



Therapeutic effects of resveratrol in a mouse model of HDM-induced allergic asthma



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ARTICLE INFO

Article history:

Received 1 September 2014

Received in revised form 29 December 2014

Accepted 12 January 2015

Available online 20 January 2015

Keywords:

Allergic asthma

Resveratrol

Syk

Airway inflammation

ABSTRACT

Asthma is an inflammatory disease of the lungs characterized by airway remodeling. In this study, we examined whether resveratrol exerts protective effects on allergic asthma in a murine model. To investigate the effects of resveratrol on allergic airway inflammation in house dust mite (HDM)-induced mouse asthma and explore its mechanism, a chronic asthma mouse model was established by intranasally administering extracts of HDM (25 µg of protein in 10 µl of saline) for 5 days/week for up to 7 consecutive weeks. Resveratrol (50 mg/kg body weight), dexamethasone (1 mg/kg body weight) or a vehicle was administered orally 1 h before antigen challenges for up to 2 weeks. Compared with the HDM-induced mice, the level of TNF-α of the BALF in the resveratrol + HDM-treated mice had obviously decreased. Histological examination of the lung tissue revealed that the resveratrol treatments attenuated the fibrotic response and airway inflammation. In addition, resveratrol inhibited the expression of the Syk protein and degranulation in mast cells. The presented findings collectively suggest that resveratrol has a therapeutic effect on mouse allergic asthma, and its mechanism of action might be related to reducing the production of the Syk protein.

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1. Introduction

Asthma is an inflammatory disease of the lungs characterized by airway hyperresponsiveness (AHR), airway remodeling and increased infiltration of leukocytes into the airways [1,2]. In asthma, inflammatory processes are mediated by a sequence of intercellular signals whose hyper-activation and/or dysregulation results in the onset and persistence of inflammatory diseases [3]. Various intercellular signals play a pivotal role during the process, some of which are mediated by soluble ligands such as cytokines, whereas others are mediated by protein kinases through receptor molecules and costimulatory methods, in which activated protein kinases contribute to various downstream pro-inflammatory cytokines and inflammatory mediators [4].

In the pathogenesis of allergic asthma, although the development of a CD4⁺ Th2 immune response and its associated cytokines (e.g., IL-4, IL-5, and IL-13) is known to play an important role, the roles of tumor necrosis factor transforming growth factor (TGF)-β are also becoming apparent [5]. TNF-α is an important cytokine in the innate immune response, which provides immediate host defense against invading organisms before activation of the adaptive immune system [6]. TNF-α is principally produced by macrophages in response to the activation of membrane-bound pattern-recognition molecules, which detect common bacterial cell surface products such as polysaccharides, carbohydrates and lipopolysaccharides. Dysregulated TNF-α responses have been implicated in several inflammatory conditions. TNF-α induces histamine release directly from human mast cells and promotes human mast cell cytokine secretion [7]. IL-6 is a small glycoprotein (21 kDa) produced not only by cells from the innate immune system (e.g., macrophages, dendritic cells, mast cells, and neutrophils) but also by B cells and, to a lesser extent, by CD4 effector Th cells. Increased levels of IL-6 in serum have been found in asthmatic patients. In addition, increased levels of IL-6 in BALF from patients with “intrinsic” asthma compared to the levels in patients with allergic asthma have also been reported, suggesting that IL-6 may play a role in various types of asthma rather than only in patients with allergic asthma, who only account for approximately 50% of all asthmatics [8]. Tissue IL-17 expression is increased in bronchial biopsies from patients with asthma

Abbreviations: Syk, spleen tyrosine kinase; HDM, house dust mite; FcεR, Fcε receptor; DEX, dexamethasone; BAY, BAY 61-3606; BALF, bronchoalveolar lavage fluid; DMSO, dimethyl sulfoxide; RBL-2H3, rat basophilic leukemia cell; PBS, phosphate buffered physiological saline; CS, cromolyn sodium; RES, resveratrol; DNP-BSA, 2,4-dinitrophenyl bovine serum albumin.

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and stimulates bronchial fibroblasts to secrete cytokines [9]. In another study, increased sputum IL-17 was correlated with methacholine bronchial hyperreactivity [10]. TGF- β regulates the airway remodeling process in the development of the symptoms associated with decreased pulmonary function in asthmatics. Syk (p72^{syk}) is expressed in human macrophages, mast cells, T cells, B cells, basophils and eosinophils [11–13]. It plays an essential role in the activation of immune cells and lymphocyte development. In mast cells, this molecule is involved in the regulation of multiple intracellular signaling pathways via binding ITAM-bearing receptors, leading to degranulation and the release of cytokines [11].

Resveratrol, known as trans-3, 5, 4'-trihydroxystilbene, was first isolated in 1940 as a constituent of the roots of white hellebore (*Veratrum grandiflorum* O. Loes) but has since been found in various plants, including grapes, berries and peanuts [14]. Since ancient times, the grape root extracts known to contain resveratrol have been used as an analgesic and as a drug for allergic inflammation in traditional Chinese medicine. Extensive research during the last two decades has suggested that resveratrol has antioxidant and anti-inflammatory activities [15]. However, few studies documenting the actual anti-airway inflammation and anti-remodeling mechanisms of resveratrol in animal asthma models have been performed to date. In this study, we reported that resveratrol exhibits potent anti-allergic activities through the inhibition of mast cells by suppressing the expression levels of Syk and degranulation, suggesting that resveratrol has potential for the treatment of airway inflammatory diseases.

