



2-(4-{2-[(phenylthio)acetyl]carbonohydrazonoyl}phenoxy)acetamide as a new lead compound for management of allergic rhinitis

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Abstract

Objective We selected a hit compound, 2-(4-{2-[(phenylthio)acetyl]carbonohydrazonoyl}phenoxy)acetamide (PA), by a molecular docking simulation between 636,565 compounds and caspase-1 protein. We examined the effect of PA on allergic rhinitis (AR) animal model.

Methods We assessed the therapeutic effects and the regulatory mechanisms of ovalbumin (OVA)-sensitized mouse model of AR.

Results A molecular docking simulation and a kinetic assay indicated that PA regulates the caspase-1 activation through the interaction with the caspase-1 active site. In the AR animal model, PA significantly reduced the rub scoring increased by OVA. The up-regulated IgE, histamine, interleukin (IL)-1 β , and thymic stromal lymphopoietin

(TSLP) levels in the serum of OVA-sensitized mice were significantly decreased by the treatment with PA. Protein levels of IL-1 β , IL-5, IL-6, IL-13, tumor necrosis factor- α , TSLP, cyclooxygenase-2, macrophage inflammatory protein-2, and intercellular adhesion molecule-1 were also significantly inhibited by the treatment with PA in the nasal mucosa tissues of the OVA-sensitized mice. In the PA-treated mice, the number of eosinophils and mast cells infiltrated by OVA-sensitization were also reduced. In addition, PA reduced the mast cell-derived caspase-1 activity and expression in the nasal mucosa tissues of the OVA-sensitized mice.

Conclusions PA showed the possibility to regulate AR in OVA-induced AR models, suggesting that it has therapeutic potential for the management of AR as a lead compound.

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Keyword 2-(4-{2-[(phenylthio)acetyl]carbonohydrazonoyl}phenoxy)acetamide · Molecular docking simulation · Allergic rhinitis · TSLP · Caspase-1

Introduction

Allergic rhinitis (AR) is a commonplace inflammatory disease characterized by inflammation of the nasal mucosa tissue, common manifestation of allergic diseases, affecting approximately 500 million people worldwide [1]. It is described by symptoms, such as sneezing, itching, lacrimation, and clear rhinorrhea. These responses are caused by an allergic reaction to an allergen, such as pollen, dust, food, and certain animals. Allergic reactions were mainly mediated by mast cells [2]. Mast cells activated by antigen exaggerated the allergic inflammatory process

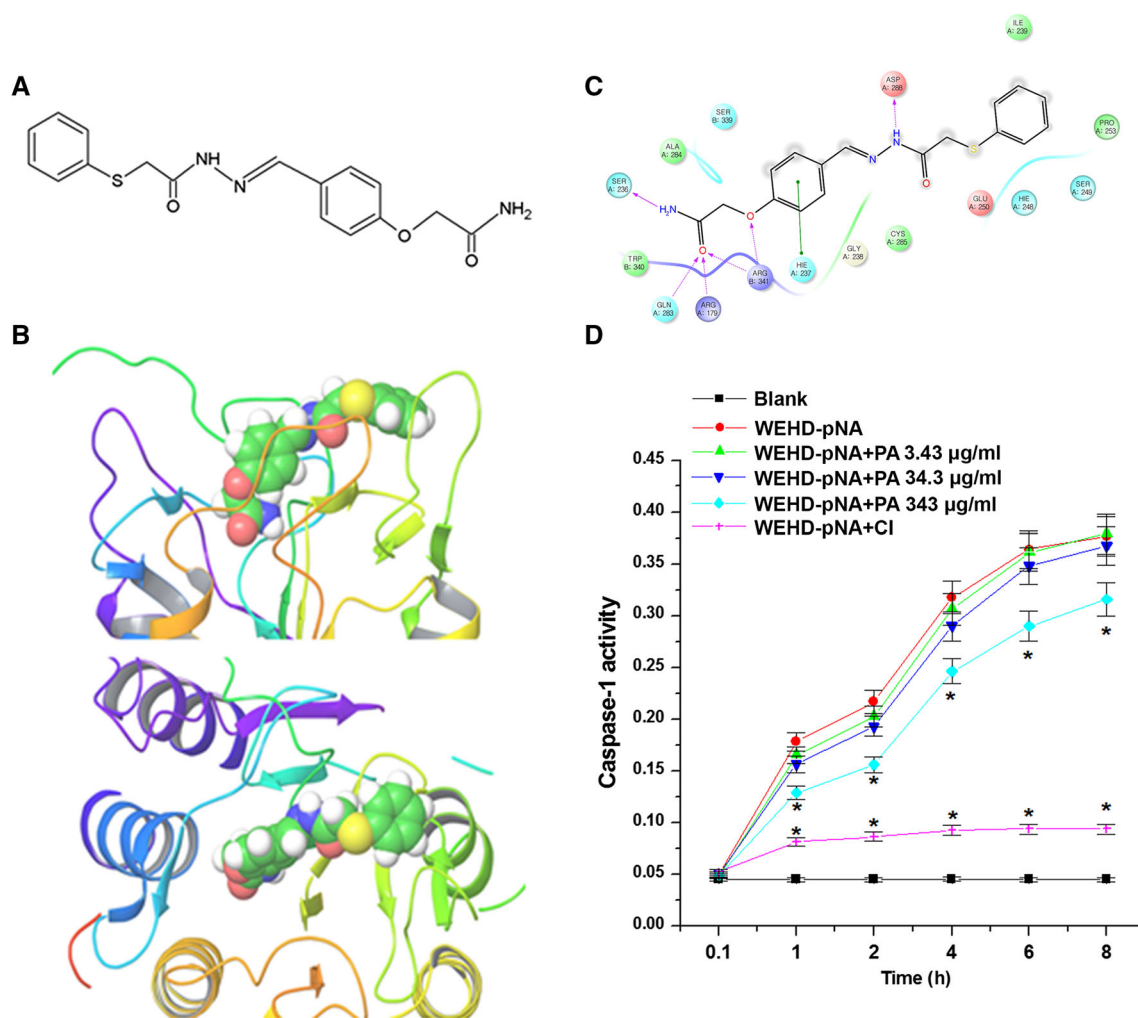


Fig. 1 Chemical structure of PA and molecular docking of the PA and caspase-1 interaction. **a** Chemical structure of PA. **b** Side- and top-view for the docked poses of the PA upon caspase-1. The PA was rendered by space-filling model and carbon atoms were colored as green. **c** Representative snapshot of interaction diagram for the PA/caspase-1 complex. The key interactions were indicated by lines.

through the release of histamine and pro-inflammatory cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, IL-8, and thymic stromal lymphopoietin (TSLP) [3].

