



Protocatechuic acid suppresses ovalbumin-induced airway inflammation in a mouse allergic asthma model

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ABSTRACT

Protocatechuic acid (PCA) has been isolated from the leaves of *Ilex chinenses* and has numerous pharmacologic effects, including anti-inflammatory and antitumoral activities. This study aims to evaluate the antiasthma activity of PCA and investigate its possible molecular mechanisms. BALB/c mice were sensitized and challenged to ovalbumin (OVA). Then mice were intraperitoneally (i.p.) injected with PCA 1 h before OVA challenge. We found that PCA treatment at 15 or 30 mg/kg significantly decreased OVA-induced airway hyper-responsiveness (AHR) to inhaled methacholine. Type 2 helper T cell (Th2) cytokines in bronchoalveolar lavage (BAL) fluid, such as interleukin-4 (IL-4), interleukin 5 (IL-5) and interleukin-13 (IL-13), and serum OVA-specific immunoglobulin E (IgE) levels, were also reduced by PCA. Moreover treatment with PCA markedly decreased the number of inflammatory cells in BALF and attenuated OVA-induced mRNA expression of CCL11, CCR3, Muc5ac, acidic mammalian chitinase (AMCase), chitinase 3-like protein 4 (Ym2) and E-selectin in lung tissues. Lung histopathological studies showed that PCA inhibited inflammatory cell infiltration and mucus hypersecretion compared with the OVA-induced mice group. We then investigated the possible molecular mechanisms which might be implicated in PCA activity. Our results suggested that the protective effect of PCA might be mediated by the inhibition of the extracellular signal-regulated protein kinase (ERK), p38 Mitogen-activated protein kinase (MAPK) phosphorylation and the nuclear factor-κB (NF-κB) activation.

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1. Introduction

Allergic asthma is a chronic airway disorder characterized by airway inflammation, mucus hypersecretion, and AHR [1]. The incidence, morbidity, and mortality of the disease have been increasing at an alarming rate in industrialized countries around the world over the last few decades. Cumulative evidence showed that these inflammatory responses are contributed by Th2 cells, together with other inflammatory cells such as eosinophils, macrophages and neutrophils, as well as inflammatory cytokines and chemokines [1,2]. IL-4, IL-5 and IL-13, three cytokines secreted from Th2 cells, have been shown to play a pivotal role in allergic airway inflammation [3]. Airway eosinophilia, together with Th2 cytokines IL-4, IL-5 and IL-13, may ultimately contribute to AHR in asthma [4]. In addition, CCL11 is chemokine which binds to a specific receptor CCR3 that participates in eosinophil, neutrophil, macrophage recruitment and Th2 cell infiltration [5].

NF-κB plays a pivotal role in the production of Th2 cytokines and recruitment of inflammatory cells in the airways of murine asthma models [6,7]. Many studies have suggested that NF-κB activity may be involved in the pathogenesis of asthma, given that its increased activation has been demonstrated in the lung tissue following allergen challenge [8]. Therefore, NF-κB has emerged as a promising molecular target for the treatment of asthma. In addition, MAPK signaling cascade has been shown to be important in the activation of various immune cells [9]. P38, ERK has been implicated in the regulation of IL-5 and other cytokines [10]. At the time, JNK have been shown to be associated with IgE switching [11,12]. Thus, the inhibitors of these inflammatory mediators have been considered as anti-inflammatory drug candidate [13].

Protocatechuic acid (PCA) is a major benzoic acid derivative found in vegetables, nuts, brown rice, fruits, and herbal medicines. Numerous pharmacologic effects, such as antioxidative, antibacterial, and antitumor promotion activities, have been attributed to PCA [14–17]. Sung-Won Min et al. have reported that PCA could inhibit LPS-stimulated TNF-α and IL-1β secretion in RAW264.7 cells via NF-κB and MAPK pathways activation [18]. In our previous study, we have demonstrated that PCA has anti-inflammatory effects in vivo in a mouse model of acute lung injury [19]. Thus far, the effects of PCA treatment on a non-infectious mouse model of airway

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inflammation have not been evaluated. Therefore, we examined the effectiveness of PCA for reducing airway inflammatory reactions and improving asthma symptoms in an OVA-induced airway inflammation model.

2. Materials and methods

2.1. Reagent

Protocatechuic acid and dexamethasone (DEX) were purchased from the National Institutes for Food and Drug Control (Beijing, China). The IL-4, IL-5 and IL-13 ELISA kits were purchased from Biolegend (California, USA). Rabbit mAb, p-NF- κ B p65, SAPK/JNK, P-p38 MAPK, P-ERK, ERK, rabbit Ab P-SAPK/JNK, p38 MAPK and mouse mAb p-I κ B α were purchased from Cell Signaling Technology Inc (Beverly, MA, USA). OVA (Grade δ) were purchased from Sigma Chemical Co. Peroxidase-conjugated Affinipure Goat Anti-Mouse IgG (H + L) and Peroxidase-conjugated Affinipure Goat Anti-Rabbit IgG (H + L) were purchased from PTG (Chicago, IL, USA). PCA was resolved in saline. All other chemicals were of reagent grade.

2.2. Animals

BALB/c mice (female, 18–20 g each) were purchased from the Center of Experimental Animals of Baiqien Medical College of Jilin University (Jilin, China). All animals were housed in a controlled environment (24 ± 1 °C, 40–80% humidity) under specific pathogen-free conditions with water and food provided ad libitum. Before experimentation, mice were housed for a minimum of one week to adapt them to the environment. All animal experiments were performed in accordance with the guide for the Care and Use of Laboratory Animals published by the US National Institute of Health.