2. Materials and methods

2.1. Plant material

Resveratrol was isolated from the ethanol extracts of methanol extracts of *Vitis amurensis* Rupr as described previously [16], their purity was over 98%. This compound was dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO was adjusted to 0.1% (v/v) in culture media. DMSO alone served as a control in all cases.

2.2. Reagents

The Syk inhibitor BAY 61-3606 was obtained from Sigma-Aldrich (St. Louis, MO, USA). The ELISA kits for IL-6, IL-17, TGF- β and TNF- α were purchased from Biolegend Systems (San Diego, CA, USA). The rabbit anti-rat IgG primary antibodies against Syk and β -actin were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). The secondary antibodies (goat anti-rabbit IgG-HRP) were also purchased from Santa Cruz (Santa Cruz, CA, USA).

2.3. Culture and stimulation of the RBL-2H3 cells for Western blotting

The RBL-2H3 cells were purchased from the cell bank of Shanghai Science Academe and grown in Eagle's minimum essential medium supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY, USA), 2 mM L-glutamine, 100 units/ml penicillin, and 100 μ g/ml streptomycin in a humidified atmosphere of 5% CO₂ at 37 °C.

The RBL-2H3 cells were plated on a 35-mm dish (5×10^5 cells/100 μ l of medium/well) and incubated overnight with 0.1 μ g/ml DNP-specific IgE (Sigma-Aldrich, St. Louis, MO, USA) or phosphate buffered physiological saline (PBS) in complete growth medium as previously described [17]. The DNP-specific IgE-sensitized RBL-2H3 cells were incubated with 10, 1, or 0.1 μ M resveratrol or 10 μ M BAY 61-3606 for 30 min, followed by treatment with DNP-BSA (2 μ g/ml) or PBS for 12 h.

2.4. Western blotting

The cells were lysed in a non-denaturing lysis buffer (Appligen Technologies Inc., Beijing, China) and adjusted for protein content. The

cell lysates were resolved by 10% sodium dodecylsulfate electrophoresis and transferred onto nitrocellulose acetate membranes. After blocking, the membranes were incubated with the appropriate primary antibody overnight at 4 °C and then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Immuno-reactive proteins were detected with an ECL Advance Western Blotting Detection Kit (Tiangen) using a chemiluminescence detector (LAS 4000 mini, GE Healthcare Japan). The quantification of the Western blot bands was performed using ImageJ v.1.31 software.

2.5. Stimulation of the RBL-2H3 cells for degranulation

The RBL-2H3 cells (5×10^4 cells/0.1 ml/well) were plated onto a 96-well plate and incubated overnight with 0.1 μ g/ml DNP-specific IgE in a humidified 5% CO₂ incubator at 37 °C overnight. The supernatants were discarded, and the cells were washed twice with 200 μ l of PBS, and 130 μ l of releasing mixture (25 mM HEPES-KOH, pH 7.6; 116.9 mM NaCl, 5.4 mM KCl, 0.8 mM MgSO₄, 2 mM CaCl₂, 5.6 mM glucose, and 0.1% BSA) was added. The cells were incubated with 10 μ l of resveratrol with different concentrations (10, 1, or 0.1 μ M), cromdyn sodium (10 μ M) or DMSO for 10 min at 37 °C and stimulated with 10 μ l of DNP-BSA (2 μ g/ml) for 1 h at 37 °C. The resveratrol was dissolved in 1% DMSO. The final concentration of DMSO was 0.067%, and there were no effects of DMSO on this assay.

2.6. Measurement of β -hexosaminidase (β -Hex) release

A β -Hex release assay was used to evaluate the antigen-induced degranulation as previously described [17]. β -Hex is stored in granules and released in parallel with histamine after the antigen stimulation of mast cells. To determine the released β -Hex activity, 45 μ l of cell supernatant and 15 μ l of 5 mM *p*-nitrophenyl-*N*-acetyl- β -D-glucosaminide in 50 mM citrate buffer (pH 4.5) were incubated for 3 h at 37 °C. The reaction was stopped by adding 180 μ l of carbonate buffer (0.1 M NaHCO₃/Na₂CO₃, pH 10.0). The absorbance because of the formation of *p*-nitrophenol was measured at 415 nm using a microplate reader (BIO-TAK instruments, VT, USA). The β -Hex release (%) was calculated using the following equation: the β -Hex release (%) = $(S - B) / (C - B) \times 100$. *C* represents the absorbance of the control, and *B* represents the absorbance of the blank (without cells) for the control. *S* represents the absorbance of the samples.

2.7. Animals

Female Balb/c mice (6–8 weeks old) were purchased from the Animal Center of Military Medical Science and housed in environmentally controlled pathogen-free conditions throughout the experiments. All the protocols were reviewed and approved by the Institutional Animal Experimental Ethics Committee of Peking Union Medical College.

2.8. Allergen sensitization and exposure

The methods were adapted from Johnson et al. [18]. The mice were exposed to either purified HDM extract (25 μ g of protein in 10 μ l of saline; Greer Laboratories, Lenoir, NC) or saline intranasally for 5 days/week for up to 7 consecutive weeks. Resveratrol (50 mg/kg body weight), dexamethasone (1 mg/kg body weight) or a vehicle was administered orally 1 h before the antigen challenges for up to 2 weeks (see Fig. 1).

