

# Anti-asthmatic Effects of Baicalin in a Mouse Model of Allergic Asthma

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**The aim of the study was to investigate the anti-asthmatic effects of baicalin (BA) and the possible mechanisms. Asthma model was established by ovalbumin (OVA) intraperitoneal injection. A total of 60 mice were randomly assigned to six experimental groups: control, model, dexamethasone (2 mg/kg), and BA (10 mg/kg, 20 mg/kg, 40 mg/kg). Airway resistance (RI) and lung compliance (Cdyn) were measured, histological studies were evaluated by the hematoxylin and eosin staining, Th1/Th2, OVA-specific serum, and BALF IgE levels and Th17 cytokines were evaluated by enzyme-linked immunosorbent assay, and Th17 cells was evaluated by flow cytometry (FCM). Our study demonstrated that BA inhibited OVA-induced increases in RI and eosinophil count; interleukin (IL)-4, IL-17A levels, and Cdyn were recovered and increased IFN- $\gamma$  level in bronchoalveolar lavage fluid. Histological studies demonstrated that BA substantially inhibited OVA-induced eosinophilia in lung tissue and airway tissue. FCM studies demonstrated that BA substantially inhibited Th17 cells. These findings suggest that BA may effectively ameliorate the progression of asthma and could be used as a therapy for patients with allergic asthma. Copyright © 2013 John Wiley & Sons, Ltd.**

**Keywords:** baicalin; anti-asthmatic effects; Th1/Th2; Th17.

## INTRODUCTION

Asthma is associated with a wide range of symptoms that are characterized by airway inflammation, mucus hypersecretion, and airway hyperresponsiveness (AHR) (Galli *et al.*, 2008). Th2 cytokines (interleukin (IL)-4, IL-5, and IL-13) play critical roles in the pathogenesis of asthma (Venuprasad *et al.*, 2006). Of the cytokines, IL-4 and -13 stimulate immunoglobulin E (IgE) production, and IL-5 enhances eosinophil accumulation; IL-5 expressed by Th2 cells is responsible for eosinophil growth, differentiation, mobilization, recruitment, activation, and survival (Sanderson, 1992; Simon *et al.*, 2004; Lopez, 1988). IL-13 plays an important role in T-cell differentiation toward a Th2 phenotype and isotype switching of B cells to immunoglobulin IgE production (Umetsu and DeKruyff, 1997; Wills-Karp *et al.*, 1998). Th1 cells (another T helper cell, which also plays critical roles in the pathogenesis of asthma) suppress Th2 immune responses by secreting interferon IFN- $\gamma$ . IL-4 induces class switching in IgG1 and IgE, whereas IFN- $\gamma$  is involved in IgG2 $\alpha$  class switching. Accordingly, estimation of IgE production and Th1/Th2 cytokine balance is an important tool in the evaluation of asthma (Brewer *et al.*, 1999). Another T lymphocyte subset, namely Th17 cells, has been recently discovered, and it may be involved in the pathogenesis of several immune-mediated disorders (Schmidt-Weber *et al.*, 2007). Th17 cells are characterized by the production of various

cytokines, including IL-17, IL-6, tumor necrosis factor (TNF)- $\alpha$ , and IL-22. IL-17 is involved in tissue pathology in autoimmune disorders. Furthermore, anti-IL-17 reduces eosinophils infiltration in experimental murine asthma model (Hellings *et al.*, 2003). IL-17 induces recruitment and increases survival for airway macrophages (Sergejeva *et al.*, 2005). These facts have initially suggested a regulatory role for IL-17 (Schnyder-Candrian *et al.*, 2006), and Th17 cell are very important in autoimmune disorders.

Due to thousands of years of experience, Chinese herbal medicines are considered as a rich source of new therapeutic agents. Baicalin (BA) (Fig. 1.) is a flavonoid compound isolated from the root of *Scutellaria baicalensis* Georgi. BA has exhibited various biological activities, including anti-expression of macrophage migration inhibitory factor (Shi-Xue *et al.*, 2012), antihyperglycemic effects (Huan-Ting *et al.*, 2011), activity of proteasome inhibitor (Yi-Xin *et al.*, 2012) antiinflammatory, antioxidant, antibacterial, and anti-allergic properties, which appear to contribute to the treatment of a variety of inflammatory diseases such as bronchitis, asthma, hepatitis, and atopic dermatitis (Chung *et al.*, 1995; Chen *et al.*, 2001). Some studies have demonstrated that BA attenuates cigarette smoke-induced inflammatory response in chronic obstructive pulmonary disease rat models and in human type II pneumocytes, by inhibiting the production of IL-6, IL-8, and TNF- $\alpha$ , as well as the activation of NF- $\kappa$ Bp65, which is known to form a complex with some members of the class I deacetylases such as HDAC2 (Barnes and Adcock, 2009; Zeng *et al.*, 2010). However, few studies reported about anti-asthma effect of BA. The present investigation was designed to determine the effects of BA on asthma in ovalbumin (OVA)-induced mice and its underlying mechanism.