2.3. OVA-induced mouse asthma model and PCA treatment protocol

Mice were sensitized with OVA adsorbed in 100 μ g/ml of Imject Alum by i.p. injection on day 0 and day 14. On days 25–27, the mice were anesthetized again and instilled intranasally (i.n.) with OVA (100 μ g) in PBS (50 μ l). PCA (5, 15 and 30 mg/kg) and DEX (2 mg/kg) were given an i.p. injection on days 25–27, 1 h prior to OVA administration. DEX (2 mg/kg) was injected as a positive control. Control mice received the same immunization, but were exposed to phosphate-buffered saline (PBS) instead of OVA during airway challenge.

2.4. Collection of blood and bronchoalveolar lavage fluid (BALF)

Mice were anesthetized 24 h after the last PCA challenge. Blood was collected and centrifuged at 4 °C (3000 rpm) for 10 min, and the serum was stored at -80 °C for measurement of IgE. The BALF was collected by cannulating the upper part of the trachea, and then PBS was infused in three aliquots (0.5, 0.5, 0.5 ml) in a total volume of 1.3 ml. The fluid recovery rate was $87 \pm 2\%$. The sample was centrifuged, and the supernatant was stored at -80 °C until cytokine assays. Remaining cell pellets were resuspended in PBS for total cell, eosinophil, and neutrophil counts using hemacytometer and cytopspins by staining with the Wright-Giemsa staining method. A minimum of 200 cells were counted per slide.

2.5. Measurements of AHR

AHR was assessed as a change in airway function after challenge with aerosolized methacholine via the airway. Mice were anesthetized 24 h after the last OVA challenge, and a tracheotomy was performed as described [20]. The internal jugular vein was cannulated and connected to a microsyringe for intravenous administration of methacholine. Lung compliance (C_{dyn}) and airway resistance (RI)

in response to increasing concentrations of methacholine were recorded using a whole-body plethysmograph chamber (Buxco, Sharon, CT, USA) as described [21]. The results are expressed as a percentage of the respective basal values in response to phosphate-buffered saline (PBS).

2.6. OVA-specific IgE and cytokines assay

OVA-specific serum IgE levels were determined by Enzyme Linked Immunosorbent Absorbent Assay (ELISA) using samples collected 24 h after the last OVA challenge. In brief, microplate wells were coated with 1% OVA in coating buffer (0.05 M sodium carbonate–bicarbonate, pH 9.6) overnight at 4 °C. The wells were then incubated with blocking buffer (1% BSA in PBS, pH 7.2) at room temperature for 1 h and then washed. Diluted serum samples were then added to the microplate, incubated at room temperature for 2 h, washed and incubated with biotin anti-mouse IgE, followed by extravidin-peroxidase at room temperature for 30 min and with 3, 3', 5, 5'-tetramethylbenzidine substrate for 30 min. The enzymatic reaction was stopped with 2 M H₂SO₄, and the absorbance was read at 450 nm. Concentrations were determined in duplicates for each sample. Cytokine assays were performed according to the manufacturer's instructions. IL-4, IL-5 and IL-13 levels in BALF were measured using specific mouse IL-4, IL-5 and IL-13 ELISA kits (BioLegend, Inc. Camino Santa Fe, Suite E San Diego, CA, USA).

2.7. Histologic analysis

Twenty-four hours after the last inhalation exposure, lungs were fixed in 10% neutral formalin, paraffinized, cut into 3 μ m sections, and stained with hematoxylin and eosin (H&E), alcian blue-periodic acid-Schiff staining (AB-PAS) for general morphology and the identification of goblet cells in the epithelium [22,23].

2.8. RNA preparation and quantitative RT-PCR

Lung tissues were harvested at 24 h after the last OVA challenge and total RNA from lung tissues was isolated with the use of TRIzol (SIGMA-Aldrich, St. Louis, USA). Reverse transcription was carried out using the first-strand cDNA synthesis kit (Bioer, Hangzhou, China). Real-time PCR was performed on cDNA samples using the SYBY Green system (Bio-Rad; Richmond, CA, USA). The following primers were used: Muc5ac, sense 5'GTGTCGGCCGAGAAAGTTGGT3' and antisense 5'GTCCTGTTGAGCCTGGCTGTG3'; Ym2, sense 5'TCCACTTTGAACCA CATCCAAGGC3' and antisense 5'CGAGAGACTGAGACAGTTCAGGGA3; AMCase, sense 5'TGGACACACCTTCATCCTGA3' and antisense 5'CCTC AGTGGCTCCACTTCTC3'; E-selectin, sense 5'CCCTCCACAGAACCTACCA3' and antisense 5'TCAGCAGACATTGCTTCACC3'; CCL11, sense 5'AAACCAT AAACAACCTCCTC3' and antisense 5'CAATAATCCACATCTCCTT3'; CCR3, sense 5'TCTGCTGAGATGTCCCAATA3' and antisense 5'TCACCAACAAAG GCCTAG3'. The conditions were as follows: initial denaturation at 94 °C for 3 min; followed by 30 cycles of denaturation at 94 °C for 1 min; annealing at 60 °C (Muc5ac, Ym2, AMCase, E-selectin), 55 °C (CCL11, CCR3) for 30 s; extension at 72 °C for 1 min. Analysis was performed using the sequence detection software supplied with the instrument. A melting curve analysis was performed to control for the specificity of the amplification products. The gene-specific threshold cycles (C_t) of respective samples were normalized internally using the average C_t value of β -actin. The Δ C_t for all experimental samples were subtracted by the Δ C_t for the control samples ($\Delta\Delta$ C_t). The magnitude change of test gene mRNA was expressed as $2^{-\Delta\Delta C_t}$ [24].