2.9. Bronchoalveolar lavage fluid (BALF) collection and analysis

The tracheas were exposed and cannulated with a 20G blunt needle. The bronchoalveolar lavage was performed by injecting 1 ml $1 \times$ PBS and withdrawing as much fluid as possible. The procedure was repeated twice, and the BALF was pooled. The cytokines listed below in the lavage

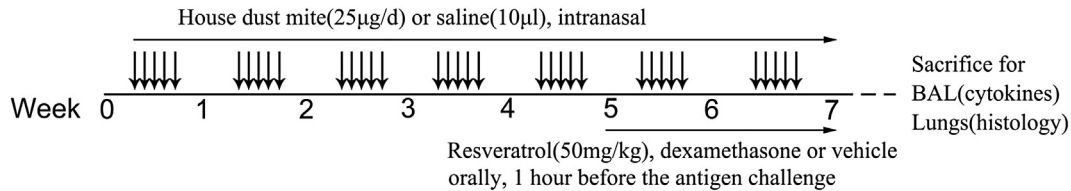


Fig. 1. Allergen sensitization and exposure protocols. A timeline of allergen immunization, exposure, and drug treatment in the chronic models is shown.

fluid were analyzed using an ELISA kit according to the manufacturer's instructions. The detection limits for these assays were 5 pg/ml for IL-6, IL-17, TGF- β , and TNF- α .

2.10. Lung histology

The histology and immunohistochemistry analyses were performed and evaluated as previously described. The right lungs from each group ($n = 6$ per group) were fixated with 4% buffered formalin solution. Serial sections of 3 μ m-thick were stained with hematoxylin and eosin (H&E) to quantify the degree of inflammation; each specimen was also stained with Masson's trichrome to identify collagen deposition using the Luna technique. A morphometric analysis of the stained lung sections was performed using a customized digital image analysis system. On the images of the H&E-stained lungs, the severity of the peribronchial inflammation was quantified from 0–5, in which 0 = no cells, 1 = a few cells, 2 = a ring of cells 1 cell-layer deep, 3 = a ring of cells 2 cells deep, 4 = a ring of cells 3–4 cells deep, and 5 = cells deep [19]. Staining with Masson's trichrome was used to determine collagen deposition in the lung. The images of Masson's trichrome stained lungs were processed using the Image-Pro Plus 6.0 software (Media Cybernetics). Briefly, three to five specimens of the Masson's trichrome-stained histological preparations of the lung lobe, in which the total length of the epithelial basement membrane of the bronchioles was 1.0–2.5 mm, were selected and the fibrotic area (stained in blue) beneath the basement membrane in 20 μ m depth was measured. The mean score of the fibrotic area divided by the basement membrane perimeter in three to five preparations of one mouse was calculated, and then the mean values of subepithelial fibrosis were calculated [20]. The slides were examined by a pathologist who was blinded to the treatment. The images of the stained cells were evaluated with 200 $\times$ magnification. At least 6 bronchioles were randomly counted in each slide, and their average scores were calculated.

2.11. Statistical analysis

All the experiments described were performed three or more times. The values are expressed as the mean $\pm$ SEM. The Student's *t*-test was used for the comparison of two independent groups. For all tests, a *P* value < 0.05 was considered statistically significant.

3. Results

3.1. Effects of resveratrol on airway inflammation in mice

To evaluate the possible anti-inflammatory or anti-remodeling effects of resveratrol, lung tissues were collected 24 h after the last challenge. The lung sections were separately stained with H&E. Compared to the control animals who received normal saline only, the HDM challenge for 7 weeks caused more pulmonary inflammation ($P < 0.05$). In the resveratrol + HDM-challenged mice, the pulmonary inflammation was significantly attenuated compared to the pulmonary inflammation in the HDM-treated mice ($P < 0.05$) (see Fig. 2).

3.2. Effects of resveratrol on airway fibrosis in mice

Remodeling of the small airways of the lungs is a common feature of patients with asthma. This remodeling takes the form of epithelial cell hyperplasia and subepithelial fibrosis. Masson's trichrome stains were used to evaluate the presence and distribution of collagen in the lungs of the mice. Collagen deposition is a hallmark of fibrosis. As shown in

