



Matrine attenuates allergic airway inflammation and eosinophil infiltration by suppressing eotaxin and Th2 cytokine production in asthmatic mice



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ABSTRACT

Ethnopharmacological relevance: Matrine has been isolated from *Sophora flavescens*, and found to show anti-inflammatory effects in macrophages and anti-cachectic effects in hepatomas. The present study investigated whether matrine suppressed eosinophil infiltration and airway hyperresponsiveness (AHR) in mice, and decreased the inflammatory response of tracheal epithelial cells.

Materials and methods: BALB/c mice were sensitized and challenged with ovalbumin to induce allergic asthma in mice. These asthmatic mice were given various doses of matrine by intraperitoneal injection. Additionally, activated human tracheal epithelial cells (BEAS-2B cells) were treated with matrine, and evaluated for levels of proinflammatory cytokines and chemokines.

Results: We found that matrine significantly decreased AHR, and suppressed goblet cell hyperplasia, eosinophil infiltration, and inflammatory response in the lung tissue of asthmatic mice. Matrine also reduced the levels of Th2 cytokines and chemokines in bronchoalveolar lavage fluid, and suppressed OVA-IgE production in serum. Furthermore, matrine treatment of activated BEAS-2B cells decreased production of proinflammatory cytokines and eotaxins, as well as suppressed ICAM-1 expression and thus adhesion of eosinophils to inflammatory BEAS-2B cells in vitro.

Conclusions: Our findings suggest that matrine can improve allergic asthma in mice, and therefore has potential therapeutic potential in humans.

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1. Introduction

Asthma is a complex chronic lung disease, and its associated morbidity has been on the rise in developed and developing countries (Busse and Lemanske, 2001). Asthma attacks are characterized by wheezing, coughing, difficulty breathing, and shortness of breath (Barrios et al., 2006; Poon et al., 2012). Without rapid treatment, an asthma attack can cause airway constriction, preventing the patient from getting enough oxygen to perform

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vital physiological functions and potentially leading to suffocation, shock, and death (Poon et al., 2012).

Recent studies have determined that asthma is an allergic lung inflammatory disease, and that it is exacerbated by Th2 activity (Hamid and Tulic, 2009; Agrawal and Shao, 2010). Activated Th2 cells secrete more IL-4, IL-5, and IL-13, thus causing airway hyperresponsiveness (AHR), eosinophil infiltration, goblet cell hyperplasia, and excessive mucus secretion (Bosnjak et al., 2011). Furthermore, Th2 cells induce B cells to secrete IgE, which activates mast cells to release more allergic and inflammatory mediators (Larché et al., 2003). These findings indicate that regulating Th2 cell activity may be an opportunity to improve asthma development.

Sophora flavescens (Leguminosae) is an evergreen perennial shrub that grows mainly in China and Taiwan (Sun et al., 2012).

In traditional Chinese medicine, the *Sophora flavescens* root is used as a diuretic and to treat pyogenic infection, jaundice, dysentery, and fever (Kim et al., 2012). The combination of *Sophora flavescens* with other herbs can also reportedly improve or treat allergic diseases, including asthma and atopic dermatitis (Hong et al., 2009; Kelly-Pieper et al., 2009). These previous findings suggest that *Sophora flavescens* likely contains some active compound that can improve asthma.

Over recent years, several pure compounds have been isolated from *Sophora flavescens*, including matrine, oxymatrine, kurarinone, and multiple sophoraflavanones (Sun et al., 2012). Previous studies have found matrine to be effective against *Cryptosporidium parvum* and hepatitis B virus infection (Li et al., 2005; Chen and Huang, 2012; Ma et al., 2013). Matrine also reportedly possesses anti-tumor activities in erythroleukemia, gastric cancer, breast cancer, and hepatoma (Jiang et al., 2007; Li et al., 2010, 2013; Sun et al., 2012). Furthermore, matrine has shown anti-inflammatory action, inhibiting pro-inflammatory cytokine production in lipopolysaccharide-stimulated mouse macrophages (Zhang et al., 2011).

We hypothesized that matrine might be able to improve the inflammatory response in asthma. To test this, asthmatic mice were given matrine by intraperitoneal injection and we evaluated whether this treatment improved asthma symptoms, including by suppression of AHR, eosinophil infiltration, and Th2-associated cytokine levels.

2. Materials and methods

2.1. Animals

We obtained 6- to 8-week-old female BALB/c mice from the National Laboratory Animal Center in Taiwan. Mice were kept and maintained in conventional animal housing on a 12-h light/dark cycle at the Animal Center of Chang Gung University. Before starting the experiment, the mice were given at least 1 week to adapt to the experimental environment. The care and housing of experimental animals were approved in accordance with the guidelines of the Laboratory Animal Care Committee of Chang Gung University of Science and Technology and Chang Gung University.

2.2. Sensitization, airway allergen challenge, and drug treatment

Fig. 1 illustrates the experimental protocol and shows the chemical structure of matrine ($\geq 99\%$ by HPLC). Matrine were dissolved in normal saline, and were purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA). On days 1–3 and 14 of the experiment, mice were sensitized by intraperitoneal injection with 50 μ g ovalbumin (OVA) (Sigma, St. Louis, MO, USA) emulsified with aluminum hydroxide (Thermo, Rockford, IL, USA) in 200 μ L normal saline, as described previously (Huang and Liou, 2012) (Fig. 1). On days 14, 17, 20, 23, and 27, mice were subjected to a 20-min airway challenge with 2% OVA in 3 mL normal saline, by inhalation through an ultrasonic nebulizer (DeVilbiss Pulmo-Aide 5650D, USA). Mice were randomly divided into 5 groups of 12 mice each: normal control mice that were sensitized and challenged with normal saline (N group); OVA-sensitized mice that were sensitized and challenged with OVA, and then given intraperitoneal injection of normal saline (OVA group); and OVA-sensitized mice that were given 5, 10, or 20 mg/kg matrine by an intraperitoneal injection 1 h before each inhalation challenge and AHR assay (groups M5, M10, and M20, respectively).

