

ORIGINAL ARTICLE

The Iron Chelator and Anticancer Agent Dp44mT Relieves Allergic Inflammation in Mice With Allergic Rhinitis

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Abstract— Our previous study showed that an iron chelator and anticancer agent Di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone (Dp44mT) has an antiinflammatory effect in human mast cells. However, antiinflammatory effect of Dp44mT remains unclear in animal models. In this study, we assessed whether administration of Dp44mT could relieve clinical symptoms of ovalbumin (OVA)-induced allergic rhinitis (AR) mice. After administration of Dp44mT, number of sneezes was significantly decreased, and levels of histamine and IgE were suppressed in serum of AR mice. Also, serum levels of interleukin (IL)-1 β , thymic stromal lymphopoietin (TSLP), and tumor necrosis factor (TNF)- α increased by OVA challenge were significantly lowered by administration of Dp44mT. T helper type 1 (Th1) cytokine interferon- γ level was significantly increased by administration of Dp44mT, whereas Th2 cytokines such as IL-4, IL-5, and IL-13 were significantly reduced by administration of Dp44mT. In intranasal tissues of AR mice, levels of IL-1 β , TSLP, TNF- α , and IL-6 and activities and protein levels of caspase-1 were significantly reduced by administration of Dp44mT. Interestingly, administration of Dp44mT reduced number of infiltrated eosinophils and mast cells through the inhibition of macrophage inflammatory protein-2 and intercellular adhesion molecule-1 in intranasal tissues of AR mice. In conclusion, these results indicate that Dp44mT also has potential antiinflammatory effects *in vivo* as well as *in vitro*.

KEY WORDS: allergic rhinitis; Dp44mT; anticancer agent; Th1/Th2 cytokines; inflammatory cytokine; caspase-1.

INTRODUCTION

Allergic rhinitis (AR) is one of the most common types of allergy and its prevalence rate is constantly increasing [1]. Many people with AR have also [allergic conjunctivitis](#), [atopic dermatitis](#), or [asthma](#) [2]. AR is an IgE-mediated inflammatory reaction characterized by one or more nasal clinical symptoms including itching, rhinorrhea, nasal discharge, and sneezing [3]. AR stimulates a systemic increase of inflammation by various immune cells [4]. Within minutes of various allergens exposure, mast cells release proteases, chemokines, histamine, and inflammatory cytokines [4]. Th1 cell (secreting IFN- γ) is mainly participated in cell-mediated immune responses, whereas Th2 cell

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(secreting IL-4, IL-5, and IL-13) fulfills strategic roles in humoral and allergic immune responses [5]. A quantitative and functional disturbance of Th1 or Th2 cytokines is important for the pathogenesis of AR [5]. Some of Th2 and inflammatory cytokines induce the clinical symptoms of AR, whereas others stimulate activation of various inflammatory cells, including eosinophils, basophils, mast cells, and neutrophils [4, 6]. In addition, inflammatory cytokines such as thymic stromal lymphopoietin (TSLP), tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6 increase the expression of macrophage inflammatory protein-2 (MIP-2) and intercellular adhesion molecule-1 (ICAM-1) and facilitate the infiltration of inflammatory cells into intranasal tissues of AR [7]. Therefore, to effectively treat AR, it is important to alleviate the inflammatory response.

Di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone (Dp44mT) is one of the most effective iron chelators developed in terms of its selective antitumor activity [8]. Recent data showed that inflammation is a critical component of tumor progression [9]. Growth of cancers was induced at inflammatory zone around tumor. Also, inflammatory cells were infiltrated in this area [9]. So, we hypothesized that anticancer drugs will have antiinflammatory effects. Previous our study showed that Dp44mT has an antiinflammatory effect by inhibition of mast cell activation [10]. However, antiinflammatory effect of DP44mT remains unclear in animal models. Therefore, we investigated whether the Dp44mT has an antiallergic inflammatory effect in AR mouse model.

MATERIALS AND METHODS

Reagents

Avidin peroxidase, dexamethasone (DEX), Dp44mT (purity $\geq 98\%$), ovalbumin (OVA), and other reagents were purchased from Sigma (St. Louis, MO, USA). Antimouse IgE/IL-1 β /TSLP/TNF- α /IL-4/IL-5/IL-13/IFN- γ /MIP-2/ICAM-1 antibody (Ab), biotinylated antimouse IgE/IL-1 β /TSLP/TNF- α /IL-4/IL-5/IL-13/IFN- γ /MIP-2/ICAM-1 Ab, and recombinant mouse IgE/IL-1 β /TSLP/TNF- α /IL-4/IL-5/IL-13/IFN- γ /MIP-2/ICAM-1 were purchased from BD Pharmingen (San Diego, CA, USA). Ab for caspase-1 and GAPDH were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

OVA-Induced AR Animal Model

Four-week-old female BALB/c (Dae-Han Experimental Animal Center, Eumsung, Republic of Korea) 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)-18-022]. Sensitization was performed three times (at 1, 5, and 14 days) by intraperitoneally (ip) injecting 100 μ g of OVA emulsified in 100 μ L 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 μ L PBS. Negative controls were challenged intranasally with PBS in an identical manner. Dp44mT (1, 10, and 100 μ g/kg), DEX (5 mg/kg), or a control vehicle (distilled water) was orally administered before OVA intranasal challenge on days 15 to 24. Nasal rub scoring was evaluated by counting the number of nasal itching motion (nasal rubbing) for 10 min after OVA intranasal challenge at 9 days. It was measured by a trained observer who was blind to the experimental treatments. Five mice were assigned to each group.