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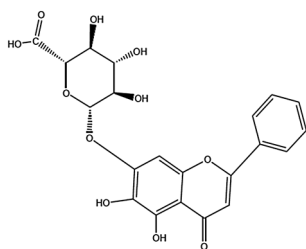


Figure 1. Chemical structure of BA.

## MATERIALS AND METHODS

**Chemicals and reagents.** BA powder (pure:98.7%) was purchased from National Institutes for Food and Drug Control (Beijing, China) and BA solution (4 mg/ml) was prepared in distilled water, and the pH of the solution was adjusted to 7.35 using 1 M sodium hydroxide. Dexamethasone was purchased from Xiansheng drug Store (Nanjing, China). OVA (Sigma Chemical Co., St Louis, MO), aluminium hydroxide (Pierce Biotechnology, Rockford, USA), Wright–Giemsa staining (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kits ((R&D, Minneapolis, MN, USA), RPMI1640 media (Gibco, USA), cell disrupter (Beckman, USA), fluorescein isothiocyanate (FITC) antihuman CD4, and phycoerythrin (PE) anti-mice IL-17A (eBioscience).

**Animals.** Specific pathogen-free female BALB/c mice, aged 6–8 weeks, which were routinely screened serologically for relevant respiratory pathogens, were purchased from Experimental Animal Center of China Pharmaceutical University (Nanjing, China). Animal experiment was carried out in accordance with the Guidelines for Animal Experimentation of China Pharmaceutical University. A total of 60 female BALB/c mice obtained from Experimental Animal Center of China Pharmaceutical University (Nanjing, China). Mice were maintained in an animal facility under standard laboratory conditions for 1 week prior to experiments and provided with water and standard chow ad libitum. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and animal handling followed the dictates of the National Animal Welfare Law of China.

**Sensitization, airway challenge, and treatment.** Mice were divided into six groups (each of 10 animals): (i) normal control, (ii) asthma-model control, (iii) asthma treated with dexamethasone (2 mg/kg, administered by gavage), (iv, v, vi) asthma treated with BA (10, 20, 40 mg/kg, administered by gavage). Allergic asthma was induced by OVA (Grade V) in six of the groups, using the method described by Oh *et al.* (Oh *et al.*, 2006). Mice were immunized via intraperitoneal injection with 10 µg chicken OVA and 2 mg aluminum hydroxide in 200 µl phosphate buffered saline (PBS) (pH 7.4), on days 0 and 14. Mice were exposed to a 1% (w/v) OVA solution in PBS, for 20 min, using an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan) on days 28, 29, and 30 after initial sensitization. Animals were sacrificed 48 h after the last challenge (thus on day 32) to

characterize the suppressive effects of BA. A schematic diagram of the treatment schedule is shown in Fig. 2.

**Determination of airway resistance (RI) and lung compliance (C<sub>dyn</sub>).** Mice were anesthetized, and tracheotomies were performed as described (Bao *et al.*, 2007). The internal jugular vein was cannulated and connected to a microsyringe for intravenous methacholine administration. Airway resistance (RI) and lung compliance (C<sub>dyn</sub>) in response to increasing concentrations of methacholine (0.125–1.00 mg/ml) were recorded using a whole-body plethysmograph chamber (Buxco, Sharon, CT, USA) as described (Teran *et al.*, 1999). RI is defined as the pressure driving respiration divided by flow. C<sub>dyn</sub> refers to the distensibility of the lung and is defined as the change in volume of the lung produced by a change in pressure across the lung. The results are expressed as the percentage of the respective basal values.

**Collection of bronchoalveolar lavage (BAL) fluid.** Mice were sacrificed using an overdose of 50 mg/kg of pentobarbital 48 h after the last challenge, and tracheotomy was performed. After instilling ice-cold PBS (0.5 ml) into a lung, BAL fluid was obtained by three successive aspirations (total volume 1.5 ml) via tracheal cannulation (Djukanovic *et al.*, 1990). BAL fluid (BALF) samples were centrifuged at 1500 rpm for 10 min at 4 °C, the supernatants were stored in –80 °C for analysis of cytokine concentrations, and the pellet was resuspended in 100 µl of saline, centrifuged onto slides and stained for 8 min with Wright–Giemsa staining. The slides were quantified for differential cell count by counting a total of 200 cells/slide at 40 magnification.

