



Immunopharmacology and inflammation

Anti-allergic and anti-inflammatory effects of the Bcl-2 inhibitor ABT-737 on experimental allergic rhinitis models

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ABSTRACT

The anti-cancer agent ABT-737 is designed specifically to inhibit anti-apoptotic proteins of the Bcl-2 family. The development of cancer has long been associated with inflammation. Here, we assess the anti-allergic and anti-inflammatory effects and the underlying molecular mechanisms of ABT-737 on allergic rhinitis (AR) using in vitro and in vivo models. In the in vitro model, the ABT-737 treatment diminished the levels of several inflammatory cytokines in this case vascular endothelial growth factor (VEGF), thymic stromal lymphopoietin (TSLP), interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α (TNF- α) by inhibiting caspase-1 and NF- κ B activation in an activated human mast cell line, here HMC-1 cells. These mechanistic observations were validated in ovalbumin-sensitized AR mice. In an AR animal model, ABT-737 significantly diminished clinical symptoms of AR and the levels of AR biomarkers, specifically IgE, histamine, hypoxia-inducible factor-1 α , VEGF, TSLP, IL-1 β , IL-4, IL-5, IL-6, IL-13, TNF- α , intercellular adhesion molecule-1, and macrophage inflammatory protein-2. In addition, ABT-737 reduced the degree of caspase-1 activity compared to that in AR mice. Simultaneously, ABT-737 diminished the recruitment of mast cells and eosinophils into nasal mucosa tissues compared to the levels in AR mice. In conclusion, we identified new anti-allergic and anti-inflammatory effects of ABT-737. These results imply that ABT-737 can ameliorate allergic inflammatory diseases such as AR.

1. Introduction

Allergic rhinitis (AR), widely known as an inflammatory disorder affecting nasal mucosa tissues, is characterized by intense activation and infiltration of inflammatory cells, including mast cells, eosinophils, and the other types (Pawankar et al., 2011). Although AR is not life-threatening disease, it deteriorates the quality of life and places an economic burden on those suffering from it due to its clinical symptoms, which include sneezing, rhinorrhea, itching, and nasal congestion (Ramirez-Jimenez et al., 2012). These clinical conditions are widely known to be induced by inflammatory mediators including histamine, leukotrienes, and inflammatory cytokines, which are secreted by mast cells, eosinophils and basophils (Pawankar et al., 2011).

The Bcl-2 protein is an inhibitor of apoptosis that has been recognized to play an important role as well in a wide range of biological processes, including autophagy, DNA repair, and drug resistance (Heath-Engel et al., 2008; Levine et al., 2008). In addition, Bcl-2 plays an important role in inflammation by increasing the expression levels of inflammatory mediators (Chand et al., 2018). As a key mediator of angiogenesis and vascular permeability, hypoxia-inducible factor (HIF)-

1 α also promotes the expression of inflammatory mediators, including vascular endothelial growth factor (VEGF), its expression is increased after an allergic challenge in the nasal mucosa, and it increases allergic inflammatory responses (Benson et al., 2002; Sumbayev and Nicholas, 2010; Zhou et al., 2012). Previous work reported that HIF-1 α and VEGF play pathogenic roles in allergic inflammation, and reductions of HIF-1 α and VEGF expression levels lead to anti-allergic effects (Benson et al., 2002). The expressions of HIF-1 α and VEGF are induced by Bcl-2 (Trisciuglio et al., 2010). Ottina et al. (2015) showed that apoptosis-inducing drugs have potential as anti-inflammatory agents. Accordingly, we hypothesized that the inhibition of Bcl-2 regulates AR through a reduction of the inflammatory mediators HIF-1 α , VEGF, and inflammatory cytokines. In this study, we investigated the anti-allergic and anti-inflammatory effects of a Bcl-2 inhibitor, ABT-737, on the activated human mast cell line, HMC-1 cells and in an AR animal models.

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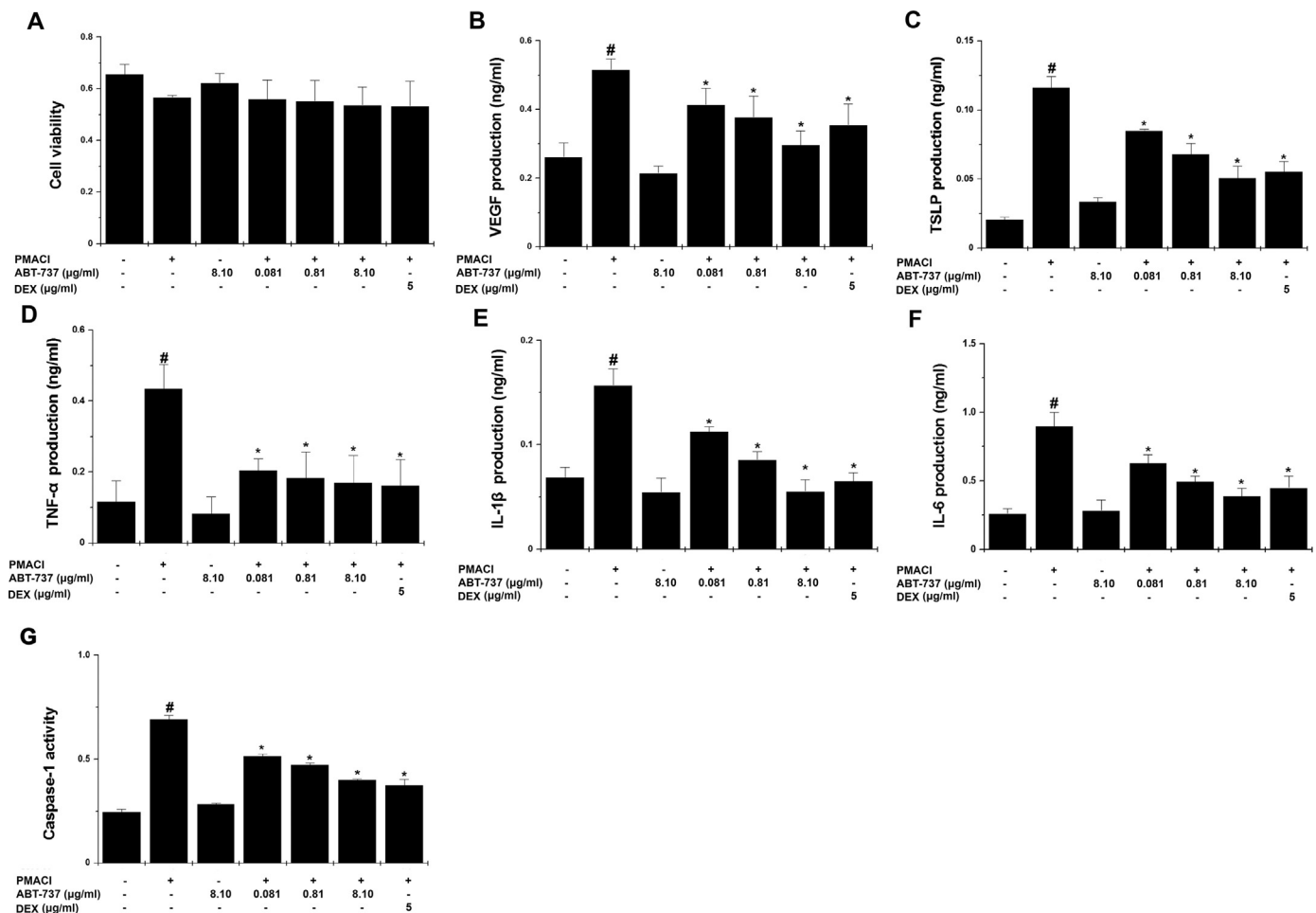