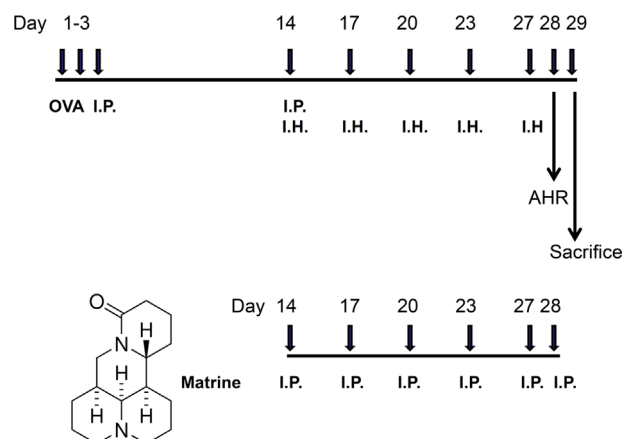


Fig. 1. Sensitization and OVA allergen challenge protocol: Mice were sensitized by intraperitoneal (IP) injection with OVA on days 1–3 and 14. Mice were then subjected to airway challenge with 2% OVA inhalation (IH) on days 14, 17, 20, 23, and 27. Matrine (in normal saline) was injected intraperitoneally 1 h before each IH and the measurement of airway hyperresponsiveness.

2.3. Measurement and analysis of AHR

On day 28, mice were assessed for AHR by evaluation of their airway function after aerosolization with methacholine, as described previously (Lin et al., 2011). Briefly, mice were challenged 24 h after the last OVA inhalation. They inhaled increasing doses of methacholine (0–40 mg/mL) for 3 min, and were then placed into the whole-body plethysmography chamber (Buxco Electronics, Troy, NY, USA). Results were recorded as enhanced pause values (Penh).

2.4. Bronchoalveolar lavage fluid and cell count

On day 29, all mice were sacrificed and bronchoalveolar lavage fluid (BALF) was collected as described previously (Liou and Huang, 2011). Briefly, the mouse trachea was intubated and the lungs were flushed with 1 mL normal saline. The supernatants were obtained and assayed for cytokine and chemokine productions. Cells were stained with Liu stain solution (Polysciences, Inc. Taipei, Taiwan) to determine the cell counts in BALF.

2.5. Histologic analysis of lung

Lung tissues were fixed in formalin, embedded in paraffin, and cut into 6- μ m sections. Some slides were stained with hematoxylin and eosin (HE) for eosinophil infiltration assay, while other slides were stained with the periodic acid-Schiff (PAS) stained system (Sigma) to measure goblet cells hyperplasia as described previously (Huang and Liou, 2012).

2.6. Serum collection and splenocyte cultures

Serum samples were collected to measure OVA-specific antibodies as previously described (Shen et al., 2011). Splenocytes (5×10^6 cells/mL) were cultured in RPMI 1640 medium (Invitrogen-Gibco™, Paisley, Scotland) with 100 μ g/mL OVA for 5 days. Then the supernatants were obtained and assayed for cytokine concentrations.

2.7. Enzyme-linked immunosorbent assay (ELISA)

The BALF and cell culture supernatants were assayed using ELISA kits for IL-4, IL-5, IL-6, IL-8, IL-13, tumor necrosis factor- α (TNF- α), eotaxin-1, eotaxin-2, and intercellular adhesion molecule

1 (ICAM-1) according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA), as previously described (Shen et al., 2011). Serum OVA-specific antibodies – including IgG1, IgG2a, and IgE – were measured using a specific ELISA kit (BD Biosciences, San Diego, CA, USA). Serum samples from OVA-sensitized mice were pooled, and OVA-IgG1 and OVA-IgG2a standard curves were constructed for measuring the units of OVA-specific IgG1 and IgG2a according to OD₄₅₀ readings. Additionally, serum was diluted 5-fold with PBS and directly used to assay OVA-IgE levels by OD₄₅₀.

2.8. Measurement of cell viability

Cell viability was assayed as previously described (Chang et al., 2012). Briefly, human bronchial epithelial cells (BEAS-2B) were plated in 96-well plates at 5×10^4 cells/well, treated with varying concentrations of matrine, and incubated for 24 h at 37 °C with 5% CO₂. The plate was then washed, and incubated with 100 µL MTT (5 mg/mL, Sigma) at 37 °C for 4 h. The supernatant was removed from the plate, isopropanol was added to dissolve the crystals, and the OD₅₇₀ readings were obtained using a microplate reader (Multiskan FC, Thermo, Waltham, MA, USA).

2.9. Culture and matrine treatment of BEAS-2B cells

BEAS-2B cells were seeded in DMEM/F12 medium (Invitrogen-Gibco™) in 24-well plates. The cells were pre-treated with matrine (100–400 µM, dissolved in ddH₂O) for 1 h, and then cultured with 10 ng/mL TNF-α for 24 h, or with 10 ng/mL TNF-α and 10 ng/mL IL-4 for 48 h. Next, the supernatants were collected and assayed for cytokine and chemokine levels using ELISA kits.

2.10. Cell-cell adhesion assay

TNF-α-activated BEAS-2B cells were treated with various concentrations of matrine (100–400 µM) in 6-well plates for 24 h, as previously described (Huang and Liou, 2012). HL-60 cells were treated with 0.5 mM butyric acid for 7 days, leading the cells to differentiate into eosinophil-like cells, as previously described (Chuang et al., 2003). HL-60 cells were then treated with calcein-AM solution (Sigma), and co-cultured with BEAS-2B cells for 1 h. A fluorescence microscope (Olympus, Tokyo, Japan) was used to observe the adherence of the HL-60 cells to BEAS-2B cells.