2.9. Western blot analysis of MAPK and NF- κ B

Mouse lungs were excised at 24 h after the last OVA challenge. The lung tissues were homogenized in liquid nitrogen and incubated in

lysis buffer in addition to protease and phosphatase inhibitors (Roche, Basel, Switzerland) to obtain extracts of lung protein. The samples were loaded to 10% SDS-PAGE gels and were transferred to a polyvinylidene difluoride (PVDF) membrane. The blots were incubated with specific antibodies against ERK, JNK and p38 antibodies, phospho-specific antibodies to p-ERK, p-JNK, p-p38, p-NF- κ B p65 and p-I κ B (Beverly, MA, USA) in 5% bovine serum albumin (BSA). Membrane was then washed three times for 5 min each with TTBS. The primary antibody was probed with HRP-conjugated goat anti-rabbit (1:7000) and HRP-conjugated goat anti-mouse (1:7000) for 1 h, washed three times in TTBS and processed with supersignal

west pico chemiluminescent substrate. β -actin western blot was performed as an internal control of protein loading.

2.10. Statistical analysis

All values were expressed as the mean $\pm$ standard error of the mean (SEM). Differences between mean values of normally distributed data were assessed by the one-way analysis of variance (ANOVA; Dunnett's t-test) and the Student's t-test. Statistical differences were considered significant if $p < 0.05$.

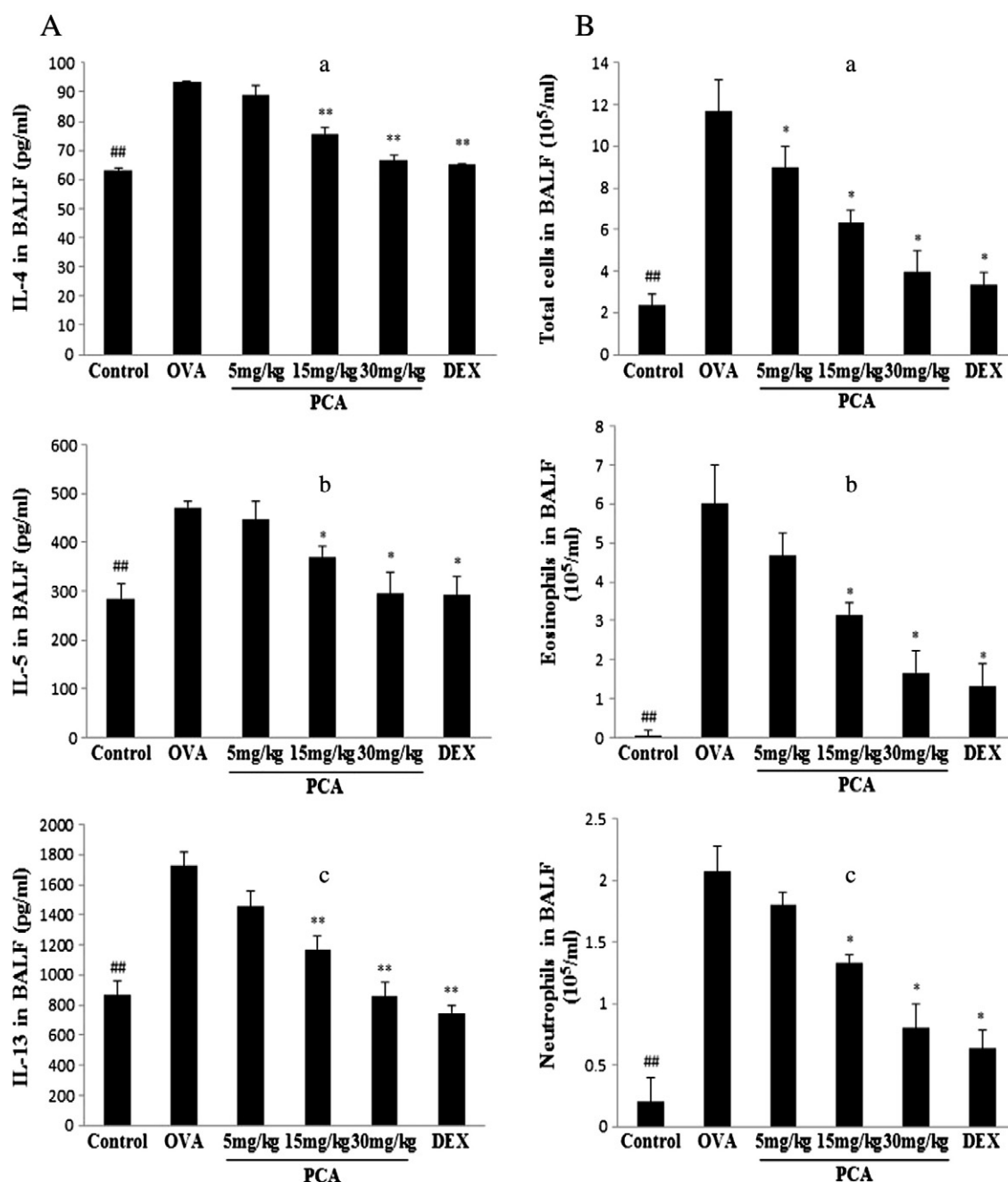


Fig. 1. Effects of protocatechuic acid (PCA) on OVA-challenged mice. Bronchoalveolar lavage fluid (BALF) was collected 24 h after the last challenge with OVA. (A) Effects of PCA on secretion of cytokines in the BALF of OVA-challenged mice: (a) the secretion of IL-4 in BALF; (b): the secretion of IL-5 in BALF; (c) the secretion of IL-13 in BALF. (B) Effects of PCA on cell accumulation in BALF of OVA-challenged mice: (a) total cell counts; (b) numbers of eosinophils; (c) numbers of neutrophils. The cytokines and cell counts in BALF from control, OVA, dexamethasone (DEX) and PCA group was evaluated. Control, normal control; OVA, OVA-induced mice; PCA (5, 15 and 30 mg/kg), PCA (5, 15 and 30 mg/kg) + OVA-induced mice; DEX (2 mg/kg), DEX (2 mg/kg) + OVA-induced mice. The values represent the mean $\pm$ SEM of three independent experiments ($n = 6$ in each group). $p^{\#} < 0.05$, $p^{\#\#} < 0.01$ vs. control group, $p^* < 0.05$, $p^{**} < 0.01$ vs. OVA.