Caspase-1 is a member of the caspase family of proteases and expressed constitutively in myeloid cells and exists in a zymogenic form [4, 5]. Initially known as IL-1 β converting enzyme (ICE), caspase-1 converted pro-IL-1 β into mature form [4, 5]. Activated caspase-1 also disseminates and coordinates essential factors of the innate inflammatory response, such as neutrophil recruitment, fever, cytotoxic NK cell activation, and endothelial activation [6]. In addition, activation of caspase-1 induces inflammatory response via stimulation of inflammatory

d Catalytic activity of recombinant caspase-1 (2 units) was measured by WEHD-pNA (caspase-1 substrate), PA (3.43, 34.3, and 343 μ g/ml), or caspase-1 inhibitor (500 nM) for various times. The color development is monitored at 405 nm. Each value was calculated from three independent experiments performed in duplicate. * $p < 0.05$, compared with WEHD-pNA. CI caspase-1 inhibitor

cytokine [5]. Therefore, this result implies that caspase-1 is an attractive target molecule for allergic inflammatory diseases.

The search for anti-allergic inflammatory substances from chemical compound has attracted much interest in recent years. Thus, we also screened the interaction between 636,565 compounds and caspase-1 protein to find excellent anti-inflammatory compounds using computer-aided docking simulation. As a result, we selected a 2-(4-{2-[(phenylthio) acetyl]carbonohydrazonyl}phenoxy)acetamide (PA) (Fig. 1A) as a hit compound. And then, we evaluated the regulatory effects of PA on ovalbumin (OVA)-induced AR animal model.

Materials and methods

Reagents

Dexamethasone (DEX), OVA, bicinchoninic acid (BCA), O-phthaldialdehyde (OPA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and other reagents were obtained from Sigma (St. Louis, MO, USA). Recombinant human caspase-1 was purchased from BioVision, Inc. (Milpitas, CA, USA). Antibodies for cyclooxygenase-2 (COX-2), caspase-1, c-Kit, and tubulin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Fetal bovine serum (FBS), iscove's modified dulbecco's medium (IMDM), and streptomycin were obtained from Gibco BRL (Grand Island, NY, USA).

Computational method

Initial structure of the caspase-1 was taken from Protein Data Bank (PDB id. 1RWK) and processed by molecular modeling tool in Maestro software (ver. 2015-3, Schrodinger Inc.). The starting configurations of PA were prepared with builder module of the Maestro. Molecular docking simulation job of the PA was performed by Glide module [7] for a binding site of caspase-1 with ligand-flexible mode. For the docking simulation, molecular grid was defined for the binding site of caspase-1 using the Receptor Grid Generation tool in Glide with the 15 Å of cubic box. In the docking mode, 2.5 kcal/mol of energy window and distance-dependent dielectric constant ($\epsilon = 1$) was applied to pose sampling process. The highest-scoring docked pose for the PA with caspase-1 was ranked according to the extra precision Glide *XP* mode.

Surface plasmon resonance assay

To convince the direct binding between caspase-1 and PA, we conducted surface plasmon resonance using a Biacore 2000 instrument (Biacore/GE Healthcare Bio-Sciences, Piscataway, NJ). In brief, PBS buffer was used for the sample dilution and analysis. The CM5 dextran sensor chip was activated with equal amounts of 0.2 M N-ethyl-N-(3-diethylaminopropyl)-carbodiimide and 0.05 M N-hydroxysuccinimide. Recombinant human caspase-1 was immobilized in 10 mM sodium acetate buffer (pH 4.0) and then 1 M ethanolamine-hydrochloride (pH 8.0) to deactivate any excess N-hydroxysuccinimide esters. This coupling resulted in 8000 response units of the immobilized proteins per flow cell. To evaluate the binding, each PA was diluted in PBS with 5 % DMSO buffer, analyzed at

flow rate of 5 ml/min. An activated- and blocked flow cell without immobilized ligand was used to evaluate non-specific binding. The immobilized surface was regenerated for subsequent measurements using 1 ml of 10 mM glycine (pH 2.5). The regeneration solution was passed over the immobilized surface until the surface plasmon resonance signal reached the initial background value before protein injection. For all of the samples, response curves were also recorded on control surfaces. The results were calculated after subtraction of the control values using the BIAevaluation 3.0 software (Biacore).

Culture of human mast cell line, HMC-1 cells

The HMC-1 cells were kindly provided by Eichi Morri (Osaka University, Japan). HMC-1 cells were cultured in IMDM supplemented with 100 unit/ml penicillin, 100 µg/ml streptomycin and 10 % heat-inactivated FBS at 37 °C 5 % CO₂ and 95 % humidity.

Cell viability assay

To estimate the cell viability, the MTT assay was performed. Briefly, cells were treated with PA (3.43, 34.3, and 343 µg/ml) for 24 h. MTT (5 mg/ml) was treated and then the cells were cultured at 37 °C for 4 h. The crystallized MTT was dissolved in DMSO and the absorbance measured using an ELISA reader at 540 nm.

OVA-induced AR animal model

4-Week-old female BALB/c (Charles River Laboratories, Inc., Wilmington, MA, USA) mice were maintained under pathogen-free conditions. Mouse care and experimental procedures were performed with the approval of the animal care committee of Kyung Hee University [KHUASP (SE)-12-019]. Sensitization was performed three times (at 1, 5, and 14 day) by intraperitoneally (ip) injecting 100 µg of OVA emulsified in 100 µl phosphate-buffered saline (PBS) containing 20 mg aluminum hydroxide (Sigma Chemical Co, St. Louis, MO, USA). Intranasal challenges were performed using 1.5 mg of OVA in 2 µl PBS and negative controls were challenged intranasally with PBS on days 15–24. PA (3.43, 34.3, and 343 mg/kg), DEX (5 mg/kg) or a control vehicle (distilled water) was administered orally and PA (34.3 and 343 mg/kg) was also ip injection on days 15–24 before intranasal OVA challenge. Nasal rub scoring was evaluated by counting the number of nasal itching motion (nasal rubbing) for 10 min after OVA intranasal challenge provocation at 10 days. It was measured by a

trained observer who was blind to the experimental treatments. Five mice were allocated to each group.

Histamine assay

The histamine content in the serum was measured by the OPA spectrofluorometric procedure of Oh et al. [8]. The fluorescent intensity was measured at 440 nm (excitation at 360 nm) in spectrofluorometer.

Enzyme-linked immunosorbent assay (ELISA)

Cytokines levels in serum, nasal mucosa tissues, and spleens were analyzed in accordance with the manufacturer's specification (BD Biosciences Pharmingen, San Diego, CA, USA). Cytokine levels in nasal mucosa tissues and spleen were divided according to the total protein levels, which were estimated using a BCA protein assay method.

Western blot analysis

Western blot analysis was used for nasal mucosa tissue extracts. Western blotting was performed as previously described [8].