2.11. Statistical analysis

All data were assessed by one-way analysis of variance (ANOVA), followed by the Tukey–Kramer post-hoc test for multiple comparisons. The values were expressed as mean ± SEM. Differences for which p was < 0.05 were considered statistically significant.

3. Results

3.1. Matrine treatment improved AHR in OVA-sensitized mice

The results of our AHR analysis showed that OVA-sensitized mice inhaled more methacholine, and that the Penh values increased along with increasing methacholine dose (Fig. 2A). At 20 mg/mL inhaled methacholine, the matrine-treated groups exhibited significantly decreased Penh values compared with the OVA group (M5, 0.71 ± 0.10 , $p < 0.01$; M10, 0.59 ± 0.06 , $p < 0.01$; M20, 0.50 ± 0.04 , $p < 0.01$ vs. OVA, 1.77 ± 0.53). Even at 40 mg/mL inhaled methacholine, all matrine-treated groups showed lower Penh values compared with the OVA group (M5, 1.69 ± 0.21 , $p < 0.05$; M10, 1.13 ± 0.24 , $p < 0.01$; M20, 0.58 ± 0.09 , $p < 0.01$ vs. OVA, 5.16 ± 0.76). Overall, treatment with all three different doses of matrine (M5, M10, and M20 groups) showed significant ability to suppress AHR in asthmatic OVA-sensitive mice.

3.2. Effects of matrine on inflammatory cells in BALF

The evaluation of inflammatory cells in BALF indicated that matrine suppressed the infiltration of inflammatory cells in asthmatic mice (Fig. 2B). Intraperitoneal injection of matrine in OVA-sensitized asthmatic mice significantly decreased total cells and eosinophils compared with in the OVA group (total cells: M5: $5.6 \times 10^5 \pm 4.9 \times 10^4$, $p < 0.01$; M10: $4.2 \times 10^5 \pm 3.5 \times 10^4$, $p < 0.01$; M20: $3.7 \times 10^5 \pm 2.1 \times 10^4$, $p < 0.01$ vs. OVA: $1.1 \times 10^6 \pm 7.2 \times 10^4$) (eosinophils: M5: $2.5 \times 10^5 \pm 2.3 \times 10^4$, $p < 0.01$; M10: $1.3 \times 10^5 \pm 1.2 \times 10^4$, $p < 0.01$; M20: $1.2 \times 10^5 \pm 1.0 \times 10^4$, $p < 0.01$ vs. OVA: $5.5 \times 10^5 \pm 7.0 \times 10^4$).

3.3. Effects of matrine on OVA-induced eosinophilia and goblet cell hyperplasia in lung tissue

HE staining revealed eosinophil infiltration in lung tissue. OVA-sensitive mice exhibited more eosinophil infiltration between the bronchus and blood vessels (Fig. 3). However, the matrine-treated asthmatic mice exhibited significantly reduced eosinophil infiltration in lung biopsy sections. Furthermore, PAS staining results

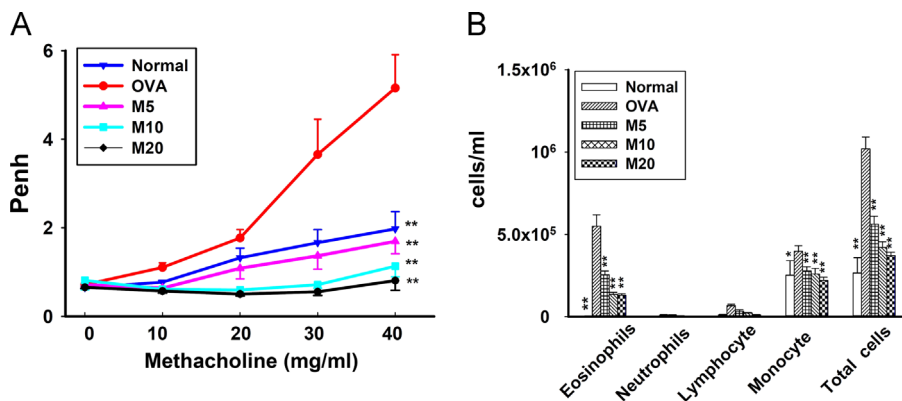


Fig. 2. The effect of matrine on AHR and cell counts of BALF in asthmatic mice. (A) Mice inhaled increasing doses of methacholine (10–40 mg/mL), and AHR was measured and shown as Penh values. ($n=12$ mice/group in three independent experiments). (B) Inflammatory cells and total cells were calculated in BALF. All data are presented as mean ± SEM. * $p < 0.05$ compared with the OVA control group. ** $p < 0.01$ compared with the OVA control group.