3. Results

3.1. PCA reduced OVA-induced BAL fluid Th2 cytokine levels

For measurement of Th2 cytokines, we used ELISA to determine if PCA (5, 15 or 30 mg/kg) and DEX (2 mg/kg) treatment could affect cytokine secretion in the BALF. OVA challenge led to significant increases in the levels of IL-4, IL-5 and IL-13 in BALF, compared with control. In the PCA-treatment (15 or 30 mg/kg) and DEX-treatment groups, cytokine elevation was significantly suppressed in relation to the OVA-challenged group (Fig. 1A).

3.2. PCA reduces inflammatory cells in BAL fluids

BALF was collected 24 h after the last OVA challenge, and total cell, neutrophil and eosinophil counts were performed. Inflammatory cell levels in BALF were significantly elevated in OVA-challenged mice vs. PBS control mice. PCA (15 or 30 mg/kg) and DEX (2 mg/kg) treatment reduced the number of total cells, neutrophils and eosinophils. PCA treatment at 5 mg/kg reduced the number of total cells (Fig. 1B).

3.3. PCA decreases the level of IgE in serum

Because Th2 cytokines promote airway inflammation in asthma through increased IgE levels, we investigated the expression of IgE in serum. As shown in Fig. 2, the levels of serum IgE were found to be significantly increased in OVA mice compared with those of control mice. However, administration of PCA (15 or 30 mg/kg) and DEX (2 mg/kg) significantly decreased the serum IgE levels.

3.4. PCA reduced AHR in OVA-induced mouse asthma

Asthma is characterized by a non-specific AHR and is defined as excessive narrowing of airways in response to a variety of contractile agonists including OVA. Airway hyperresponsiveness was assessed by measuring Cdyn and RI in response to increasing doses of methacholine administration in mechanically ventilated mice. Cdyn refers to the distensibility of the lung and is defined as the change in volume of the lung produced by a change in pressure across the lung. RI is defined as the pressure driving respiration divided by flow. OVA-challenged mice developed AHR, typically reflected by high RI and low Cdyn. Treatment with PCA (15 or 30 mg/kg) and DEX (2 mg/kg) reduced RL (* p <0.05, ** p <0.01) and stored Cdyn (* p <0.05, ** p <0.01) in OVA-challenged mice in response to

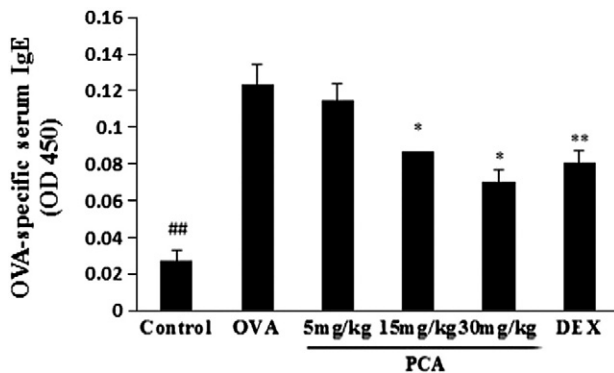


Fig. 2. Effects of PCA on OVA-specific IgE in serum of OVA-challenged mice. Serum was collected 24 h after the final OVA challenge in mice. Each sample was analyzed using ELISA. Treatment with PCA (15 or 30 mg/kg) and DEX (2 mg/kg) reduced IgE in OVA-induced mice. Control, normal control; OVA, OVA-induced mice; PCA (5, 15 and 30 mg/kg), PCA (5, 15 and 30 mg/kg) + OVA-induced mice; DEX (2 mg/kg), DEX (2 mg/kg) + OVA-induced mice. The values represent the mean $\pm$ SEM of three independent experiments ($n=6$ in each group) $p^{\#}<0.05$ vs. control group, $p^{*}<0.05$ vs. OVA.

methacholine. PCA and DEX treatment significantly reduced AHR compared with that in the OVA group (Fig. 3).

3.5. PCA ameliorates pathological changes of OVA-induced asthma

H&E and AB-PAS staining were performed on the lung tissues to analyze the effects of PCA on histological feature of asthma. The lung tissue obtained from OVA-induced asthmatic mice was characterized by peribronchial inflammation due to leukocyte infiltration, mucus overproduction and goblet cell hyperplasia compared with the normal tissue. PCA (15 or 30 mg/kg) and DEX (2 mg/kg) treatment significantly inhibited the OVA-induced inflammatory infiltration, goblet cell hyperplasia and mucus overproduction in a mice model of asthma (Fig. 4).

3.6. PCA inhibits the expression of OVA-induced inflammatory gene, chemokines and chemokines receptor expression

As CCL11, CCR3, Muc5ac, AMCase, Ym2 and E-selectin are closely associated with the pathogenesis of asthma, we examined them in the lungs. Twenty four hours after the last challenge with OVA, lungs were harvested for Real time PCR analysis. OVA challenge led to significant increases in the levels of CCL11, CCR3, Muc5ac, AMCase, Ym2 and E-selectin compared with control. Treatment with PCA (15 or 30 mg/kg) and DEX (2 mg/kg) demonstrated the suppression of CCL11, CCR3, Muc5ac, AMCase, Ym2 and E-selectin (Fig. 5).