**Histological assessment.** Lung tissues and airway tissues were detached from the mice and fixed with Carnoy's solution overnight at 4 °C. The fixed tissues were embedded in paraffin and cut into 4-µm sections with amicrotome (Leica, Nussloch, Germany). The sections were placed on slide glasses, deparaffinized, and stained with hematoxylin and eosin (Sigma, Korea) in order to examine the cells that had infiltrated into the peribronchial connective tissues and airway tissues. Peribronchial and airway cells count based on a five-point scoring system was performed as previously described to estimate the severity of leukocyte infiltration (Duan *et al.*, 2004). The scoring system was: 0, no cells; 1, a few cells; 2, a ring of

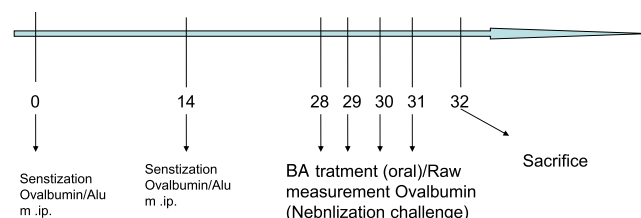


Figure 2. Experimental protocol for induction of airway inflammation, and treatment with BA, using a mouse model. Mice were divided into four groups and sensitized via intraperitoneal injection of 10 µg OVA emulsified in 2 mg aluminum hydroxide in 100 µl PBS buffer (pH 7.4) on days 0 and 14. Each mouse was challenged through the airway with OVA (1%, w/v, in PBS) for 20 min, using an ultrasonic nebulization, on days 28, 29, and 30 after initial sensitization. Mice were given 10, 20, 30 mg/kg/day of BA, orally, on each of days 28 to 30. Animals were sacrificed 48 h after the last challenge.

cells 1 cell layer deep; 3, a ring of cells 2–4 cell layers deep; and 4, a ring of cells more than 4 cell layers deep.

**ELISA detection of BALF cytokines IL-4, IFN- $\gamma$ , and IL-17A.** BALF levels of IL-4, IFN- $\gamma$ , and IL-17A were measured by ELISA according to the manufacturer's instructions (R&D, Minneapolis, MN, USA). The lower detection limits of these assays were 7.8 pg/ml (IL-4), 3.9 pg/ml (IFN- $\gamma$ ), and 3.9 pg/ml (IL-17A). All measurements were performed in duplicate. Briefly, the BALF samples were added in duplicate to 96-well plates with 100  $\mu$ l per well. The appropriate biotinconjugated antibodies were added to each well. The samples were incubated at room temperature for 2 h. The wells were then aspirated, and each well was washed five times. The substrate solutions were added to each well and were incubated for 30 min at room temperature in the dark. The optical density (O.D.) of each well was determined using a microplate reader (Bio-Rad Model 680, USA) that was set to 450 nm. A standard curve was created of the average of the O.D. duplicate readings. The results were calculated using Excel 2007 (Microsoft office, USA).

**Measurement of OVA-specific serum and BALF levels of IgE.** OVA-specific serum and BALF IgE levels were determined by ELISA using samples collected 48 h after the last OVA challenge, as described previously (Jain *et al.*, 2002). In brief, a 96-well microtitre plate was coated with OVA (10 mg/ml) and then treated with mouse sera followed by biotin-conjugated rat anti-mouse IgE (Pharmingen, San Diego, CA). Then, avidinehorseradish peroxidase (HRP) solution was added to each well. Units are O.D. readings at 405 nm.

**Flow cytometric analysis of Th17 cells.** The spleen was removed from each sacrificed mouse and placed in a tube containing RPMI1640 media (Gibco). A single cell suspension was prepared by disrupting the spleen using Cell disrupter (Beckman); cells were equally distributed into tubes and washed once in PBS. For Th17 analysis cells were incubated with FITC antihuman CD4 at 4 °C for 30 min. Following surface staining, cells were fixed and permeabilized according to the manufacturer's instructions and then stained with PE anti-mice IL-17A (eBioscience) for Th17 detection.

**Statistical analysis.** The statistical significance of any difference was determined by one-way ANOVA. The data are expressed as the means  $\pm$  SD. The SPSS statistical software package (Version 10.0, Chicago, IL) was used for the statistical analysis. A p-value less than 0.05 was considered statistically significant, and  $p < 0.01$  was considered to be statistically very significant.

## RESULTS

### Effect of BA on airway resistance (RI) and lung compliance (Cdyn)

Measurements of airway resistance (RI) and lung compliance (Cdyn) during methacholine-induced constriction are shown in Fig. 3. The RI values of the OVA challenged