Histamine Assay

Histamine levels were measured from the serum of mice according to the manufacturer's specifications (Oxford Biomedical Research, Oxford, MI, USA).

Enzyme-Linked Immunosorbent Assay

Cytokine levels in spleen, intranasal tissues, and serum were analyzed according to the manufacturer's specification (BD Pharmingen, San Diego, CA, USA). Cytokine levels of intranasal tissues and spleens were divided according to the total protein concentrations, which were measured using a bicinchoninic acid (Pierce, Rockford, IL, USA).

RNA Isolation and Quantitative Real-Time PCR

RNA isolation, cDNA synthesis, and quantitative real-time PCR were accomplished as previously described [11]. Target messenger RNA (mRNA) levels were normalized *versus* GAPDH. Data were analyzed using the $\Delta\Delta$ CT method.

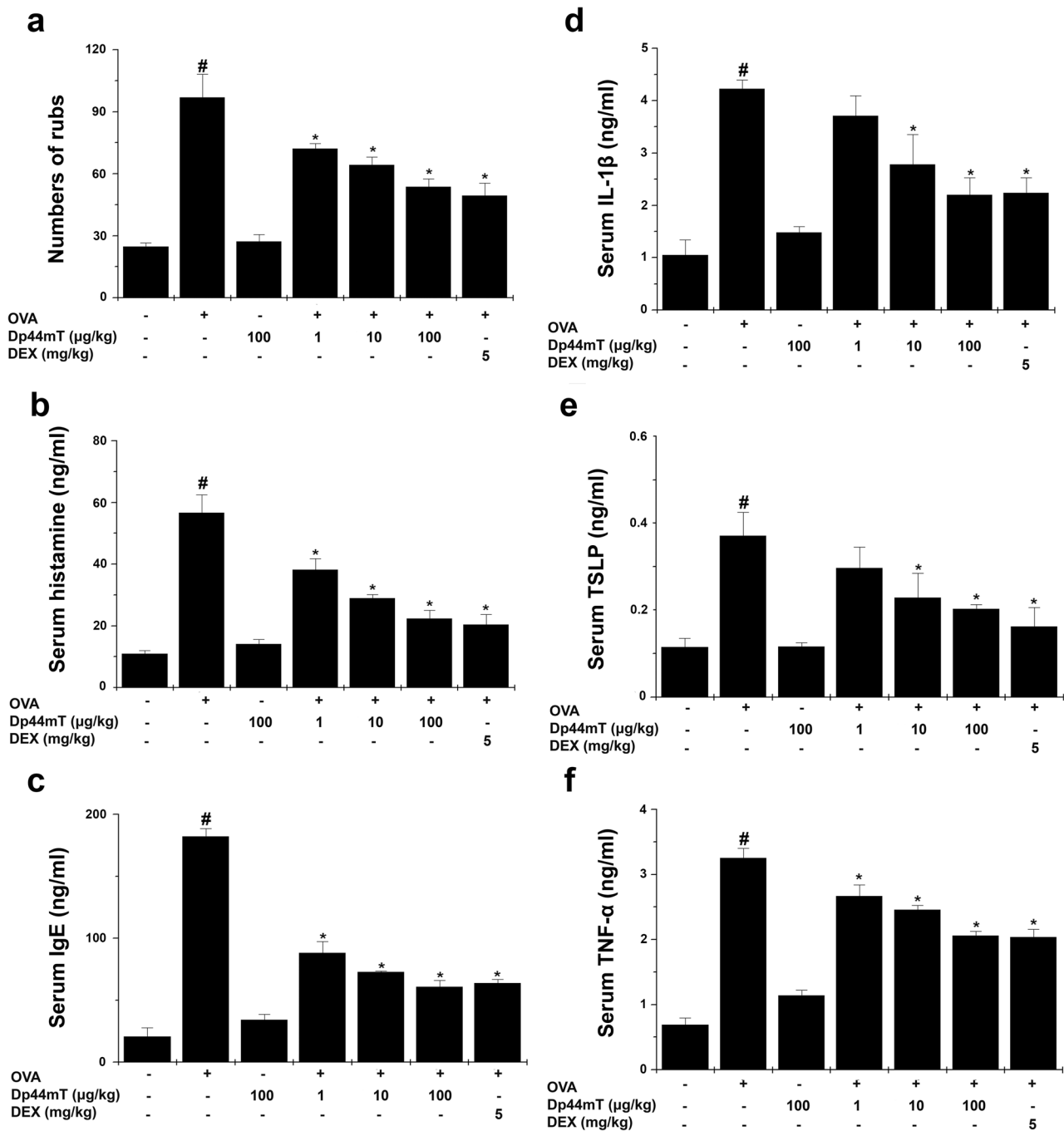


Fig. 1. Alleviate effect of Dp44mT on OVA-induced AR biomarkers in serum. We sensitized mice on days 1, 5, and 14 by ip injections of 100 μg OVA emulsified in 20 mg of aluminum hydroxide and we challenged mice with 1.5 mg OVA. Mice received orally Dp44mT (1, 10, 100 μg/kg) or DEX (5 mg/kg) before the OVA intranasal challenge for 10 days. **(a)** The number of the nasal rubs that occurred in the 10 min after the OVA intranasal challenge. Serum was isolated from blood and then assayed for **(b)** histamine, **(c)** IgE, **(d)** IL-1β, **(e)** TSLP, and **(f)** TNF-α in the serum were measured by the ELISA method. [#]*P* < 0.05, significantly different from the normal control mice. ^{*}*P* < 0.05, significantly different from the OVA-challenged mice. *N* = 5. *DEX* dexamethasone.

Western Blot Analysis

Western blot analysis was used for protein extracts of intranasal tissues. Western blotting was performed as previously described [12].

Caspase-1 Assay

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

Infiltration of Eosinophils and Mast Cells