Fig. 1. Suppressive effects of ABT-737 on PMACI-induced cytokine expression and caspase-1 activation in HMC-1 cells. HMC-1 cells were pretreated with ABT-737 (0.081, 0.81, 8.10 μg/ml) or DEX (5 μg/ml) for 1 h and then stimulated with PMACI for 8 h. (A) Cell viability was evaluated by a MTT assay. Levels of (B) VEGF (C) TSLP (D) TNF-α, (E) IL-1β, and (F) IL-6 in the cell supernatants were measured by the ELISA method. (G) HMC-1 cells were pretreated with ABT-737 (0.081, 0.81, 8.10 μg/ml) or DEX (5 μg/ml) for 1 h and then stimulated with PMACI for 2 h. Caspase-1 activities were analyzed with caspase-1 colorimetric assay kit. [#]*P* < 0.05; significantly different from un-stimulated cells. **P* < 0.05; significantly different from PMACI-stimulated cells. PMACI, PMA and A23187; DEX, dexamethasone.

2. Material and methods

2.1. Materials

ABT-737 was purchased from APEX BIO (Boston, MA, USA). Dexamethasone (DEX), Dimethyl sulfoxide (DMSO), ovalbumin (OVA), phorbol 12-myristate 13-acetate (PMA), A23187, O-phthalaldehyde, avidin peroxidase (AP), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), bicinchoninic acid (BCA), and other reagents were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS), iscove's modified dulbecco's medium (IMDM), and streptomycin were purchased from Gibco BRL (Grand Island, NY, USA). Anti-mouse IgE/VEGF/TSLP/TNF-α/IL-1β/IL-4/IL-5/IL-6/IL-13/MIP-2/ICAM-1 antibodies (Ab), biotinylated anti-mouse IgE/VEGF/TSLP/TNF-α/IL-1β/IL-4/IL-5/IL-6/IL-13/MIP-2/ICAM-1Ab, recombinant mouse IgE/VEGF/TSLP/TNF-α/IL-1β/IL-4/IL-5/IL-6/IL-13/MIP-2/ICAM-1, anti-human VEGF/TSLP/TNF-α/IL-1β/IL-6 Ab, biotinylated anti-human VEGF/TSLP/TNF-α/IL-1β/IL-6 Ab, and recombinant human VEGF/TSLP/TNF-α/IL-1β/IL-6 were purchased from Pharmingen (Sandiego, CA, USA). Ab for HIF-1α, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), NF-κB, poly ADP ribose polymerase (PARP), phosphorylated (p)IκBα, caspase-1, and α-Tubulin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The caspase-1 assay kit was supplied by R&D Systems Inc. (Minneapolis, MN, USA). Caspase-1 inhibitor was purchased from Merck Millipore (Burlington, MA, USA).

2.2. Culture of HMC-1 cells

HMC-1 cell was generously provided by Eichi Morri (Osaka University, Japan). HMC-1 cells were grown 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. ABT-737 was prepared by dissolving with DMSO and diluted with phosphate-buffered saline (PBS) with reference to the study of Mason et al. (2009). HMC-1 cells (3×10^5 cells/ml) were treated with ABT-737 (0.081, 0.81, and 8.1 μg/ml) or DEX (5 μg/ml) for 1 h prior to stimulation with PMA and calcium ionophore A23187 (PMACI).

2.3. Cell viability assay

To estimate the cell viability, the MTT assay was performed. Briefly, cells were treated with ABT-737 or DEX. 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. The plates were read at 540 nm.

2.4. Enzyme-linked immunosorbent assay (ELISA)

HMC-1 cells (3×10^5) were treated with ABT-737 (0.081, 0.81, and 8.10 μg/ml) or DEX (5 μg/ml) for 1 h prior to stimulation with PMACI incubated for 8 h. Cytokines of serum, mucosa, and cell supernatants were measured by the ELISA. The ELISA was performed by coating 96-

