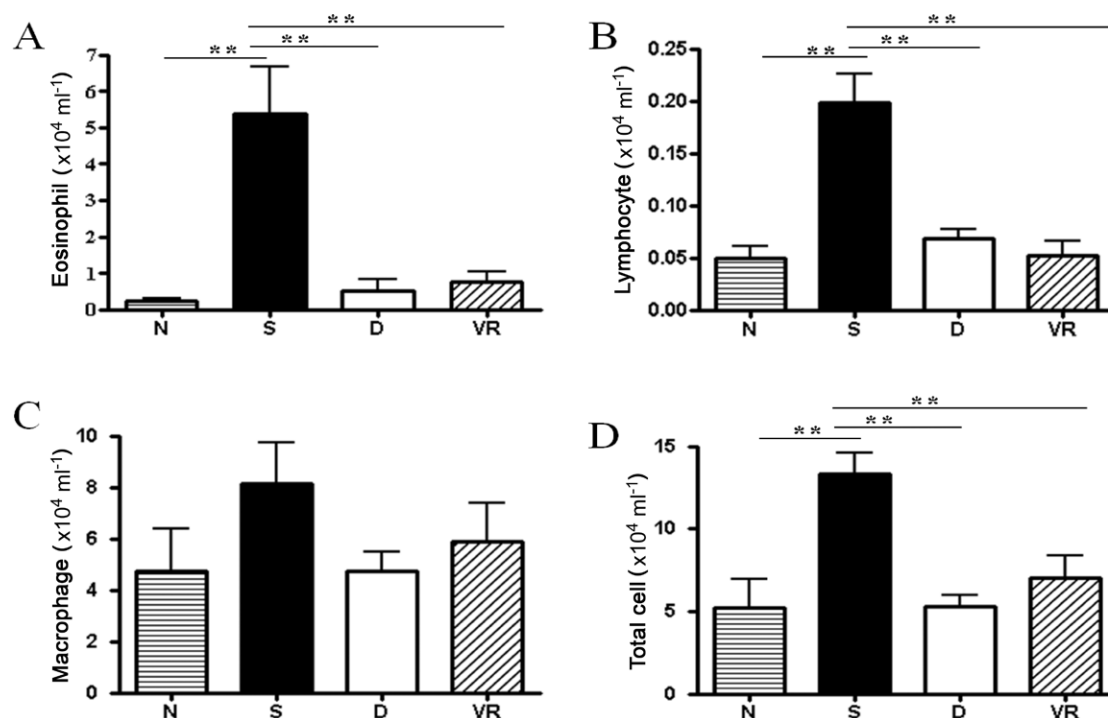


Fig. 2. Effects of VR extracts on the recruitment of inflammatory cells into BAL fluid in OVA-induced allergic asthmatic mice. The VR- and dexamethasone-treated groups received 100mg kg⁻¹ VR and 10mg kg⁻¹ dexamethasone orally between days 14 and 17. Normal and OVA-immunized mice were treated with saline on the same days. The BAL cells were separated using a hemacytometer and then stained with Diff-Quick, after which ~500 cells were counted. This experiment used the following six groups of mice: N, normal mice; S, OVA + saline-treated mice (control); D, OVA + 10mg kg⁻¹ dexamethasone-treated mice; VR, OVA + VR 100mg kg⁻¹ extract-treated mice. The number of eosinophils (A), lymphocytes (B), macrophages (C) and total cells (D). Values shown are the means $\pm$ SEM. ***P* < 0.01 indicates statistically significant differences between normal mice and the asthma control group, or between the asthma control group and dexamethasone- or VR-treated group.

Treatment with VR extract and dexamethasone significantly suppressed the elevated levels of IL-4 (**P* < 0.05), IL-5 and TNF- α (***P* < 0.01). The level of IL-13 was decreased, but not significantly, by VR extract treatment (Fig. 3).

Decreases in the level of IgE in serum in mice treated with VR extract

Because IgE is associated with the T_H2-cell-mediated immune response in the pathogenesis of allergic inflammation, the effects of treatment with VR extract on the total IgE and OVA-specific IgE in the serum were investigated. In the asthmatic control mice, the total IgE and OVA-specific IgE levels in the serum were significantly up-regulated when compared with normal mice (***P* < 0.01). Additionally, treatment with VR extract and dexamethasone led to significant down-regulation of the secretion of total IgE levels in the serum (**P* < 0.05). However, OVA-specific IgE was not significantly decreased in the serum by the VR extract treatment (Fig. 4).