Intranasal tissues were immersed in 10% neutral formaldehyde. Tissues were embedded in paraffin and sectioned anterior to posterior at 4- μ m thickness. Appearance of inflammatory cell infiltration was observed in sections stained by hematoxylin and eosin (H&E; for eosinophils) and alcian blue and safranin O (A&S; for mast cells). All tissue sections were investigated blindly with respect to the source of the tissue, and counts were determined at five different intranasal areas for each group.

Statistical Analysis

Treatment effects were analyzed using one-way ANOVA followed by Turkey and the independent *t* test. All data are expressed as the mean $\pm$ standard deviation (SD). Statistical significance was accepted for *P* values < 0.05.

RESULTS

Alleviate Effect of Dp44mT on OVA-Induced AR Biomarkers in Serum

The previous our study showed that Dp44mT has an antiinflammatory effect by inhibition of mast cell activation [10]. Here, we investigated whether the Dp44mT has an antiallergic inflammatory effect in AR mouse model. As shown in Fig. 1, administration of Dp44mT significantly reduced the numbers of rubs compared with the OVA-challenged AR group (Fig. 1a; *P* < 0.05). In sequence, we analyzed the levels of histamine and IgE in the serum. The levels of serum histamine and IgE released by OVA challenge were significantly lowered by the administration of Dp44mT (Fig. 1b, c; *P* < 0.05). Also, the levels of serum IL-1 β ,

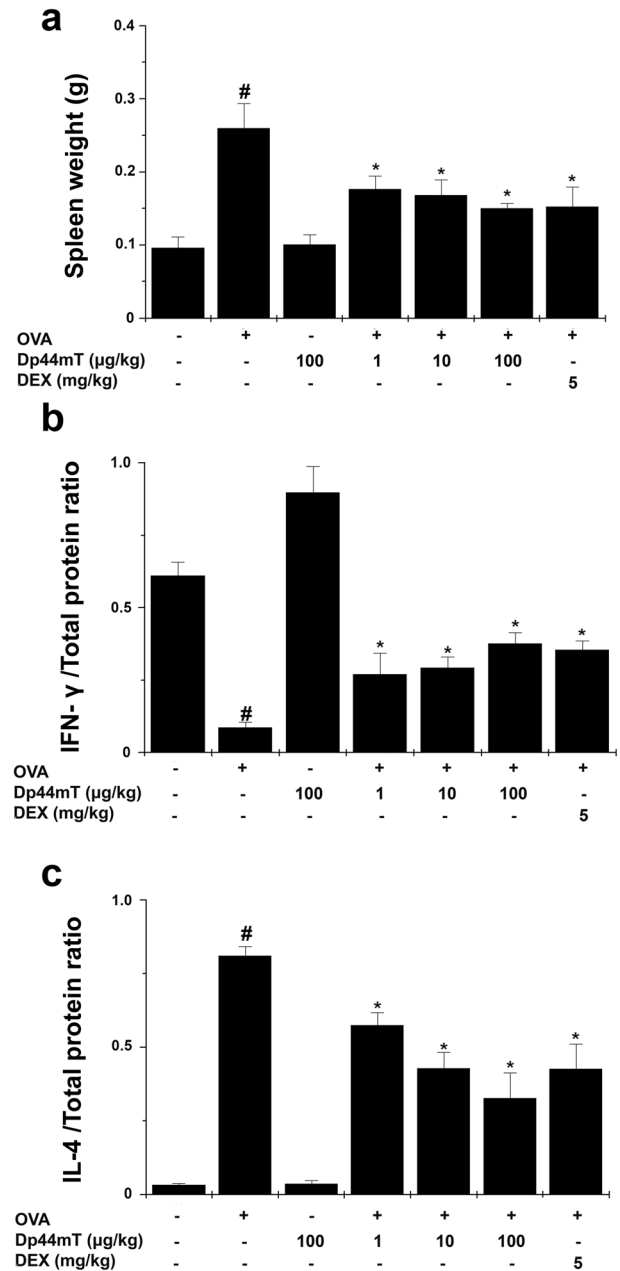


Fig. 2. Alleviate effect of Dp44mT on OVA-induced Th1/Th2 cytokine imbalance in spleen. **a** Spleen weight, **b** IFN- γ , and **c** IL-4 were measured by the ELISA method. All parameters measured in the spleen homogenate were presented as a ratio to the total protein levels of intranasal tissues. [#]*P* < 0.05, significantly different from the normal control mice. ^{*}*P* < 0.05, significantly different from the OVA-challenged mice. *N* = 5. DEX dexamethasone.

