

RESEARCH ARTICLE

Pinellia ternata Breitenbach attenuates ovalbumin-induced allergic airway inflammation and mucus secretion in a murine model of asthma

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Abstract

Objective: *Pinellia ternata* is an important plant in traditional Chinese medicine. This study describes the anti-inflammatory effects of a water extract of *P. ternata* (PTE) in allergic airway inflammation in a model of asthma in mice.

Materials and methods: BALB/c mice were sensitized with ovalbumin (OVA) and, upon an OVA aerosol challenge, developed airway eosinophilia, mucus hypersecretion, elevations in cytokine, chemokine, and immunoglobulin levels and overexpression of inducible nitric oxide (iNOS).

Results: Intragastric administration of PTE significantly attenuated OVA-induced influx of total leukocytes, eosinophils, neutrophils, macrophages and lymphocytes into lungs, and attenuated levels of interleukin (IL)-4, IL-13 and tumor necrosis factor- α (TNF- α), in a dose-dependent manner. PTE also significantly reduced the plasma levels of total and OVA-specific immunoglobulin (Ig)E release into the airspace. Histological studies showed that PTE inhibited OVA-induced lung tissue eosinophilia and airway mucus production. Moreover, in whole lung tissue lysates, immunohistology showed that PTE markedly attenuated the OVA-induced increase in mucin 5AC and iNOS expression.

Conclusions: These results indicate that PTE has protective effects against allergic airway inflammation.

Keywords

Asthma, cytokine, muc 5AC, OVA, *Pinellia ternata* Breitenbach

History

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Introduction

Allergic diseases are the most common inflammatory diseases, and their prevalence and incidence have increased in many developed and developing countries¹. Allergic asthma is a chronic, reversible airway inflammatory disease of great public health importance in children and adults. Although the exact mechanism is not clear, allergic asthma appears to result from allergen-specific type 2 T helper (Th2) lymphocyte proliferation with concomitant excessive production of Th2 cytokines including interleukin (IL)-4, IL-5, IL-13 and IL-25². These alterations induced secretion of allergen-specific immunoglobulin (Ig)E, overproduction of bone marrow eosinophils, airway eosinophilia, mucus secretion by goblet cells and smooth muscle contraction. Collectively, these were contributing to airway hyperreactivity (AHR)^{3–5}. Cumulative evidence has revealed that these inflammatory responses are mediated by Th2 cells together with mast cells, B cells and eosinophils, as well as a number of inflammatory cytokines and chemokines^{3,6,7}. Therefore, Th2 responses to allergens play a central role in both the induction and the orchestration of inflammatory responses in

asthmatic airways^{8–10}. Several researchers considered the modulation of Th2 responses as an important strategy for controlling asthma¹¹.

Pinellia ternata is a monocotyledonous perennial herbaceous plant widely used for thousands of years in China as an important herb in traditional Chinese medicine. It has been applied for anti-emetic, anti-tussive, sedative and anti-inflammatory purposes¹². In addition, *P. ternata* is effective in removing dampness-phlegm¹³. Considering these properties of *P. ternata*, it is considered to be effective for asthma treatment. However, there was no study on effects of *P. ternata* against asthma.

In the present study, we examined the protective effect of *P. ternata* extract (PTE) on expression of mucin 5AC and inducible nitric oxide synthase (iNOS) in lung tissues of ovalbumin (OVA)-induced airway inflammation in our model of asthma and determined biochemical variables in the plasma and in bronchoalveolar lavage fluid (BALF) including inflammatory cells, cytokines, chemokines and IgE.

Materials and methods

Chemicals and reagents

Commercially available enzyme-linked immunosorbent assay (ELISA) kits for IL-4 (KMC0042), IL-13 (KMC0051) and tumor necrosis factor (TNF)- α (KMC3012) were purchased from Invitrogen (Carlsbad, CA), and those for total IgE

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(MCA2475KZZ) and OVA-specific IgE (107008) were obtained from Serotec (Oxford, UK). OVA, aluminum hydroxide, *o*-phenylenediamine dihydrochloride, tissue lysis/extraction reagent plus protease inhibitor, and hematoxylin and eosin solution were purchased from Sigma-Aldrich (St. Louis, MO). Protein assay reagent was purchased from Bio-Rad Laboratories, Inc. (Hercules, CA). Reagents used in the histological examination, namely Diff-Quik Stain reagents and periodic acid-Schiff (PAS) reagents, were purchased from Dade Behring (B4132-1A; Deerfield, IL) and IMEB (San Marcos, CA). Antibodies used for western blotting, namely anti-mucin 5AC and anti-iNOS, were purchased from Abcam (Cambridge, UK). The anti- β -actin antibody and horseradish peroxidase-conjugated secondary antibody were purchased from Cell Signaling (Boston, MA) and Jackson ImmunoResearch (West Grove, PA), respectively. The enhanced chemiluminescence (ECL) kit was purchased from Amersham (Buckinghamshire, UK).

Preparation of *P. ternata* Breitenbach water extract

Soluble components of dried *P. ternata* Breitenbach (100 g) were extracted once with distilled water (1 L) by refluxing for 2 h. The extract was filtered through a filter paper, the water was evaporated and the residue was freeze-dried (9.30 g). The yield was 9.30% of the original dry weight of the herbs.

Animals

Six-week-old pathogen-free female BALB/c mice were obtained from the Orient Co. (Seoul, Korea). Mice were maintained in an animal facility under standard laboratory conditions for 1 week prior to experiments, and were provided with water and standard chow *ad libitum*. All experimental procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and animal handling followed the dictates of the National Animal Welfare Law of Korea.