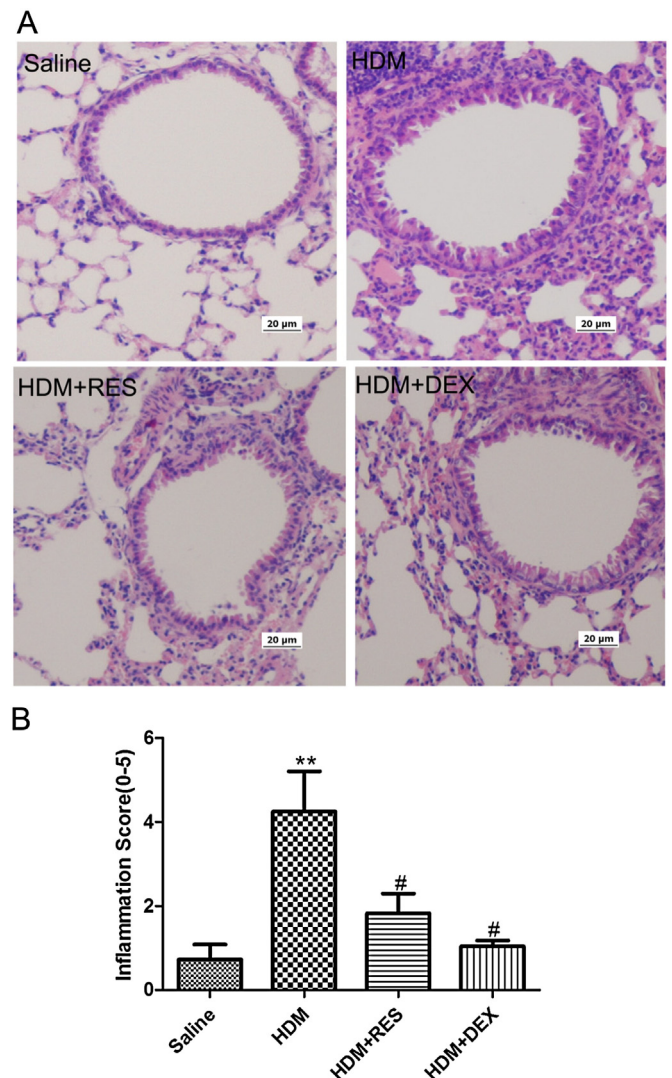


Fig. 2. Effects of resveratrol on airway inflammation in mice. (A) A histological examination of lung tissue from each group ($n = 6$ per group) was performed 24 h after the last HDM challenge. The lung tissues were fixed, sectioned at 4 μ m thickness, and stained with H&E solution (magnification $\times 200$). (B) The scoring of the extent of inflammation by the quantitative analysis of inflammatory-cell infiltration in the lung sections was based on the method of Chen et al. [32]. Saline, saline treated-mice; HDM, HDM-sensitized/challenged mice; HDM + RES, RES (50 mg/kg) + HDM-sensitized/challenged mice; DEX, dexamethasone (1 mg/kg) + HDM-sensitized/challenged mice. ** $P < 0.01$, compared with saline, and # $P < 0.05$, compared with HDM.

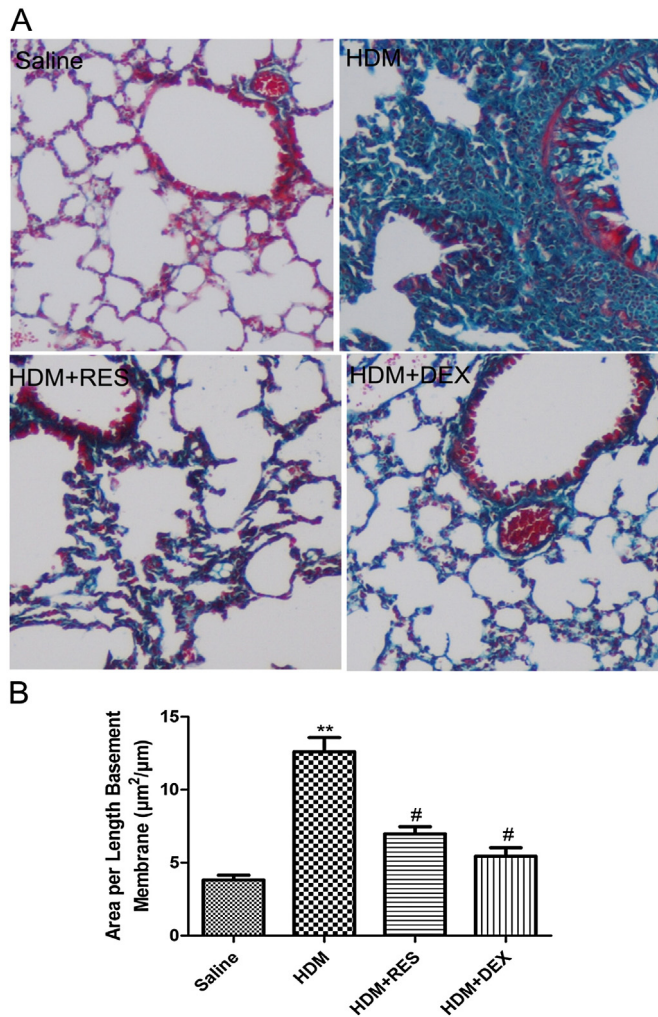


Fig. 3. Effects of resveratrol on airway fibrosis in mice. (A) A histological examination of lung tissue from each group ($n = 6$ per group) was performed 24 h after the last HDM challenge. The lung tissues were fixed, sectioned at 4 µm thickness, and stained with Masson's trichrome solution (magnification $\times 200$). (B) The scoring of the extent of airway fibrosis by the quantitative analysis of collagen deposition in the lung sections was based on the method of Chen et al. [32]. Saline, saline treated-mice; HDM, HDM-sensitized/challenged mice; HDM + RES, RES (50 mg/kg) + HDM-sensitized/challenged mice; DEX, dexamethasone (1 mg/kg) + HDM-sensitized/challenged mice. ** $P < 0.01$, compared with saline, and # $P < 0.05$, compared with HDM.