Prevention of inflammation of lung tissue by VR extract in an asthmatic mouse model

To evaluate the effect of VR extract on the inflammatory histological features of the OVA-induced asthmatic model, lung tissues were stained with hematoxylin and eosin. The results of the histological examination of lung tissues paralleled the cell

numbers in the BAL fluid. A marked influx of inflammatory cells into the airway and blood vessels was detected in the lung sections of OVA-induced asthmatic mice, but not in normal mice (***P* < 0.01; Fig. 5A, B and E). Additionally, VR extract treatment led to a marked reduction in the infiltration of inflammatory cells into the airway of OVA-induced asthmatic mice that was similar to the results observed in dexamethasone-treated mice (***P* < 0.01; Fig. 5C, D, and E). These results demonstrated that treatment with VR extract inhibits OVA-induced inflammation in lung tissue in a fashion similar to dexamethasone.

Decrease in goblet cell hyperplasia induced by VR extract in an asthmatic mouse model

To evaluate the effects of VR extract on goblet cell hyperplasia, lung tissues were stained using PAS. The overproduction of goblet cell hyperplasia was detected in the bronchial airway epithelium of OVA-induced asthmatic mice, when compared with normal mice (***P* < 0.01; Fig. 6A, B and E). Additionally, OVA-induced asthmatic mice that received VR extract showed a large reduction in the number of goblet cells in the bronchial epithelium compared with those that received 10mg kg⁻¹ dexamethasone (***P* < 0.01; Fig. 6C, D and E). These results demonstrate that VR extract may be effective in inducing airway remodeling in OVA-induced asthmatic mice.