Experimental procedure

Mice were divided into five groups (of seven animals each), and airway inflammation was induced by chicken ovalbumin (OVA, Grade III, Sigma) in mice from three of the groups, using the method described by Oh et al.¹⁴. Briefly, mice were immunized via intraperitoneal injection with 20 μ g OVA and 2 mg aluminum hydroxide in 200 μ L phosphate-buffered saline (PBS; pH 7.4), on days 0 and 14 (Table 1). Mice were given the following treatments once daily by oral

gavage from days 18 to 23: saline in groups 1 (saline sensitization/challenge, Saline/Saline) and 2 (OVA sensitization/challenge, OVA/saline), montelukast (OVA sensitization/challenge + montelukast 30 mg/kg, OVA/Mon) in group 3 and PTE (PTE 23.3 mg/kg or 46.6 mg/kg + OVA sensitization/challenge) in groups 4 and 5. Montelukast and PTE were dissolved in PBS and application volume is 5 mL/kg. Groups 1 and 2 received an equivalent volume of PBS. At the time of oral administration, mice received an airway challenge with a 1% (w/v) OVA solution in saline using an ultrasonic nebulizer (NE-U12; Omron, Tokyo, Japan) for 20 min on days 21, 22 and 23 after initial sensitization except for the mice receiving the control saline/saline treatment (Table 1). Animals were sacrificed 48 h after the final challenge (day 25) to characterize the effects of PTE. Montelukast is a cysteinyl leukotriene 1 receptor antagonist¹⁵ and useful clinical responses to this agent have been obtained in patients with aspirin-sensitive asthma, nocturnal exacerbation of asthma and allergic asthma. These data have resulted in successful commercialization of the drug¹⁶. In the present work, montelukast was used as a positive control.

Body weights were measured once a week. The absolute weights of the liver and kidneys were measured, and their relative weight (percentage of body weight) was calculated. Complete gross observation was performed on all euthanized animals.

Inflammatory cell counts in BALF

Following OVA challenge, BALF samples were obtained from the mice and processed, and inflammatory cells were counted as described previously¹⁷. In brief, mice were sacrificed by intraperitoneal injection of pentobarbital (50 mg/kg; Hanlim Pharm. Co., Seoul, Korea) 48 h after the last challenge, and a tracheostomy was performed. To obtain BALF, ice-cold PBS (0.5 mL) was infused into the lung and withdrawn via tracheal cannulation three times (total volume 1.5 mL). Total inflammatory cell numbers were assessed by counting cells in at least five squares of a hemocytometer after exclusion of dead cells by Trypan blue staining. To determine differential cell counts, 100 μ L of BALF was centrifuged onto slides using a Cytospin (Hanil Science Industrial, Seoul, Korea) (200 g, 4 °C, 10 min). The slides were dried, and the cells were fixed and stained using Diff-Quik® staining reagent (B4132-1A; IMEB Inc., Deerfield, IL), according to the manufacturer's instructions. The supernatant obtained from BALF was stored at -70 °C for the biochemical analysis.

Table 1. Experimental procedure.

Groups	Sensitization (Days 0 and 14)	Drug treatment (Once daily from day 18–23)	Inhalation (Days 21, 22 and 23)	Sacrifice
Saline/Saline (Group 1)	Saline (i.p.)	Saline (p.o.)	Saline	Day 25
OVA/Saline (Group 2)	OVA/Alum (i.p.)	Saline (p.o.)	1% OVA	Day 25
OVA/Mon (Group 3)	OVA/Alum (i.p.)	Montelukast (30 mg/kg, p.o.)	1% OVA	Day 25
OVA/PTE-1 (Group 4)	OVA/Alum (i.p.)	PTE (23.3 mg/kg, p.o.)	1% OVA	Day 25
OVA/PTE-2 (Group 5)	OVA/Alum (i.p.)	PTE (13.5 mg/kg, p.o.)	1% OVA	Day 25

OVA/Alum: 20 μ g of OVA and 2 mg of aluminum hydroxide dissolved in 0.2 mL of PBS.

Measurement of total IgE and OVA-specific IgE levels in plasma

BALF and plasma were collected and stored at -70°C after centrifugation (300 g, 10 min). Total IgE and OVA-specific IgE levels in plasma were measured using ELISA. Complementary capture and detection antibody pairs for mouse IgE antibodies (Serotec, Oxford, UK) and the IgE ELISA was performed according to the manufacturer's directions.

Measurement of cytokines in BALF

The levels of IL-4, IL-13 and TNF- α in BALF were measured using a specific mouse ELISA kit (BioSource International, Camarillo, CA). ELISA experiments were performed according to the manufacturer's instructions.

Western blotting

The lung tissue was homogenized (1/10 w/v) in tissue lysis/extraction reagent containing protease inhibitors (Sigma-Aldrich) using an IKA T10 Basic (IKA Works, Staufen, Germany). Homogenates were centrifuged at 12 000 g for 25 min at 4°C . Protein concentrations in the supernatant fractions were determined using Bradford reagent (Bio-Rad Laboratories, Inc.). Equal amounts of total lung protein (30 μg) were heated at 100°C for 5 min and then loaded onto 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels, followed by transfer to a nitrocellulose membrane (at 100 V for 2 h). Nonspecific binding sites on the membranes were blocked for 1 h with Tris-buffered saline containing 0.05% Tween 20 (TBST) plus 5% skimmed milk and were incubated with anti-mucin 5AC, iNOS and anti- β -actin overnight at 4°C . The membranes were washed thrice with TBST, and then incubated with a 1:10 000 dilution of horseradish peroxidase-conjugated secondary antibody in TBS for 1 h at room temperature. The membranes were again washed thrice with TBST, and specific binding sites were determined using an ECL kit.

Lung tissue histopathology

After BALF was obtained, lung tissue was fixed in 4% paraformaldehyde for 24 h at 4°C . Tissues were embedded in paraffin, sectioned at 4 μm thickness, and stained with hematoxylin and eosin solution (hematoxylin, Sigma MHS-16; eosin, Sigma HT110–1–32) and PAS (IMEB) to assess mucus production. Tissues were subsequently mounted and coverslipped using Dako mounting medium (Invitrogen). For immunohistochemistry, paraffin sections were deparaffinized, dehydrated, washed in PBS with 0.3% Triton X-100, and preincubated for 10 min at room temperature with 10% goat serum to block nonspecific staining. Subsequently, slides were incubated with primary mouse anti-rabbit mucin 5AC antibody (1:200 dilution) overnight at 4°C . After removal of primary antibodies, sections were washed and incubated with biotinylated secondary antibody at 37°C for 1 h, followed by an avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA) for 1 h at room temperature. Excess secondary antibody was removed, and sections were washed with PBS and incubated with 0.05% diaminobenzidine

(1:200; Millipore, Billerica, MA) for further 10 min. Sections were rinsed in PBS to terminate the reaction, and coverslipped for microscopic examination.