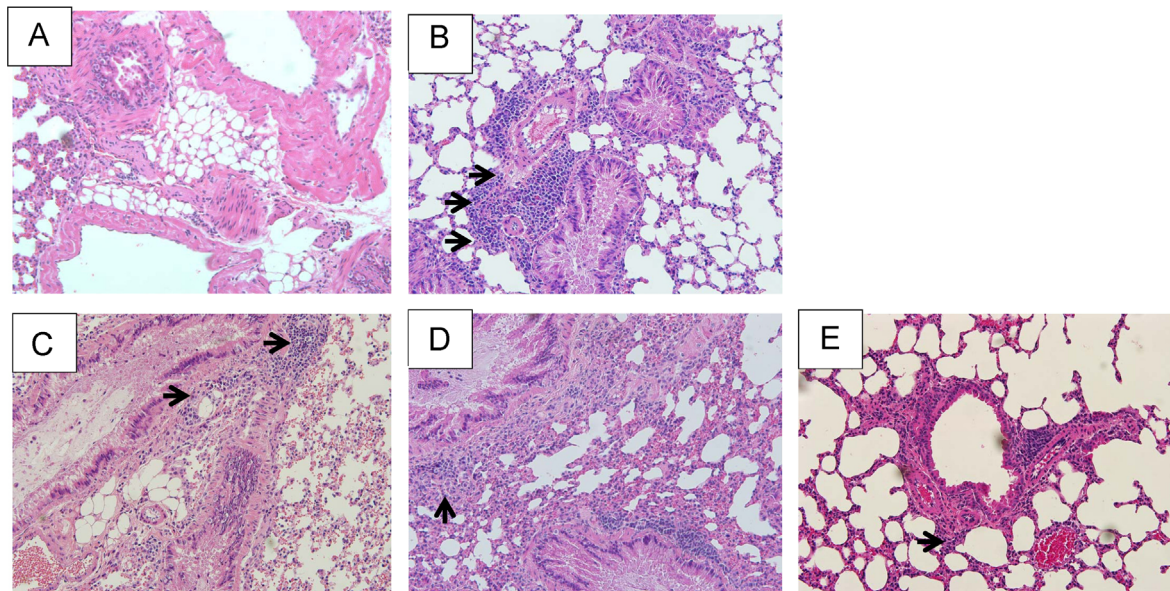


Fig. 3. The effect of matrine on eosinophil infiltration of lung tissue in OVA-sensitized mice. Hematoxylin and eosin staining of lung sections (6 μ m) from the normal (A), OVA (B), M5 (C) M10 (D), and M20 (E) groups. 200 $\times$ magnification. Eosinophils are indicated by arrows.

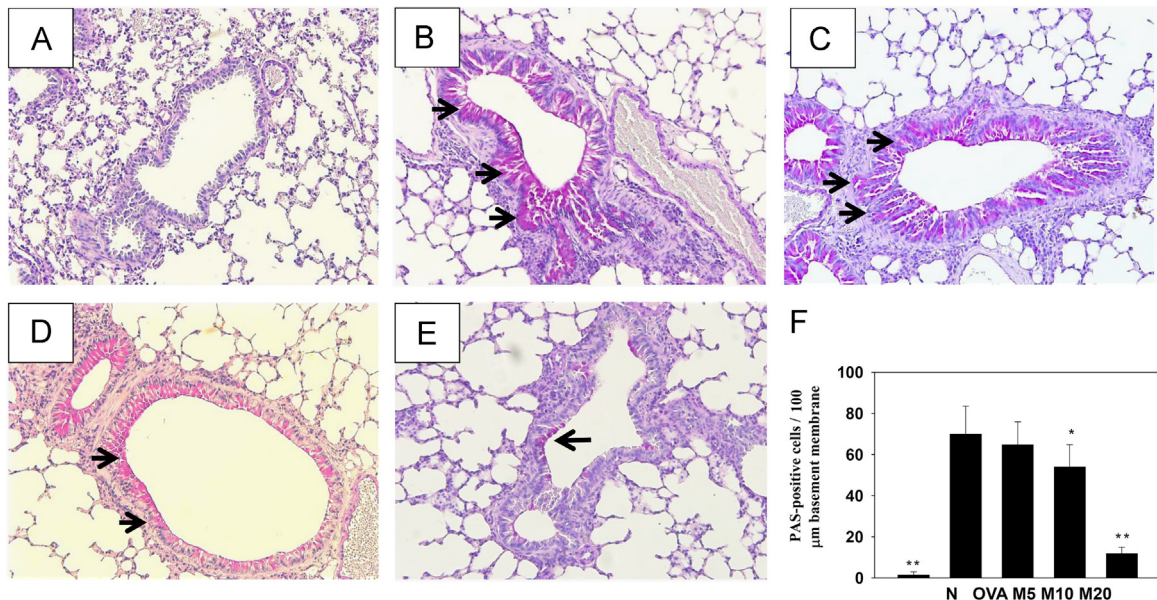


Fig. 4. The effects of matrine on goblet cell hyperplasia in lung tissue of OVA-sensitized mice. Lung sections (6 μ m) were stained with PAS stain to observe goblet cell hyperplasia in the normal (A), OVA (B), M5 (C) M10 (D), and M20 (E) groups. 200 $\times$ magnification. Goblet cells are indicated by arrows. (F) The PAS-positive cells per 100 μ m of basement membrane were also analyzed. All data are presented as mean $\pm$ SEM. * p < 0.05 compared with the OVA control group. ** p < 0.01 compared with the OVA control group.

showed that matrine treatment significantly inhibited goblet cell hyperplasia compared with in OVA-sensitive mice (Fig. 4).

3.4. Effects of matrine on cytokine and chemokine levels in BALF

Cytokine and chemokine productions in BALF were determined using ELISA (Fig. 5). We found that OVA-sensitized mice treated with high-dose matrine (M20 group) exhibited lower IL-4 levels (M5: 81.1 ± 11.0 pg/mL, $p=0.42$; M10: 83.4 ± 7.6 pg/mL, $p=0.19$; M20: 49.2 ± 10.2 pg/mL, $p < 0.05$ vs. OVA: 97.7 ± 16.5 pg/mL). In addition, the M10 and M20 groups showed significantly reduced levels of IL-5, IL-13, TNF- α , IL-6, and eotaxin-1 compared with the OVA group.