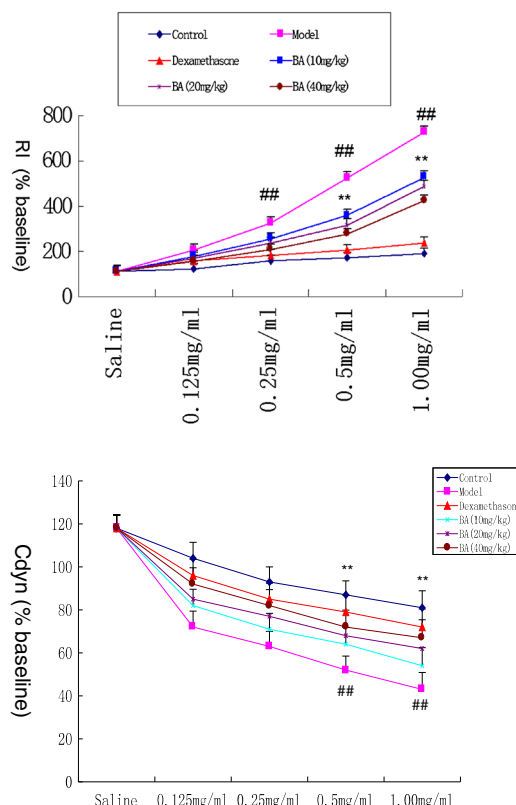


**Figure 3.** Effect of BA on airway resistance (RI) and lung compliance (Cdyn). Values are expressed as means  $\pm$  SDs. Compared with control: #  $P < 0.05$ , ##  $P < 0.01$ ; Compared with model: \*  $P < 0.05$ , \*\*  $P < 0.01$ . Columns, mean of six independent experiments; bars, SD.

group was significantly higher than that of the control group when concentrations of 0.250–1.00 mg ml<sup>-1</sup> methacholine. OVA-sensitized and-challenged mice treated with BA, especially BA (40 mg/kg), displayed a reduced mean RI upon exposure to 0.5–1.0 mg ml<sup>-1</sup> compared with the response of the OVA-sensitized and -challenged group. Positive control mice given with dexamethasone showed an RI decrease similar to that achieved using BA; the Cdyn values of the OVA-challenged group was significantly lower than that of the PBS control group when concentrations of 0.500–1.00 mg ml<sup>-1</sup> methacholine, OVA-sensitized and-challenged mice treated with BA, especially BA (40 mg/kg), displayed an increased mean Cdyn upon exposure to 0.5–1.0 mg ml<sup>-1</sup> compared with the response of the OVA-sensitized and -challenged group. Positive control mice given with dexamethasone showed a Cdyn increase similar to that achieved using BA.

### Effect of BA on OVA-induced eosinophilia and BAL fluid composition

We examined changes in total cell levels in the BAL fluid to determine the effects of BA on asthma. Suppression of eosinophilia by BA in OVA-challenged mice was estimated by counting of cells recruited to the BAL fluid 48 h after the last challenge. As shown in Fig. 4, OVA induced a marked influx of leukocytes into BAL fluid in the control group. Eosinophil levels markedly increased in the BAL fluid of OVA-challenged mice. OVA-sensitized and -challenged mice treated with BA displayed significantly reduced leukocytosis, a fall in absolute eosinophilia, and decreased lymphocytosis, upon asthma induction.

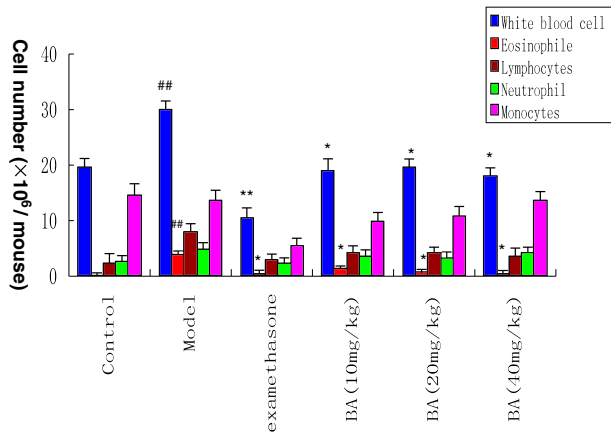


**Figure 4.** Effect of BA on OVA-induced eosinophilia and BAL fluid composition. Values are expressed as means  $\pm$  SDs. Compared with control: <sup>#</sup>  $P < 0.05$ , <sup>##</sup>  $P < 0.01$ ; Compared with model: <sup>\*</sup>  $P < 0.05$ , <sup>\*\*</sup>  $P < 0.01$ . Columns, mean of six independent experiments; bars, SD.

### Effect of BA on OVA-induced eosinophilia in lung tissue and airway tissue

Because BA inhibits inflammatory cell recruitment into the lung tissue and airway tissue, we next examined the anti-asthmatic effects of the BA. Lung tissue and airway

tissue were collected 48 h after the final OVA challenge. In OVA-induced asthmatic lung tissue and airway tissue, we observed marked infiltration of inflammatory cells into perivascular and airway, peribronchial connective tissues, and airway tissue, compared with the normal tissue. Moreover, most leukocytes were eosinophils. The control group displayed no change in the extent of inflammatory cell infiltration, similar to what was seen in dexamethasone-treated positive control mice. Eosinophilia in OVA-sensitized and -challenged mice treated with BA was significantly attenuated compared with the level seen in OVA-challenged mice as shown in Fig. 5, 5b, 5c, and 5d.