Statistical analysis

Data are expressed as means $\pm$ standard deviations (SD). Statistical comparisons were made by one-way analysis of variance (ANOVA) using SPSS software (version 16.0; Chicago, IL), followed by Duncan's new multiple range test, using $p < 0.05$ as the level of significance.

Results

Effects of PTE on inflammatory cell recruitment

To determine whether PTE has a protective effect on airway inflammation, the eosinophils, macrophages, neutrophils, monocytes and total cell numbers in BALF were measured in OVA-challenged mice untreated or treated with PTE. OVA-challenged mice displayed markedly increased total cell and eosinophil numbers in their BALF. PTE significantly attenuated cell influx to BALF. The positive drug control, montelukast, displayed similar attenuating effects to those of the lower dose of PTE on leukocyte influx into BALF (Figure 1).

Effects of PTE on liver, spleen and body weight

The development of the mice during the experimental period corresponded to that of normal mice of their species and age. Body weights were measured once a week and no significant PTE-treatment-related difference was observed in any experimental group at any time point in the experimental period (Figure 2A). There were no significant differences between any of the experimental groups in the relative liver (Figure 2B) and spleen (Figure 2C) weights.

Effects of PTE on cytokine levels

As shown in Figure 3, OVA challenge led to significant increases in the levels of IL-4 (Figure 3A) and IL-13 (Figure 3B), and decreased the level of TNF- α (Figure 3C) in BALF, compared with unchallenged controls. After the administration of PTE, the concentrations of IL-4 and IL-13 in BALF were reduced, whereas that of TNF- α increased. Moreover, montelukast (the positive control) also induced significant reductions in the levels of IL-4 and IL-13 compared with those in untreated OVA-challenged mice.

Effect of PTE on total IgE and OVA-specific IgE in plasma

As shown in Table 2, the total IgE level in plasma was much higher in the plasma of untreated OVA-challenged mice compared with unchallenged controls, while it was significantly lower in montelukast-treated mice compared with untreated OVA-challenged mice. All PTE-treated groups had significantly lower total IgE levels compared with untreated OVA-challenged mice. Similar to the total IgE level results, untreated OVA-challenged mice had significantly higher OVA-specific IgE levels compared with unchallenged controls while PTE-treated mice had significantly lower levels of OVA-specific IgE compared with untreated OVA-challenged mice.

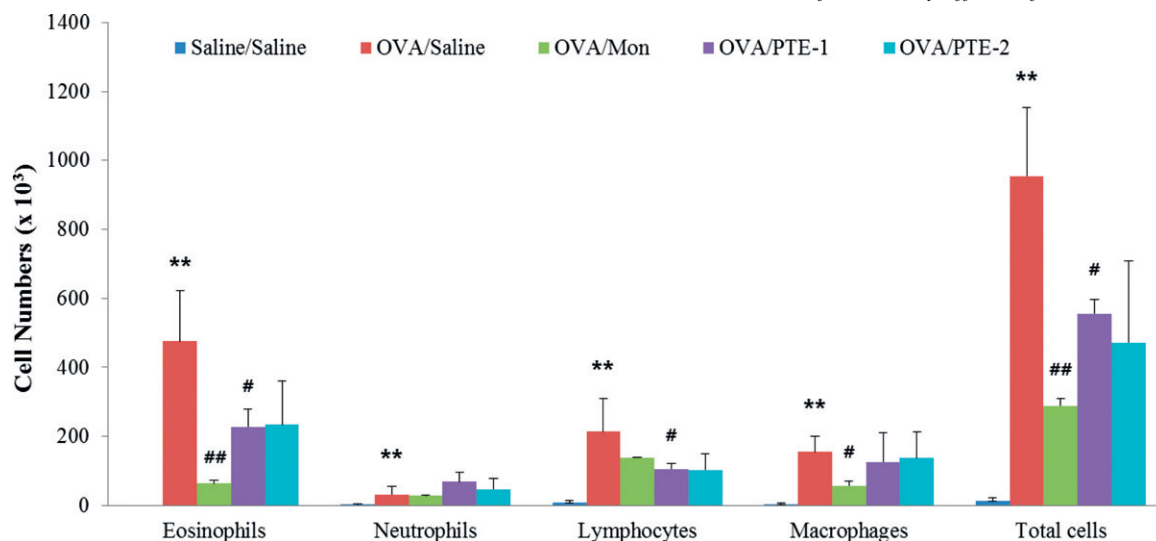


Figure 1. Effects of PTE on the recruitment of inflammatory cells into the BALF of mice 48 h after the final ovalbumin (OVA) challenge. Cell numbers were determined using a light microscope to count cells in at least five squares of a hemocytometer after excluding dead cells using Trypan blue. Cells were isolated by centrifugation and stained with Diff-Quik Stain reagent. Saline/Saline: normal control mice treated with PBS only; OVA/Saline: OVA-sensitized/challenged mice; OVA/Mon: montelukast (30 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-1: PTE (23.3 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-2: PTE (46.6 mg/kg) + OVA-sensitized/challenged mice. Values are expressed as mean $\pm$ SD. **Significantly different from Saline/Saline, $p < 0.01$; ##Significantly different from OVA/Saline, $p < 0.05$ and $p < 0.01$, respectively.