Histological examination

Tissue samples were immediately fixed with 10 % formaldehyde for 24 h at 20°C and embedded in paraffin. Sections (4 µm) of nasal mucosa tissues samples were stained with hematoxylin and eosin (H&E, for eosinophils) and alcian blue and safranin O (A&S, for mast cells), and numbers of eosinophils and mast cells on both sides of septal mucosa were counted. For this examination, sections were coded and randomly analyzed by two blinded observers.

Caspase-1 assay

Caspase-1 activity was analyzed in accordance with the manufacturer's specifications using a caspase-1 assay kit (R & D Systems, Minneapolis, Minnesota, USA).

Confocal microscopy

After de-waxing and dehydration, sections were blocked with bovine serum albumin and incubated for 60 min with anti-mouse c-Kit and anti-rabbit caspase-1 antibodies (Santa Cruz, CA, USA). The secondary antibody, fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG, tetramethylrhodamine-5-(and-6)-isothiocyanate (TRITC)-conjugated anti-rabbit IgG (Invitrogen), was then added for

30 min. A mounting medium containing 4',6-diamidino-2-phenylindole (DAPI, Vector Laboratories, Burlingame, CA, USA) was used to counterstain DNA. All specimens were examined under a confocal laser-scanning microscope. Sections were coded and randomly analyzed by two blinded observers.

Statistical analysis

Treatment effects were analyzed using one-way ANOVA and the independent *t* test. All data are expressed as the mean $\pm$ standard error of mean (SEM). Statistical significance was accepted for *p* values <0.05.

Results

Molecular docking of the PA and caspase-1 interaction

Recently, we demonstrated that caspase-1 plays a crucial role in the mast cell-mediated allergic inflammatory reactions [8]. We selected a hit compound, PA, by a molecular docking simulation between 636,565 compounds and caspase-1 protein. Binding mode of the PA upon caspase-1 was estimated using by molecular modeling approach. Figure 1b is a representative image for the complex between PA and caspase-1 from the molecular docking simulation. The PA was settled on the characteristic inhibitor binding site surrounding by four-loop region of the caspase-1. A phenoxyacetamide moiety of the PA was oriented toward inner space of the caspase-1 while thio-phenyl group was located facing outward. This geometrical feature of the PA was mainly responsible for the stable complex formation with the caspase-1 to inhibit inflammatory activity. Figure 1c contains detailed ligand–protein interaction diagram for the PA/caspase-1 complex. The PA was located around binding site of caspase-1 formed by flexible loop regions. In particular, the phenoxyacetamide moiety was key interacting site with the caspase-1 in atomic level. The phenoxy ring of the PA showed strong interaction with the His237 residue of the caspase-1. Moreover, oxygen atoms of the phenoxyacetamide moiety made multiple hydrogen bonds with Arg179, Gln283, and Arg341 residues of caspase-1. Whereas phenylthio-hydrazonoyl region of the PA was solvent-exposed only contact with hydrogen bonding to Asp288 of the caspase-1.

A caspase-1 kinetic assay was performed to estimate the binding affinity of PA for the caspase-1 active site. As shown in Fig. 1d, caspase-1 activities were up-regulated by the caspase-1 substrate (WEHD-pNA). However, PA or caspase-1 inhibitor significantly reduced the binding

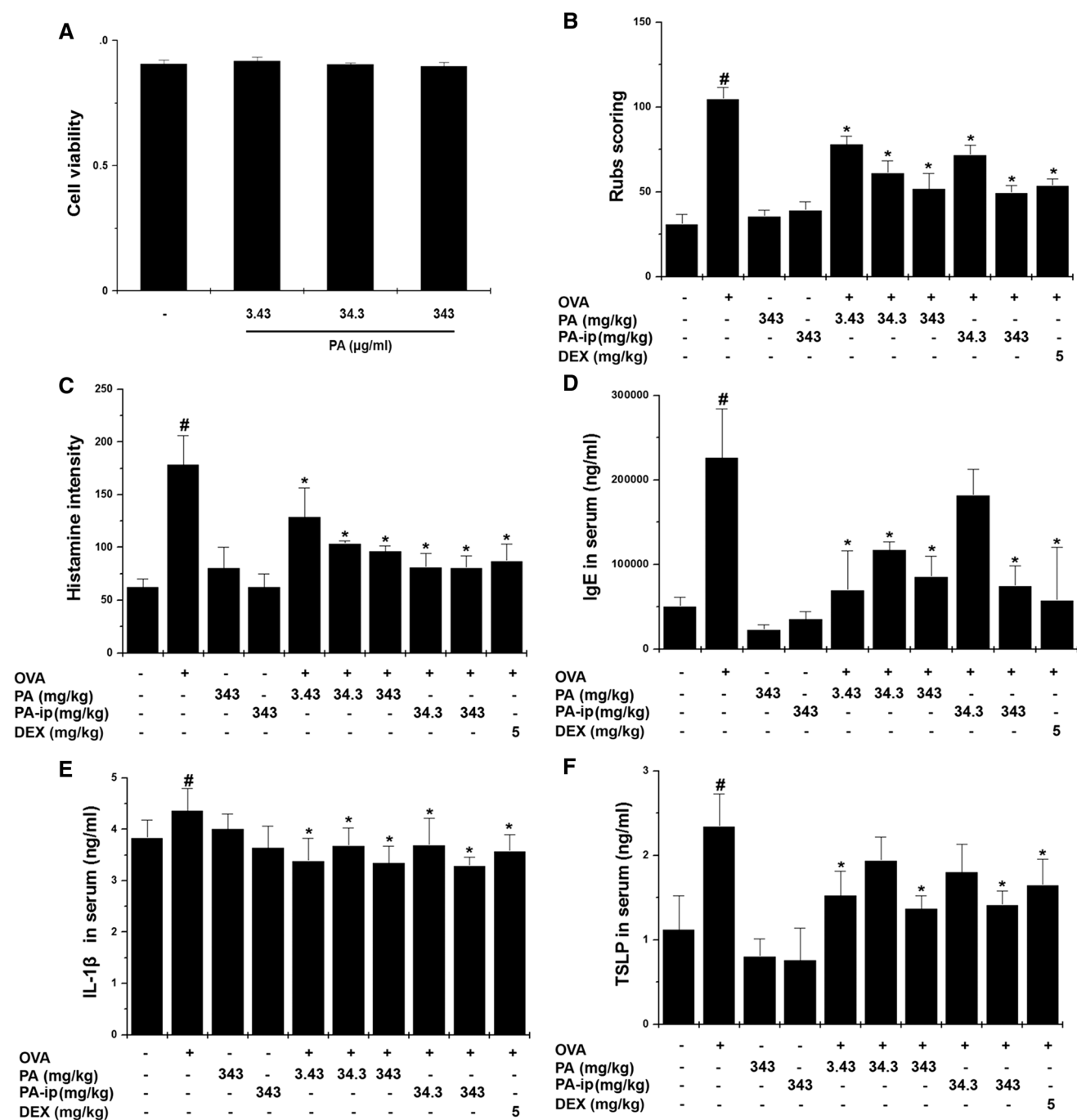


Fig. 2 Regulatory effects of PA on cytotoxicity and on clinical symptoms, histamine, IgE, IL-1β, and TSLP levels in the AR mice. **a** HMC-1 cells were treated with PA (3.43, 34.3, and 343 μg/ml) for 24 h. Cell viability was evaluated by a MTT assay. We sensitized mice on days 1, 5, and 14 by ip injections of 100 μg OVA emulsified in 20 mg of aluminum hydroxide and we challenged mice with 1.5 mg OVA. Mice received orally or intraperitoneally PA before the intranasal OVA challenge for 10 days. **b** The number of the nasal rubs