### Effect of BA on cytokine levels in BAL fluid

To determine whether BA influenced cytokine secretion into BAL fluid, IL-4, IFN- $\gamma$ , and IL-17A in the BAL fluid were measured by ELISA 48 h after the final challenge. As shown in Table 1, as for IL-4 and IL-17A, OVA challenge triggered significant increases in cytokine levels in the BAL fluid, compared with control fluid. In OVA-sensitized and -challenged mice treated with BA, the rises in cytokine levels were significantly suppressed, compared with the concentrations seen in the OVA-challenged group, specifically, BA (40 mg/kg); as for IFN- $\gamma$ , OVA challenge triggered significant decreases

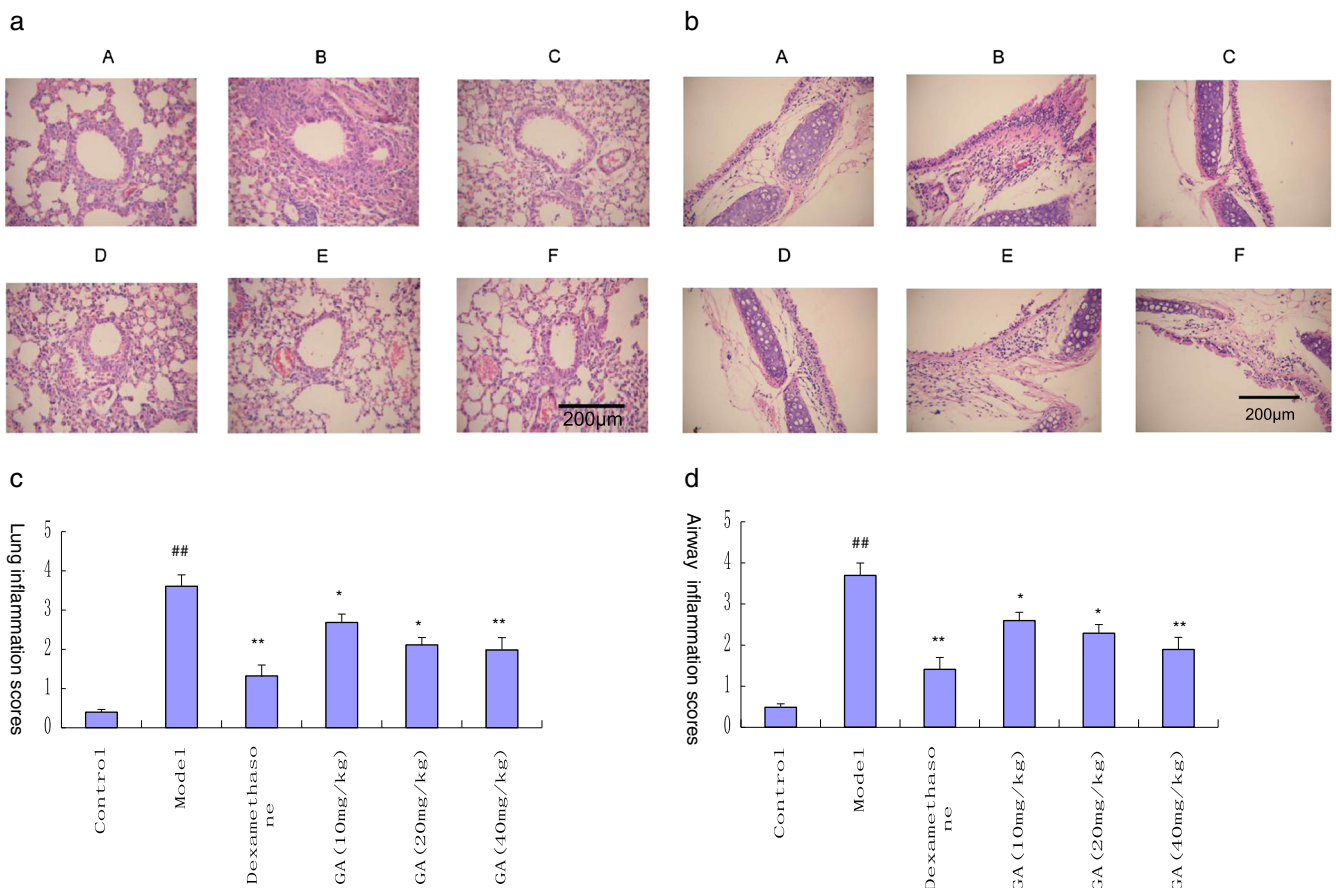


**Figure 5.** a. Pathological changes of lung tissues observed by hematoxylin and eosin (HE) staining. (Light microscopy,  $\times 200$ ): A (control); B (model); C (Dexamethasone, 2 mg/kg); D [BA (10 mg/kg)]; E [BA (20 mg/kg)]; F [BA (40 mg/kg)]. Bar = 200  $\mu$ m. b. Pathological changes of airway tissues observed by HE staining (Light microscopy,  $\times 200$ ): A (control); B (model); C (Dexamethasone, 2 mg/kg); D [BA (10 mg/kg)]; E [BA (20 mg/kg)]; F [BA (40 mg/kg)]. Bar = 200  $\mu$ m. c. Scoring of the extent of inflammation via quantitative analysis of inflammatory cell infiltration in lung sections. Values are expressed as means  $\pm$  SDs. Compared with control: <sup>#</sup>  $P < 0.05$ , <sup>##</sup>  $P < 0.01$ ; Compared with model: <sup>\*</sup>  $P < 0.05$ , <sup>\*\*</sup>  $P < 0.01$ . Columns, mean of six independent experiments; bars, SD. d. Scoring of the extent of inflammation via quantitative analysis of inflammatory cell infiltration in airway sections. Values are expressed as means  $\pm$  SDs. Compared with control: <sup>#</sup>  $P < 0.05$ , <sup>##</sup>  $P < 0.01$ ; Compared with model: <sup>\*</sup>  $P < 0.05$ , <sup>\*\*</sup>  $P < 0.01$ . Columns, mean of six independent experiments; bars, SD.

**Table 1.** Effect of BA on cytokine levels in BAL fluid

| Group         | Dose (mg/kg) | Level of IL-4(pg/ml)           | Level of IFN- $\gamma$ (pg/ml) | Level of IL-17A (pg/ml)        |