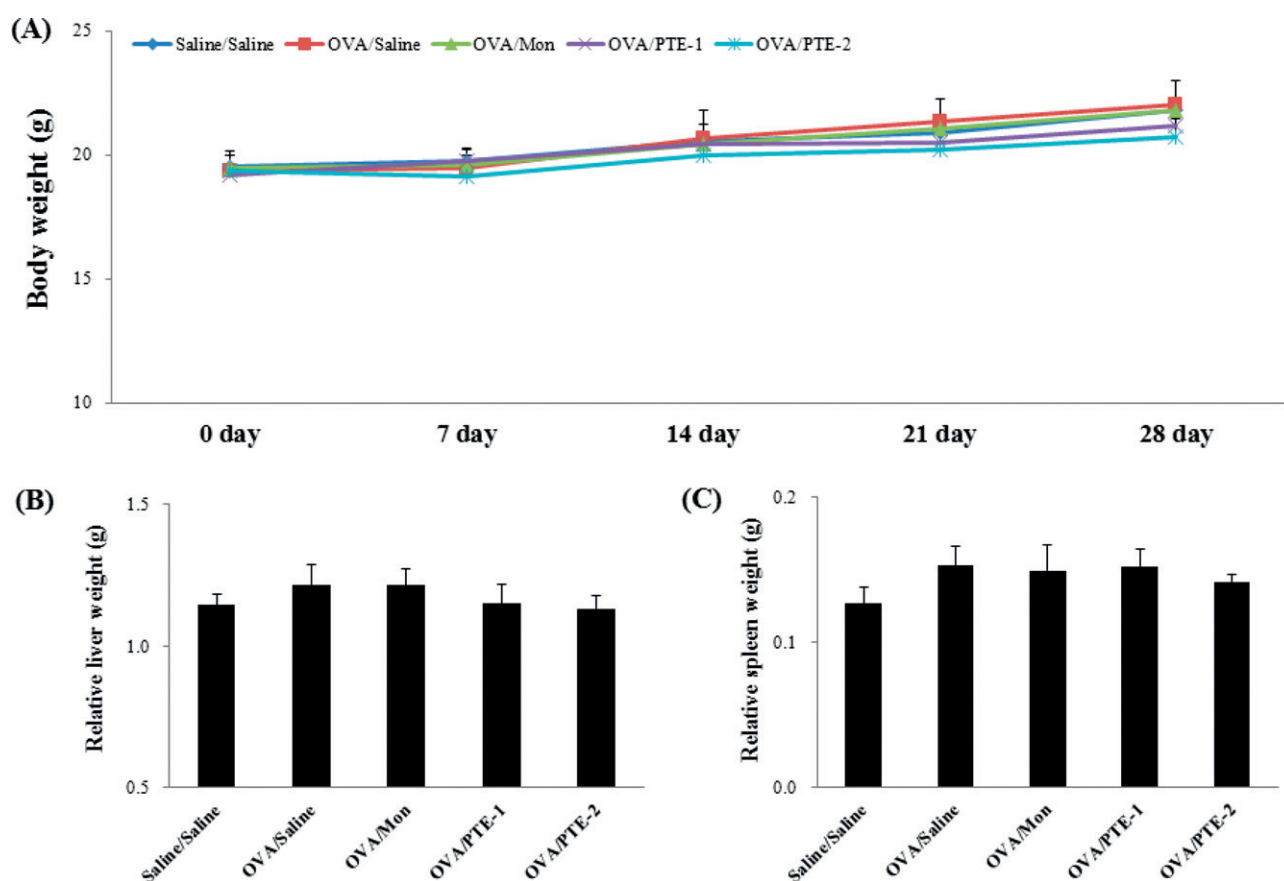


Figure 2. Effects of PTE on the body weight (A), liver (B) and spleen (C) weight. Saline/Saline: normal control mice treated with PBS only; OVA/Saline: OVA-sensitized/challenged mice; OVA/Mon: montelukast (30 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-1: PTE (23.3 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-2: PTE (46.6 mg/kg) + OVA-sensitized/challenged mice. Values are expressed as mean $\pm$ SD.

Effect of PTE on inflammatory cell infiltration and mucus production in lung tissue

Because PTE inhibits inflammatory cell recruitment into BALF, we next examined its anti-inflammatory effects. In the

OVA-induced model of asthmatic lung tissue, we observed marked infiltration of inflammatory cells into the perivascular and peribronchial areas, compared with the negative control group. Most of the inflammatory cells were eosinophils.

