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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.

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

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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 μg ovalbumin (OVA) (Sigma, St. Louis, MO, USA) emulsified with aluminum hydroxide (Thermo, Rockford, IL, USA) in 200 μL 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).

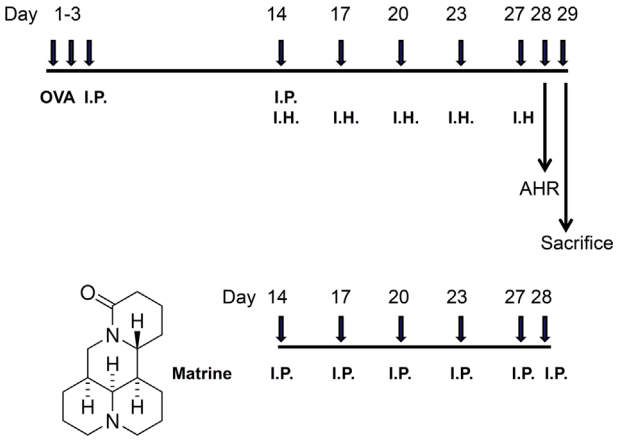

Fig. 1. Sensitization and OVA allergen challenge protocol: Mice were sensitized by intraperitoneal (IP) injection with OVA on days 1–3 and 14. Mice were then subjected to airway challenge with 2% OVA inhalation (IH) on days 14, 17, 20, 23, and 27. Matrine (in normal saline) was injected intraperitoneally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