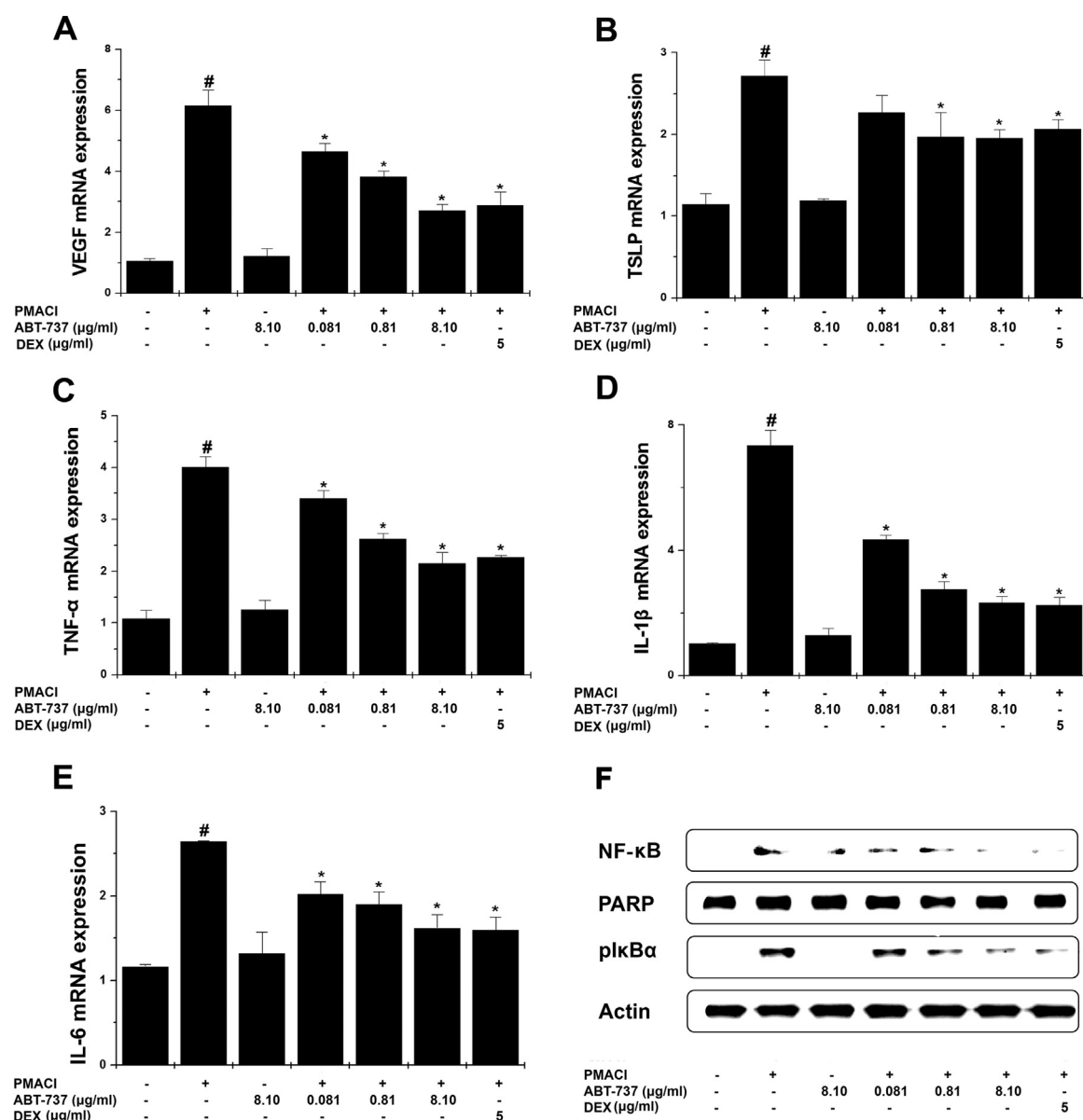


Fig. 2. Suppressive effect of ABT-737 on PMACI-induced cytokine mRNA expression and NF-κB activation in HMC-1 cells. HMC-1 cells were pretreated with ABT-737 (0.081, 0.81, 8.10 μg/ml) or DEX (5 μg/ml) for 1 h and then stimulated with PMACI for 6 h. Messenger RNA for (A) VEGF, (B) TSLP, (C) TNF-α, (D) IL-1β, and (E) IL-6 was measured using the quantitative real time-PCR method. (F) HMC-1 cells were pretreated with ABT-737 (0.081, 0.81, 8.10 μg/ml) or DEX (5 μg/ml) for 1 h and then stimulated with PMACI for 2 h. The levels of NF-κB and pIκBα were analyzed by Western blotting. Results are representative of three independent experiments. # $P < 0.05$; significantly different from un-stimulated cells. * $P < 0.05$; significantly different from PMACI-stimulated cells. PMACI, PMA and A23187; DEX, dexamethasone.

well plates with 1 μg/well of capture Ab. Before the subsequent steps in the assay, the coated plates were washed twice with 1 × PBS containing 0.05% tween-20 (PBST). All reagents and coated wells used in this assay were incubated for 2 h at room temperature. The standard curve was generated from the known concentrations of cytokine, as provided by the manufacturer. After exposure to the medium, the assay plates were exposed sequentially to each of the biotin-conjugated secondary Ab, AP, and substrate solution containing 30% H₂O₂. The plates were read at 405 nm. Appropriate specificity controls were included, and all samples were run in duplicate. Cytokine levels in the nasal mucosa were divided according to the total protein. The protein was estimated using the BCA method with the BCA protein assay kit (Pierce, Rockford, IL, USA).

2.5. RNA isolation and quantitative real-time PCR

RNA isolation and quantitative real-time PCR were accomplished as

previously described (Kim et al., 2016). Target mRNA levels were normalized versus GAPDH. Data were analyzed using the $\Delta\Delta CT$ method.

2.6. OVA-induced AR animal model

We maintained 4-week-old female BALB/c (Charles River Technology) mice under pathogen-free conditions. Mouse care and experimental procedures were performed under approval from the animal care committee of Kyung Hee University [KHUASP (SE)-17-040]. The mice were sensitized on days 1, 5, and 14 by intraperitoneal (i.p.) injection of 100 μg OVA emulsified and 20 mg aluminum hydroxide (Sigma) in a 100 μl PBS and challenged intranasally with 1.5 mg OVA in 2 μl PBS. The mice were challenged intranasally with PBS in a similar manner for the negative control. ABT-737 (0.081, 0.81, 8.10 mg/kg), DEX (0.5 and 5 mg/kg), ABT-737 (0.81 mg/kg) and DEX (0.5 mg/kg),