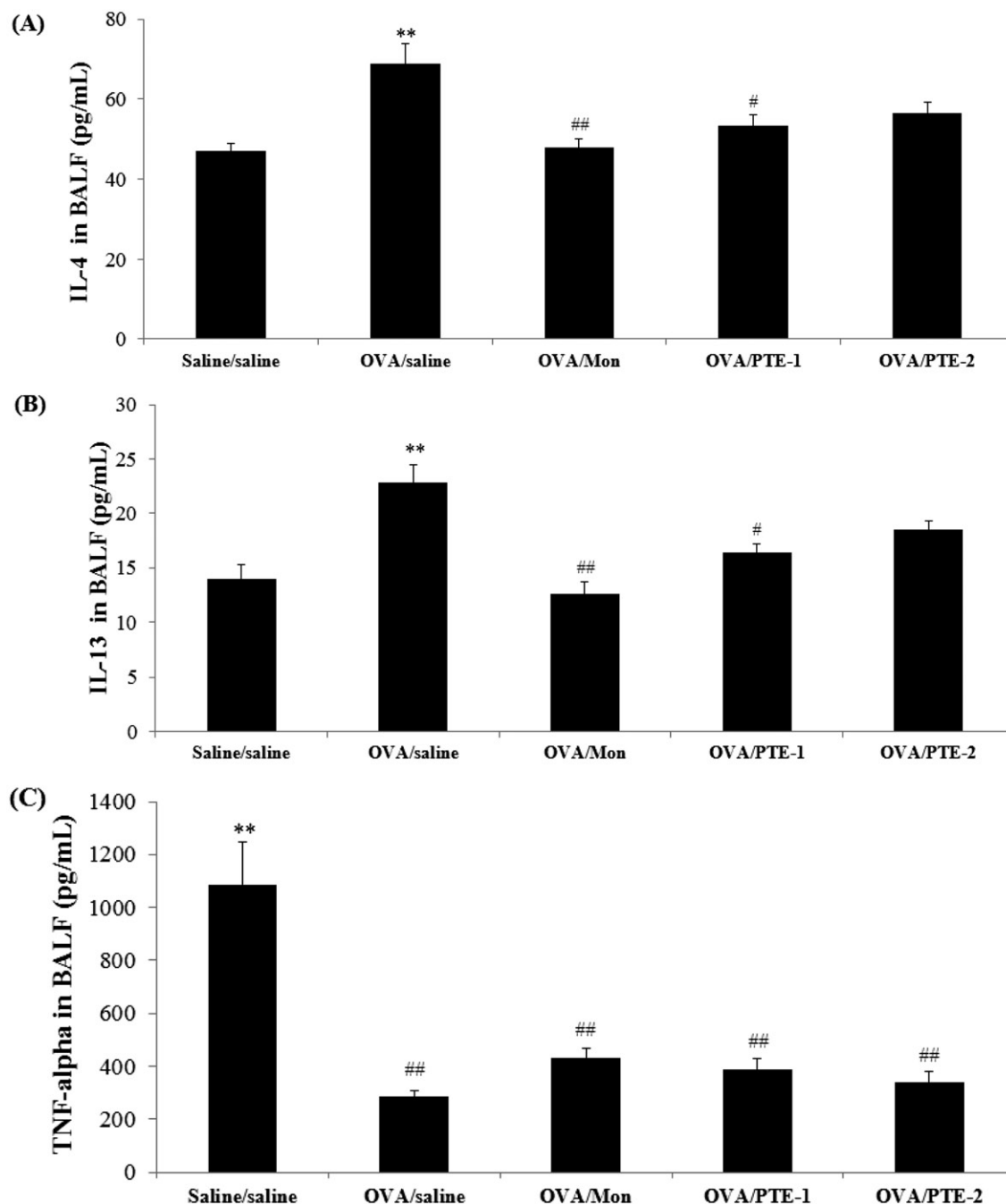


Figure 3. Effects of PTE on IL-4 (A), IL-13 (B) and TNF- α (C) cytokine levels. BALF was collected 48 h after the most recent OVA challenge. Saline/Saline: normal control mice treated with PBS only; OVA/Saline: OVA-sensitized/challenged mice; OVA/Mon: montelukast (30 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-1: PTE (23.3 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-2: PTE (46.6 mg/kg) + OVA-sensitized/challenged mice. Values are expressed as mean $\pm$ SD. **Significantly different from Saline/Saline, $p < 0.01$; ##Significantly different from OVA/Saline, $p < 0.05$ and $p < 0.01$ respectively.

Eosinophilia in the group of mice with the OVA-induced model of asthmatic lung tissue that had been treated with PTE (23.3 or 46.6 mg/kg) was significantly attenuated compared with the level seen in untreated mice after OVA challenge. Moreover, inflammatory leucocytes in lung tissue were more affected at a dose of 23.3 mg/kg than at 46.6 mg/kg of PTE (Figure 4).

Mucus hypersecretion is a characteristic of airway remodeling. To determine whether PTE-suppressed mucus overproduction caused by goblet cell hyperplasia, we stained lung sections with PAS. In mice with the OVA-induced model of asthmatic lung, mucus overproduction was clearly observed as a violet color in bronchial airways compared with scant

staining in the negative control group. In contrast, the extent of mucus staining was markedly diminished in OVA-challenged mice treated with PTE (Figure 5).

Effect of PTE on mucin 5AC expression in lung tissue

Next, we investigated whether PTE regulates mucin 5AC expression as part of the mechanism underlying protection against OVA-challenged lung mucus secretion and goblet cell hyperplasia. To determine whether the inhibitory effects of PTE on mucus secretion are related to a reduction in mucin 5AC levels, its expression was assessed using a western blot

and immunohistochemical analyses. As shown in Figure 6(A), immunohistochemical staining readily provided a quantitative measure of mucin 5AC expression in the lung. We found a predominance of mucin 5AC-stained cells in lung tissue from mice in our OVA-induced model of asthma compared with that in the PBS control group. However, the administration of PTE decreased the prevalence of stained cells in lung tissue from mice with the OVA-induced model. To quantify further, the inhibitory effect of mucin 5AC observed by immunohistochemical staining, we investigated mucin 5AC expression using the western blot analysis.

Effect of PTE on iNOS and nuclear factor-kappa B (NF-κB) immunoblotting in lung tissue

To investigate further anti-inflammatory effects of PTE in OVA-induced asthma, we measured iNOS and NF-κB in the lung by immunoblotting. As shown in Figure 7(A and B), the western blotting readily provided a quantitative measure of iNOS expression in the lung. The increased iNOS and NF-κB

expression observed in the lungs from mice with the OVA-induced model was significantly inhibited by PTE. These results may indicate that PTE treatment has anti-inflammatory effects that are related to the downregulation of iNOS and NF-κB.

Discussion

In this study, the potential effectiveness of PTE as a protective drug for asthma was examined using an OVA-induced model of airway inflammation in mice. The results show that PTE-suppressed eosinophil infiltration and excessive mucus secretion into the airway. PTE also significantly inhibited IgE, IgG1, and Th1- and Th2-type cytokine release into the lungs and blood. Moreover, PTE inhibited the expression of mucin 5AC, iNOS and NF-κB in the lung tissue.

The recruitment of eosinophils into the airways is a characteristic of asthma, and the degree of eosinophilia is correlated with the severity of the disease. Transmigration of leukocytes into the airways is regulated by cytokines such as IL-4, IL-5 and IL-13; and chemokines such as eotaxin^{18,19}. IL-4 is imperative for B cell isotype switching for the synthesis of IgE. IL-5 plays a major role in the maturation and recruitment of eosinophils into the airway²⁰. IL-13 plays a pivotal role in the effector phase of Th2 responses, such as eosinophilic inflammation and mucus hypersecretion²¹. Among these cytokines, Th2 cytokines such as IL-4, IL-5 and IL-13 are closely associated with the pathogenesis of asthma. The number of leukocytes, particularly eosinophils, in the peribronchial tissues and BALF were increased in the mice with the OVA-induced model of asthmatic lungs, and the infiltration was diminished by PTE. IL-4, IL-5 and IL-13 can be produced by various lung-resident cells, such as bronchial epithelial cells, mast cells and alveolar macrophages, and infiltrated inflammatory cells, such as lymphocytes and

Table 2. Effects of PTE on total IgE and OVA-specific IgE in plasma.

Groups	Total IgE (ng/mL)	OVA-specific IgE (ng/mL)
Saline/Saline	389.9 ± 73.8	–
OVA/Saline	9873.9* ± 994.1	82.8* ± 12.07
OVA/Mon	6993.8** ± 536.9	35.0** ± 16.5
OVA/PTE-1	6449.5** ± 2065.4	44.8** ± 18.4
OVA/PTE-2	6196.8** ± 2068.6	34.8** ± 25.0

Saline/Saline: normal control mice treated with PBS only; OVA/Saline: OVA-sensitized/challenged mice; OVA/Mon: montelukast (30 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-1: PTE (23.3 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-2: PTE (46.6 mg/kg) + OVA-sensitized/challenged mice.

*Significantly different from Saline/Saline, *p* < 0.01.