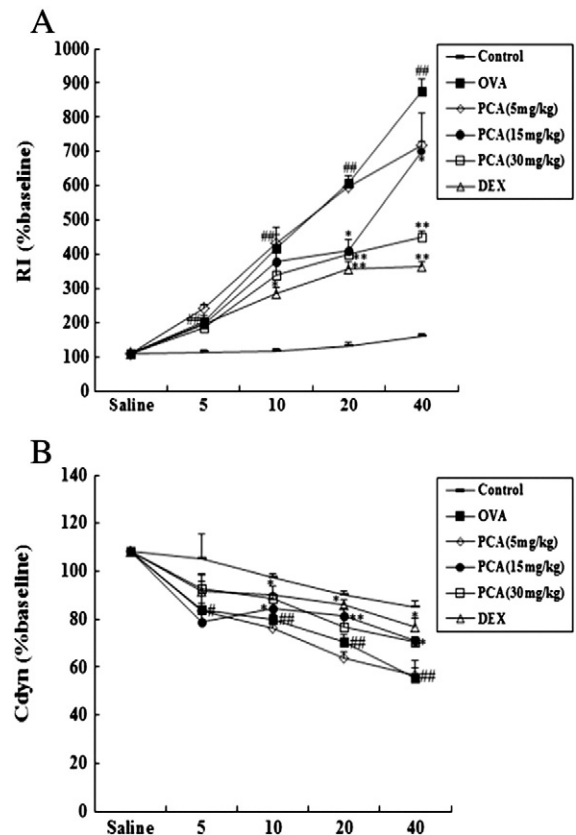


Fig. 3. Effects of PCA on airway hyper-responsiveness in mice. Airway hyperresponsiveness was assessed by percentage change from the baseline level of (A) lung resistance (RI, $n=6$ mice per treatment group) and (B) dynamic compliance (Cdyn, $n=6$ mice per treatment group). Control, normal control; OVA, OVA-induced mice; PCA (5, 15 and 30 mg/kg), PCA (5, 15 and 30 mg/kg) + OVA-induced mice; DEX (2 mg/kg), DEX (2 mg/kg) + OVA-induced mice. Mch = methacholine. $p^{\#}<0.05$, $p^{*}<0.01$ vs. control group, $p^{*}<0.05$, $p^{**}<0.01$ vs. OVA.

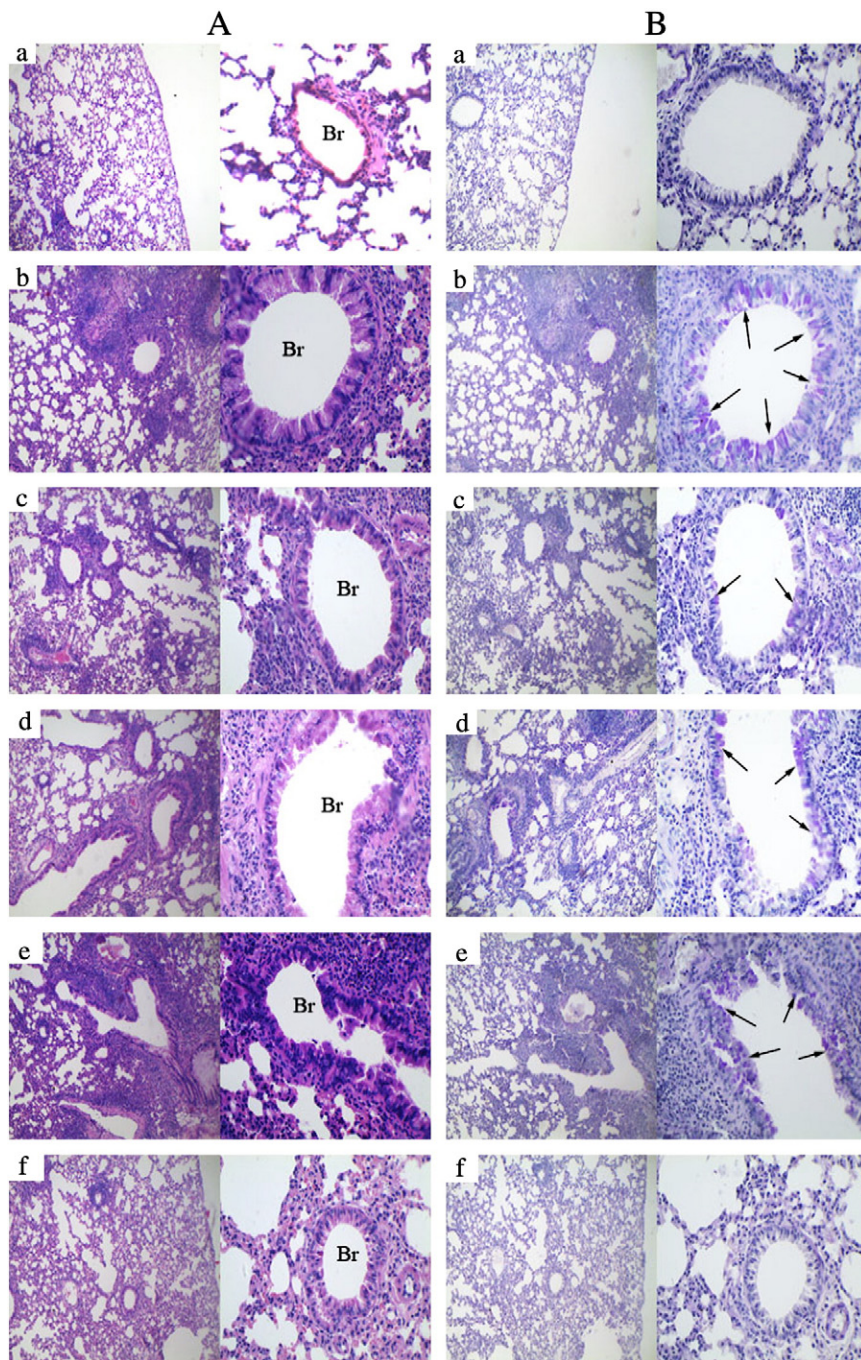


Fig. 4. Effects of PCA on airway inflammation and airway goblet cell hyperplasia. Lungs ($n=3$) from each experimental group were processed for histological evaluation at 24 h after the last challenge with OVA. Representative H&E (A) and AB-PAS (B) stained sections of lung from control mice (a); and OVA-challenged mice (b); OVA-challenged mice treated with PCA (30 mg/kg) (c); OVA-challenged mice treated with PCA (15 mg/kg) (d); OVA-challenged mice treated with PCA (5 mg/kg) (e); OVA-challenged mice treated with DEX (2 mg/kg) (f). The left panel is magnified 100 $\times$; the right panel is magnified 400 $\times$. Br, bronchi. Arrows indicate areas of alcian blue + cells.