TSLP, and TNF- α increased by OVA challenge were significantly lowered by the administration of Dp44mT (Fig. 1d–f; *P* < 0.05). However, administration of

Dp44mT alone did not affect the rub scoring, histamine, IgE, IL-1 β , TSLP, and TNF- α compared with the normal control (Fig. 1). Administration of DEX

(5 mg/kg) was used as a positive control and modulated the AR clinical symptom and various AR-related biomarkers (Fig. 1; $P < 0.05$).

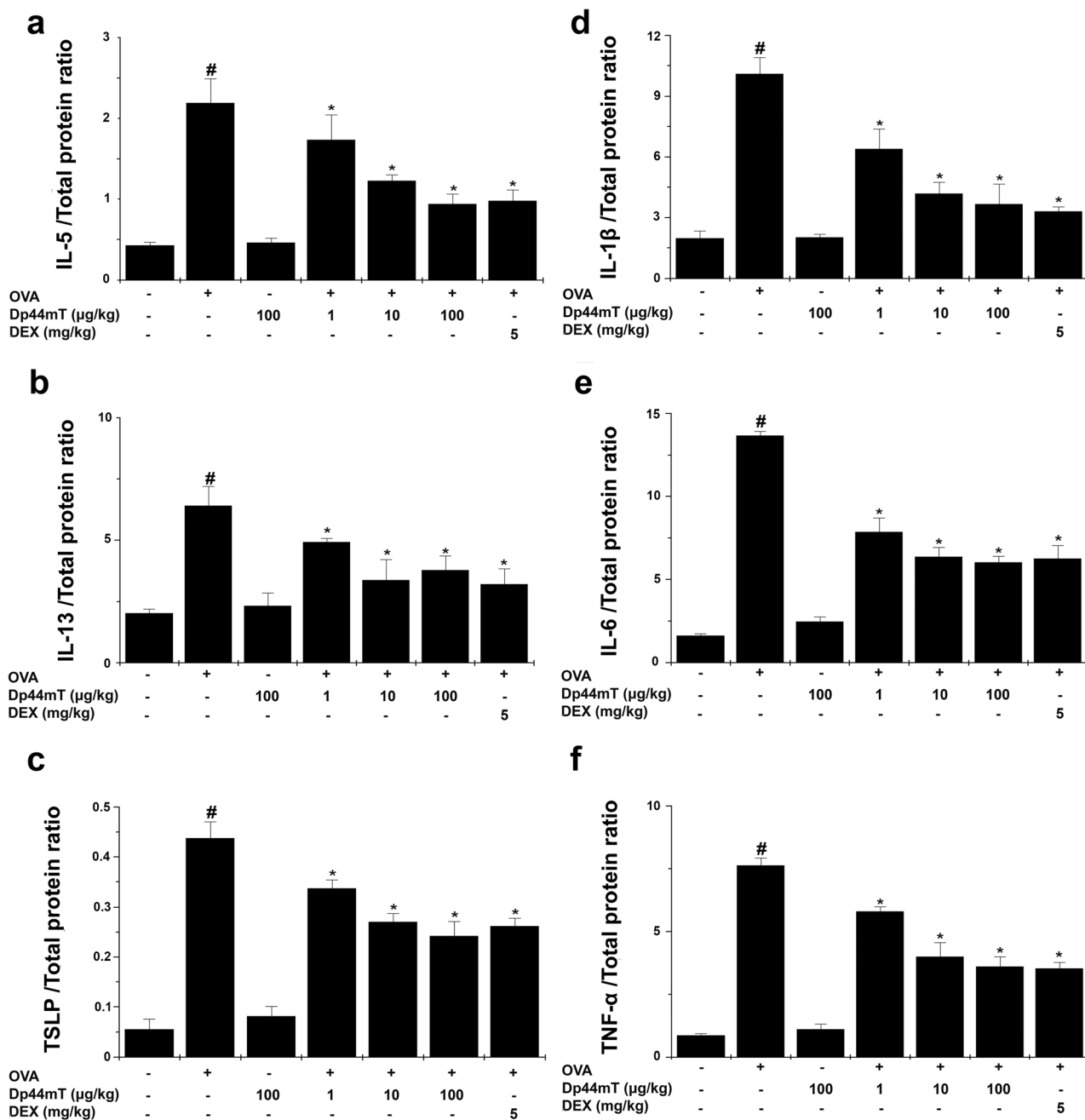


Fig. 3. Alleviate effect of Dp44mT on OVA-induced AR biomarkers in intranasal tissues. **a** IL-5, **b** IL-13, **c** TSLP, **d** IL-1 β , **e** IL-6, and **f** TNF- α were measured by the ELISA method. All parameters measured in intranasal tissue homogenate were presented as a ratio to the total protein levels of the intranasal tissue. [#] $P < 0.05$, significantly different from the normal control mice. ^{*} $P < 0.05$, significantly different from the OVA-challenged mice. $N = 5$. DEX dexamethasone.