**Significantly different from OVA/Saline, *p* < 0.05.

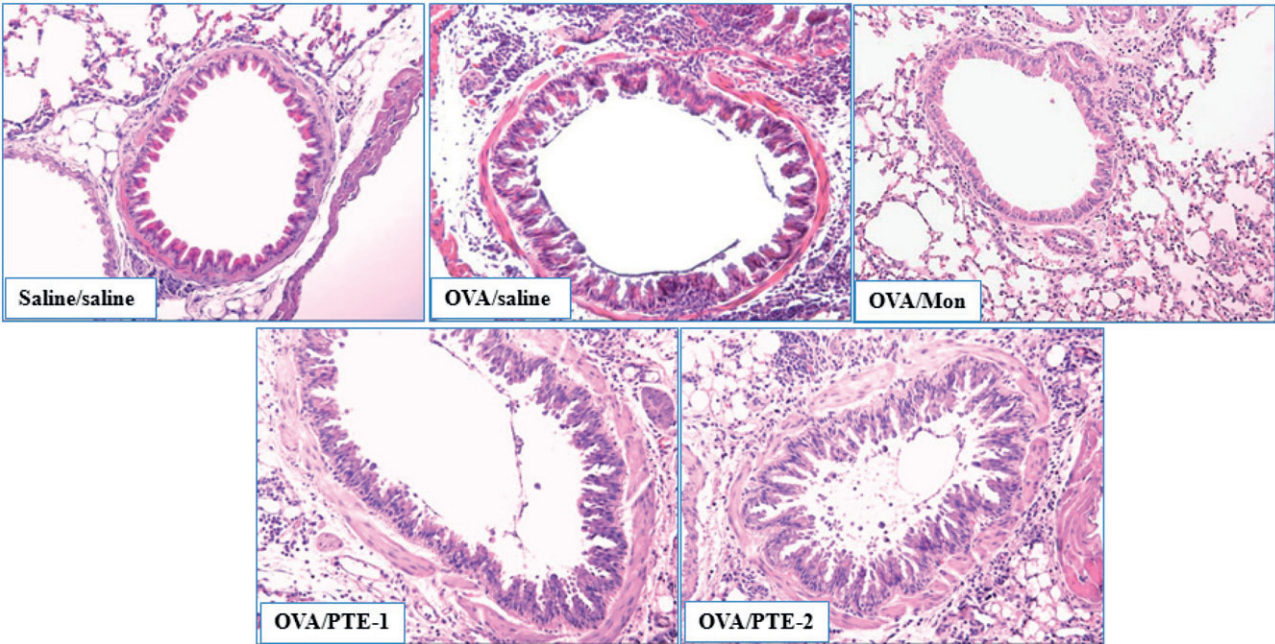


Figure 4. Effects of PTE on the recruitment of inflammatory cells into the lung tissue of mice 48 h after the final OVA challenge. Lung tissues were fixed, sectioned at 4 μm thickness, and stained with hematoxylin and eosin solution (magnification ×200). Saline/Saline: normal control mice treated with PBS only; OVA/Saline: OVA-sensitized/challenged mice; OVA/Mon: montelukast (30 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-1: PTE (23.3 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-2: PTE (46.6 mg/kg) + OVA-sensitized/challenged mice.

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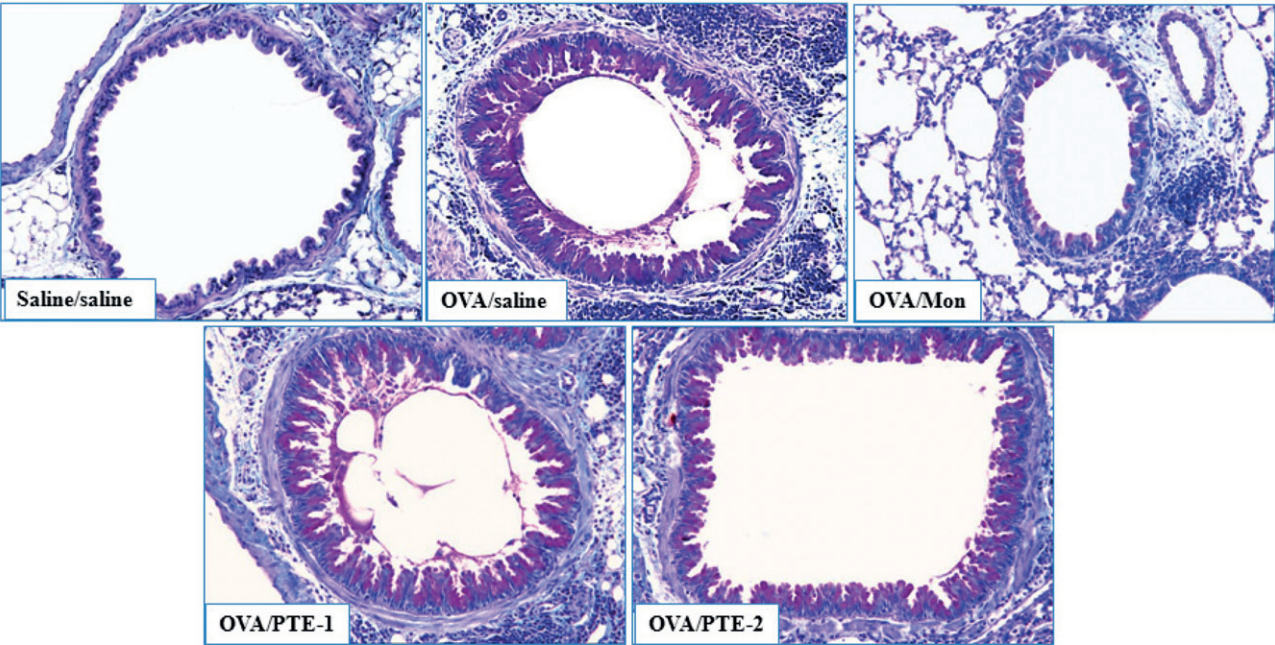


Figure 5. Effects of PTE on mucus production in the lung tissue of mice 48 h after the final OVA challenge. Lung tissue was fixed, sectioned at 4 μ m thickness and stained with PAS reagent to assess mucus production (magnification $\times 200$). Histological examination of mucus secretion in lung tissue 48 h after the final OVA challenge. Saline/Saline: normal control mice treated with PBS only; OVA/Saline: OVA-sensitized/challenged mice; OVA/Mon: montelukast (30 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-1: PTE (23.3 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-2: PTE (46.6 mg/kg) + OVA-sensitized/challenged mice.

