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Research article

Regulation of hypothalamic-pituitary-adrenal axis activity and immunologic function contributed to the anti-inflammatory effect of acupuncture in the OVA-induced murine asthma model

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HIGHLIGHTS

- We evaluated the regulatory role of acupuncture in airway inflammation and the HPA axis activity in murine asthma model.
- Our results demonstrated that acupuncture was effective in suppression of AHR and airway inflammation in serum.
- The HPA axis activity was regulated by acupucnture.
- Our findings may provide support to better understand the contribution of acupuncture to regulation of inflammation and HPA axis activity in asthma.

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ABSTRACT

Asthma is a complex inflammatory disease of the airways and acupuncture is one of the effective therapies widely used to treat asthma in China. The aim of the study was to evaluate the regulatory role of acupuncture in airway inflammation and the hypothalamic-pituitary-adrenal (HPA) axis activity in OVA-induced murine asthma model. Our results demonstrated that acupuncture was effective in suppression of AHR, inhibition of total leukocyte, neutrophil, lymphocyte and eosinophil counts in BALF, attenuation of airway inflammation and TNF-α, IL-1β, IL-5 and eotaxin secretion. Furthermore, the HPA axis activity was also regulated by acupuncture, which included promotion of adrenocorticotropic hormone and cortisol secretion in the plasma. Our findings revealed that acupuncture could attenuate airway inflammation and regulate HPA axis and immunologic function in the OVA-induced murine asthma model, which may provide support to better understand the contribution of acupuncture to the regulation of airway inflammation and HPA axis activity in asthma.

1. Introduction

Asthma is a chronic inflammatory disorder affecting 300 million individuals worldwide [1]. Airway hyperresponsiveness (AHR) and chronic airway inflammation are two key features of asthma, and have been shown to be involved in the development of asthma pathogenesis. Both genetic and environmental factors are likely involved in asthma pathogenesis [2], which make the complexity and heterogeneity of asthma, and the latter in turn make both the clinical management and investigation of asthma challenging [3]. Inhaled corticosteroids (ICS) and β-adrenergic agonists are significantly effective in relieving inflammation and control symptoms in asthma [4], and most asthmatic individuals respond well to the currently available treatments of ICS and β-adrenergic agonists. However, side effects such as easy bruising, osteoporosis, cataracts and glaucoma, and adrenal suppression may occur in a population of asthmatics [1].

Acupuncture treatment of asthma has a long history, assured curative effects and is widely used in clinic in China. Previous studies have identified that acupuncture has the potential to reg-

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ulate gene expression profiles in asthma, which include immune response and steroid hormone related pathways [5]. Furthermore, acupuncture is believed to elicit profound psychophysical responses by harmonizing or balancing the energy and blood flow through the body [6]. The hypothalamus-pituitary-adrenal (HPA) axis is considered to be a central integrative system being crucial in the successful physiological adaptation of the organism to stress [7], and HPA axis activation was reported to be associated with asthma pathogenesis [7,8]. The activation of the HPA axis involves the release of corticotropin-releasing hormone (CRH) by the paraventricular nucleus of the hypothalamus, stimulating the production of adrenocorticotropic hormone (ACTH) in the anterior lobe of the pituitary gland. Secreted ACTH in the peripheral circulation stimulates the adrenal cortex, releasing glucocorticoids and, in particular, cortisol into the blood [9,10]. It is commonly known that glucocorticoids inhibit the activity of the HPA axis by negative feedback on CRH and ACTH secretion [11]. However, the data on the regulation of HPA axis by acupuncture is scarce and the potential role of acupuncture on the HPA axis in the OVA-induced murine asthma model has not been explored. In this study, we aimed to investigate the role of acupuncture in airway inflammation and the HPA axis activity in OVA-induced murine asthma model.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Committee on the Ethics of Animal Experiments of Fudan University. Female BALB/c mice (six-week-old, 14–17 g) were purchased from Shanghai SLAC Laboratory Animal Co. Ltd and raised with food and water freely available.

2.2. Reagents

Ovalbumin (OVA), aluminum hydroxide (Al(OH)3), pentobarbital sodium and methacholine (Mch) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Adrenocorticotropic hormone (ACTH) and cortisol (CORT) ELISA kits were purchased from mybiosource (San Diego, California, USA) and cayman (Michigan, USA) separately. Inflammatory cytokine Bio-Plex kit was purchased from Bio-Rad (San Diego, California, USA). Sterile acupuncture needles (Hwato, 13 mm long, 0.25 mm in diameter) were purchased from Suzhou medical supplies co., LTD (Suzhou, Jiangsu, China). Trizol reagent and SYBR Green kit and RevertAid TM First Strand cDNA Synthesis kit were purchased from Thermo Fisher (Massachusetts, USA).