Fig. 3, the enhanced collagen deposition was readily appreciated in the lungs of the HDM-challenged mice compared to the control animals that received normal saline ($P < 0.05$). In the resveratrol + HDM-treated mice, a small amount of wispy blue staining collagen was observed in and near the airway wall, and loosely packed collagen was observed in the bronchovascular bundles of the dexamethasone (1 mg/kg) + HDM-sensitized/challenged mice ($P < 0.05$).

3.3. Effects of resveratrol on cytokine levels in BALF

To determine the effects of resveratrol on cytokine release in HDM-induced asthmatic mice, the levels of cytokines (IL-6, IL-17, TGF- β and TNF- α) in BALF were measured by ELISA 24 h after the last challenge. As shown in Fig. 4, the HDM challenge induced an elevated level of cytokines to $35,073 \pm 37,637$ pg/ml (IL-6), 179 ± 199 pg/ml (IL-17), $12,793 \pm 6493$ pg/ml (TNF- α) and $141,523 \pm 41,911$ pg/ml (TGF- β) in the BALF compared to the control BALF (IL-6, 1376 ± 855 pg/ml; IL-17, 7 ± 10 pg/ml; TNF- α , 4442 ± 3205 pg/ml; and TGF- β , $111,642 \pm 37,768$ pg/ml). In the resveratrol + HDM-treated group, the cytokine elevation was suppressed; the following decreases were observed

compared to the HDM-challenged group: $8816 \pm 15,901$ pg/ml in IL-6 ($P = 0.228$), 41 ± 42 pg/ml in IL-17 ($P = 0.19$), 1443 ± 1471 pg/ml in TNF- α ($P = 0.009$) and $115,281 \pm 43,963$ pg/ml in TGF- β ($P = 0.351$). Dexamethasone also caused a reduction in the abovementioned cytokines. In addition, the recruited cells in BALF were counted 24 h after the last challenge in this experiment (data not shown). Chronic HDM challenges did not cause a marked influx of inflammatory cells into the BALF (saline treated group VS HDM treated group: total cells, neutrophils, lymphocytes, macrophages and eosinophils, not statistically significant).

3.4. Effects of resveratrol on IgE-induced Syk and degranulation in mast cells

RBL-2H3 cells are a type of mast cell isolated from rats; they have the properties of mast cells. The expression of Syk in the cells was analyzed using an immunoblot analysis. The cells were stimulated with the DNP-BSA antigen after they were primed with DNP specific IgE. As shown in Fig. 5, resveratrol significantly suppressed IgE-induced expression of Syk in a dose-dependent manner in the RBL-2H3 cells. As expected, the degranulation events downstream of Syk were inhibited with a similar potency by resveratrol. The results indicated that resveratrol has an effect on inhibiting the expression of Syk proteins and degranulation in mast cells.

4. Discussion

The suppression of inflammatory responses and the identification of new target inhibitors to alleviate the occurrence of asthma and airway remodeling have been the primary emphasis over recent decades in the treatment of asthma. In the present study, we showed that resveratrol significantly modulates the pulmonary environment of cytokines (IL-6, IL-17, TGF- β and TNF- α) in BALF compared with the environment in HDM-induced mice. In lung histopathological studies using hematoxylin and eosin and Masson's trichrome staining, resveratrol inhibited inflammatory cell infiltration and airway remodeling compared with HDM-challenged mice. In addition, resveratrol suppressed the total Syk levels and degranulation in mast cells.

Asthma has a wide clinical spectrum, ranging from a mild disease to a severe, intractable disease. In its severe form, asthma is characterized by airway remodeling and a significant loss of lung function. The histopathologic features of this process include epithelial changes, subepithelial fibrosis (thickening of the basement membrane), and inflammatory cell infiltration. Subepithelial fibrosis is reportedly a potential marker for disease severity [21]. Important mediators involved in the process of airway remodeling are TGF- β and IL-17 [22]. TGF- β represents a profibrotic cytokine that is produced by a number of cells, including macrophages, epithelial cells, fibroblasts, and eosinophils. We have previously demonstrated an association between the level of expression of TGF- β and the severity of disease [1]. In this study, the oral administration of resveratrol to HDM-challenged mice decreased lung TGF- β levels in BALF compared to the HDM-treated mice (Fig. 4D). Correspondingly, we found that compared to HDM-treated mice, the histopathological findings revealed that significant collagen deposition induced by an airway challenge with HDM was markedly attenuated upon resveratrol administration; this was observed using Masson's trichrome stains (Fig. 3). These effects may be related to the inhibition of TGF- β synthesis by resveratrol, resulting in a decrease in the levels of collagen deposition responsible for airway remodeling.

The severity of asthma is correlated with the level of IL-17 cytokines found in the lung, sputum, BALF, or serum of patients [23]. In vitro, IL-17 stimulates human bronchial fibroblasts to synthesize and release higher amounts of IL-6, which are Th2 profibrotic cytokines. IL-6 is a pleiotropic cytokine that, together with TNF- α and IL-1 β , has traditionally been considered more of a biomarker of ongoing inflammation than a regulatory cytokine with the potential to modulate the immune response [24].