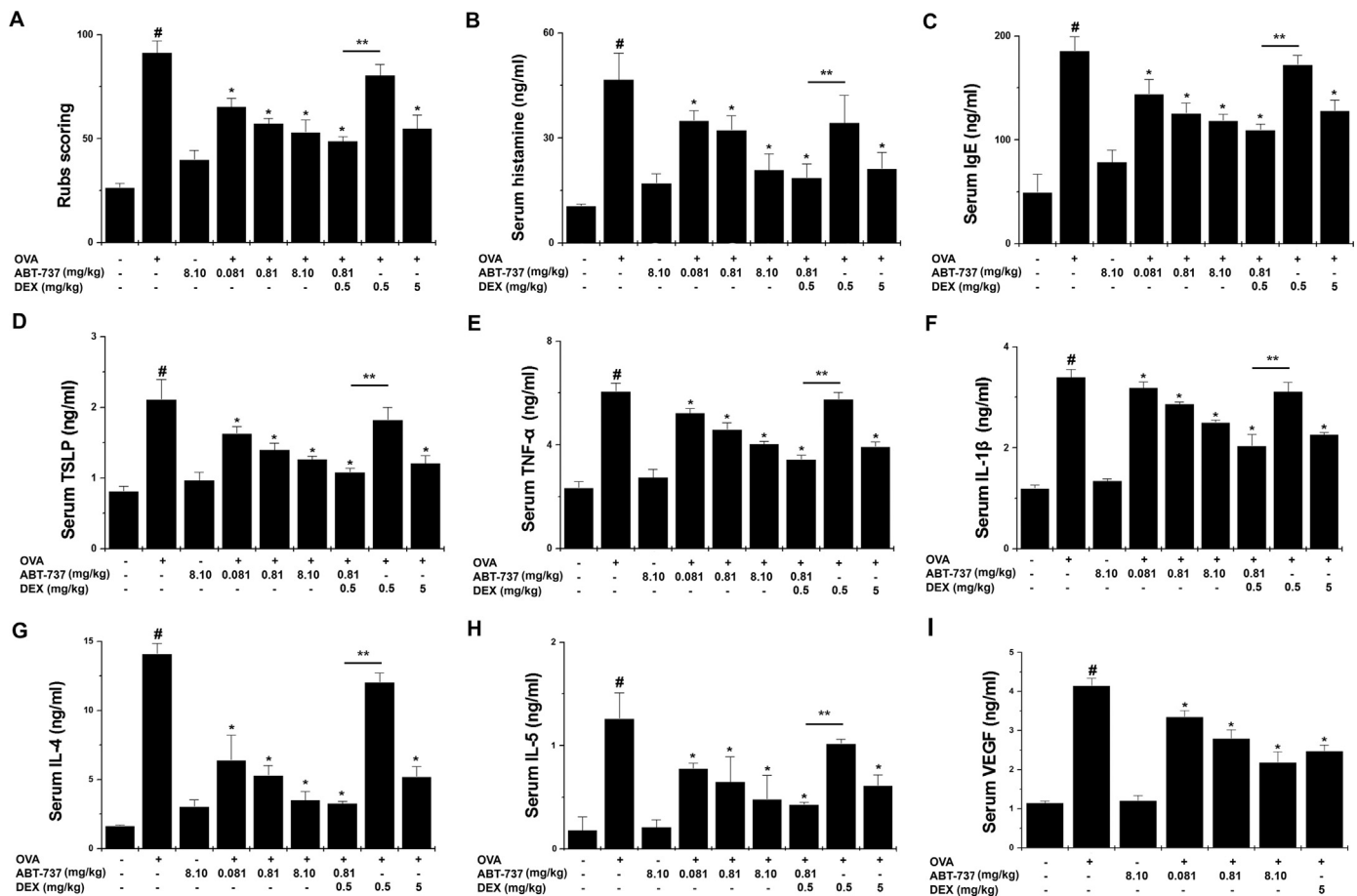


Fig. 3. Suppressive effect of ABT-737 on the clinical symptoms and AR biomarkers in the serum of AR mice. We sensitized mice on days 1, 5, and 14 by ip injections of 100 μ g OVA emulsified in 20 mg of aluminum hydroxide and we challenged mice with 1.5 mg OVA. Mice received orally ABT-737 (0.081, 0.81, 8.10 mg/kg), DEX (0.5, 5 mg/kg), or ABT-737 (0.81 mg/kg) and DEX (0.5 mg/kg) before the intranasal OVA challenge for ten days. (A) The number of the nasal rubs that occurred during the 10 min after the OVA intranasal provocation. Serum was isolated from blood and then assayed for (B) histamine, (C) IgE, (D) TSLP, (E) TNF- α , (F) IL-1 β , (G) IL-4, (H) IL-5, and (I) VEGF in the serum were measured by the ELISA method. [#] $P < 0.05$; significantly different from OVA-unsensitized mice. ^{*} $P < 0.05$; significantly different from OVA-sensitized mice. ^{**} $P < 0.05$; significantly different from the DEX 0.5 mg/kg-treated mice. $N = 5$. DEX, dexamethasone.

caspase-1 inhibitor (10 μ M), or a control vehicle (PBS) was orally administered for 10 days before the intranasal (i.n.) OVA challenge. Nasal symptoms were evaluated by counting the number of nasal rubs that occurred in the 10 min after OVA i.n. provocation at the 10 day after the challenge. The number of mice in each group was 5.

2.7. Histamine assay

The histamine was measured from serum according to the manufacturer's specifications using the histamine assay kit supplied by Oxford Biomedical Research (Oxford, MI, USA).

2.8. Western blot analysis

Nasal mucosa tissue extracts were prepared by a detergent lysis procedure. Samples were heated at 95 $^{\circ}$ C for 5 min, and briefly cooled on ice. Following the centrifugation at 15,000g for 10 min, 50 μ g aliquots were resolved by 10% SDS-PAGE. The resolved proteins were electrotransferred to nitrocellulose membranes in 25 mM Tris, pH 8.5, 200 mM glycerol, 20% methanol at 25 V. Blots were blocked for at least 2 h with PBST containing 5% nonfat dry milk and then incubated with primary Ab for 1 h at room temperature. Blots were developed by peroxidase-conjugated secondary Ab, and proteins were visualized by enhanced chemiluminescence procedures (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions.

2.9. Caspase-1 assay

Caspase-1 activity was measured according to the manufacturer's specifications using a caspase assay kit (R & D system). Equal amounts of the total protein were quantified by a BCA protein quantification kit (Sigma) in each lysate. Catalytic activity of caspase-1 from the tissue extracts was measured by the proteolytic cleavage of WEHD-pNA (Biovision, Inc. California, USA). The plates were read at 405 nm.

2.10. Histological examination

Tissue samples were immediately fixed with 10% formaldehyde and embedded in paraffin. The sections of the nasal mucosa sample were 4 μ m thick. Each section was stained with Giemsa (for eosinophils) and alcian blue and safranin O (A&S, for mast cells) after de-waxing and dehydration. The numbers of eosinophils and mast cells on both sides of the septal mucosa were counted. Sections were coded and randomly analyzed by two blinded observers.

2.11. Statistical analysis

In vitro data are representative of three independent experiments conducted in duplicate and are expressed as mean $\pm$ standard errors of means (S.E.M.) and *in vivo* data are represented as the mean $\pm$ S.E.M. The statistical values of the results were performed by an independent *t*-test and an ANOVA with a Tukey's multiple range tests. The results were

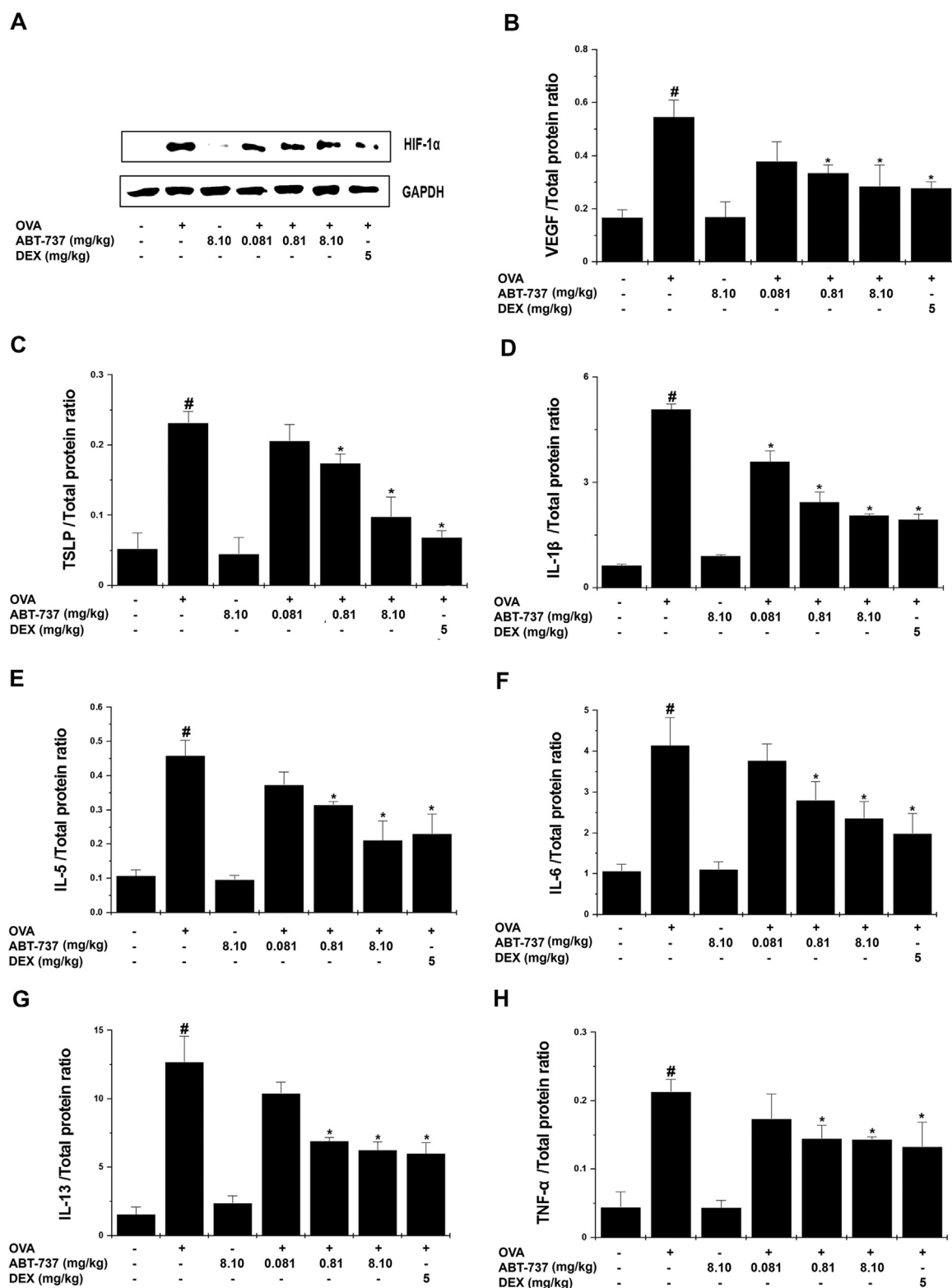


Fig. 4. Suppressive effect of ABT-737 on the protein levels of HIF-1 α and inflammatory cytokines in the nasal mucosa tissues of AR mice. (A) The levels of HIF-1 α were analyzed by Western blotting. Results are representative of three independent experiments. (B) VEGF, (C) TSLP, (D) IL-1 β , (E) IL-5, (F) IL-6, (G) IL-13, and (H) TNF- α 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 OVA-unsensitized mice. ^{*} $P < 0.05$; significantly different from OVA-sensitized mice. $N = 5$. DEX, dexamethasone.