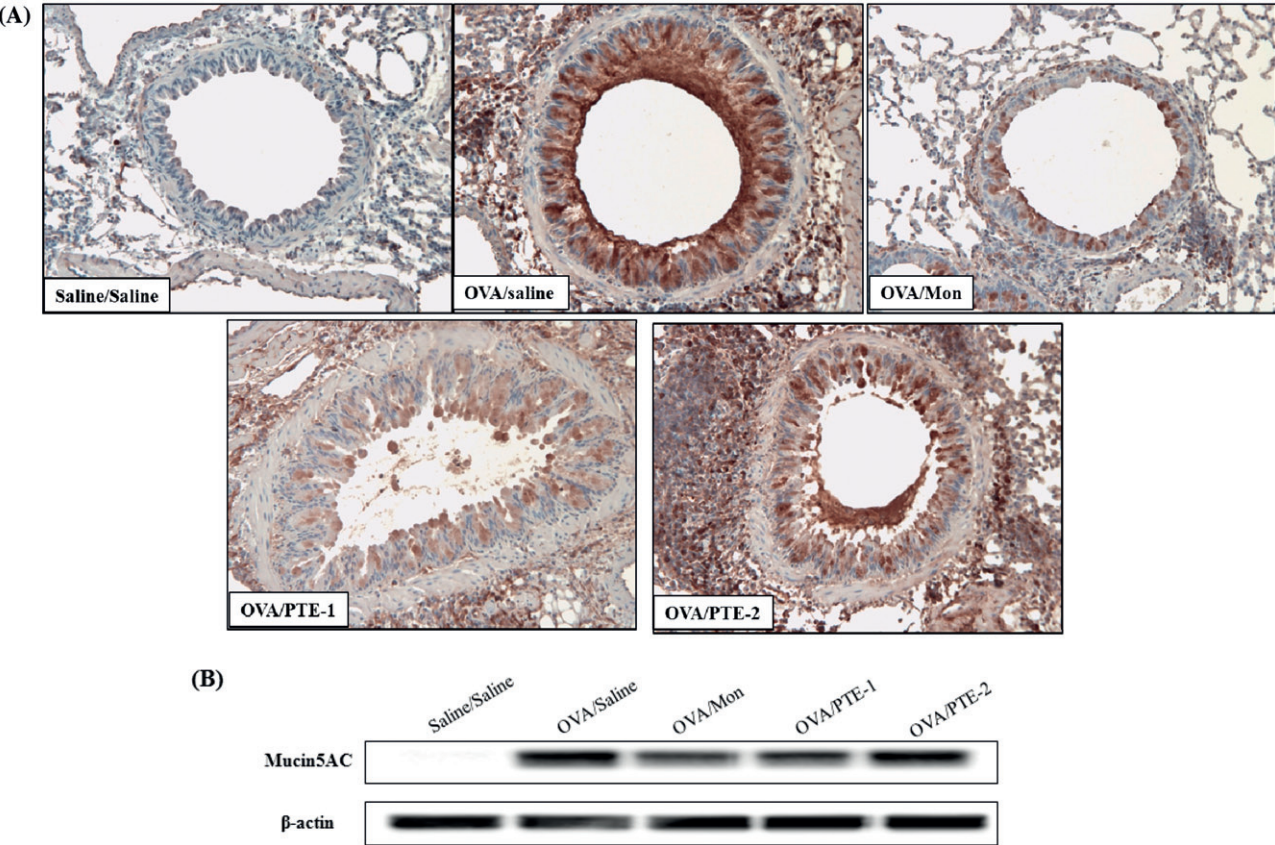


Figure 6. Effects of PTE on the mucin 5AC expression in the lung tissue of mice 48 h after the final OVA challenge. (A) Immunohistochemical analysis. (B) Mucin 5AC immunoblotting. (C) Relative units of mucin 5AC protein expression. Relative units are expressed as relative ratios of mucin 5AC to β -actin. Saline/Saline: normal control mice treated with PBS only; OVA/Saline: OVA-sensitized/challenged mice; OVA/Mon: montelukast (30 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-1: PTE (23.3 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-2: PTE (46.6 mg/kg) + OVA-sensitized/challenged mice. Values are expressed as mean $\pm$ SEM. **Significantly different from Saline/Saline, $p < 0.01$; #Significantly different from OVA/Saline, $p < 0.05$.

Figure 6. Continued.

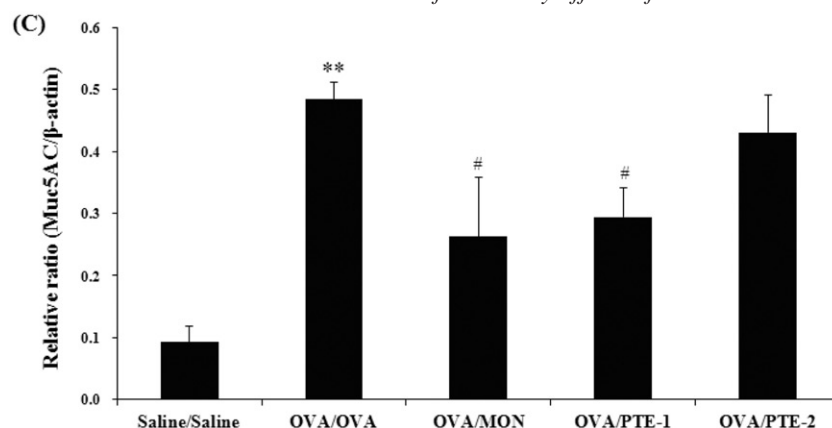
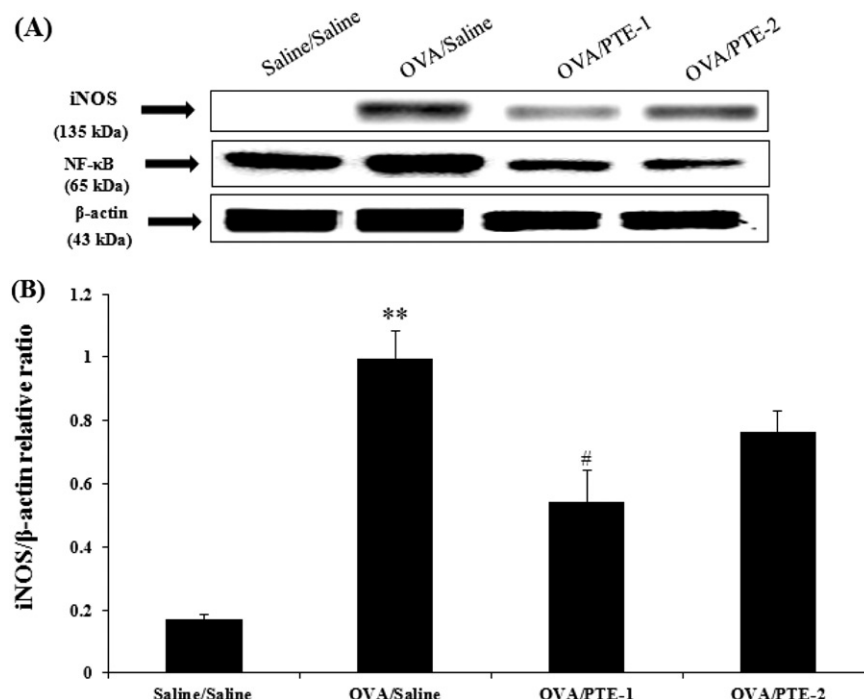