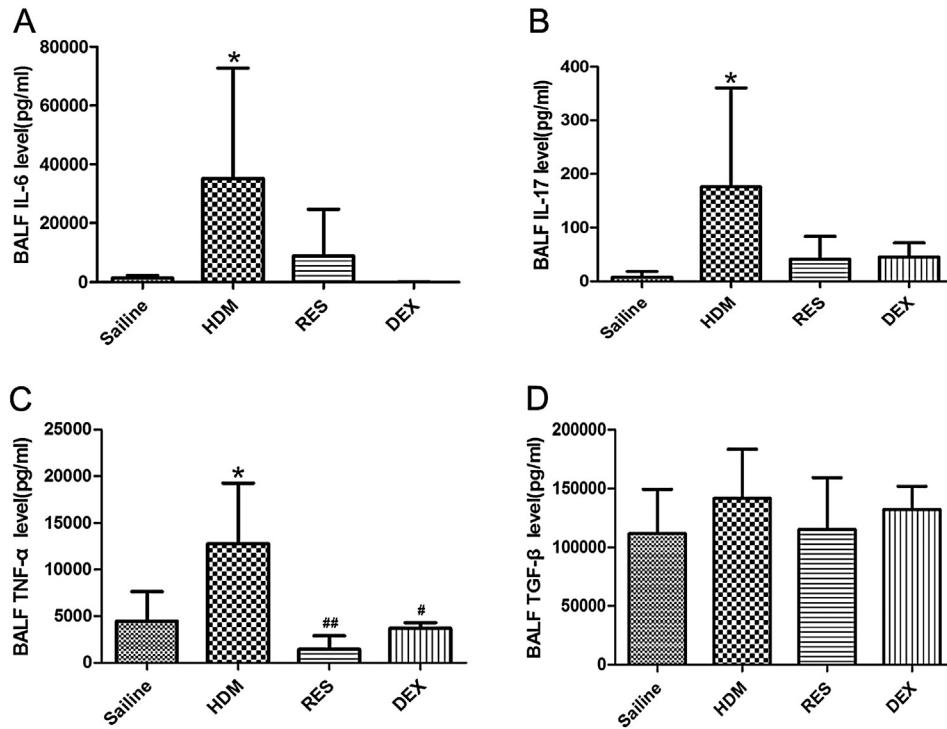


Fig. 4. Effects of resveratrol on cytokine levels in BALF. BALF was collected 24 h after the last HDM challenge in the mice. Each sample was analyzed with an ELISA ($n = 5$). (A) IL-6 levels in BALF, (B) IL-17 levels in BALF, (C) TGF-β levels in BALF, and (D) TNF-α levels in BALF. Saline, saline treated-mice; HDM, HDM-sensitized/challenged mice; HDM + RES, RES (50 mg/kg) + HDM-sensitized/challenged mice; DEX, dexamethasone (1 mg/kg) + HDM-sensitized challenged mice. * $P < 0.05$, compared with saline; # $P < 0.05$ and ## $P < 0.01$, compared with HDM.

TNF-α is increased in the airways of severe, corticosteroid refractory patients with asthma and is implicated in airway pathology, whereas in healthy subjects, the inhalation of TNF-α triggers airway constriction,

hyperresponsiveness, and sputum neutrophilia. In this study, we observed a considerable upregulation of the expression of TNF-α in the HDM-challenged mice compared with the controls. However, the oral