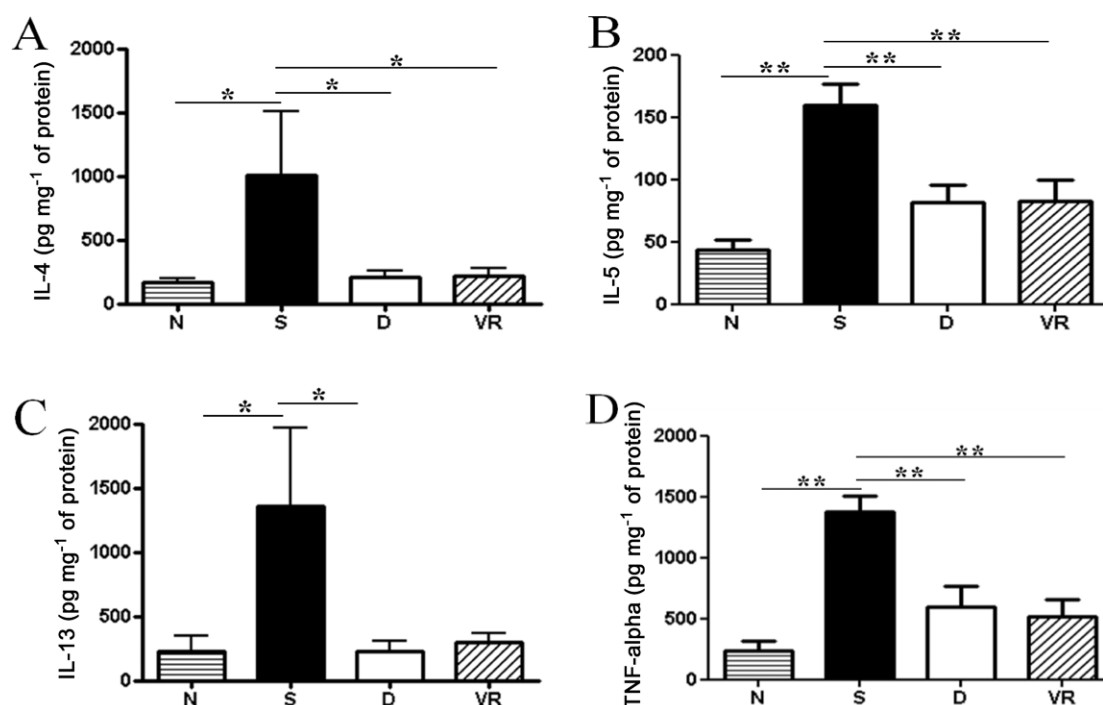


Fig. 3. Effects of VR extract on the levels of IL-4 (A), IL-5 (B), IL-13 (C) and TNF- α (D) in BAL fluid. BAL fluid was collected and the cells were eliminated. The cytokine levels were determined using an ELISA kit. N, normal mice; S, OVA + saline-treated mice (control); D, OVA + 10 mg kg⁻¹ dexamethasone-treated mice; VR, OVA + VR 100 mg kg⁻¹ extract-treated mice. Values shown are the means $\pm$ SEM. * P < 0.05 and ** P < 0.01 indicate statistically significant differences between normal mice and the asthma control group, or between the asthma control group and dexamethasone- or VR-treated group.

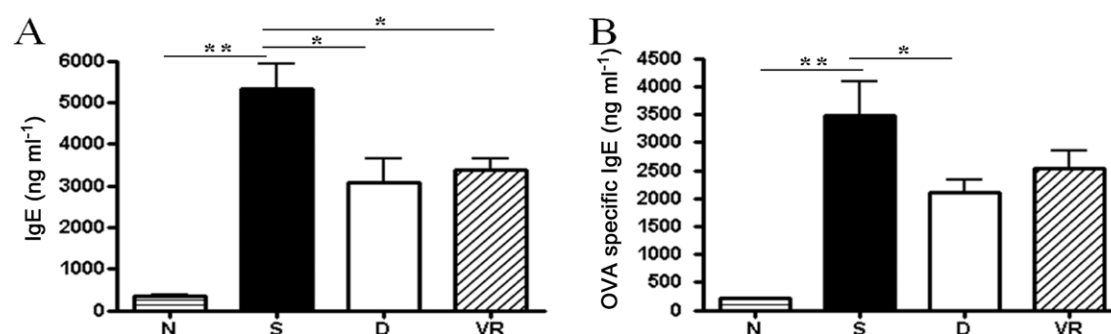


Fig. 4. Effects of VR extract on total IgE and OVA-specific IgE in serum. The serum levels of total IgE (A) and OVA-specific IgE (B) were determined using an ELISA kit. N, normal mice; S, OVA + saline-treated mice (control); D, OVA + 10 mg kg⁻¹ dexamethasone-treated mice; VR, OVA + VR 100 mg kg⁻¹ extract-treated mice. Values shown are the means $\pm$ SEM. * P < 0.05 and ** P < 0.01 indicate statistically significant differences between normal mice and the asthma control group, or between the asthma control group and dexamethasone- or VR-treated group.

Inhibition of increased airway smooth muscle actin by VR extract in an asthmatic mouse model

To investigate the effect of VR extract on airway SMA, lung tissues were subjected to immunohistochemistry analyses. Marked increases in the mass production of SMA-positive cells were observed in the lung tissues of OVA-induced asthmatic mice when compared with normal mice (** P < 0.01; Fig. 7A, B and E). All groups treated with VR showed decreased SMA immunoreactivity when compared with OVA-induced asthmatic mice (** P < 0.01; Fig. 7B, D and E), which was similar to the results seen

in the dexamethasone-treated group (** P < 0.01; Fig. 7B, C and E). These results demonstrated that VR extract may be effective in inducing airway remodeling in OVA-induced asthmatic mice, producing results similar to dexamethasone.

Decline of AHR induced by VR extract

Penh values were measured to analyze the effect of VR extract in lung function. The OVA-induced asthmatic mice group had higher Penh values than the normal group. In the VR extract group, there was a marked decrease in Penh values with 50