|---------------|--------------|--------------------------------|--------------------------------|--------------------------------|
| Control       | 0            | 44.82 $\pm$ 0.63               | 66.62 $\pm$ 0.48               | 17.21 $\pm$ 1.13               |
| Model         | 0            | 86.53 $\pm$ 0.40 <sup>#</sup>  | 43.69 $\pm$ 0.34 <sup>#</sup>  | 31.22 $\pm$ 1.09 <sup>#</sup>  |
| Dexamethasone | 2            | 52.41 $\pm$ 0.16 <sup>**</sup> | 62.82 $\pm$ 0.29 <sup>**</sup> | 18.37 $\pm$ 1.23 <sup>**</sup> |
| BA            | 10           | 58.47 $\pm$ 0.22 <sup>**</sup> | 58.88 $\pm$ 0.16 <sup>*</sup>  | 21.46 $\pm$ 1.33 <sup>**</sup> |
| BA            | 20           | 57.00 $\pm$ 0.27 <sup>**</sup> | 57.69 $\pm$ 0.18 <sup>*</sup>  | 20.29 $\pm$ 1.21 <sup>**</sup> |
| BA            | 40           | 53.24 $\pm$ 0.29 <sup>**</sup> | 55.25 $\pm$ 0.18 <sup>*</sup>  | 19.11 $\pm$ 1.04 <sup>**</sup> |

Values are expressed as means  $\pm$  SDs. Compared with control: <sup>#</sup>  $P < 0.05$ , <sup>##</sup>  $P < 0.01$ ; Compared with model: <sup>\*</sup>  $P < 0.05$ , <sup>\*\*</sup>  $P < 0.01$ . Columns, mean of six independent experiments; bars, SD.

in cytokine levels in the BAL fluid, compared with control fluid. In OVA-sensitized and-challenged mice treated with BA, the rises in cytokine levels were significantly strengthened, compared with the concentrations seen in the OVA-challenged group, specifically, BA (40 mg/kg).

### Effect of BA on OVA-specific serum and BALF levels of IgE

The cross-linking of allergen-specific IgE on the surfaces of mast cells upon allergen challenge is relevant to the initiation of the early asthmatic reaction. The serum and BALF levels of OVA-specific immunoglobulins were measured 48 h after the final airway challenge. We determined that sensitization and challenge with OVA resulted in increased serum and BALF levels of OVA-specific IgE, when compared with the control mice. The treatment of sensitized mice with BA resulted in reductions in OVA-specific IgE (Fig. 6). These results