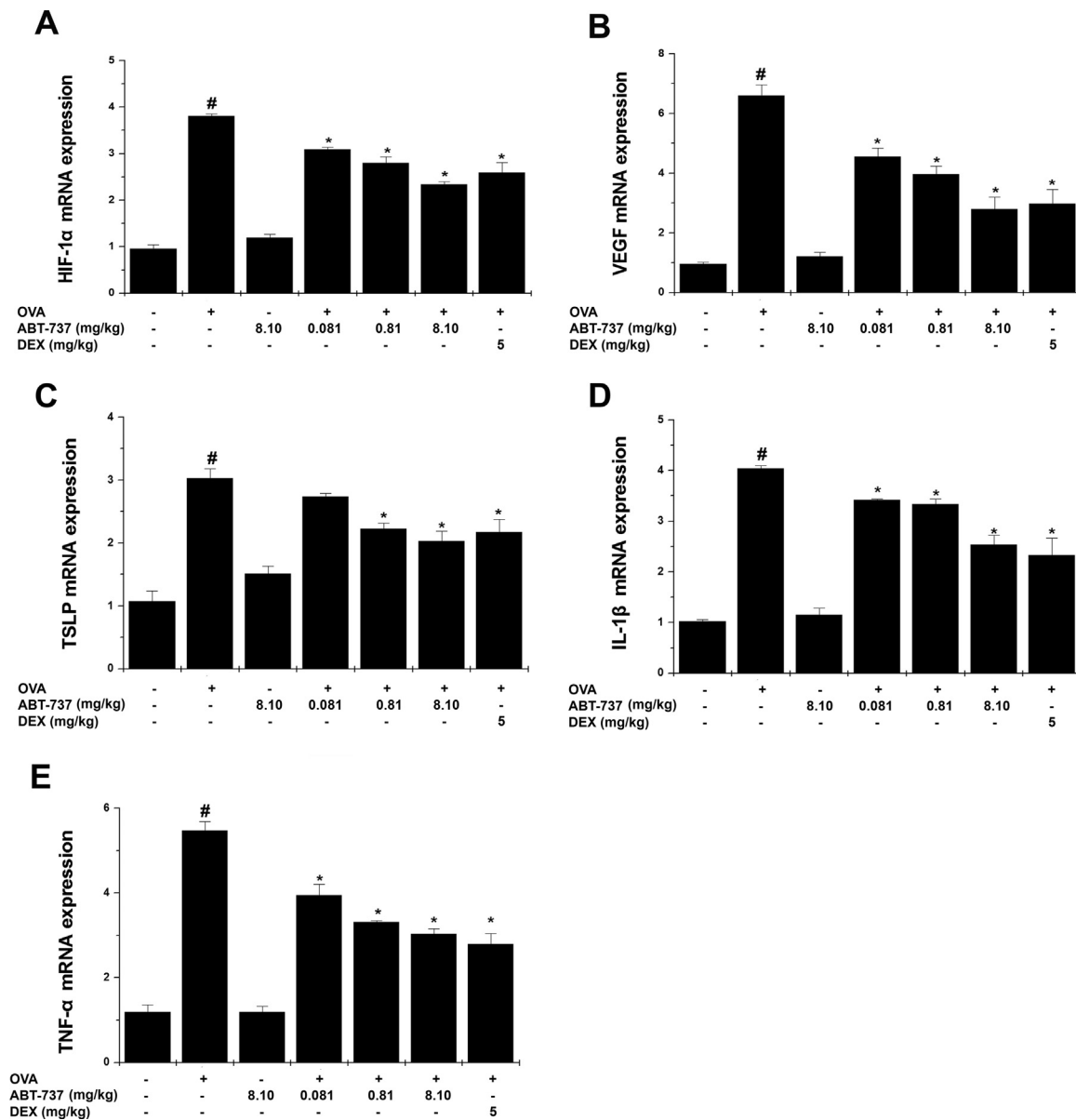


Fig. 5. Suppressive effect of ABT-737 on the mRNA levels of HIF-1 α and inflammatory cytokine in nasal mucosa tissues of AR mice. Messenger RNA levels of (A) HIF-1 α (B) VEGF, (C) TSLP, (D) IL-1 β , and (E) TNF- α were measured using the quantitative real time-PCR method. [#] $P < 0.05$; significantly different from OVA-unsensitized mice. ^{*} $P < 0.05$; significantly different from OVA-sensitized mice. $N = 5$. DEX, dexamethasone.

considered significant at a value of $P < 0.05$.

3. Results

3.1. Suppressive effects of ABT-737 on PMACI-induced cytokine expression, caspase-1, and NF- κ B activation in HMC-1 cells

Initially, we sought to investigate the anti-allergic and anti-inflammatory effects of ABT-737 in an in vitro model. First, we utilized MTT assay to investigate the potential cytotoxicity of ABT-737 in HMC-1 cells. ABT-737 at 0.081–8.1 μ g/ml did not show cytotoxicity (Fig. 1A). Next, we investigated the effect of ABT-737 on PMACI-induced inflammatory cytokine production by means of ELISA method. As shown in Fig. 1B–F, up-regulation of VEGF, TSLP, TNF- α , IL-1 β , and IL-6 by PMACI was significantly down-regulated by an ABT-737 treatment ($P < 0.05$). However, ABT-737 alone did not affect the inflammatory cytokine production compared to a media control (Fig. 1B–F). As shown in Fig. 1B–F, ABT-737 effectively reduced

inflammatory cytokine production in activated HMC-1 cells. The production of inflammatory cytokine was induced by the activation of caspase-1 (Jeong et al., 2018). Thus, we investigated whether ABT-737 could affect the activation of caspase-1 by PMACI stimulation. ABT-737 reduced caspase-1 activation in activated HMC-1 cells (Fig. 1G). Furthermore, we investigated the effect of ABT-737 on PMACI-induced inflammatory cytokine mRNA expression levels by means of quantitative real time-PCR. Treatment of ABT-737 significantly down-regulated the PMACI-induced mRNA expression levels of VEGF, TSLP, TNF- α , IL-1 β , and IL-6 (Fig. 2A–E, $P < 0.05$). Next, we examined whether ABT-737 could regulate the activation of NF- κ B (a transcription factor of inflammatory cytokines) and the phosphorylation of I κ B α (a NF- κ B inhibitor) in PMACI-treated HMC-1 cells. As shown in Fig. 2F, ABT-737 diminished NF- κ B activation and I κ B α phosphorylation caused by PMACI. DEX (5 μ g/ml), used as a positive control, significantly regulated inflammatory cytokine levels and the activation of caspase-1 and NF- κ B (Figs. 1 and 2, $P < 0.05$).

