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Anti-asthmatic effects of matrine in a mouse model of allergic asthma

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ABSTRACT

The aim of the study was to investigate the anti-asthmatic effects of matrine and the possible mechanisms. Asthma model was established by ovalbumin-induced. A total of 50 mice were randomly assigned to five experimental groups: control, model, dexamethasone (2 mg/kg) and matrine (50 mg/kg, 100 mg/kg). Airway resistance (Raw) was measured, histological studies were evaluated by the hematoxylin and eosin (HE) staining, interleukin-4 (IL-4) and interleukin-13 were evaluated by enzyme-linked immunosorbent assay (ELISA), IL-4 and IL-13 signal protein STAT6 was measured by western blotting. Our study demonstrated that matrine inhibited OVA-induced increases in Raw and eosinophil count; IL-4 and IL-13 were recovered. Histological studies demonstrated that matrine substantially inhibited OVA-induced eosinophilia in lung tissue. Western blotting studies demonstrated that matrine substantially inhibited STAT6 protein level. These findings suggest that matrine may effectively ameliorate the progression of asthma and could be used as a therapy for patients with allergic asthma.

1. Introduction

Allergic diseases of the airway such as asthma or allergic rhinitis are on the rise throughout the world, especially in western countries [1]. One of the most important diseases is bronchial asthma, that is defined as a chronic inflammation of the bronchial airways and characterized by reversible airway obstruction, increased mucus production and infiltration of the airway with eosinophils, neutrophils, mast cells and T-lymphocytes [2].

Interleukin (IL)-4 and IL-13, expressed by T-helper type 2 (Th2) cells, are key cytokines in the pathogenesis of atopy and atopic asthma [3,4]. Both IL-4 and IL-13 promote acute inflammatory processes and underlying structural changes to the airways, and their receptors are expressed on a number of cell types. Both IL-4 and IL-13, which share the IL-4R a subunit in their cognate receptors, activate signal transducer and activator of transcription factor-6 (STAT-6) [5]. STAT-6 is required for IL-4 production from antigen-stimulated murine splenocytes [6]. Moreover, activation of STAT-6 is critical for the differentiation of naive T-cells into Th2 effector cells, and STAT-6 regulates IL-4- and IL-13-induced production of Th2 chemokines, including eotaxin, from airway epithelial cells, fibroblasts and smooth muscle cells [7,8]. The IL-4/IL-13/STAT-6 pathway plays a key role in asthma pathogenesis.

Matrine (Mat) and oxymatrine are the major alkaloids of *S. Flavescens*. They have similar molecular structure and also have similar wide spectrum of pharmacological effects, including anti-inflammatory, anti-allergy, anti-virus and antitumor, and are ancillary drugs used clinically in China for protection of liver function and treatment of tumors [9].

Hence, we hypothesized that Mat, which have protective effects in ovalbumin-sensitized asthma *in vivo*, might also be associated with the inhibition of inflammation and immunoregulatory. The present study was undertaken to evaluate the protective effects of Mat and to elucidate the mechanism underlying these protective effects in mice. Our results indicate that Mat pretreatment significantly protected against ovalbumin-sensitized asthma through regulating IL-4/IL-13/STAT-6 pathway.

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2. Materials and methods

2.1. Materials

Mat powder (pure:98.7%) was purchased from National Institutes for Food and Drug Control (Beijing, China) and Mat solution (4 mg/ml) was prepared in distilled water. Dexamethasone was purchased from Xiansheng drug Store (Nanjing, China). Ovalbumin (OVA) was obtained from Sigma Chemical Co., St Louis, MO, aluminium hydroxide from Pierce Biotechnology, Rockford, USA, Wright–Giemsa staining from Nanjing Jiancheng Bioengineering Institute, Nanjin, China, ELISA kits from R&D, Minneapolis, MN, USA, and anti-mouse STAT6 from eBioscience.

2.2. Animals

Specific pathogen-free female BALB/c mice, aged 6–8 weeks, which were routinely screened serologically for relevant respiratory pathogens, were purchased from the 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 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.

2.3. Sensitization, airway challenge and treatment

Mice were divided into five groups (each of 10 animals): (1) normal control, (2) asthma-model control, (3) asthma treated with dexamethasone (2 mg/kg, administered by gavage) (4, 5) asthma treated with Mat (50, 100, administered by gavage). Allergic asthma was induced by ovalbumin (Grade V) in five of the groups, using the method described by Oh et al. [10]. Mice were immunized via intraperitoneal (i.p.) 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 GA. A schematic diagram of the treatment schedule is shown in Fig. 1.

2.4. Measurement of airway resistance (Raw)

The forced oscillation technique was used to measure respiratory mechanics. Regular ventilation was interrupted and a computer generated volume signal that consisted of waveforms of mutually primed frequencies was delivered to the airway opening. Piston displacement and cylinder pressure were measured. Impedance values were obtained before and after the delivery of increasing concentrations of methacholine

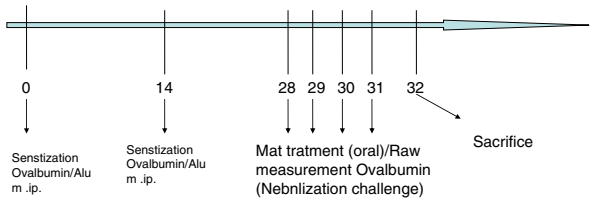

Fig. 1. Sensitization, challenge treatment protocols for the different groups in this study.

aerosols. Prior to the start of the methacholine concentration response curve, two total lung capacity breaths were delivered. Methacholine (Sigma) dissolved in saline was given via an ultrasonic nebuliser (Hudson RCI, Teleflex Medical) in increasing concentrations (0.125 mg ml^{-1}, 0.25 mg ml^{-1}, 0.5 mg ml^{-1}, and 1 mg ml^{-1}). After delivery of each aerosol, forced oscillations were delivered every 15 s, over a 5-min duration. In between impedance measurements, regular ventilation was resumed. The peak response for each variable was determined.

2.5. 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 volume1.5 ml) via tracheal cannulation [11]. 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.

2.6. 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 [12]. The scoring system was: 0, no cells; 1, a few cells; 2, a ring of 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.

2.7. Enzyme-linked immunosorbent assay detection of BALF cytokines IL-4 and IL-13

BALF levels of IL-4 and IL-13 were measured by ELISA according to the manufacturer's instructions (R&D, Minneapolis, MN, USA). All measurements were performed in duplicate. Briefly, the BALF samples were added in duplicate to 96-well