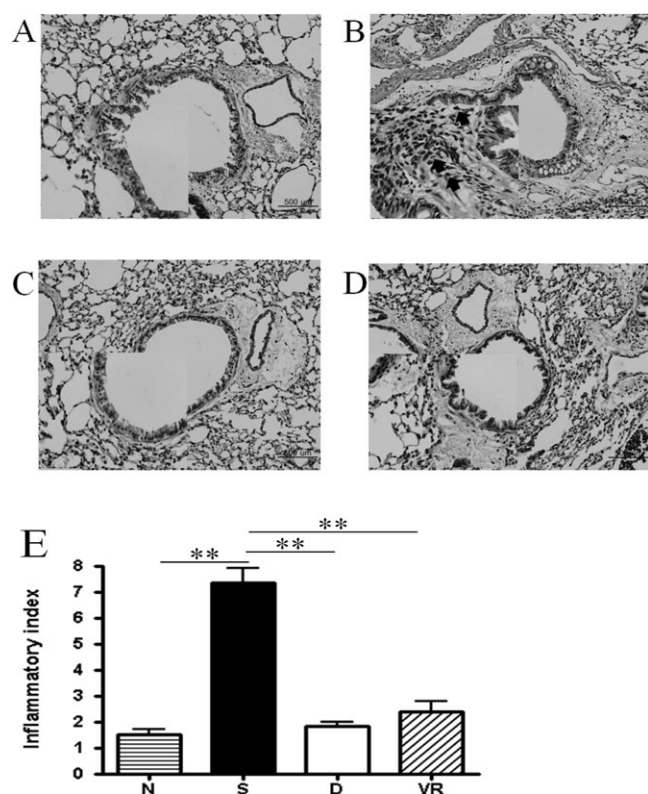


Fig. 5. Effects of VR extract on cell infiltration in lung tissues. Lung tissue from the mice of the respective groups was stained with hematoxylin and eosin ($\times 200$). Lung tissue of normal mice (A), lung tissue of OVA + saline-treated mice (B), lung tissue of OVA + 10 mg kg^{-1} dexamethasone-treated mice (C), lung tissue of OVA + 100 mg kg^{-1} VR extract-treated mice (D), the histological appearance was scored for the presence of lung tissue inflammation by analyzing inflammatory cell infiltration and these inflammation scores were added (E). Shown by arrows are the inflammatory cells. Scale bar = $500 \mu\text{m}$. Values shown are the means $\pm$ SEM. $**P < 0.01$ indicates statistically significant differences between normal mice and the asthma control group, or between the asthma control group and dexamethasone- or VR-treated group.

and 100 mg ml^{-1} doses of methacholine relative to the OVA-induced asthmatic mice group ($**P < 0.01$; Fig. 8).

Analysis of casticin in the VR extract and serum samples

Casticin was used as the standard for the quantitative analysis. The average casticin content in VR was $0.267 \pm 0.012 \text{ mg g}^{-1}$ ($n = 3$) (data not shown), and the amount of casticin in serum samples was $1.8 \pm 0.23 \text{ ng g}^{-1}$ ($n = 3$). Figure 9 shows the chromatogram of the casticin standard and serum samples.

Discussion

Asthma is characterized by chronic inflammation of the airways and airway remodeling as a result of overproduction of inflammatory cells (16). It is believed that production of the T_H2 cytokines IL-4, IL-5 and IL-13 and secretion of the pro-inflammatory cytokine TNF- α are responsible for eosinophil