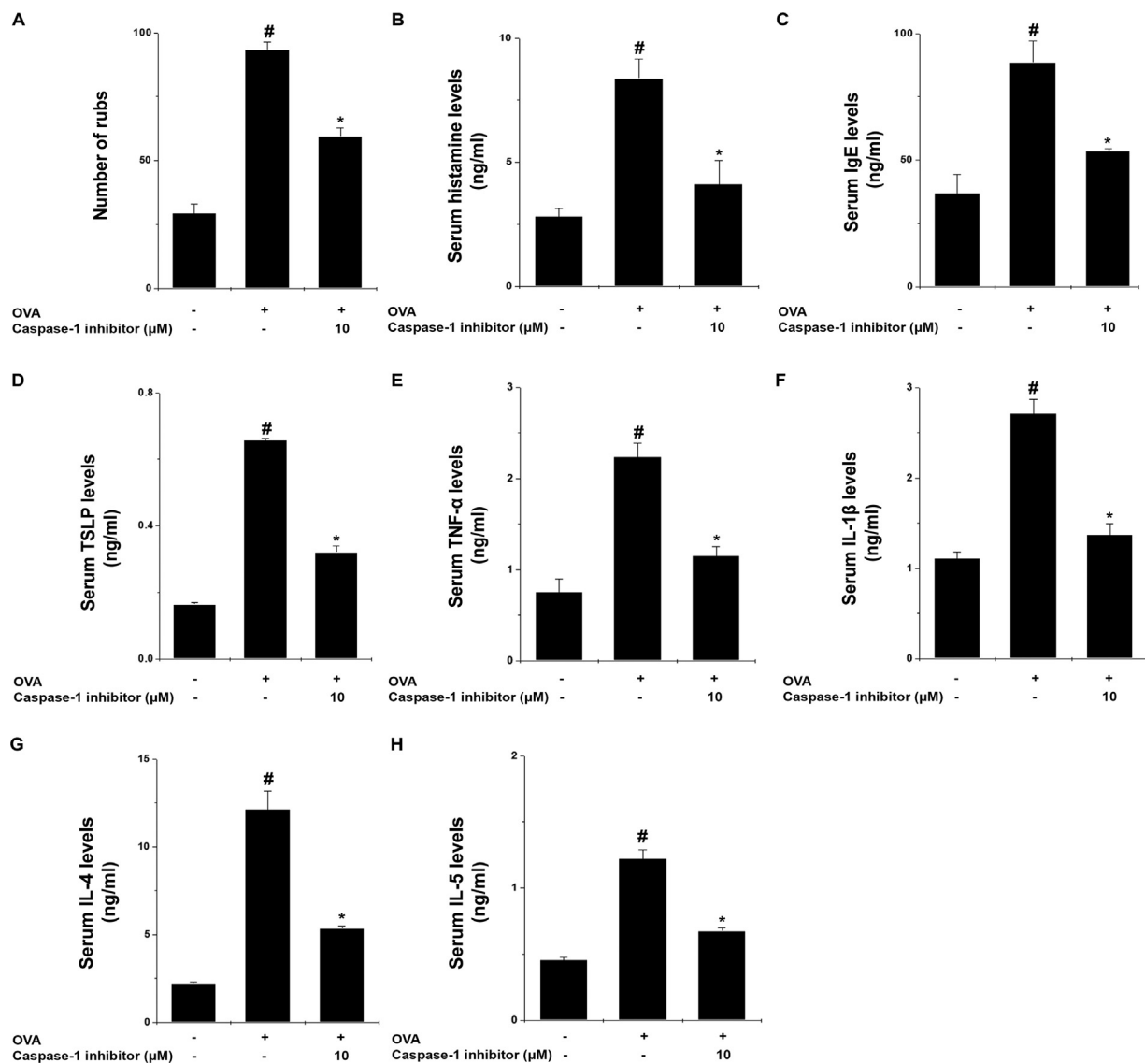


Fig. 6. Suppressive effect of caspase-1 inhibitor in AR mice. (A) The number of the nasal rubs that occurred during the 10 min after the OVA intranasal provocation. Serum was isolated from blood and then assayed for (B) histamine, (C) IgE, (D) TSLP, (E) TNF- α , (F) IL-1 β , (G) IL-4, and (H) IL-5 in the serum were measured by the ELISA method. [#] $P < 0.05$; significantly different from OVA-unsensitized mice. ^{*} $P < 0.05$; significantly different from OVA-sensitized mice. $N = 5$.

3.2. Suppressive effect of ABT-737 on clinical symptoms and AR biomarkers in the serum of AR mice

The mechanistic observations of ABT-737 were validated in OVA-sensitized AR mice. The OVA-sensitized mice showed significantly increased rubs score compared to those of OVA-unsensitized mice. In addition, the increased rubs scores were significantly decreased by the administration of ABT-737 (Fig. 3A, $P < 0.05$). The levels of serum histamine and IgE up-regulated by OVA were significantly down-regulated by the administration of ABT-737 (Fig. 3B and C, $P < 0.05$). In addition, ABT-737 significantly down-regulated the levels of serum TSLP, TNF- α , IL-1 β , IL-4, IL-5, and VEGF in OVA-sensitized mice (Fig. 3D–I, $P < 0.05$). Next, we sought to investigate the synergistic effects of ABT-737 and DEX on AR. It was found that a combination of ABT-737 (0.81 mg/kg) and DEX (0.5 mg/kg) has greater effects than ABT-737 (0.81 mg/kg) or DEX (0.5 mg/kg) alone (Fig. 3).

3.3. Suppressive effect of ABT-737 on the levels of inflammatory mediators in the nasal mucosa tissues of AR mice

Inflammatory cytokines (TSLP, IL-1 β , IL-5, IL-6, and IL-13)

contribute to the initiation and maintenance of allergic responses (Finkelman and Urban, 2001). Moreover, allergic airway inflammation is modulated by the HIF-1 α /VEGF pathways (de Souza Alves et al., 2013). Mo et al. (2014) detected increased HIF-1 α and VEGF levels in the nasal mucosa tissues of patients with AR. OVA-sensitized mice showed up-regulated expressions of HIF-1 α compared with OVA-unsensitized mice, whereas this increase was suppressed by the administration of ABT-737 (Fig. 4A). In this study, the levels of VEGF up-regulated by OVA in nasal mucosa tissues were significantly down-regulated by the administration of ABT-737 (Fig. 4B, $P < 0.05$). The levels of TSLP, IL-1 β , IL-5, IL-6, IL-13, and TNF- α up-regulated by OVA in the nasal mucosa tissues were also significantly down-regulated by the administration of ABT-737 (Fig. 4C–H, $P < 0.05$). Subsequently, we sought to investigate the effect of ABT-737 on the OVA-induced inflammatory cytokine mRNA expression via a quantitative real time-PCR. These results showed that ABT-737 significantly reduced OVA-induced mRNA expression levels of HIF-1 α , VEGF, TSLP, IL-1 β , and TNF- α (Fig. 5, $P < 0.05$).