3.7. PCA inhibits the MAPK and NF- κ B activation in a mouse asthma model

Many studies have demonstrated that NF- κ B activation is involved in the chronic feature of airway inflammation in asthma. And the MAPK pathway has been reported to play a major role in biological responses, including cytokine expression. Thus we investigated the effect of PCA on MAPK and NF- κ B activation in a mouse model of asthma. The lung tissues were collected 24 h after the final airway OVA challenge, and total and nuclear proteins were extracted. We examined the levels of phosphorylated ERK, JNK, p38, I κ B- α and nuclear p-NF- κ B p65; and the levels of ERK, JNK, p38 in mouse lung tissues.

As shown in Fig. 6, PCA (15 or 30 mg/kg) and DEX (2 mg/kg) suppressed phosphorylation of I κ B- α in lung parenchyma, and attenuated nuclear localization of p-NF- κ B p65. Furthermore, treatment with PCA (15 or 30 mg/kg) inhibited the phosphorylation level of ERK and p38 signal pathways, but not JNK. We found that PCA (5 mg/kg) blocked ERK phosphorylation and DEX (2 mg/kg) had significant effect on ERK, p38 and JNK activities.

4. Discussion

Asthma is one of the most common chronic disorders of the airways that affect adults and children of all ages [25]. Although the