3.5. Effects of matrine on OVA-specific antibody production in serum, and cytokine levels in splenocyte culture supernatant

Serum analysis revealed that the M20 group exhibited significantly reduced production of OVA-IgE and OVA-IgG1, but no changes in OVA-IgG2a levels (Fig. 6A–C). Analysis of splenocyte culture supernatant showed that matrine treatment did not significantly reduce IL-4, IL-5, or IL-13 levels compared to in the OVA group (Fig. 6D–F).

3.6. Effects of matrine on inflammatory mediators in activated BEAS-2B cells

The MTT assay showed that treatment with ≤ 800 μ M matrine did not significantly affect cell viability in BEAS-2B cells (data not

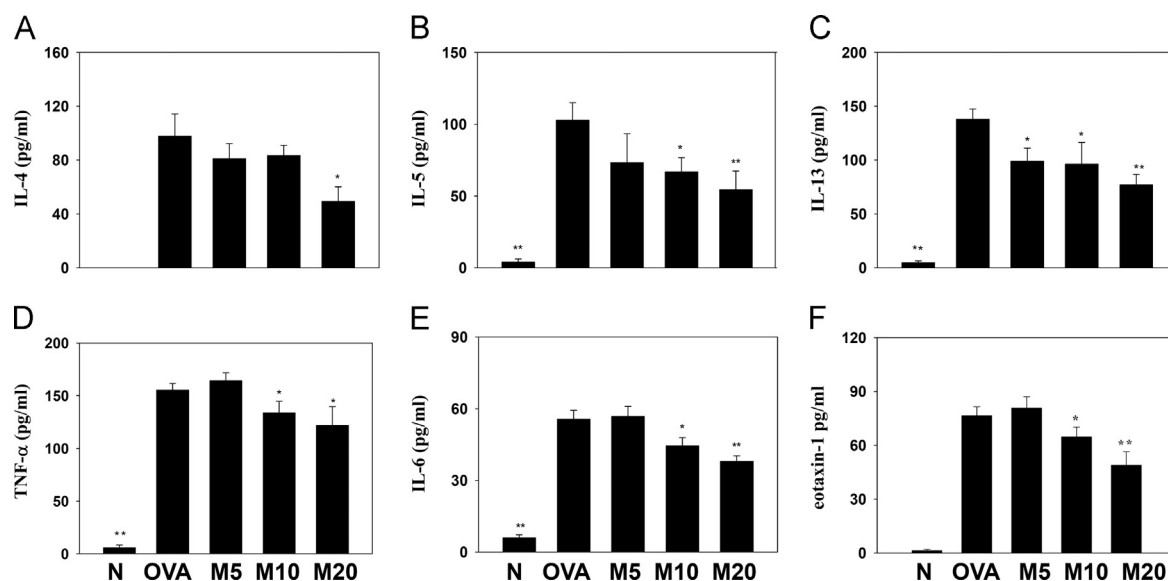


Fig. 5. The effects of matrine on the cytokine and chemokine levels in BALF. The concentrations of IL-4 (A), IL-5 (B), IL-13 (C), TNF-α (D), IL-6 (E), and eotaxin-1 (F) were measured. All data are presented as mean ± SEM. * $p < 0.05$ compared with the OVA control group. ** $p < 0.01$ compared with the OVA control group.

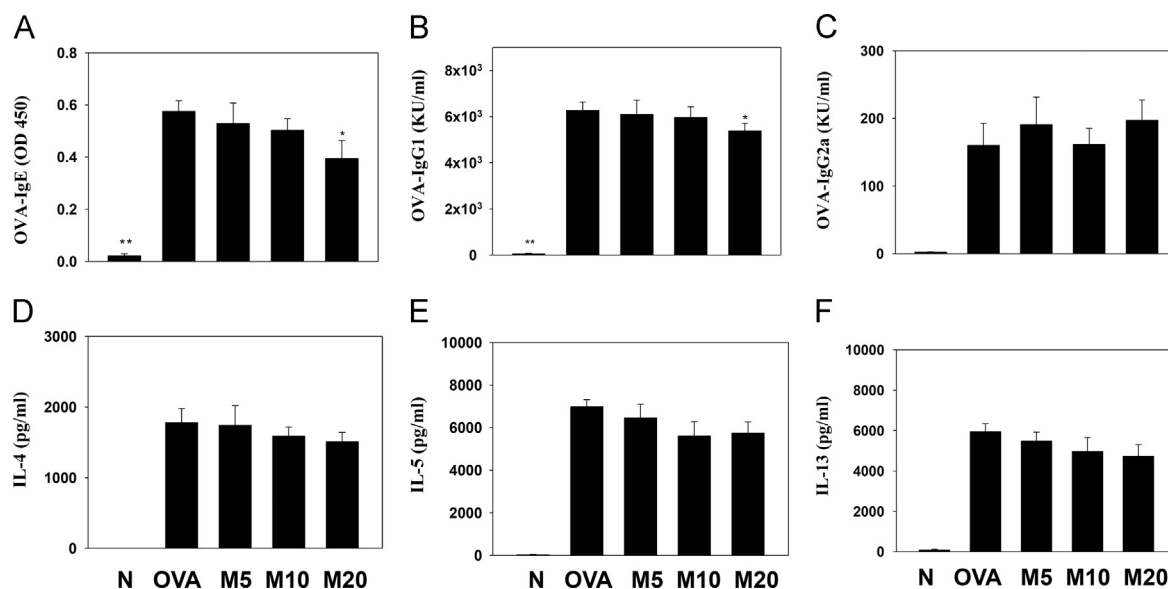


Fig. 6. Matrine affected the levels of OVA-specific antibodies in serum, including OVA-IgE (A), OVA-IgG1 (B), and OVA-IgG2a (C). Matrine also affected the levels of cytokines produced by OVA-activated splenocytes, including IL-4 (D), IL-5 (E), and IL-13 (F). All data are presented as mean ± SEM. * $p < 0.05$ compared with the OVA control group. ** $p < 0.01$ compared with the OVA control group.