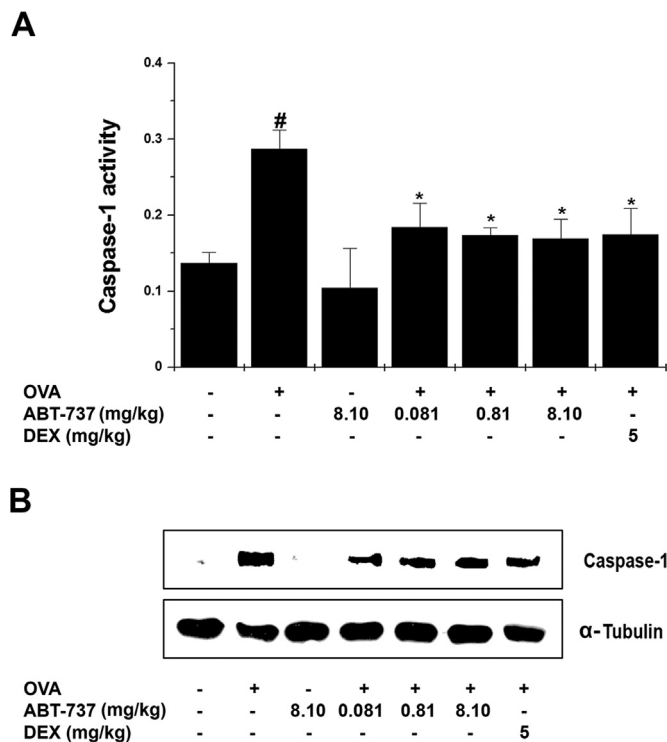


Fig. 7. Suppressive effect of ABT-737 on caspase-1 activation in the nasal mucosa tissues of AR mice. (A) Caspase-1 activity in the nasal mucosa tissues was measured by a caspase-1 assay kit. (B) The protein levels of caspase-1 were assayed by Western blotting. Results are representative of three independent experiments. [#] $P < 0.05$; significantly different from OVA-unsensitized mice. ^{*} $P < 0.05$; significantly different from OVA-sensitized mice. $N = 5$. DEX, dexamethasone.

3.4. Suppressive effect of ABT-737 on caspase-1 activation in the nasal mucosa tissues of AR mice

The activation of caspase-1 induces allergic inflammation (Jeong et al., 2018). Therefore, we sought to investigate the role of caspase-1 in AR using a caspase-1 inhibitor. As shown in Fig. 6, the rub scores, serum histamine, IgE, and cytokines up-regulated by OVA were significantly down-regulated by the caspase-1 inhibitor.

In Fig. 1F, ABT-737 significantly reduced caspase-1 activities in activated HMC-1 cells. Thus, we sought to confirm the effect of ABT-737 on OVA-caused caspase-1 activation using a caspase-1 assay kit. As shown in Fig. 7A, OVA-caused caspase-1 activation was significantly down-regulated by the administration of ABT-737 ($P < 0.05$). The levels of the caspase-1 active form were also analyzed by Western blotting. Similar to the outcomes shown in Fig. 7A, the administration of ABT-737 down-regulated the levels of the caspase-1 active form (Fig. 7B).

3.5. Suppressive effect of ABT-737 on the infiltration of eosinophils and mast cells into the nasal mucosa tissues of AR mice

Mast cell activation is central to the pathogenesis of allergic diseases, including anaphylaxis, AR, and allergic asthma (Stone et al., 2010). Moreover, blood and tissue eosinophilia are hallmarks of helminth infection, asthma, and AR (Stone et al., 2010). MIP-2 and ICAM-1 are chemotactic molecules associated with eosinophils and mast cells (Song et al., 2012). First, we investigated the effect of ABT-737 on the levels of MIP-2 and ICAM-1. The levels of MIP-2 and ICAM-1 in OVA-sensitized AR mice were significantly higher than those in the nasal tissues of the OVA-unsensitized mice (Fig. 8A and B, $P < 0.05$). The up-regulated levels of MIP-2 and ICAM-1 were down-regulated by the

administration of ABT-737 (Fig. 8A and B, $P < 0.05$). Next, we confirmed the regulatory effects of the administration of ABT-737 on the influx of immune cells into the nasal tissues of AR. The number of eosinophils in the ABT-737-administrated mice was significantly down-regulated compared to that in OVA-sensitized AR mice (Fig. 8C and E). In addition, the number of mast cells in the nasal tissues of OVA-sensitized AR mice was significantly lowered by the administration of ABT-737 (Fig. 8D and F).

4. Discussion

In this study, we showed that ABT-737 significantly reduced inflammatory cytokine production by blocking caspase-1 and NF- κ B activation in activated HMC-1 cells. In addition, ABT-737 alleviated AR clinical symptoms and AR biomarkers, and a co-treatment with ABT-737 and DEX had synergistic effects in an AR animal model.

Mast cells play a critical role in conditions with involve allergic inflammation including atopic dermatitis and AR via multiple mechanisms (Han et al., 2014; Pawankar et al., 2011). Mast cell-derived inflammatory cytokines such as TSLP, TNF- α , IL-1 β , and IL-6 contribute to initiation and maintenance of allergic responses (Finkelman, 2001). The activation of caspase-1 and NF- κ B induces the production and expression of inflammatory cytokines (Jeong et al., 2018). In this study, we showed that ABT-737 diminished VEGF, TSLP, TNF- α , IL-1 β , and IL-6 protein production and mRNA expression levels by inhibiting caspase-1 and NF- κ B activation. Therefore, we suggest that ABT-737 has an anti-inflammatory effect which acts through the inhibition of mast cell activation.

The pathogenesis of AR is complex and is characterized by the activation of many cytokines and immune cells. IL-4 expression is related to the differentiation of Th2 lymphocytes, the synthesis of IgE, the upregulation of IgE receptors, and the hypersecretion of mucus (Dabbagh et al., 1999; Pawankar et al., 2011; Seder et al., 1992). IL-5 plays a role in eosinophilic inflammation (Foster et al., 1996). IL-13 is related to the differentiation of B lymphocytes and isotype switching of the antibody to IgE (Cocks et al., 1993). TSLP or TSLP-induced Th2 cytokine also induces allergic inflammatory diseases (Han et al., 2014). Thus, clinical trials to assess the efficacy of anti-IL-1 β , anti-IL-6, anti-TSLP, and anti-TNF- α drugs have been conducted in relation to inflammatory disorders.

HIF-1 α expression is significantly increased in mice with allergic airway inflammation and in humans with AR and asthma (Huerta-Yepez et al., 2011). Several studies have shown that increased HIF-1 α expression causes the overproduction of VEGF, which results in the leakage of plasma proteins, inflammatory mediators, and inflammatory cells into the extravascular space of the airway (Kim et al., 2010; Lee et al., 2008). In addition, pro-inflammatory cytokines and chemokines were up-regulated by HIF-1 α in activated HMC-1 cells and the transcriptional activity of HIF-1 α is regulated by Bcl-2 (Jeong et al., 2003; Trisciuglio et al., 2010). In addition, the production of inflammatory cytokines is induced by the activation of caspase-1 (Jeong et al., 2018). Hence, the activation of caspase-1 can induce inflammation and high levels of caspase-1 are associated with inflammatory diseases risk factors (Boost et al., 2007). Recent evidence has demonstrated that pharmacological interference with cytokine production signaling pathways decreases inflammatory reactions and may therefore serve as a basis for preventive and therapeutic approaches (Nam et al., 2017). In this study, we showed that the administration of a caspase-1 inhibitor or ABT-737 alleviated AR clinical symptoms and lowered the AR-related biomarker levels in AR mice. Therefore, we suggest that ABT-737 undergoes anti-allergic and anti-inflammatory reactions by blocking both HIF-1 α and caspase-1.