Alleviate Effect of Dp44mT on OVA-Induced Th1/Th2 Cytokine Imbalance in Spleen

Spleen is a central organ in regulating the inflammation-related immune [13]. IFN- γ plays a key role in primary immune responses [14]. IL-4 as Th2 cytokine has been known to regulate IgE-mediated allergy [15]. Therefore, we estimated whether administration of Dp44mT could influence balance of Th1/Th2 cytokine in spleen. As shown in Fig. 2a, b, spleen weight and IL-4 levels increased by OVA challenge were significantly lowered by the administration of Dp44mT or dexamethasone. However, IFN- γ levels decreased by OVA challenge were significantly increased by the administration of Dp44mT or dexamethasone (Fig. 2c; $P < 0.05$).

Alleviate Effect of Dp44mT on OVA-Induced AR Biomarkers in Intranasal Tissues

Th2 cytokines including IL-5 and IL-13 have been known to exaggerate allergic reactions [15].

Therefore, we investigated whether administration of Dp44mT could alleviate levels of Th2 cytokines in the intranasal tissues. The levels of IL-5 and IL-13 increased by OVA challenge were significantly lowered by the administration of Dp44mT (Fig. 3a, b; $P < 0.05$). Inflammatory cytokines, such as IL-1 β , TSLP, TNF- α , and IL-6, are involved in AR inflammatory reactions [16]. The levels of inflammatory cytokines, including TSLP, IL-1 β , TNF- α , and IL-6, increased by OVA challenge were significantly lowered by the administration of Dp44mT (Fig. 3c–f; $P < 0.05$). In sequence, we also measured the mRNA levels of TSLP, IL-1 β , TNF- α , and IL-6 in the intranasal tissues. The mRNA levels of these inflammatory cytokines in the intranasal tissues were significantly lowered by the administration of Dp44mT (Fig. 4; $P < 0.05$). Dexamethasone significantly reduced the levels of Th2 and inflammatory cytokines (Figs. 3 and 4; $P < 0.05$).