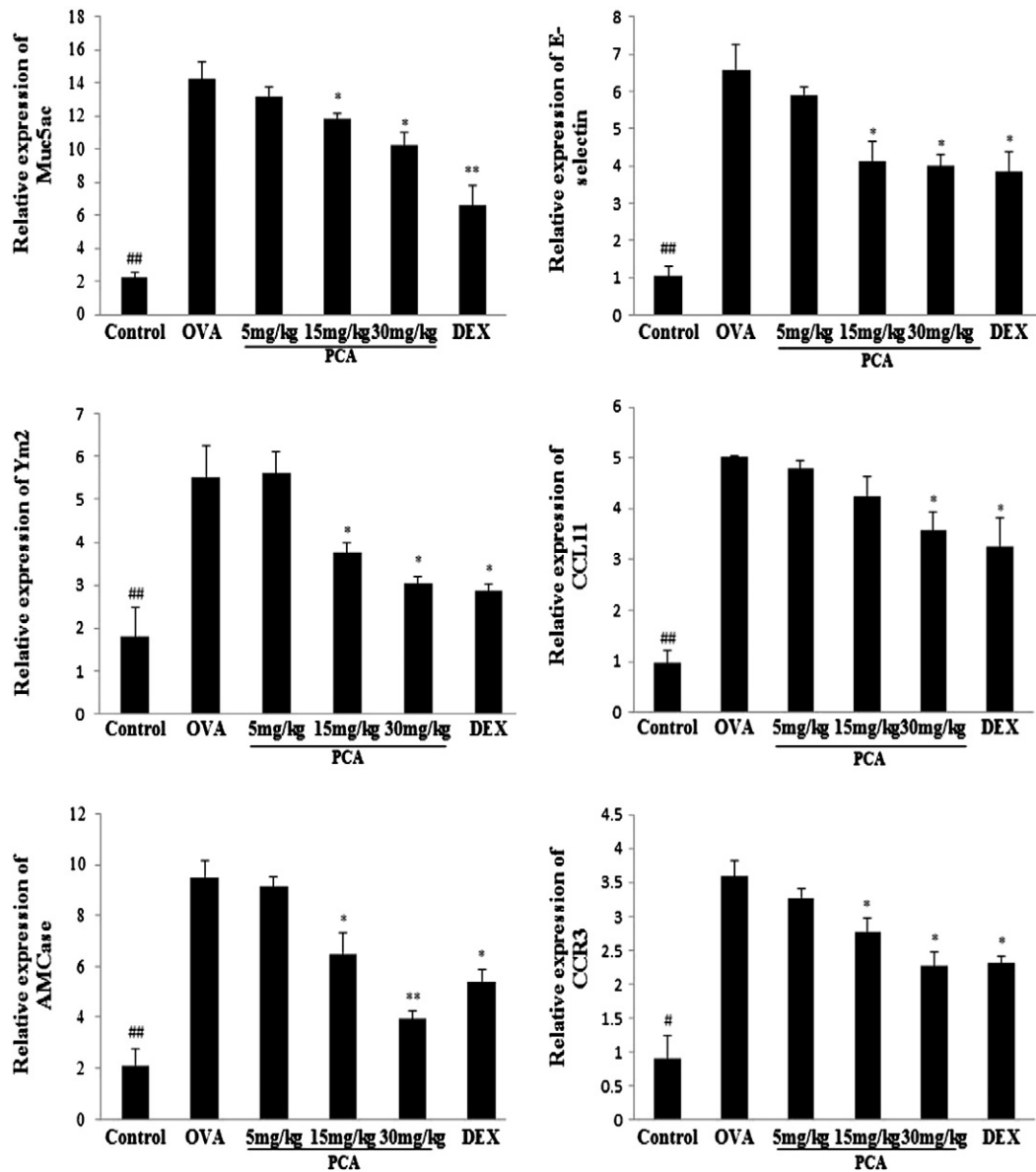


Fig. 5. Effects of PCA on OVA-induced inflammatory gene expression of OVA-challenged mice. Lung tissues were collected 24 h after the last OVA challenge. The mRNA in whole lung extracts was measured by real-time PCR. Treatment with PCA (15 or 30 mg/kg) and DEX (2 mg/kg) reduced mRNA expression in the lung of OVA-induced mice. Control, normal control; OVA, OVA-induced mice; PCA (5, 15 and 30 mg/kg), PCA (5, 15 and 30 mg/kg) + OVA-induced mice; DEX (2 mg/kg), DEX (2 mg/kg) + OVA-induced mice. The experiments were repeated for three times with similar pattern of results. The values represent the mean $\pm$ SEM of 6 animals in each group. $p^* < 0.05$, $p^{**} < 0.01$ vs. control group, $p^* < 0.05$, $p^{**} < 0.01$ vs. OVA.

pathophysiology of asthma has been extensively investigated, there is still no cure for this disease. This study is the first to provide experimental evidence demonstrating that PCA inhibits OVA-induced airway inflammation in a mouse model of asthma.

Th2 lymphocytes play an important role in the initiation and progression of allergic diseases, including asthma, by releasing IL-4, IL-5 and IL-13 [26]. These cytokines may affect the expression of inflammatory molecules both on the endothelial cells of the bronchial circulation and the airway epithelial cells, and may also promote eosinophil and lymphocyte recruitment [27]. Specifically, IL-4 and IL-13 play critical roles in eosinophil infiltration into lung tissue and stimulate mucus hypersecretion and AHR [28,29]. IL-4 especially induces isotype switching in B cells, from IgG to IgE production [30]. IL-5 is essential for eosinophils activation and survival, and in development of AHR [31]. Because IgE levels are dependent on IL-4 and IL-5 and is considered as an additional index of Th2 cytokine secretion in the pathogenesis of asthma, we measured the IgE level.

IgE has been a key target in developing antiasthma strategies and clinical trials involving the neutralization of IgE have generated satisfactory outcomes in treating patients with asthma [1,32]. In our study, the administration of PCA reduced the expression of Th2 cytokines and IgE. Therefore, the reduction of IgE and Th2 cytokines may account for the improvement in OVA-induced asthma.

Treatment with PCA abrogated OVA-induced inflammatory parameters, such as marked infiltration of inflammatory cells into the lung tissues, mucin hypersecretion and goblet cell hyperplasia. We argue that the suppression of NF- κ B and MAPK activation may be principal therapeutic mechanism of PCA. Several lines of evidence suggest a central role of NF- κ B in the pathogenesis of asthma. NF- κ B activation within airway epithelium is necessary to fully induce the recruitment of inflammatory cells [33]. NF- κ B induces multiple pro-inflammatory genes that contribute to allergic asthma [6]. It has been reported that the bronchial epithelium displayed robust and rapid NF- κ B p65 nuclear translocation compared with the controls