Mast cells and eosinophils regulate local immune and inflammatory responses, and their accumulation in blood and tissue is associated with several inflammatory diseases (Bradding et al., 2006; Tian et al., 2017). Infiltrations of mast cells and eosinophils were increased by ICAM-1

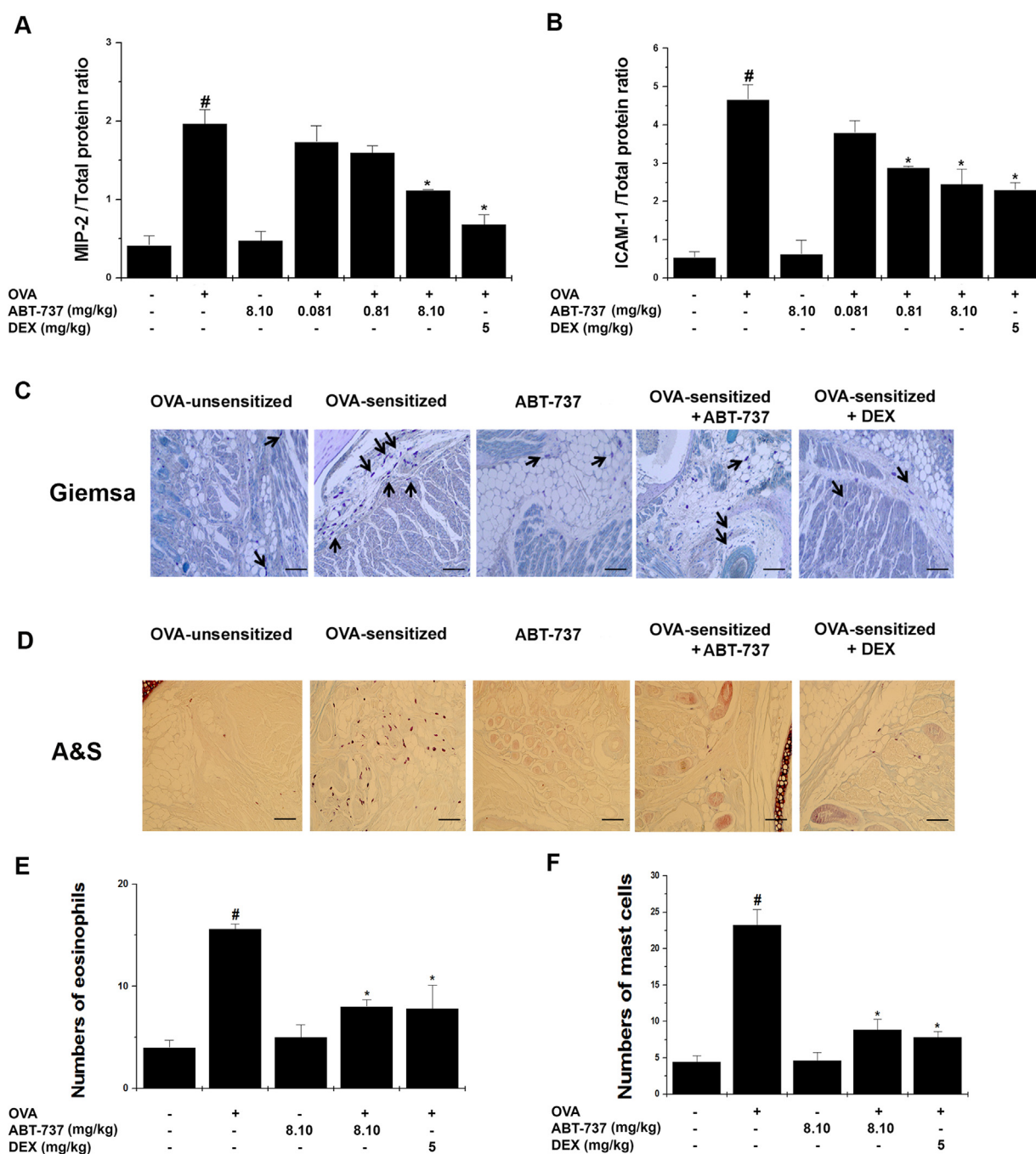


Fig. 8. Suppressive effect of ABT-737 on infiltration of eosinophils and mast cells into the nasal mucosa tissues of AR mice. Levels of (A) MIP-2 and (B) ICAM-1 in the nasal mucosa tissues were measured by the ELISA method. All parameters measured in the tissue homogenate were presented as a ratio to the total protein levels in the nasal tissue. (C) Nasal mucosa stained with Giemsa (for eosinophils) and (D) A&S (for mast cells). (Original magnification $\times 400$, scale bar = 100 μ m). 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 $\pm$ S.E.M. [#] $P < 0.05$; significantly different from OVA-unsensitized mice. ^{*} $P < 0.05$; significantly different from OVA-sensitized mice. N = 5. DEX, dexamethasone.

and MIP-2 (Gonzalo et al., 1996). Recent study reported that higher expression levels of the Bcl-2 protein in inflammatory cells from mice with allergic lung tissue were observed in mouse models of both eosinophilic and neutrophilic airway inflammation and that Bcl-2 inhibitors proved effective in reducing either eosinophilic or neutrophilic airway inflammation in mouse models (Tian et al., 2017). Depletion of Bcl-2 family proteins was shown to have protective effects against allergic diseases, such as anaphylaxis (Ottina et al., 2015). The Bcl-2 inhibitor ABT-737 is efficient when used to alleviate eosinophilic or corticosteroid-resistant neutrophilic airway inflammation by inducing the

apoptosis of immune cells, such as eosinophils, neutrophils, Th2 cells, Th17 cells, and dendritic cells (Tian et al., 2017). Chand et al. (2018) reported that the intranasal instillation of ABT-737 decreased lipopolysaccharide-induced neutrophilic inflammation in mice. In this study, we showed that ABT-737 inhibited the infiltration of mast cells and eosinophils into the nasal mucosa tissues of AR mice. Therefore, we suggest that ABT-737 undergoes anti-allergic and anti-inflammatory reactions through the inhibition of inflammatory cell infiltration.

The results of this study also show that a combined therapy of ABT-737 and DEX was more effective than a monotherapy. The usage of low

doses of ABT-737 and DEX may be feasible for the treatment of AR, and such a regimen can prevent associated side effects. Therefore, these results imply that the use of anti-cancer drugs is a new therapeutic approach for the treatment of allergic diseases.

In conclusion, our findings provide *in vitro* and *in vivo* evidence that the anti-cancer agent ABT-737 significantly diminishes allergic inflammatory reactions by reducing mast activation levels and alleviating AR clinical symptoms and AR biomarkers. These results imply that ABT-737 ameliorates the allergic inflammatory diseases such as AR.

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Conflict of interest

The authors state no conflict of interest.

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