2.3. Asthma model establishment and acupuncture treatment

Forty mice were randomly divided into four groups (N = 10 each group), including normal control group (NC), OVA-induced asthma group (A), asthma model combined with acupuncture treatment group (AA) and asthma model combined with sham acupuncture treatment group (ASA). Murine asthma model was induced by multiple OVA sensitization and challenge. As shown in Fig. 1, mice were sensitized with 20 μg OVA and 2 mg Al(OH)3 in 0.2 ml of saline solution by intraperitoneal injection on day 0 and day7. From day 14 to day 40, mice were challenged by inhalation of 3% (w/v) OVA solution for 30 min every other day for four weeks. Briefly, mice were placed in a plexiglas chamber and nebulized with an ultrasonic nebulizer (402AI, Yuyue medical equipment Co. Ltd., Jiangsu, China). Mice in NC group were sensitized and challenged with saline.

In traditional Chinese medicine (TCM), there are fourteen meridians which include 365 acupoints. According to the TCM theory, the acupuncture points, GV14 (Dazhui, between the C7 and T1 vertebrae), bilateral BL12 (Fengmen, foveola laterally between the T2 and T3 vertebrae), and bilateral BL13 (Feishu, foveola laterally between the T3 and T4 vertebrae) were selected for acupuncture treatment [5]. GV14 which has the function of regulating the vital Qi of the body belongs to the Governor Meridian. BL12 and BL13 which could regulate the local lung function, belong to the Urinary Bladder Meridian of Foot-Taiyang. From day 14 and 1 h before the OVA challenge, acupuncture treatment was performed with a fixation device in an awakened state. Sterile needles were inserted into the acupoints with a depth of approximately 3 mm and withdrawn after the needle retaining time of 30 min. During the needle retaining time, manual manipulations were performed every 10 min. GB30 (Huantiao) belongs to the Gallbladder Meridian of foot-Shaoyang, and is considered to have no regulatory effect on the lung function. Therefore, distal irrelevant acupoints of bilateral GB30 was selected as sham acupuncture treatment in the same way.

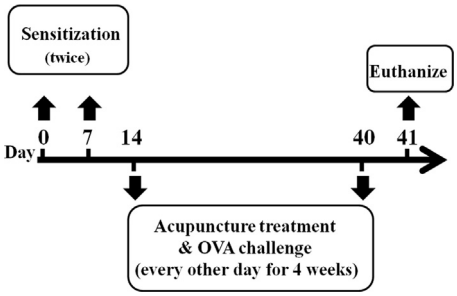

Fig. 1. Protocol for murine asthma model establishment and acupuncture treatment.

2.4. Measurement of AHR

Within 24 h of the last OVA challenge, AHR was investigated with an invasive method under anaesthesia using Buxco pulmonary system (Buxco Electronics Inc., NY, USA). Tracheostomy was performed and the tracheal tube was inserted. Mice were put into the body plethysmograph chamber and the inserted tracheal tube was connected to the ventilator. After a stable baseline airway pressure (<5% variation over 2.5 min) was reached, PBS and Mch (3.125, 6.25, and 12.5 mg/mL) were administrated to mice by nebulization. The airway resistance (R_L) and lung dynamic compliance (Cdyn) were recorded. Results were expressed as the percentage change in baseline.

2.5. Hematoxylin and eosin (HE) staining of the lung tissue

The right lung of mice were harvested after bronchoalveolar lavage (BALF) collection, fixed in 4% paraformaldehyde, dehydrated in alcohol gradient, embedded in paraffin and sliced at 4 μm of thickness. Lung sections were then stained with H&E. Six mice from each group and six arbitrarily selected fields in each mouse were photographed at 200× magnification using an optical microscope (ECLIPSE 80i, Nikon, Japan).

2.6. Inflammatory cell counts in BALF

BALF was performed by lavaging the left lung through the tracheal cannula using 0.3 ml aliquots of sterile PBS twice (total volume 0.6 ml), then centrifuged at 500g for 10 min, and the cell pellet was resuspended with 100 μl PBS for inflammatory cell counts using hemavet 950 instrument (Drew scientific group).