that occurred in the 10 min after the OVA intranasal provocation. **c** Serum was isolated from blood and then assayed about histamine. **d** IgE **e** IL-1β and **f** TSLP in the serum were measured by the ELISA method. [#]*p* < 0.05; significantly different from the OVA-unsensitized mice. ^{*}*p* < 0.05; significantly different from the OVA-sensitized mice, *n* = 5. PA orally administration, PA-ip intraperitoneally administration, DEX dexamethasone

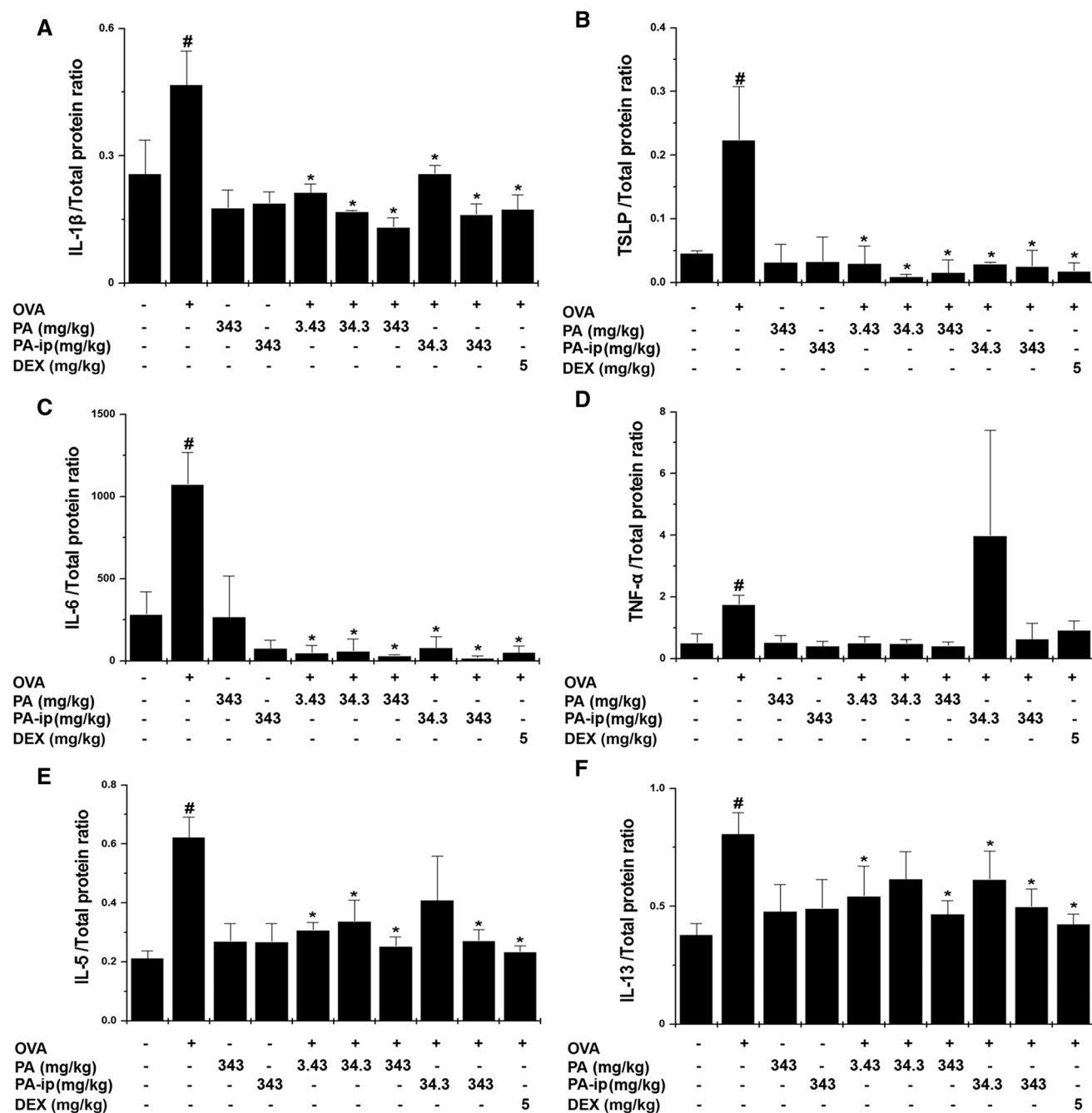


Fig. 3 Regulatory effects of PA on inflammatory cytokines of the nasal mucosa tissues. **a** IL-1 β , **b** TSLP, **c** IL-6, **d** TNF- α , **e** IL-5, and **f** IL-13 were measured by the ELISA method. All parameters measured in the tissue homogenate were presented as a ratio to the

total protein levels in the nasal tissue. # $p < 0.05$; significantly different from the OVA-unsensitized mice. * $p < 0.05$; significantly different from the OVA-sensitized mice, $n = 5$. PA orally administration, PA-ip intraperitoneally administration, DEX dexamethasone

affinity of WEHD-pNA with the caspase-1 active site. To confirm the binding of PA to caspase-1, we also performed the surface plasmon resonance analysis using Biacore. PA (0.1 mM) was applied to a recombinant human caspase-1-immobilized CM5 dextran sensor chip. The PA binding affinity for the caspase-1 protein was not detected (data not shown).