shown). In all experiments, we used 100 μM–400 μM matrine. To evaluate the anti-inflammatory effects of matrine, the BEAS-2B cells were activated with TNF-α (Fig. 7). The results showed that matrine significantly suppressed levels of IL-6 (matrine 100 μM: 2453.8 ± 133.7 pg/mL, $p = 0.33$; matrine 200 μM: 1607.1 ± 164.3 pg/mL, $p < 0.01$; matrine 400 μM: 1244.6 ± 96.1 pg/mL, $p < 0.01$; vs. TNF-α control: 2680.8 ± 101.1 pg/mL, respectively) and IL-8 (matrine 100 μM: 6119.1 ± 186.7 pg/mL, $p = 0.37$; matrine 200 μM: 5753.7 ± 207.8 pg/mL, $p = 0.29$; matrine 400 μM: 4944.4 ± 141.2 pg/mL, $p < 0.05$; vs. TNF-α control: 6013.5 ± 167.3 pg/mL, respectively). BEAS-2B cells were also stimulated with TNF-α and IL-4, which revealed that matrine also significantly reduced eotaxin-1 (matrine 100 μM: 41.0 ± 3.8 pg/mL, $p < 0.05$; matrine 200 μM: 31.1 ± 2.1 pg/mL, $p < 0.01$; matrine 400 μM: 19.4 ± 3.1 pg/mL, $p < 0.01$; vs. control: 49.9 ± 4.6 pg/mL, respectively) and eotaxin-2 (matrine 100 μM: 24.2 ± 1.7 pg/mL, $p = 0.26$; matrine 200 μM:

22.3 ± 1.4 pg/mL, $p = 0.22$; matrine 400 μM: 19.9 ± 1.8 pg/mL, $p < 0.05$; vs. TNF-α control: 24.9 ± 2.2 pg/mL, respectively).

3.7. Matrine suppresses eosinophil adhesion to BEAS-2B cells

Previous studies have demonstrated that inflammatory BEAS-2B cell expression of ICAM-1 leads to adhesion of eosinophils (Poynter, 2012). Here we found that matrine also significantly reduced ICAM-1 expression (matrine 100 μM: 1353.1 ± 52.2 pg/mL, $p = 0.31$; matrine 200 μM: 980 ± 68.9 pg/mL, $p < 0.05$; matrine 400 μM: 557.6 ± 50.3 pg/mL, $p < 0.01$; vs. TNF-α control: 1419.9 ± 69.9 pg/mL, respectively) (Fig. 7C). Therefore, we further investigated whether matrine inhibited eosinophilic adherence to inflammatory BEAS-2B cells. HL-60 cells were stained with calcein-AM, and co-cultured with TNF-α-activated BEAS-2B cells (Fig. 8). We found that treatment with matrine suppressed the