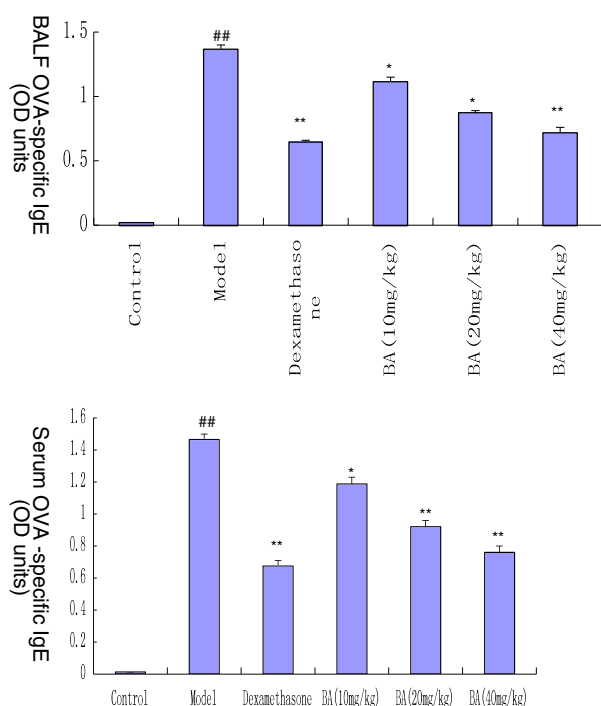


**Figure 6.** Effect of BA on OVA-specific serum and BALF levels of IgE. Values are expressed as means  $\pm$  SDs. Compared with control: <sup>#</sup>  $P < 0.05$ , <sup>##</sup>  $P < 0.01$ . Compared with model: <sup>\*</sup>  $P < 0.05$ , <sup>\*\*</sup>  $P < 0.01$ . Columns, mean of six independent experiments; bars, SD.

**Table 2.** Effect of BA on Th17 cells

| Group         | Dose (mg/kg) | Numbers of Th17 (%)<br>(n = 10/group) |
|---------------|--------------|---------------------------------------|
| Control       | 0            | 0.98 $\pm$ 0.22                       |
| Model         | 0            | 9.65 $\pm$ 1.23 <sup>#</sup>          |
| Dexamethasone | 2            | 2.59 $\pm$ 0.43 <sup>**</sup>         |
| BA            | 10           | 5.70 $\pm$ 2.70 <sup>*</sup>          |
| BA            | 20           | 4.50 $\pm$ 0.51 <sup>**</sup>         |
| BA            | 40           | 4.32 $\pm$ 1.08 <sup>**</sup>         |

Values are expressed as means  $\pm$  SDs. Compared with control: <sup>#</sup>  $P < 0.05$ , <sup>##</sup>  $P < 0.01$ ; Compared with model: <sup>\*</sup>  $P < 0.05$ , <sup>\*\*</sup>  $P < 0.01$ . Columns, mean of six independent experiments; bars, SD.

support the conclusion that BA suppressed the generation of a Th2-type immune response in this animal asthma model.

### Effect of BA on Th17 cells

As shown in Table 2 and Fig. 7, the frequencies of Th17 cells in model group (9.65  $\pm$  1.23%) were higher than those in the normal group (0.98  $\pm$  0.22%), while in dexamethasone group and treatment groups, they were significantly lower ( $P < 0.05$ ).

## DISCUSSION

Allergic asthma is a chronic airway inflammation disease. In most asthma phenotypes, increases in eosinophil levels are observed in the tissues, blood, and BAL fluid. Furthermore, Th2 cells and their secreted products aggregate into airway and lung tissues. In addition, high serum levels of IgE, persistent AHR, and goblet cell hyperplasia are observed. BA (7-glucuronic acid, 5,6-dihydroxyflavone) is a flavonoid compound purified from *Scutellaria baicalensis* Georgi, an herb used widely in traditional Chinese medicine. BA has exhibited various biological activities, including antiinflammatory, antioxidant, antiallergic, antiplatelet, antiischemic, antiviral, antitumor, and antipyresis properties (Tsai *et al.*, 2006). However, this is the first time the anti-asthmatic effects of BA are demonstrated in a mouse model of allergic asthma. We also investigated the possible mechanisms. Our results support the hypothesis that BA may be used as a therapy for patients with allergic asthma.