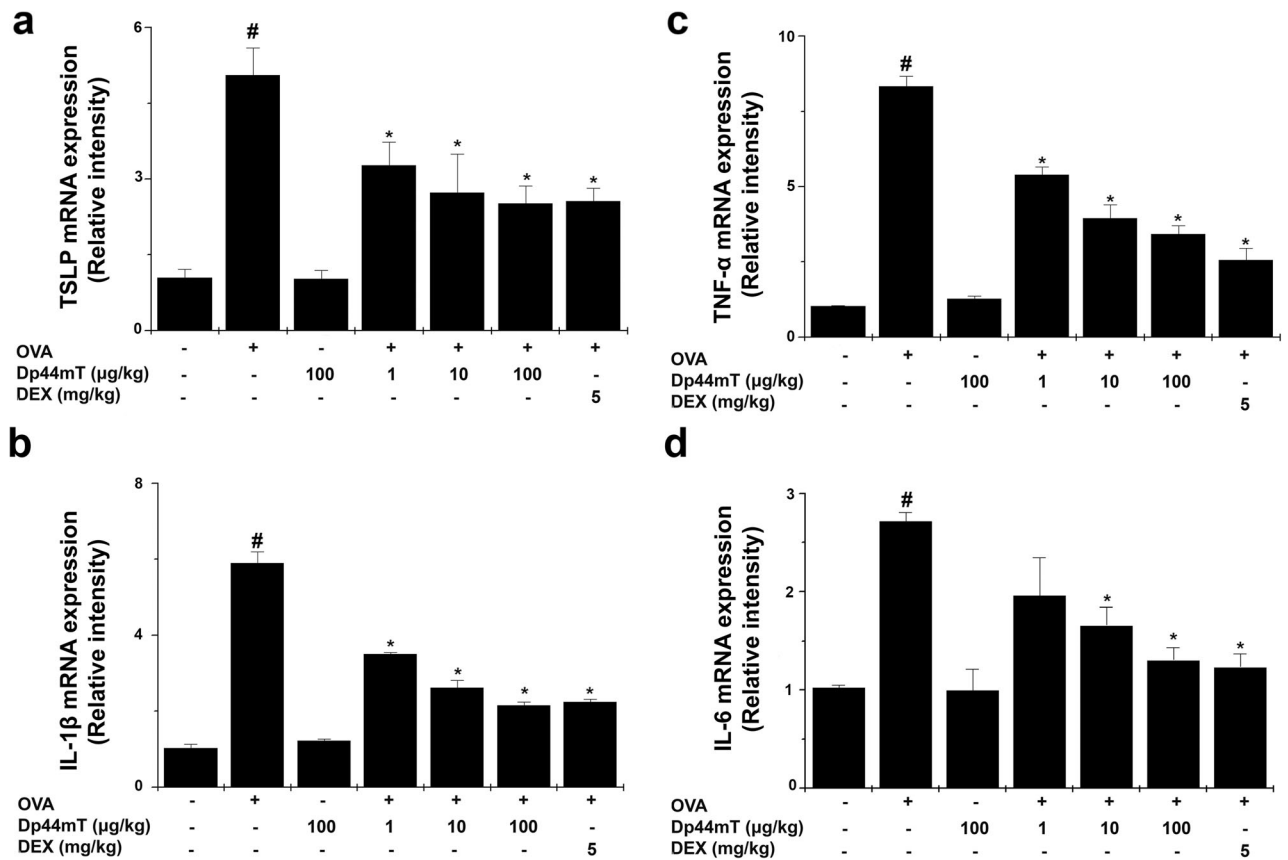


Fig. 4. Alleviate effect of Dp44mT on OVA-induced inflammatory cytokine mRNA levels in intranasal tissues. Messenger RNA levels of **a** TSLP, **b** IL-1 β , **c** TNF- α , and **d** IL-6 were measured using the quantitative real time-PCR method. # $P < 0.05$, significantly different from the normal control mice. * $P < 0.05$, significantly different from the OVA-challenged mice. $N = 5$. DEX dexamethasone.

Alleviate Effect of Dp44mT on OVA-Induced Caspase-1 Activities in Intranasal Tissues

Activation of caspase-1 can induce inflammatory reaction of AR [17]. Also, caspase-1 contributes to the secretion of IL-1 β by cleaving IL-1 β from precursors [18]. Accordingly, we measured the caspase-1 activity in the intranasal tissues using a caspase-1 assay kit. The activation of caspase-1 increased by OVA challenge was significantly lowered by the administration of Dp44mT (Fig. 5a; $P < 0.05$). The protein levels of caspase-1 active form were also analyzed using Western blotting. Similar to the Fig. 5a data, administration of Dp44mT decreased the protein levels of caspase-1 (Fig. 5b). In addition, dexamethasone significantly reduced the activity of caspase-1 (Fig. 5; $P < 0.05$).

ALLEVIATE EFFECT OF DP44MT ON OVA-INDUCED EOSINOPHILS AND MAST CELL INFILTRATION IN INTRANASAL TISSUES

From the above the results, we found that Dp44mT decreased the levels of inflammatory cytokines in AR mice. Inflammatory cytokines induce the infiltration of immune cells *via* increasing chemokines and adhesion molecules [19, 20]. Chemokine, MIP-2, and adhesion molecules, ICAM-1, are important factors in allergic inflammation [19, 20], and induce the infiltrate of eosinophils and mast cells [20, 21]. Thus, we investigated whether Dp44mT could alleviate the levels of MIP-2 and ICAM-1. The levels of MIP-2 and ICAM-1 increased by OVA challenge were significantly lowered by the administration of Dp44mT or dexamethasone (Fig. 6a,