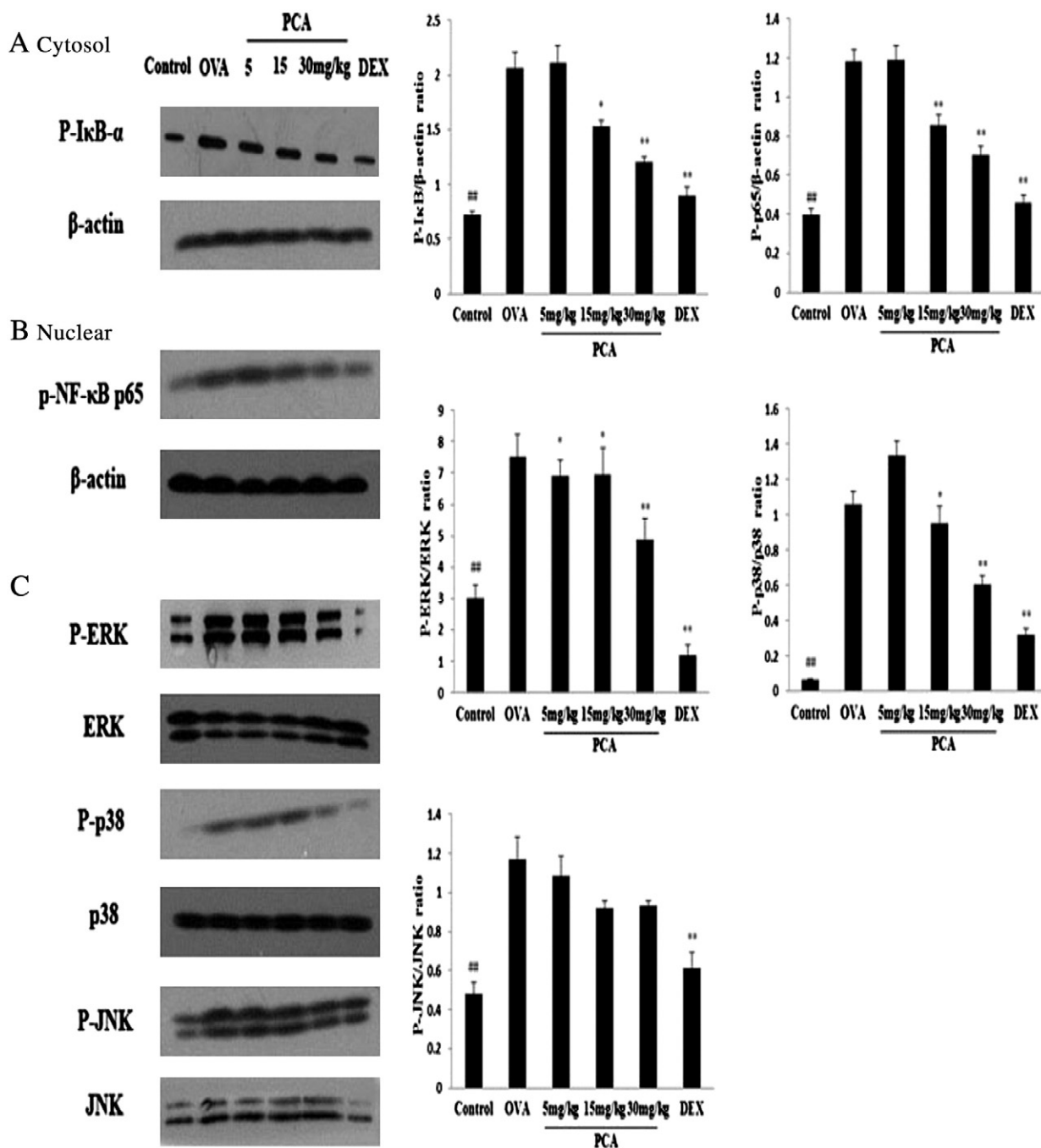


Fig. 6. Effects of PCA on NF-κB and MAPK pathway activation. Lung tissues were collected 24 h after the last OVA challenge. (A) Detection of phosphorylated IκB; (B) detection of the p-NF-κB p65 subunit; (C) detection of phosphorylated or total forms of the three MAPK molecules (ERK, p38 and JNK) by Western blot. β-actin was used as an internal control. Control, normal control; OVA, OVA-induced mice; PCA (5, 15 and 30 mg/kg), PCA (5, 15 and 30 mg/kg) + OVA-induced mice; DEX (2 mg/kg), DEX (2 mg/kg) + OVA-induced mice. Experiments were repeated three times and results were obtained. The values represent the mean ± SEM of three independent experiments (n = 6 in each group) p# < 0.05, p## < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. OVA.

in a mouse model [34]. In our study, we demonstrated that OVA-induced p-NF-κB p65 translocation from the cytoplasm to the nucleus was strongly inhibited by PCA and the phosphorylation and degradation of IκBα, required for p65 activation, were regulated in PCA-treated mice. Muc5ac gene expression is dependent on the transcriptional activity of NF-κB [35]. We observed a substantial drop in Muc5ac mRNA expression by PCA in OVA-challenged lungs. Collectively, we suggest that observed decrease in NF-κB activity may explain the therapeutic effect of PCA. In addition, MAPKs play critical roles in the regulation of cell growth and differentiation and in the control of cellular responses to cytokines and stress [36]. A recent study showed that ERK activity in the lungs of asthmatic mice was

significantly higher than normal mice [37]. In OVA-sensitized and exposed mice and guinea pigs, a p38 MAPK inhibitor effectively suppressed eosinophilic inflammation in the lungs [38]. Moreover, JNK has been shown to be associated with IgE switching [12]. Therefore, MAPK is a key mediator of allergic diseases. Our results showed that PCA can inhibit the activation of p38 and ERK, but not JNK. This suggests that the inhibition of p38 and ERK may have potential benefit in the therapy of asthma.

Eosinophils are the preliminary cells recruited to the site of allergic reaction. The cells contain large amounts of granules that induce severe airway inflammation [39]. Our findings showed that PCA prevented OVA-induced inflammatory cell infiltrating into the

airways as shown by a significant drop in total cell, eosinophil and neutrophil counts in BALF. Migration of inflammatory cells, specifically eosinophils, into the lung is a major contributor to the development of allergic airway inflammation. This observed effect of PCA treatment on eosinophils may be due to decrease in the Th2 cytokines and chemokines, such as IL-4, IL-5, IL-13 and CCL11/eotaxin and its receptor CCR3 that are responsible for the recruitment of eosinophils into the lung during asthma. In addition, eosinophils also play an important role in the development of AHR. Because AHR is absent in mice whose eosinophils are completely ablated. AHR, a useful marker of airway abnormality in asthma, has been used in epidemiological studies and is predictive of the subsequent course of disease [40,41]. Th2 cells, through the release of cytokines and chemokines, especially IL-4, IL-5 and IL-13, regulate inflammatory cell recruitment to the lung, leading to AHR [31]. Our data demonstrated that PCA inhibited OVA-induced AHR caused by inhaled methacholine. Therefore, it is reasonable to assume that the improvements of AHR observed in PCA-treated animals might be the consequence of decreased amount of Th2 cytokines at the local site.

Many chitinase proteins including AMCase and Ym2 have recently been found to be markedly elevated in allergic airway inflammation in human and in mouse asthma models [42–44]. It has reported that IL-13 induces the expression of an AMCase in airway epithelium [45]. When given intratracheally, IL-13 elevates Ym2 levels in BALF from OVA-induced mice. Our results showed that PCA markedly down-regulated AMCase and Ym2 mRNA expression in the lungs of OVA-induced mice. These may be a consequence of the major drop in IL-13 level in the airways with PCA treatment. E-selectin is pivotal for leukocyte transmigration into the airways [46]. In our study, we have demonstrated that PCA suppressed E-selectin mRNA expression in OVA-challenged lungs.

In summary, we report here for the first time that PCA effectively inhibited Th2 cytokines, IgE levels inflammatory cells recruitment, mucus overproduction and goblet cell hyperplasia. Moreover, AHR, CCL11, CCR3, Ym2, E-selectin, AMCase and Muc5ac were attenuated by PCA. PCA inhibited the activation of NF- κ B and MAPK, in turn reduced inflammatory cytokine and chemokine levels. These findings support a novel therapeutic use of PCA in the treatment of asthma. The effects of PCA (30 mg/kg) were similar to those of DEX in some measured results. Although our study has shed some light on this issue, further and more comprehensive studies are still required before the full clinical application.

Acknowledgments

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