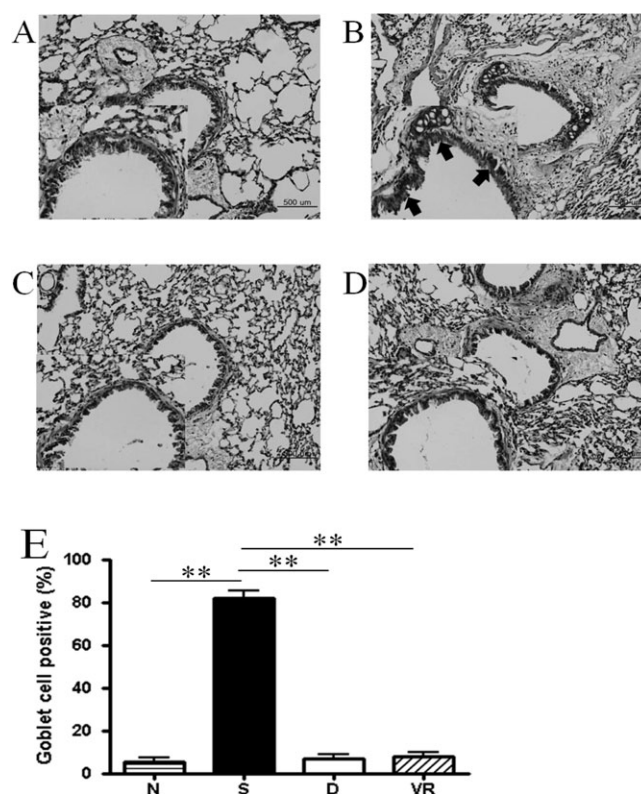


Fig. 6. Effects of VR extract on goblet cell hyperplasia in lung tissues. Lung tissue from mice in the respective groups was stained with PAS ($\times 200$). Lung tissue of normal mice (A), lung tissue of OVA + saline-treated mice (B), lung tissue of OVA + 10 mg kg^{-1} dexamethasone-treated mice (C), lung tissue of OVA + 100 mg kg^{-1} VR extract-treated mice (D), the histological appearance was scored for the presence of PAS-positive cells in epithelium and total epithelial cells were counted, and the percentage of PAS-positive cells was calculated (E). Shown by arrows are the goblet cells. Scale bar = $500 \mu\text{m}$. Values shown are the means $\pm$ SEM. $**P < 0.01$ indicates statistically significant differences between normal mice and the asthma control group, or between the asthma control group and dexamethasone or VR-treated group.

recruitment, AHR and airway remodeling due to the fact that these cytokines are found in high concentrations in allergic sites (17). IL-4 allows for the recruitment of eosinophils, and also leads to the enhanced production of IgE and up-regulation of the IgE-mediated response to inflammatory cells within the airway, while IL-5 is involved in the maturation of eosinophils (17, 18). Eosinophils are the main modulators of airway remodeling factors (19), and eosinophil-mediated damage to airways is a major mechanism underlying the pathogenesis of asthma that occurs via both inflammation and remodeling (20). Murine models have also provided important evidence demonstrating that these eosinophils are crucial regulators of airway remodeling in that eosinophil-deficient mice are protected from the characteristic features of airway remodeling (21–23).

BALB/c mice are sensitive responders to OVA and are a well-established airway inflammatory disease model of human asthma (24). OVA sensitization and subsequent OVA challenge in a murine model induces the infiltration of immune

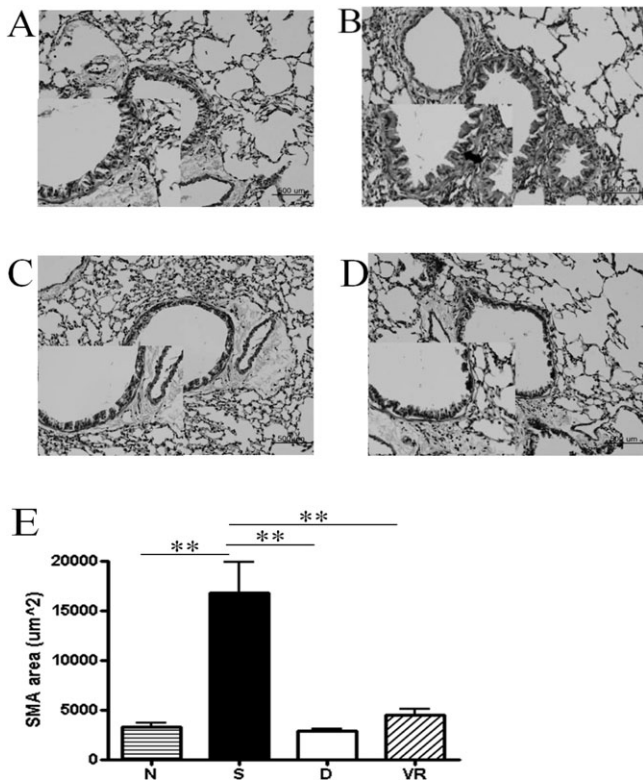


Fig. 7. Effects of VR extract on the increased airway smooth muscle actin in lung tissues. Lung tissue from mice in the respective groups was subjected to immunohistochemical staining ($\times 200$). Lung tissue of normal mice (A), lung tissue of OVA + saline-treated mice (B), lung tissue of OVA + 10mg kg⁻¹ dexamethasone-treated mice (C), lung tissue of OVA + 100mg kg⁻¹ VR extract-treated mice (D), thickness of the airway smooth muscle cell layer in μm^2 (E), shown by arrow is the smooth muscle cell layer. Scale bar = 500 μm . Values shown are the means $\pm$ SEM. $^{**}P < 0.01$ indicates statistically significant differences between normal mice and the asthma control group, or between the asthma control group and dexamethasone- or VR-treated group.

cells, including eosinophils and lymphocytes in the BAL fluid and mucus-secreting goblet cell hyperplasia (20).