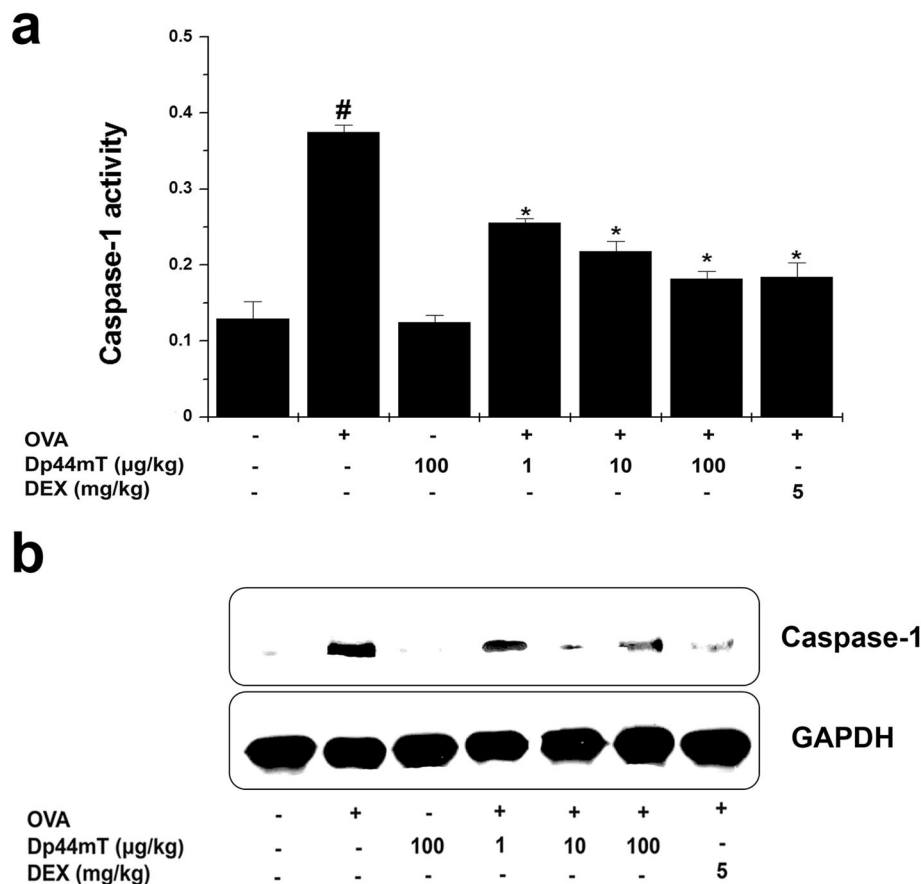


Fig. 5. Alleviate effect of Dp44mT on OVA-induced caspase-1 activation in intranasal tissues. **a** Caspase-1 activity in the intranasal tissues was measured by a caspase-1 assay kit. **b** The protein levels of caspase-1 were assayed by western blotting. Results are representative of three independent experiments. [#] $P < 0.05$, significantly different from the normal control mice. ^{*} $P < 0.05$, significantly different from the OVA-challenged mice. $N = 5$. *DEX* dexamethasone.

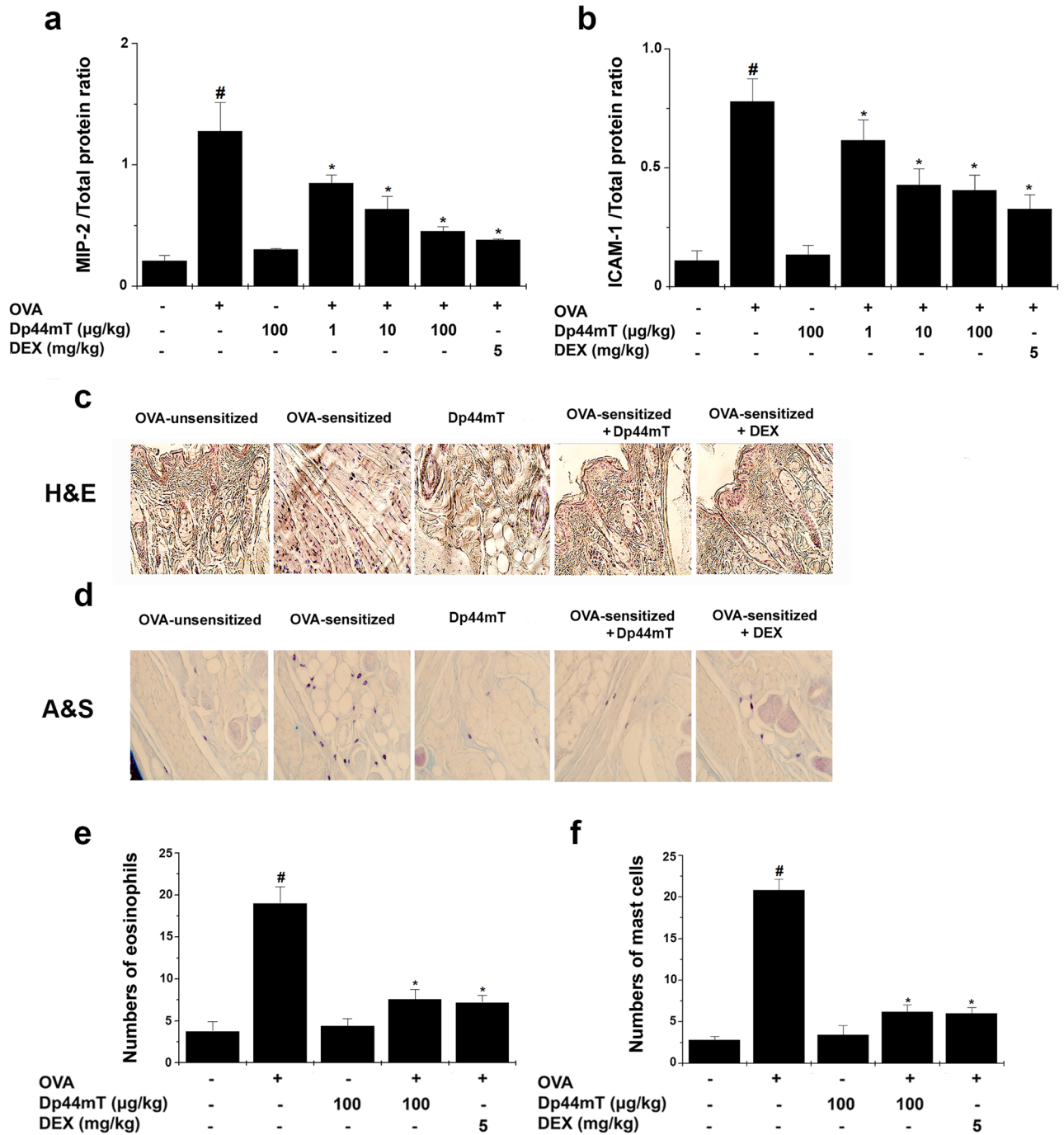


Fig. 6. Alleviate effect of Dp44mT on OVA-induced infiltration of eosinophils and mast cells in intranasal tissues. Levels of **a** MIP-2 and **b** ICAM-1 in intranasal tissues were measured by the ELISA method. All parameters measured in the tissue homogenate were presented as a ratio to the total protein levels of intranasal tissues. **c** Intranasal tissues were stained with H&E (for eosinophils) and **d** a&S (for mast cells) (original magnification $\times 400$, scale bar = 100 μm). The numbers of **e** eosinophils and **f** 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 $\pm$ SD. [#] $P < 0.05$, significantly different from the normal control mice. ^{*} $P < 0.05$, significantly different from the OVA-challenged mice. $N = 5$. DEX dexamethasone.

b; $P < 0.05$). In sequence, we examined the alleviate effects of Dp44mT on infiltration of eosinophils and mast cells into intranasal tissues. The numbers of eosinophils and mast cells increased by OVA challenge were significantly lowered by the administration of Dp44mT or dexamethasone (Fig. 6c–f; $P < 0.05$).