Regulatory effect of PA on the OVA-induced AR mice

First, to estimate the cytotoxicity of PA, we performed MTT assay on HMC-1 cells. As a result, various concentrations of PA (3.43, 34.3, and 343 $\mu\text{g/ml}$) did not affect the cell viability (Fig. 2a). Next, we examined the

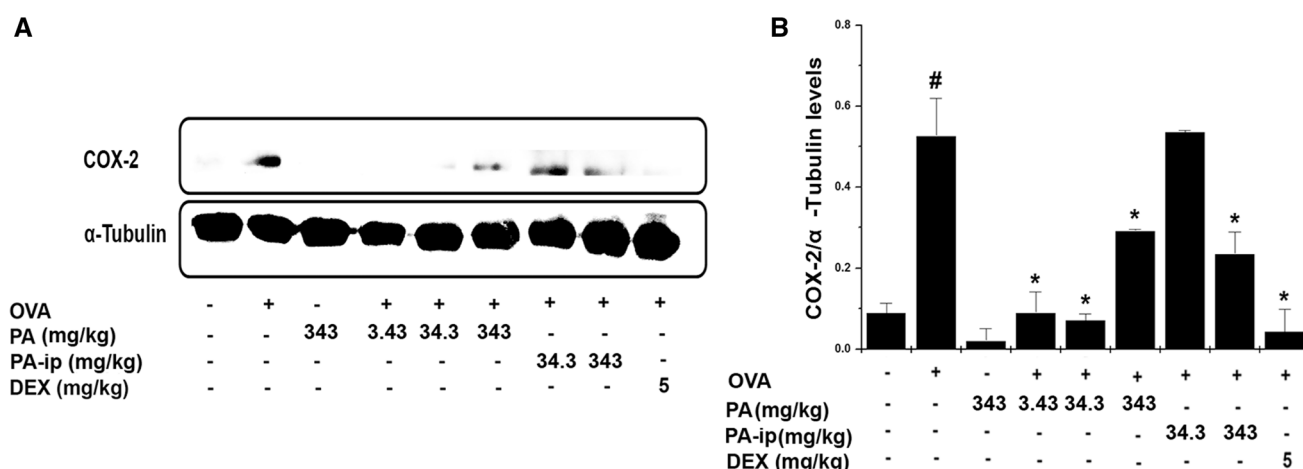


Fig. 4 Regulatory effect of PA on COX-2 levels of the nasal mucosa tissues. **a** COX-2 protein expression was evaluated by Western blotting. The results are representative tissue samples. **b** The protein levels of COX-2 were quantified by densitometry. Values were

expressed as mean $\pm$ SEM. [#] $p < 0.05$; significantly different from the OVA-unsensitized mice. ^{*} $p < 0.05$; significantly different from the OVA-sensitized mice, $n = 5$. PA orally administration, PA-ip intraperitoneally administration, DEX dexamethasone

regulatory effect of PA in the AR in vivo model. The rub scoring of nose after the OVA challenge was significantly increased compared with the OVA-unsensitized mice. Elevated rub scoring was suppressed by the treatment with PA in a dose-dependent manner (Fig. 2b, $p < 0.05$). The histamine, IgE, IL-1 β , and TSLP levels in the OVA-induced AR mice were significantly higher than those in the serum of the OVA-unsensitized mice (Fig. 2c–f, $p < 0.05$). Up-regulated levels of histamine, IgE, IL-1 β , and TSLP in the serum were down-regulated by PA (Fig. 2c–f, $p < 0.05$). DEX (5 mg/kg) was used as a positive control and significantly regulated the various allergic factors (Fig. 2, $p < 0.05$).

Regulatory effect of PA on the inflammatory cytokine levels of the nasal mucosa tissues

Pro-inflammatory cytokines, including the IL-1 β , IL-6, TSLP, and TNF- α , are crucial factors in pathogenesis of AR [9]. So, we examined the regulatory effect of PA on the inflammatory cytokines levels of the nasal mucosa tissues. OVA significantly up-regulated the cytokine levels compared with the normal control mice (Fig. 3a–d). The levels of IL-1 β , TSLP, IL-6, and TNF- α up-regulated by OVA were significantly down-regulated by PA (Fig. 3a–d, $p < 0.05$). IL-5 and IL-13 increased by OVA were also significantly down-regulated by PA (Fig. 3e–f). DEX significantly reduced the inflammatory cytokine levels (Fig. 3, $p < 0.05$).

Regulatory effect of PA on the COX-2 levels of the nasal mucosa tissue

COX-2 is involved in inflammatory response and its expression was increased in allergic patients [10]. So, we

examined the regulatory effects of PA on COX-2 expression of nasal mucosa tissues. COX-2 expression in the AR mice was significantly higher than those in the nasal mucosa tissues of the OVA-unsensitized mice (Fig. 4). Increased COX-2 expression was suppressed by PA (Fig. 4). DEX also significantly reduced the COX-2 levels (Fig. 4).

Regulatory effect of PA on the eosinophils and mast cells infiltration into the nasal mucosa tissue

Eosinophils and mast cells play a central function in the pathogenesis of a variety of allergic diseases [7, 11]. So, we examined the regulatory effects of PA on the infiltration of eosinophils and mast cells into nasal mucosa tissues. As shown in Fig. 5, numbers of eosinophils and mast cells in the nasal mucosa tissues were significantly higher in OVA-sensitized mice than in the OVA-unsensitized mice. However, these up-regulations were significantly suppressed by PA (Fig. 5, $p < 0.05$). DEX also significantly reduced the infiltration of inflammatory cells (Fig. 5, $p < 0.05$). Macrophage inflammatory protein-2 (MIP-2) and intercellular adhesion molecule-1 (ICAM-1) are chemotactic molecules for eosinophils and mast cells [12]. Subsequently, we examined the regulatory effect of PA on the levels of MIP-2 and ICAM-1 because infiltration of eosinophils and mast cells were reduced by PA (Fig. 5a–d). MIP-2 and ICAM-1 increased by OVA were also significantly down-regulated by PA (Fig. 5e–f, $p < 0.05$).

Regulatory effect of PA on the caspase-1 activity of the nasal mucosa tissue

Caspase-1 is associated with inflammation and apoptosis, activation of caspase-1 induces inflammatory response by