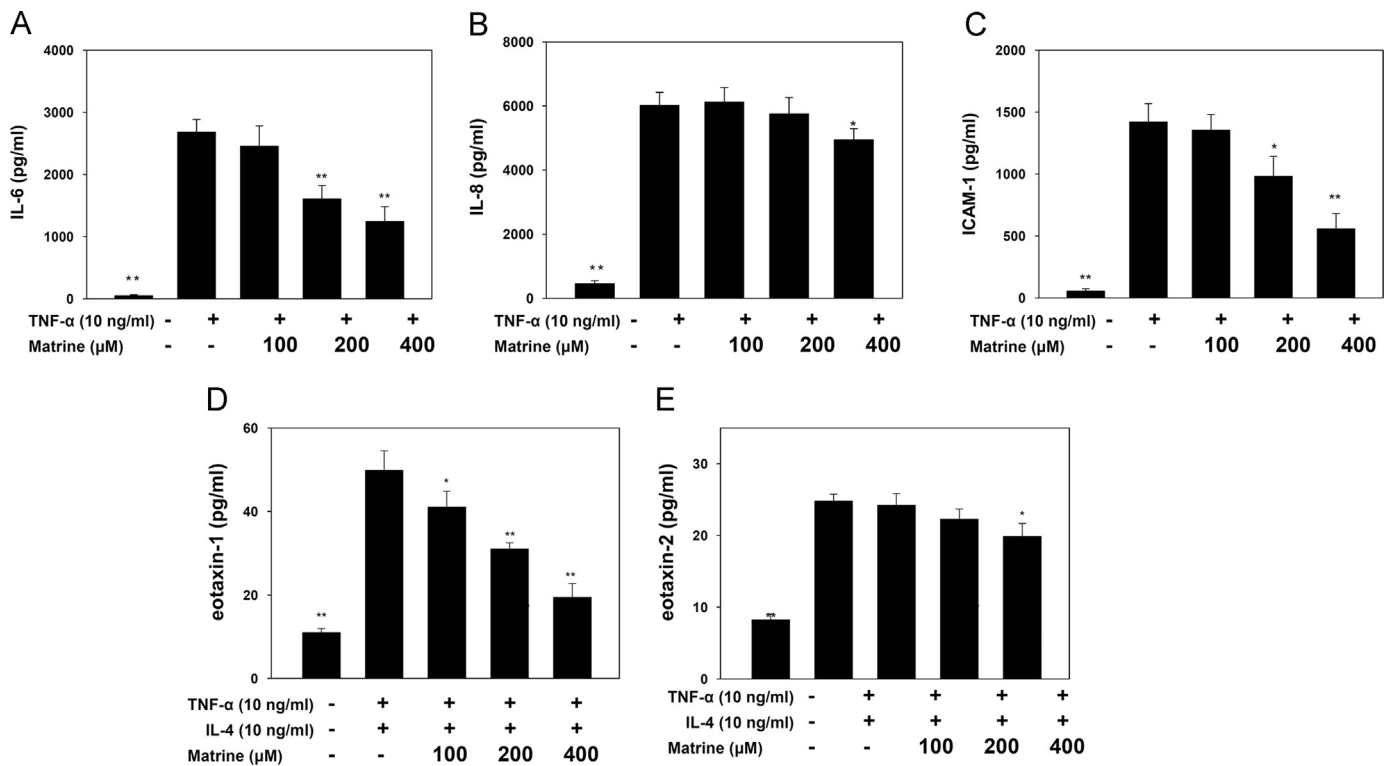


Fig. 7. Matrine affects the cytokine and chemokine productions in BEAS-2B cells. The levels of IL-6 (A), IL-8 (B), ICAM-1 (C), eotaxin-1 (D), and eotaxin-2 (E) were measured by ELISA. All data are presented as mean $\pm$ SEM. * $p < 0.05$ compared with the OVA control group. ** $p < 0.01$ compared with the OVA control group.

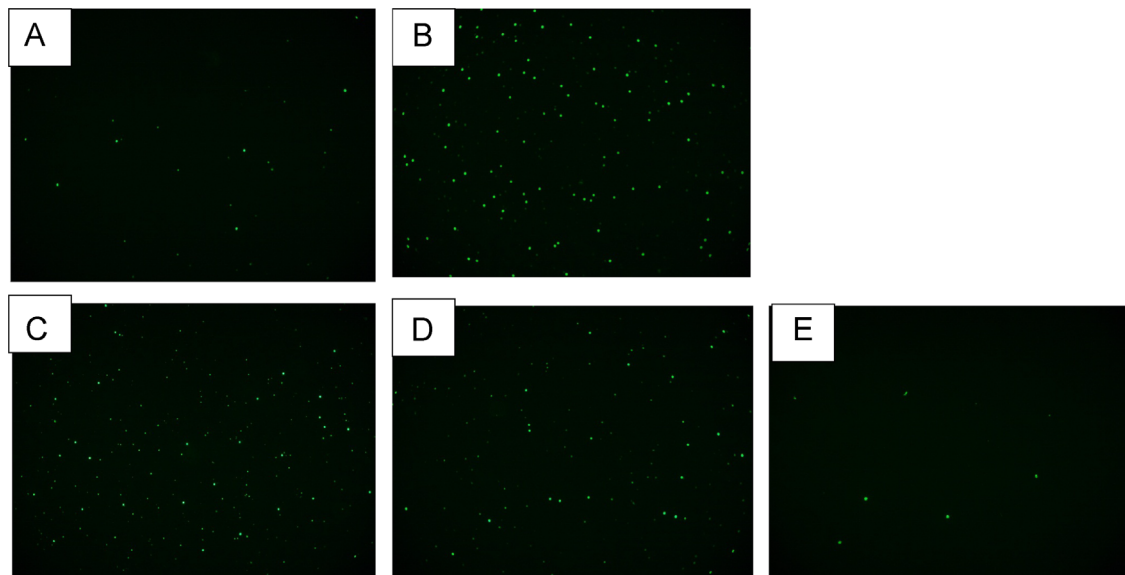


Fig. 8. Matrine inhibited the adhesion of eosinophil-like cells (HL-60) to active BEAS-2B cells. HL-60 cells were labeled with calcein-AM and mixed with BEAS-2B cells, and then observed using fluorescence microscopy. Representative images show the adherence of HL-60 cells to normal (A) and TNF- α -activated BEAS-2B cells (B), as well as HL-60 cells treated with 100 μ M matrine (C), 200 μ M matrine (D), and 400 μ M matrine (E).

adherence of HL-60 cells to BEAS-2B cells compared with in the TNF- α -activated group.

4. Discussion

The worldwide prevalence of asthma is rising, increasing the importance of improving the treatment and control of asthma symptoms in clinical medicine (Lemanske and Busse, 2010). Asthma attacks may be induced by allergens, seasonal changes,

or excessive exercise, and patients often experience a dry cough, shortness of breath, and difficulty breathing (Barrios et al., 2006; Cohn et al., 2004). Pathological features of asthma include AHR to severe shortness of breath, and eosinophil infiltration for increasing lung inflammation (Poon et al., 2012). Additionally, tracheal goblet cell hyperplasia causes increased mucus secretion, further obstructing airways and causing severe breathing difficulties and even death (Poynter, 2012).

Steroids and bronchodilators are commonly used drugs for asthma treatment (Castro-Rodriguez and Rodrigo, 2012). However,

some patients seek to improve asthma symptoms using alternative medicine, including Chinese herbal medicine, yoga, homeopathy, and urine therapy (Mark, 2007). Ding Chuan Tang and Xiaoqing Dragon Tang are popular asthma treatments in Chinese medicine (Chan et al., 2006; Wang et al., 2012), and clinical studies have confirmed that they can modulate Th2 cells and improve asthma (Ko et al., 2004; Chan et al., 2006). However, these two complex herbal formulas contain ephedra and thus ephedrine, which can cause excitement and unwanted side effects (Fleming, 2008). Other Chinese herbal formulas, single agents, or pure compounds that have been reported to improve and treat asthma include ASHMI, MSSM-002, CVT-E002, Danggui Buxue Tang, *Gynostemma pentaphyllum*, and viscolin (Srivastava et al., 2003; Busse et al., 2010; Liou et al., 2010; Ebeling et al., 2011; Lin et al., 2011; Shen et al., 2011). ASHMI is a composition of three herbal formulas – *Sophora flavescens*, *Glycyrrhiza uralensis*, and *Ganoderma lucidum*, (Kelly-Pieper et al., 2009) – and can reportedly suppress AHR and eosinophil infiltration in asthmatic mice, as well as decrease serum IgE and Th2-associated cytokine levels in splenocytes (Busse et al., 2010). A double-blind trial in humans found that oral administration of ASHMI significantly ameliorates FEV1 and peak expiratory flow, and suppresses serum IgE levels (Kelly-Pieper et al., 2009). Furthermore, a successful Phase I clinical trial of ASHMI in humans was recently completed (Kelly-Pieper et al., 2009). We hypothesized that *Sophora flavescens* of ASHMI contained the active compound that suppresses asthma.