In recent years, VR extract was reported to have an effect on anti-DNP IgE-induced TNF- α production (12) and to have inhibitory activity against T-lymphocyte proliferation (25). In this study, we investigated whether VR extract has an inhibitory effect against allergic inflammation in the OVA-induced asthmatic murine model. VR contains four flavonoids: casticin, artemetin, quercetagenin and 5,3'-dihydroxy-6,7,4'-trimethoxyflavanone (14). The quality of VR extracts was evaluated in terms of casticin, and the amount of casticin in the mouse serum samples treated with VR extracts was quantified. The amount of casticin in serum samples was very small, but could be quantitatively analyzed (Fig. 9).

Since the migration of inflammatory cells into the lungs is inevitable in allergic and asthmatic disorders, we tested whether VR extract inhibited the infiltration of inflammatory cells into the BAL fluid in OVA-challenged mice. The levels of each inflammatory cell type (eosinophils and lymphocytes) were reduced significantly (Fig. 2). We further examined changes in inflammatory cell recruitment in lung tissues of

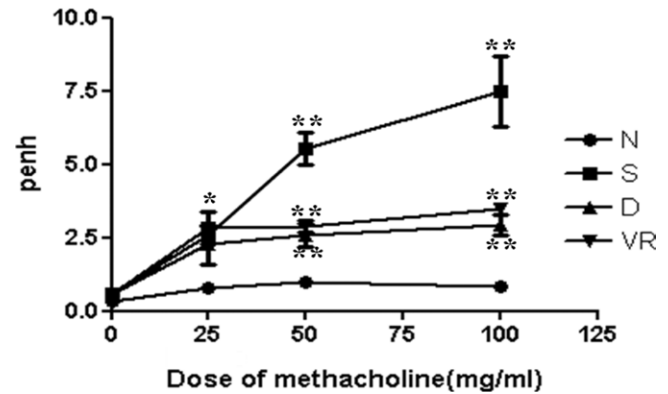


Fig. 8. Effects of VR extract on the AHR. N, normal mice; S, is OVA-induced asthmatic mice; D, OVA + 10mg kg⁻¹ dexamethasone-treated mice; VR, OVA + VR 100mg kg⁻¹ extract-treated mice. Values shown are the means $\pm$ SEM. $^{**}P < 0.01$ indicates statistically significant differences between normal mice and the asthma control group, or between the asthma control group and dexamethasone- or VR-treated group.

OVA-challenged mice using hematoxylin and eosin staining. Inflammatory cell recruitment into the lung tissue sections isolated from OVA-challenged mice was observed, and treatment with VR extract resulted in a marked decrease in inflammatory cell infiltration (Fig. 5). We also observed an increase in T_H2 and pro-inflammatory cytokines following the allergen challenge that was prevented by treatment with VR extract (Fig. 3). The suppression of IL-4 and IL-5 secretions led to a reduction in total IgE levels in response to treatment with VR extract in the serum of the asthmatic mice (Fig. 4). These results suggest that the suppressive effects of VR extract on OVA-induced airway eosinophilia are due to modulation of the T_H2 response. Because of the strong effects of VR extract on allergic responses, we measured the alteration in lung functions in OVA-induced asthmatic mice that were treated with VR extract. We confirmed that oral administration of VR extract resulted in a significant reduction in AHR (Fig. 8). Asthma is an inflammatory disorder that involves structural changes in the lungs. One such change is goblet cell hyperplasia, which leads to the obstruction of the airway through excessive mucus production. Another structural change is an increase in SMA-positive cell mass (20). Pulmonary fibrosis is increased by many pathologic agents, and SMA is localized near the fibrotic foci of the airway (26). In this study, the inhibition of both goblet cell hyperplasia and SMA mass was observed in response to treatment with VR extract when compared with OVA-induced asthmatic mice (Figures 6 and 7). We also detected an inhibitory effect of VR on IL-13 and TNF- α , both of which have a role in airway remodeling (Fig. 3C and D). These results suggested that treatment with VR extract can prevent the development of airway remodeling. Our results demonstrate that VR extract is a promising therapeutic candidate that is able to prevent the development of airway remodeling and interferes with eosinophilic inflammation and hyperplasia. These effects result from the inhibition of the T_H2 response, indicating that VR extract may have pharmacological effects that would be useful in the treatment of asthma.