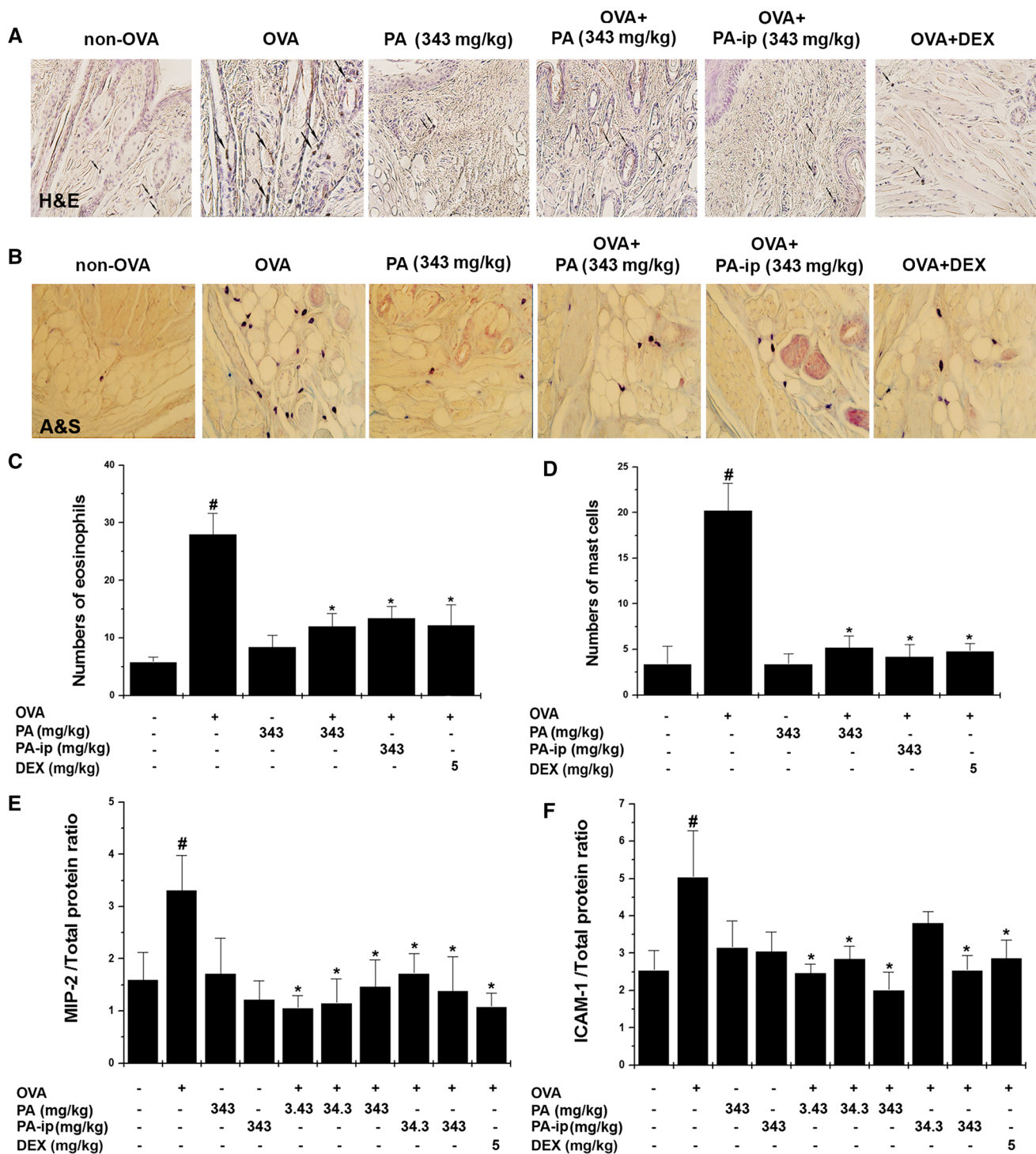
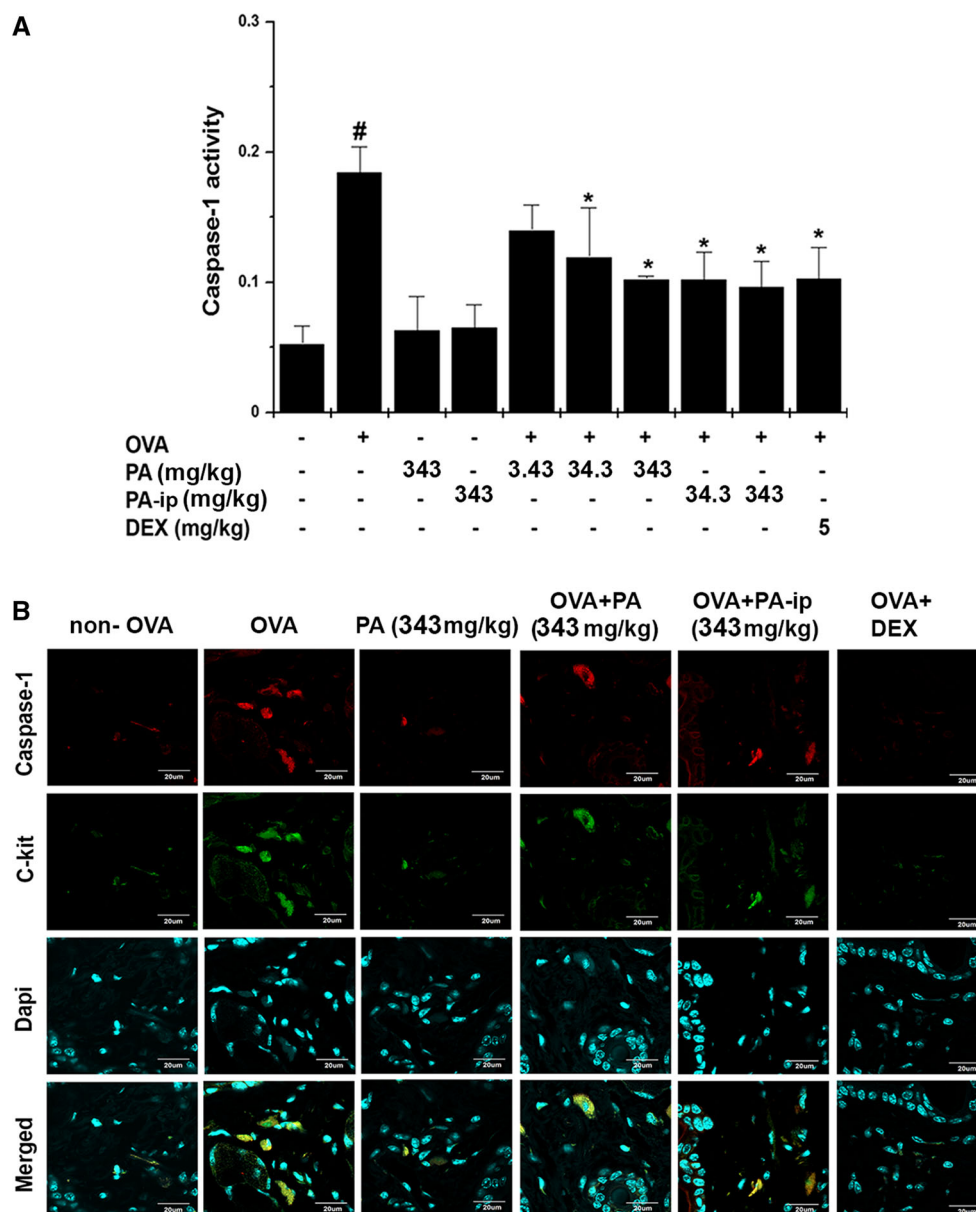