DISCUSSION

In this study, we showed that Dp44mT suppressed the levels of histamine, IgE, Th2 cytokines, and inflammatory cytokines and also suppressed the activation of caspase-1 and infiltration of eosinophils and mast cells. So, we suggested that Dp44mT has potential antiinflammatory effects on OVA-induced AR model.

AR is an inflammatory disorder of the nasal mucosa tissues by interaction of IgE and allergen [22]. Mast cells are involved in early-phase inflammatory response such as AR, which is marked by degranulation and the production of pro-inflammatory mediators, including histamine [22]. Histamine is a major mediator of AR and triggers the mucous glands causing the secretion of mucous [22]. Also, AR is characterized by disequilibrium of Th cells with a predominance of Th2 cytokines including IL-4, IL-5, and IL-13, but normal levels of Th1 cytokines including the IFN- γ [23]. IFN- γ has antiinflammatory properties and is essential in order to control the inflammatory conditions [24, 25]. Th2 cytokines, IL-4, IL-5, and IL-13 are produced by Th2 cells, basophils, eosinophils, or mast cells and elevated under allergen challenge [26, 27]. In this study, we showed that administration of Dp44mT alleviated the AR clinical symptoms and AR biomarkers. In addition, administration of Dp44mT markedly increased the Th1 cytokine and decreased Th2 cytokines in the OVA-induced AR mice. Therefore, these results demonstrated that Dp44mT has an antiinflammatory effect through the regulation of Th1/Th2 balance in AR mice.

IL-1 β and TNF- α are pro-inflammatory cytokines and interact with other cytokines to modulate allergic inflammation [28]. And TSLP is a key factor in allergen-induced inflammatory reactions [29]. In the OVA-induced allergy model, TSLP is enhanced and correlated with the number of infiltrated inflammatory cell [29]. Levels of TSLP are increased by IL-1 β and TNF- α in various immune cell types and tissues [30]. The other inflammatory cytokine, IL-6, is a regulator

of pulmonary inflammation and a crucial component of biologic homeostasis [28]. The production of IL-1 β and TSLP was increased by caspase-1, and the caspase-1 activation was upregulated in allergic inflammatory reaction [10]. Inhibition of allergic inflammatory reactions was found in caspase-1-deficient AR mice [31]. In addition, caspase-1 activation or increased IL-1 β promotes the allergic inflammatory reactions by infiltration of eosinophils into intranasal tissues [32]. In this study, we showed that administration of Dp44mT markedly alleviated the levels of inflammatory cytokines and activation of caspase-1 in the OVA-challenged AR mice. Therefore, these results demonstrated that Dp44mT has an antiinflammatory effect through the regulation of inflammatory cytokines and caspase-1 in AR animal model.

The pathophysiology of AR is associated with the presence of inflammatory cells including eosinophils and mast cells in intranasal tissues [22]. Also, AR is characterized by an inflammatory infiltrate made up of immune cells including the mast cells, eosinophils, and basophils [22]. The role of intranasal mast cells in the immediate hypersensitivity reaction of AR patients has been well verified [33]. In sequence, the eosinophil is a multifunctional leukocyte involved in inflammatory reactions and immune modulating responses [34]. Also, eosinophils are considered to constitute a hallmark of inflammation of AR [35]. Infiltration of inflammatory cells was induced by MIP-2 and ICAM-1 [36]. MIP-2 activates eosinophils in allergic inflammatory process and is rapidly upregulated at inflammatory tissues [37, 38]. In addition, MIP-2 and ICAM-1 induce inflammatory tissue destruction in a wide variety of allergy [39]. In this study, we showed that administration of Dp44mT markedly alleviates the levels of ICAM-1 and MIP-2 and the infiltrations of eosinophils and mast cells in the OVA-challenged AR mice. Therefore, these results demonstrated that Dp44mT has an antiinflammatory effect through alleviating the precipitation of inflammatory cells and levels of chemokine and adhesion molecules in AR animal model.

In this study, we demonstrated that Dp44mT suppressed the levels of histamine, IgE, Th2 cytokines, and inflammatory cytokines and also suppressed the activation of caspase-1 and infiltration of eosinophils and mast cells. In conclusion, we suggested that Dp44mT has a potential antiinflammatory effect on OVA-induced AR mice.

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COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed in studies involving murine tissues were in accordance with the ethical standards of the institution and/or national research.

Conflict of Interest. The authors declare that they have no conflicts of interest.

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