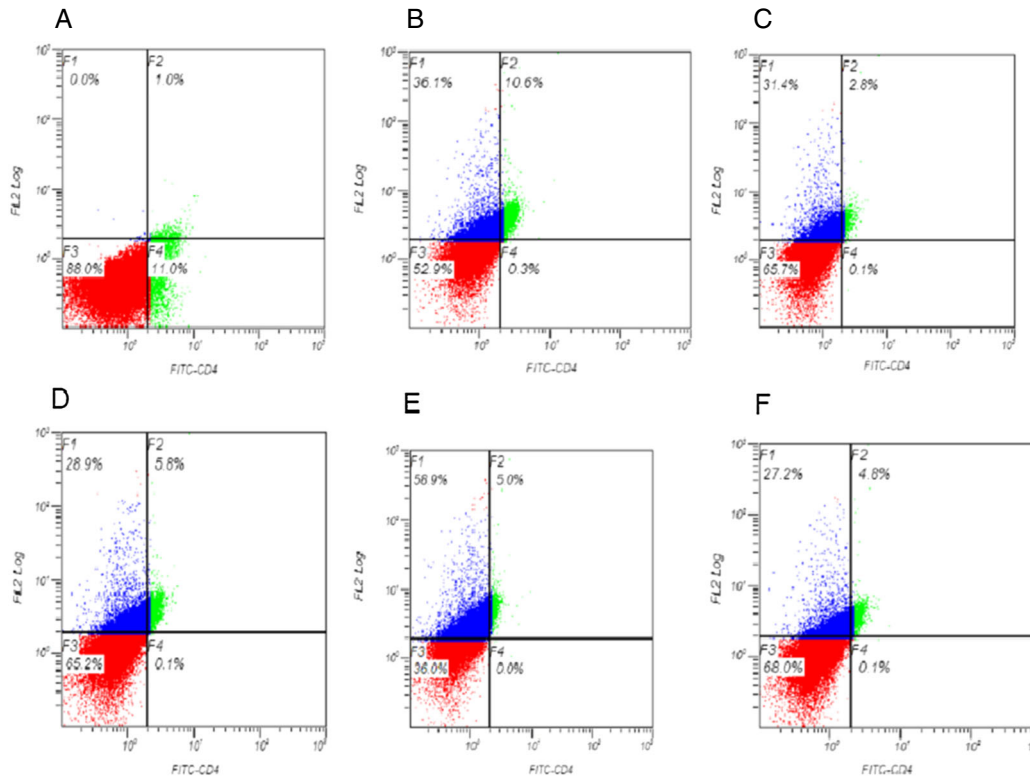


**Figure 7.** Effect of BA on Th17 cells. Values are expressed as means  $\pm$  SDs. Compared with control: # $P < 0.05$ , ## $P < 0.01$ . Compared with model: \* $P < 0.05$ , \*\* $P < 0.01$ . Columns, mean of six independent experiments; bars, SD.

In animal models, OVA challenges induced a significant increase in the total serum IgE and BALF IgE (Hamelmann *et al.*, 1999; Holgate *et al.*, 2005). Our data showed that the serum and BALF concentrations of IgE were significantly reduced in allergic mice after BA administration. This result suggests that BA has an effect on the allergic asthma that developed in an IgE-dependent manner.

Asthma is defined as a variable level of airway obstruction usually accompanied by AHR. Bronchoconstriction attributable to contraction or hypertrophy of airway smooth muscle, and inflammation within the airway leads to decreased lung function (Wenzel, 2006). AHR is a measure of the bronchial constriction commonly observed in individuals with asthma. We monitored the effects of BA on AHR by measuring RI and Cdyn. These experiments disclosed that BA inhibited the OVA-induced AHR in response to inhaled methacholine.

The mechanisms of recruitment of inflammatory cells associated with and presumably causing AHR have been well-studied. Migration of inflammatory cells, specifically eosinophils and lymphocytes, into the lung is a major contributor to the development of allergic airway inflammation (Elsner and Kapp, 1999). A rise in the number of eosinophils in the BAL fluid is a characteristic of asthma. The present results clearly demonstrate that BA significantly reduces eosinophil numbers in the lung tissue and airway tissue.

Allergic asthma is recognized as a TH2-mediated immune system disease. The induction of allergen-specific TH1 cells can attenuate allergic asthma. Several studies have shown that administration of TH1 cytokines, such as IFN- $\gamma$ , at the time of sensitization can inhibit the induction of AHR and TH2-driven inflammation

(Gavett *et al.*, 1995; Li *et al.*, 1996; Sur *et al.*, 1996). Asthma and inflammation are associated with enhanced production of IL-4 and decreased production of IFN- $\gamma$ . Our data show that BA significantly suppresses IL-4 cytokine levels and significantly increases IFN- $\gamma$  cytokine levels.

Recently, T-helper17 (Th17) cells have been described as two distinct subsets from Th1 and Th2 cells that play opposite roles *in vivo* (Brewer *et al.*, 1999). Th17 cells expressing retinoic acid receptor-related orphan receptor  $\gamma$ t (ROR $\gamma$ t) play crucial roles in induction of autoimmune tissue injuries and inflammation by producing cytokines such as IL-17A, TNF- $\alpha$ , and IL-6. Th17 cells may be important in the development/prevention of inflammatory and autoimmune diseases such as asthma. Our data show that BA significantly suppresses IL-17A and Th17 cells.

In summary, BA effectively suppressed IL-4, improved IFN- $\gamma$ , and inhibited both the recruitment of eosinophils and mucus overproduction, in mice with OVA-induced asthma. Airway inflammation and bronchial hyperresponsiveness were attenuated in the presence of BA. Moreover, BA effectively suppressed IL-17A and Th17 cells. Our findings support the possible use of BA as a therapeutic drug for patients with allergic asthma.

### Acknowledgements

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### Conflict of Interest

There are no financial and commercial conflicts of interest in this study.

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