Fig. 5 Regulatory effect of PA on infiltration of eosinophils and mast cells into the nasal mucosa tissues. **a** Nasal mucosa stained with H&E (for eosinophils = arrow) and **b** A&S (for mast cells). **c** Eosinophils and **d** mast cells were counted by two individuals. Afterwards, five randomly selected tissue sections per mouse were counted. The absolute number of cell was counted as the mean $\pm$ S.E.M. **e** MIP-2 and **f** ICAM-1 were measured by the ELISA method. All parameters

measured in the tissue homogenate were presented as a ratio to the total protein levels in the nasal tissue. [#] $p < 0.05$; significantly different from the OVA-unsensitized mice. ^{*} $p < 0.05$; significantly different from the OVA-sensitized mice. PA orally administration, PA-ip intraperitoneally administration, DEX dexamethasone (original magnification $\times 400$, scale bar = 100 μ m)

Fig. 6 Regulatory effect of PA on caspase-1 activation of the nasal mucosa tissues.

a Caspase-1 activity of nasal mucosa tissues was measured by a caspase-1 assay kit. **b** The caspase-1 and c-Kit from nasal mucosa tissues were examined using a confocal laser-scanning microscope. The two tissue sections were randomly selected per mouse. The data are representative tissue samples. PA orally administration, PA-ip intraperitoneally administration, DEX dexamethasone (original magnification $\times 400$, scale bar = 20 μm)



stimulation of inflammatory cytokine [13]. Therefore, we investigated the regulatory effect of PA on caspase-1 activity of nasal mucosa tissues using a caspase-1 assay kit. PA significantly down-regulate the OVA-induced caspase-1 activity (Fig. 6a, $p < 0.05$). When PA was orally administered, the maximum inhibition rate was about 62.19 % at 343 mg/kg and IC_{50} value was 44.17 mg/kg. Also, we previously reported that mast cell-derived caspase-1 activation induced the inflammatory cytokines production in allergic reaction [11]. So, we performed immunohistochemistry for mast cell and caspase-1 to examine the regulatory effects of PA on mast cell-derived caspase-1 expression of nasal mucosa tissues. As shown in Fig. 6b, PA down-regulated the expression of mast cell-derived caspase-1.

Discussion

In this study, we showed that PA suppressed the levels of histamine and inflammatory cytokines and also suppressed the levels of COX-2 and activation of caspase-1. So, we suggested that PA has a beneficial effect in treating OVA-induced AR.

The AR symptoms include itchy nose, nasal congestion, lacrimation rhinorrhea, and sneezing [14]. These symptoms are mainly originated from other inflammatory cells and mast cells and triggered by inflammatory cell-derived IL- 1β , TSLP, TNF- α , IL-4, IL-6, IL-8, and IL-13 [8, 15]. IL- 1β is a powerful pro-inflammatory cytokine and a known lymphocyte activating factor, which enhances B cells activation and leads to IgE production [16]. TNF- α has

been reported to increase the migration of Th2 cells and to induce the inflammation of nasal mucosa tissues [17]. TSLP is a crucial factor of innate and adaptive immune responses related to Th2 mediated cytokines, which include inflammation and AR [18]. IL-4 elevation is associated with upregulation of IgE receptors, synthesis of IgE, hypersecretion of mucus, and differentiation of Th2 lymphocytes [19, 20]. IL-5 acts an important role in eosinophil-mediated inflammatory reaction and eosinophil influx into the airway [20]. IL-13 participates in the isotype switching of antibody to IgE and differentiation of B lymphocytes [21]. Therefore, these inflammatory cytokines are essential factor in initiation and maintenance of allergic responses [16]. COX-2 plays important roles in regulating several aspects of airway function and the levels of COX-2 were increased by inflammatory cytokines in the allergic diseases including AR and asthma [11, 22]. Also, Smith et al. showed that COX-2 selective inhibitors have an anti-inflammatory effect [23]. In this study, we showed that PA markedly inhibited the levels of these inflammatory mediators in the OVA-sensitized AR mice. Therefore, these results demonstrated that PA has an anti-inflammatory effect by suppressing the levels of inflammatory mediators.

Allergic mucosal inflammation is described by influx of eosinophils, T lymphocytes, and mast cells [24]. Eosinophils are important effector cells in immune responses against infections and contribute to the pathology of allergic inflammatory response [24, 25]. The mediators released by eosinophils play a vital role in the development of rhinitis [26, 27]. Mast cells are one of the most crucial cells in allergic responses, such as AR, atopic dermatitis, and asthma [28]. Mast cell contained proteases, histamine, and other potent chemical mediators implicated in a wide variety of inflammatory and immunologic process [28]. Inflammatory proteins like ICAM-1 and MIP-2 play critical roles in AR [29]. ICAM-1 is a chemotactic molecule for eosinophils and mast cells and is expressed in the nasal mucosal epithelial cells of AR patients, and has been proposed as a marker of allergic inflammation [12]. MIP-2 is also expressed in the nasal mucosal epithelial cells of AR and belongs to the chemotactic cytokines family, and is a chemotactic factor for neutrophils [12]. In this study, we showed that PA markedly inhibited the infiltration of eosinophils and mast cells and the levels of ICAM-1 and MIP-2 in the OVA-sensitized AR mice. Therefore, these results demonstrated that PA suppressed the precipitation of inflammatory cells by regulating chemokine levels.

Caspase-1 acts a vital function in several important inflammatory diseases and in innate immunity [6]. Caspase-1 activation mediated the inflammatory reactions [30]. In AR animal model, the levels of caspase-1 was higher than normal control mice [31]. The levels of caspase-1 in

allergic patients are also higher than those in normal individuals [31]. The caspase-1 elevated the COX-2-dependent inflammatory reactions, whereas caspase-1 deficiency reduced the expression of COX-2 [32]. The activation of caspase-1 also promotes production of IL-1 β and IL-6 [32]. In addition, TSLP is expressed and produced via the activation of caspase-1 in mast cells [33]. Thus, the suppression of caspase-1 activation is related with a reduction in inflammation. We found that PA significantly inhibited the caspase-1 activation in AR mice. These results suggest that PA down-regulated the AR by blocking the caspase-1 pathway.

A surface plasmon resonance assay was performed to confirm the binding affinity of PA for the caspase-1 active site. However, in this study, the direct binding affinity between caspase-1 and PA was not observed. A surface plasmon resonance assay was affected by various factors. Molecular size can also affect the surface plasmon resonance assay. Molecular weights of PA and caspase-1 are 343 and 20 or 10 kDa, respectively. In this study, we showed that PA significantly inhibited the binding affinity of WEHD-pNA with the caspase-1. Therefore, we suggest that PA down-regulated the caspase-1 activity, although the direct binding affinity between PA and caspase-1 is not known at this time. To elucidate the direct interaction between caspase-1 and PA, further detailed studies are required.