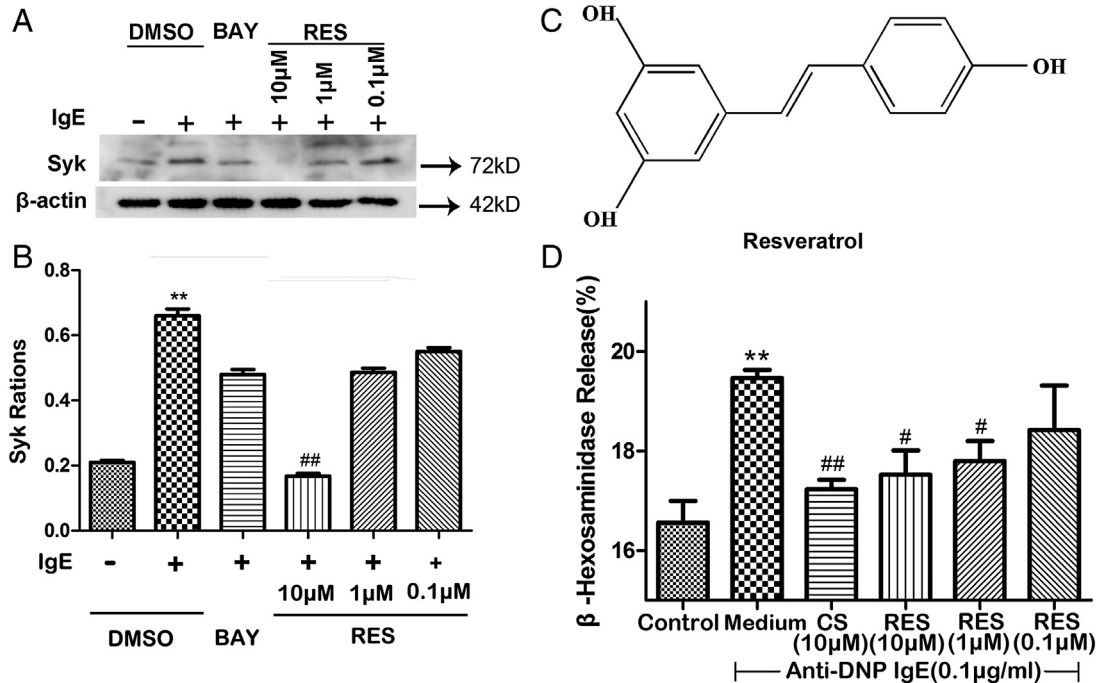


Fig. 5. Resveratrol suppressed Syk expression in RBL-2H3 cells. A. Western blots using Syk antibodies. DNP-specific IgE-sensitized RBL-2H3 cells were preincubated for 30 min with resveratrol, BAY (BAY 61-3606, 10 μM) or DMSO as indicated, and were then treated with DNP-BSA (2 μg/ml) or PBS for 12 h. B. The relative amounts of Syk proteins were quantified using ImageJ. ** $P < 0.01$, compared with DMSO; ## $P < 0.01$, compared with the DNP-specific IgE-sensitized group. C. The chemical structure of resveratrol. D. DNP-specific IgE-sensitized RBL-2H3 cells were pre-incubated for 10 min with different concentrations of resveratrol or cromolyn sodium (CS) and then stimulated with 10 μl of DNP-BSA (2 μg/ml) for 1 h at 37 °C. The released β-hexosaminidase was assayed. ** $P < 0.01$, compared with the control; # $P < 0.05$ and ## $P < 0.01$, compared with the medium group. The data are the means ± SE ($n = 3$).

administration of resveratrol to the HDM-challenged mice significantly decreased TNF- α level in BALF.

Syk (p72^{Syk}) is expressed in macrophages, mast cells, T cells, B cells, basophils and eosinophils [25]. It plays an essential role in the activation of immune cells and lymphocyte development. In mast cells, this molecule is involved in the regulation of multiple intracellular signaling pathways via binding ITAM-bearing receptors, leading to the release of allergic mediators and degranulation. Important downstream targets of Syk include PLC γ , the activation of which results in Ca²⁺ mobilization [26]. Syk also induces the activation of LAT and cPLA2, which in turn regulate transcription factors necessary for cytokine gene expression (IL-6, IL-17, TGF- β and TNF- α) [25]. Previous studies have reported that resveratrol is a major Syk inhibitor and inhibits the activation of Syk kinase in mast cells [25,27]. In this study, resveratrol significantly suppressed the IgE-induced expression of Syk and degranulation in a dose-dependent manner in the RBL-2H3 cells. These effects could be related to the inhibition of cytokine synthesis and the activation of immune cells.

Resveratrol has been detected in trees, in a few flowering plants, in peanuts, and in grapevines. Numerous in vitro studies have described the different biological effects of resveratrol [28]. Its major properties of interest are its anti-oxidative, anti-inflammatory, and estrogenic effects, and anticancer and chemopreventative activities [29,30]. Recently, resveratrol has been suggested to have many properties that could be beneficial in the treatment of several chronic inflammatory diseases such as chronic obstructive pulmonary disease (COPD) and arthritis [31]. In this study, resveratrol reduced the levels of cytokines (IL-6, IL-17, TGF- β and TNF- α) in BALF and airway remodeling in an HDM-induced asthmatic mouse model. Furthermore, in terms of safety, resveratrol had no effect on body weight in mice, but dexamethasone caused significant weight loss compared to the HDM-challenged group. These results provide further evidence that the oral administration of resveratrol is suitable for the treatment of allergic diseases such as asthma.

Acknowledgments

This work was supported by the Jiangxi Province Education Office Youth Scientific Research Fund (No.GJJ14226).