Matrine was isolated from *Sophora flavescens* (Ma et al., 2013). In the present study, we found that matrine significantly suppressed AHR and eosinophil infiltration, as well as decreased tracheal goblet cell hyperplasia in lung tissue. Furthermore, matrine treatment inhibited Th2-associated cytokine and chemokine levels in the BALF of asthmatic mice. However, only treatment with high-dose matrine (M20 group) decreased OVA-IgE production, and none of the tested doses of matrine decreased Th2 cytokine secretion from splenocyte culture. Our results in this experimental model suggested that matrine improved local lung immune responses of asthma, but did not modulate systemic immune responses. We further found that in vitro treatment of human tracheal epithelial cells with matrine significantly suppressed the levels of proinflammatory cytokines (IL-6) and chemokines (IL-8, eotaxin-1, and eotaxin-2). Matrine treatment of tracheal epithelial cells also decreased ICAM-1 expression and thus adhesion to eosinophils. Our results clearly demonstrate that matrine can decrease the local lung inflammatory response and pathological manifestations of asthma.

Previous studies have shown that excessive secretion of Th2 cytokines induces more severe inflammatory cell hyperplasia and lung infiltration in cases of asthma (Poon et al., 2012). This suggests that modulation of Th2 cytokines could help ameliorate asthma symptoms. IL-5 stimulates the differentiation of bone marrow cells to form eosinophils, which are then activated and induced by eotaxins to migrate into inflammatory tissue (Rothenberg and Hogan, 2006). Inflammatory tracheal epithelial cells can also express ICAM-1 to promote adhesion of activated eosinophils in the lung (Poynter, 2012). Our present results in asthmatic mice showed that administration of matrine by intraperitoneal injection significantly decreased IL-5 production in BALF, thus reducing eosinophil proliferation and differentiation. Matrine can also suppress tracheal epithelial cell secretion of eotaxins, reduce ICAM-1 expression to block eosinophil adhesion, and reduce eosinophil infiltration into the lung in asthmatic mice. Previous studies have found that activated eosinophils secrete eosinophil peroxidase (EPO) and eosinophil cationic protein (ECP), which increase severe inflammation and injury in lung tissue (Rothenberg and Hogan, 2006; Poon et al., 2012). Our results did not confirm whether matrine suppresses bone marrow cell

differentiation from eosinophils, but we found that matrine treatment reduced eosinophil infiltration in the lungs and BALF.

A previous study showed that allergy-sensitive mice with knockout of IL-13 or IL-9 did not exhibit high AHR (Frew, 2010). Here we found that intraperitoneal injection of matrine significantly decreased IL-13 levels in BALF, and also decreased AHR (Penh value) in OVA-sensitized mice compared to asthmatic mice that were not treated with matrine. Surprisingly, the matrine-treated asthmatic mice also exhibited lower Penh values compared to in normal mice. At a high dose of 40 mg/mL methacholine, we found that the respiratory behavior in asthmatic mice treated with 5–20 mg/mg matrine scarcely showed any breathing difficulties, indicating that matrine can ameliorate AHR and decrease the breathing difficulties associated with an acute asthma attack. However, matrine did not modulate Th2 cytokine productions of splenocytes in the systemic immune responses. These findings provide further evidence that matrine mainly improves local pathological airway and lung symptoms in asthmatic mice.

Smooth muscle cell proliferation causes airway narrowing in asthmatic patients (Poynter, 2012). Additionally, substantial tracheal goblet cells hyperplasia in asthmatic patients leads to greater mucus secretion and hence greater airway obstruction, leading to breathing difficulties and even death (Barrios et al., 2006). It is thought that IL-4 and IL-13 induce tracheal goblet cell hyperplasia (Venkayya et al., 2002; Hamid and Tulic, 2009). Here we found that matrine significantly reduced goblet cells hyperplasia in lung tissue. We propose that matrine acts to decrease excess mucus secretion from goblet cells by suppressing IL-13 and IL-4 productions in BALF. Additionally, B cells secrete IgE antibodies to bind mast cells, causing histamine release and thus acute inflammatory reaction (Poon et al., 2012), and we found that asthmatic mice that were given 40 mg/kg matrine exhibited suppressed serum IgE levels.

We next used human tracheal epithelial cells (BEAS-2B cells) to investigate whether matrine could decrease local airway inflammation in vitro. We found that matrine treatment reduced proinflammatory cytokine and chemokine levels, as well as decreased adhesion of eosinophils to BEAS-2B cells. These results provide further confirmation that matrine inhibits local inflammation and airway symptoms in asthmatic mice.

Matrine has been previously found to have anti-inflammatory effects (Zhang et al., 2011) and tumor proliferation inhibiting effects, including in lung and liver cancer (Sun et al., 2012). Here we present the first evidence that matrine inhibits asthma symptoms in mice. Future investigations will further test the usefulness of matrine for asthma treatment, as well as evaluate other pure compounds from *Sophora flavescens*, including oxymatrine and sophoraflavanone G, to determine if they can also improve asthma symptoms.

5. Conclusion

Our results demonstrate that matrine suppressed eosinophil infiltration, AHR, and airway inflammation by suppressing Th2 cytokines and eotaxin in asthmatic mice.

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