In summary, our findings demonstrated that PA has an anti-allergic inflammatory effect by improving rhinitis symptoms and inhibiting the release of allergic mediators in OVA-induced AR mice. Also, PA suppressed the precipitation of inflammatory cells and the activation of caspase-1. These results suggest that PA as a new lead compounds, might be useful for the treatment of allergic inflammatory diseases such as AR.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Howarth PH, Salagean M, Dokic D. Allergic rhinitis: not purely a histamine-related disease. *Allergy*. 2000;64:7–16.
2. Kramer MF, Jordan TR, Klemens C, Hilgert E, Hempel JM, Pfrogner E, Rasp G. Factors contributing to nasal allergic late phase eosinophilia. *Am J Otolaryngol*. 2006;27:190–9.
3. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, Zhang Y, Elias JA. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest*. 1999;103:779–88.

4. Franchi L, Eigenbrod T, Munoz-Planillo R, Nunez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat Immunol*. 2009;10:241–7.
5. Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS, Flavell RA. Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. *Science*. 1995;267:2000–3.
6. Shamaa OR, Mitra S, Gavriliu MA, Wewers WD. Monocyte caspase-1 is released in a stable, active high molecular weight complex distinct from the unstable cell lysate-activated caspase-1. *PLoS One*. 2015;10:e0142203.
7. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE, Francis P, Shenkin PS. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J Med Chem*. 2004;47:1739–49.
8. Oh HA, Ryu JG, Cha WS, Kim HM, Jeong HJ. Therapeutic effects of traditional Korean medicine, Jeechool-Whan in allergic rhinitis model. *TANG*. 2012;2:e9.
9. Grzelewska-Rzymowska I, Pietrzkowicz M. Role of tumor necrosis factor alpha in allergic inflammation and airway hyperresponsiveness. *Pol Merkur Lekarski*. 2004;16:173–8.
10. Profita M, Sala A, Bonnano A, Riccobono L, Siena L, Melis MR, Di Giorgi R, Mirabella F, Gjomarkaj M, Bonsignore G, Vignola AM. Increased prostaglandin E2 concentrations and cyclooxygenase-2 expression in asthmatic subjects with sputum eosinophilia. *J Allergy Clin Immunol*. 2003;112:709–16.
11. Oh HA, Kim HM, Jeong HJ. Distinct effects of imperatorin on allergic rhinitis: imperatorin inhibits caspase-1 activity in vivo and in vitro. *J Pharmacol Exp Ther*. 2011;339:72–81.
12. Spencer LA, Szela CT, Perez SA, Kirchhoffer CL, Neves JS, Radke AL, Weller PF. Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially. *J Leukoc Biol*. 2009;85:117–23.
13. Lee SH, Stehlik C, Reed JC. Cop, a caspase recruitment domain-containing protein and inhibitor of caspase-1 activation processing. *J Biol Chem*. 2001;276:34495–500.
14. Mandhane SN, Shah JH, Thennati R. Allergic rhinitis: an update on disease, present treatments and future prospects. *Int Immunopharmacol*. 2011;11:1646–62.
15. Rosenberg HF, Dyer KD, Foster PS. Eosinophils: changing perspectives in health and disease. *Nat Rev Immunol*. 2013;13:9–22.
16. Finkelman FD, Urban JF Jr. The other side of the coin: the protective role of the TH2 cytokines. *J Allergy Clin Immunol*. 2001;107:772–80.
17. Artis D, Humphreys NE, Bancroft AJ, Rothwell NJ, Potten CS, Grencis RK. Tumor necrosis factor alpha is a critical component of interleukin 13-mediated protective T helper cell type 2 responses during helminth infection. *J Exp Med*. 1999;190:953–62.
18. Ziegler SF. The role of thymic stromal lymphopoietin (TSLP) in allergic disorders. *Curr Opin Immunol*. 2010;22:795–9.
19. Liu X, Li M, Wu Y, Zhou Y, Zeng L, Huang T. Anti-IL-33 antibody treatment inhibits airway inflammation in a murine model of allergic asthma. *Biochem Biophys Res Commun*. 2009;386:181–5.
20. Seder RA, Paul WE, Davis MM. Groth de St, Fazekas B. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4 + T cells from T cell receptor transgenic mice. *J Exp Med*. 1992;176:1091–8.
21. Pawankar R, Okuda M, Yssel H, Okumura K, Ra C. Nasal mast cells in perennial allergic rhinitis exhibit increased expression of the Fc epsilonRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J Clin Invest*. 1997;99:1492–9.
22. Pang L, Pitt D, Petkova D, Knox AJ. The COX-1/COX-2 balance in asthma. *Clin Exp Allergy*. 1998;28:1050–8.
23. Smith WL, Dewitt DL, Garavito RM. Cyclooxygenase: structure, cellular and molecular biology. *Annu Rev Biochem*. 2000;69:145–81.
24. Melvin TA, Ramanathan M Jr. Role of innate immunity in the pathogenesis of allergic rhinitis. *Curr Opin Otolaryngol*. 2012;20:194–8.
25. Isobe Y, Kato T, Arita M. Emerging roles of eosinophils and eosinophil-derived lipid mediators in the resolution of inflammation. *Front Immunol*. 2012;3:270.
26. Sollberger G, Strittmatter GE, Garstkiewicz M, Sand J, Beer HD. Caspase-1: the inflammasome and beyond. *Innate Immun*. 2014;20:115–25.
27. Pawankar R, Lee KH, Nonaka M, Takizawa R. Role of mast cells and basophils in chronic rhinosinusitis. *Clin Allergy Immunol*. 2007;20:93–101.
28. Gilfillan AM, Beaven MA. Regulation of mast cell responses in health and disease. *Crit Rev Immunol*. 2012;31:475–529.
29. Rothenberg ME, Hogan SP. The eosinophil. *Annu Rev Immunol*. 2006;24:147–74.
30. Oh HA, Kim HM, Jeong HJ. Alleviation of allergic rhinitis symptoms with Pyeongwee-San extract (KMP6). *Immunopharmacol Immunotoxicol*. 2012;34:35–142.
31. Grzegorzczak J, Kowalski ML, Pilat A, Iwaszkiewicz J. Increased apoptosis of peripheral blood mononuclear cells in patients with perennial allergic asthma/rhinitis: relation to serum markers of apoptosis. *Mediat Inflamm*. 2002;11:225–33.
32. Cunha TM, Talbot J, Pinto LG, Vieira SM, Souza GR, Guerrero AT, Sonogo F, Verri WA Jr, Zamboni DS, Ferreira SH, Cunha FQ. Caspase-1 is involved in the genesis of inflammatory hypernociception by contributing to peripheral IL-1 β maturation. *Mol Pain*. 2010;6:63.
33. Moon PD, Kim HM. Thymic stromal lymphopoietin is expressed and produced by caspase-1/NF- κ B pathway in mast cells. *Cytokine*. 2011;54:239–43.