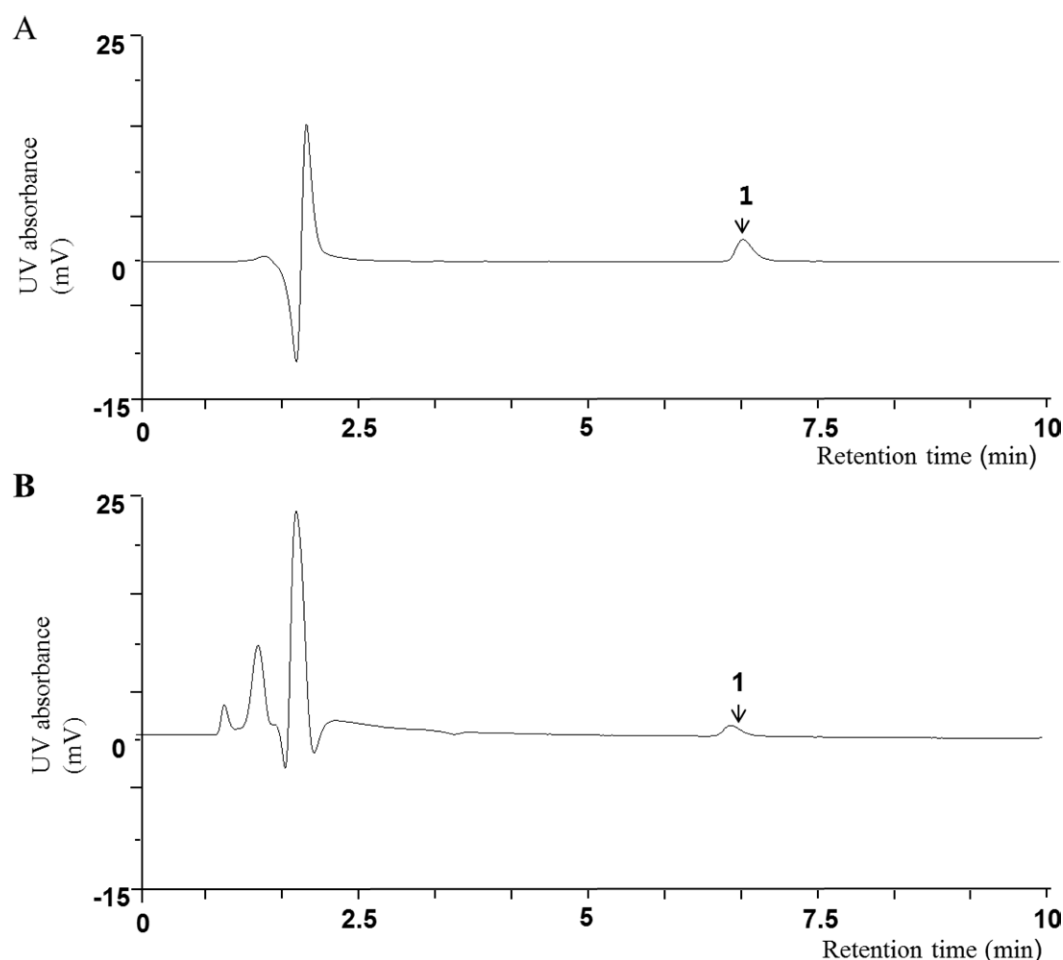


Fig. 9. Chromatograms of castin (A) and casticin in serum (B). Peak 1: casticin.

Funding

Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (No. 2009-0063466); the Ministry of Knowledge Economy (MKE), Korea Institute for Advancement of Technology (KIAT), Jeju Leading Industry Office through the Leading Industry Development for Economic Region (A002200703).

Acknowledgements

The authors would like to thank Youngwoo Cho for his assistance with grammar.

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