References

- [1] Liu R, Bai J, Xu G, Xuan L, Zhang T, Meng A, et al. Multi-allergen challenge stimulates steroid-resistant airway inflammation via NF-kappaB-mediated IL-8 expression. *Inflammation* 2013;36:845–54.
- [2] Barnes PJ. New drug for asthma. *Nat Rev Drug Discov* 2004;3:831–44.
- [3] Pj B. New drugs for asthma. *Nat Rev Drug Discov* 2004;3:831–44.
- [4] Wong WF. Inhibitors of the tyrosine kinase signaling cascade for asthma. *Curr Opin Pharmacol* 2005;5:264–71.
- [5] Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Lavolette M, et al. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF- β , IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol* 2003;111:1293–8.
- [6] Berry Mike, Brightling Christopher, Ian Pavord AW. TNF- α in asthma. *Curr Opin Pharmacol* 2007;7:279–82.
- [7] Choi Il-Whan, Sun-Kim, Kim Young-Suk, Ko Hyun-Mi, Im Suhn-Young, Kim Jae-Hong, et al. TNF- α induces the late-phase airway hyperresponsiveness and airway inflammation through cytosolic phospholipase A2 activation. *J Allergy Clin Immunol* 2005;116: 537–43.
- [8] Rincon M, Irvin CG. Role of IL-6 in asthma and other inflammatory pulmonary diseases. *Int J Biol Sci* 2012;8:1281–90.
- [9] Souwer Y, Szegedi K, Kapsenberg ML, de Jong EC. IL-17 and IL-22 in atopic allergic disease. *Curr Opin Immunol* 2010;22:821–6.
- [10] Nembrini Chiara, Marsland Benjamin J, Kopf M. IL-17-producing T cells in lung immunity and inflammation. *J Allergy Clin Immunol* 2009;123:986–94.
- [11] Siraganian Reuben P, de Castro Rodrigo O, Barbu Emilia A, Zhang J. Mast cell signaling: the role of protein tyrosine kinase Syk, its activation and screening methods for new pathway participants. *FEBS Lett* 2010;584:4933–40.
- [12] Geahlen RL. Syk and pTyr'd: signaling through the B cell antigen receptor. *Biochim Biophys Acta* 2009;1793:1115–27.
- [13] Tang Bo, Zhou Jing, Park Jeoung-Eun, Cullins David, Yi Ae-Kyung, Andrew H, et al. T cell receptor signaling induced by an analog peptide of type II collagen requires activation of Syk. *Clin Immunol* 2009;133:145–53.
- [14] Lee M, Kim S, Kwon OK, Oh SR, Lee HK, Ahn K. Anti-inflammatory and anti-asthmatic effects of resveratrol, a polyphenolic stilbene, in a mouse model of allergic asthma. *Int Immunopharmacol* 2009;9:418–24.
- [15] Bellaver Bruna, Guerini Souza Débora, Onofre Souza Diogo, Quincozes-Santos A. Resveratrol increases antioxidant defenses and decreases proinflammatory cytokines in hippocampal astrocyte cultures from newborn, adult and aged Wistar rats. *Toxicol Vitro* 2014;28:479–84.
- [16] Wang D-G, Liu W-Y, Chen G-T. A simple method for the isolation and purification of resveratrol from *Polygonum cuspidatum*. *J Pharm Anal* 2013;3:241–7.
- [17] Sanderson MP, Gelling SJ, Rippmann JF, Schnapp A. Comparison of the anti-allergic activity of Syk inhibitors with optimized Syk siRNAs in Fc epsilon RI-activated RBL-2H3 basophilic cells. *Cell Immunol* 2010;262:28–34.
- [18] Johnson Jill R, Wiley Ryan E, Fattouh Ramzi, Swirski Filip K, Gajewska Beata U, Anthony J, et al. Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med* 2004;169:378–85.
- [19] Klopfeisch R. Multiparametric and semiquantitative scoring systems for the evaluation of mouse model histopathology – a systematic review. *BMC Vet Res* 2013;123: 1–15.
- [20] Masato Komai HT, Masuda Taisei, Nagao Koichi, Ishizaki Masayuku, Sawada Masatsugu, Nagai Hiroichi. Role of Th2 responses in the development of allergen-induced airway remodeling in a murine model of allergic asthma. *Br J Pharmacol* 2003;138:912–20.
- [21] Chakir Jamila, Shannon Joanne, Molet Sophie, Fukakusa Motonori, Elias Jack, Lavolette Michel, et al. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF- β , IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol* 2003;111:1293–8.
- [22] Makiinde T, Murphy RF, Agrawal DK. The regulatory role of TGF-beta in airway remodeling in asthma. *Immunol Cell Biol* 2007;85:348–56.
- [23] Agache I, Ciobanu C, Agache C, Anghel M. Increased serum IL-17 is an independent risk factor for severe asthma. *Respir Med* 2010;104:1131–7.
- [24] Neveu Wendy A, Allard Jenna L, Raymond Danielle M, Bourassa Lorraine M, Burns Stephanie M, Bunn Janice Y, et al. Elevation of IL-6 in the allergic asthmatic airway is independent of inflammation but associates with loss of central airway function. *Respir Res* 2010;28:1–10.
- [25] Stenton GR, Ulanova M, Dery RE, Merani S, Kim MK, Gilchrist M, et al. Inhibition of allergic inflammation in the airways using aerosolized antisense to Syk kinase. *J Immunol* 2002;169:1028–36.
- [26] Manetz TS, Gonzalez-Espinosa C, Arudchandran R, Xirasagar S, Tybulewicz V, Rivera J. Vav1 regulates phospholipase cgamma activation and calcium responses in mast cells. *Mol Cell Biol* 2001;21:3763–74.
- [27] Lu Y, Li Y, Jahng Y, Son JK, Chang HW. Citreorosein inhibits degranulation and leukotriene C(4) generation through suppression of Syk pathway in mast cells. *Mol Cell Biochem* 2012;365:333–41.
- [28] Knobloch J, Sibbing B, Jungck D, Lin Y, Urban K, Stoelben E, et al. Resveratrol impairs the release of steroid-resistant inflammatory cytokines from human airway smooth muscle cells in chronic obstructive pulmonary disease. *J Pharmacol Exp Ther* 2010; 335:788–98.
- [29] Wood Lisa G, Wark Peter AB, Garg ML. Antioxidant and anti-inflammatory effects of resveratrol in airway disease. *Antioxid Redox Signal* 2009;13:1535–48.
- [30] Mikula-Pietrasik J, Kuczmarska A, Kucinska M, Murias M, Wierzbowski M, Winckiewicz M, et al. Resveratrol and its synthetic derivatives exert opposite effects on mesothelial cell-dependent angiogenesis via modulating secretion of VEGF and IL-8/CXCL8. *Angiogenesis* 2012;15:361–76.
- [31] Lee KY, Jung JY, Lee MY, Jung D, Cho ES, Son HY. Diospyros blancoi attenuates asthmatic effects in a mouse model of airway inflammation. *Inflammation* 2012; 35:623–32.
- [32] Qiuhua Chen YY, Huang Yingzi, Pan Chun, Liu Ling, Qiu Haibo. Angiotensin-(1–7) attenuates lung fibrosis by way of Mas receptor in acute lung injury. *J Surg Res* 2013;185:740–7.