Figure 7. Effects of PTE on iNOS and NF-κB expression in the lung tissue of mice 48 h after the final OVA challenge. iNOS and NF-κB (A) immunoblotting. (B) Relative units of protein expression. Relative units are expressed as relative ratios of iNOS or NF-κB to β-actin. Saline/Saline: normal control mice treated with PBS only; OVA/Saline: OVA-sensitized/challenged mice; OVA/PTE-1: PTE (23.3 mg/kg) + OVA-sensitized/challenged mice; OVA/PTE-2: PTE (46.6 mg/kg) + OVA-sensitized/challenged mice. Values are expressed as mean ± SEM. **Significantly different from Saline/Saline, $p < 0.01$; #Significantly different from OVA/Saline, $p < 0.05$.



eosinophils. Our present findings show that PTE attenuated OVA-induced inflammatory cell infiltration into the airways as shown by significant drops in total cell count, eosinophils, macrophages and lymphocytes in BALF, as well as in tissue eosinophilia in lung sections. Our results show that PTE significantly reduced the levels of IL-4 and IL-13 in BALF and IgE in plasma from mice with the OVA-induced model of asthma. Therefore, the observed reductions in IL-4 and IL-13 in BALF and IgE in plasma from PTE-treated mice may be the result of inhibition of airway-resident inflammatory cells. These data show that the anti-inflammatory effect of PTE is, at least in part, mediated through a direct suppressive action on T lymphocytes. TNF-α is also an important chemoattractant for the recruitment of eosinophils into the lungs^{22,23}. Taken together, these findings suggest that the observed reduction in airway eosinophilia by PTE may be a result of combined inhibitory effects on the production of Th2-type cytokines, such as IL-4 and IL-13; and Th1-type cytokines, such as TNF-α. Endogenous NO is considered to be a key

marker of physiological deterioration of airway functions and is closely implicated in airway diseases, such as asthma, adult respiratory distress syndrome and lung fibrosis^{24,25}. Increased exhaled NO is associated with increased iNOS expression in the lung epithelium of asthma patients²⁶. Excessive NO may recruit eosinophils into the airway and shift the balance toward Th2 cells, thus exacerbating inflammation of the airway. Therefore, potent iNOS inhibitors have long been considered to be an effective treatment of airway inflammation.

We also demonstrated a dramatic reduction in airway mucus production in PTE-treated mice compared with untreated mice in our OVA-induced model. Cumulative data indicate that PTE treatment inhibited IL-4 and IL-13 production and goblet cell hyperplasia and mucin production in the mouse model.

Mucin 5AC gene expression is also dependent on the transcriptional activity of NF-κB^{27,28}. We also observed a substantial reduction in mucin 5AC immunoreactivity in

histology and immunoblotting of lungs from mice with the OVA-induced model of asthma. As such, the marked decrease in mucus production in the lungs of PTE-treated mice may be attributable to a significant reduction in Th2 cytokine levels, together with an inhibition of NF- κ B activity. Mucin 5AC gene expression is dependent on the transcriptional activity of NF- κ B^{29,30}. In addition, selective ablation of NF- κ B function in the airway epithelium has been shown to reduce OVA-induced mucus production in mice³¹. In this study, we showed that PTE markedly suppressed the OVA-induced iNOS expression in the lungs, which may be the result of inhibition of downstream NF- κ B activity and the reduced level of IL-13 in the allergic airways.

IL-4 and IL-13 play critical roles in goblet cell hyperplasia and mucin 5AC expression in mice³⁰. The increased secretion of mucus and the hyperplasia of goblet cells in lung tissue were significantly decreased by PTE. Moreover, the IgE level in blood was markedly inhibited. These results indicate that the suppressive effect of PTE on leukocytosis and mucus hypersecretion into the airway may be associated with the inhibitory effect of PTE on the secretion of cytokines and IgE levels. Taken together, these findings suggest that the marked decrease in mucus production in the lungs of PTE-treated mice may be attributable to significant reductions in IL-4 and IL-13 levels and a direct inhibitory action on NF- κ B in the airway epithelium. Anti-inflammatory effect of PTE was considered to be related with its active components. Previous study reported that nine compounds were isolated in PTE. Of nine components, (E)-p-coumaryl alcohol, 3,4-dihydroxycinnamyl alcohol, ferulic acid, sachalide and coniferin were effectively inhibited TNF- α production induced by lipopolysaccharide. Therefore, anti-inflammatory effect of PTE in this study was considered to be closely associated with these components.

In conclusion, this study provides a scientific basis for the traditional use of PTE for the treatment of various inflammatory diseases including allergic asthma. Further work to isolate the active constituents of PTE that contribute to its potential anti-inflammatory action is therefore justified.

Declaration of interest

No competing financial interests exist. This research was part of a project (Evaluation of Herb–Herb Interaction by Array Methods) funded by the Korea Institute of Oriental Medicine.

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