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EXPERIMENTAL STUDY

Effect of Gubenfangxiao decoction on respiratory syncytial virus-induced asthma and expression of asthma susceptibility gene orosomucoid 1-like protein 3 in mice

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Abstract

OBJECTIVE: To investigate the effect of Gubenfangxiao decoction (GBFXD) on respiratory-syncytial-virus (RSV) -induced asthma and the expression of asthma susceptibility gene, orosomucoid 1-like protein 3 (ORMDL3) in mice.

METHODS: Seventy-two female BALB/c mice were randomly assigned to normal, model, GBFXD high dose, GBFXD moderate dose, GBFXD low dose and montelukast groups. An asthma model was induced via intraperitoneal injection and aerosol inhalation of ovalbumin (OVA) and repeated intranasal instillation of RSV in all mice, except those in the normal group. All treatments were administered at the first onset of asthma (within 8 weeks of model establishment) and the mice were euthanized after 28 days of treatment. The levels of transforming growth factor-β (TGF-β) and interleukin-6 (IL-6) in bronchoalveolar lavage fluid (BALF) of the mice were measured and the expression of asthma susceptibility gene ORMDL3 in lung tissue was determined using real-time polymerase chain reaction (RT-PCR) and western blotting.

RESULTS: Expression of ORMDL3 and levels of TGF-β and IL-6 were significantly higher in the model group ($P < 0.05$, $P < 0.01$) compared with the normal mice. Levels of ORMDL3, TGF-β and IL-6 were significantly lower in all three GBFXD treated groups ($P < 0.05$) compared with the model group. However, the levels in the GBFXD treatment groups did not differ significantly from the montelukast group.

CONCLUSION: GBFXD had a therapeutic effect in this experimental model. The functional mechanism of GBFXD may involve multiple factors, including alleviation of airway inflammation, down-regulation of asthma susceptibility gene ORMDL3 and inhibition of airway remodeling.

Key words: Asthma; ORMDL3 protein, mouse; Airway remodeling; Gubenfangxiao decoction

INTRODUCTION

Bronchial asthma is chronic inflammation of the airway, with both genetic and environmental factors implicated in its pathogenesis.[1,2] Recurrent asthma attacks involve infiltration of inflammatory cells and abnormal changes to the structure of the airway. The mechanism underlying airway remodeling has yet to be determined. Recently, the first asthma-specific, ge-

nome-wide association study (GWAS) identified orosomucoid 1-like protein 3 (ORMDL3) as a candidate asthma risk gene,[3] in multiple ethnic groups.[4-6] However, the function of ORMDL3 remains unknown and further studies are needed to elucidate the mechanisms of ORMDL3 and its association with asthma. Gubenfangxiao decoction (GBFXD) is prepared using a formula containing eleven Chinese herbs. The formula was designed by Professor Jiang Yuren for the management of asthma and has been used in Jiangsu Province Hospital of Traditional Chinese Medicine (TCM) for more than 30 years. Our previous studies showed that GBFXD could protect against airway inflammation and inhibit airway hyperresponsiveness.[7]

The aim of the present study was to investigate the effect of GBFXD on respiratory-syncytial-virus (RSV) -induced asthma and the expression of the asthma susceptibility gene ORMDL3, in mice.

METHODS

Drug and reagents

GBFXD was prepared from a TCM formula consisting of Huangqi (*Radix Astragali Mongolici*), Dangshen (*Radix Codonopsis*), Baizhu (*Rhizoma Atractylodis Macrocephalae*), Fuling (*Poria*), calcining Muli (*Concha Ostreae*), Chantui (*Periostracum Cryptotympanae*), Chenpi (*Pericarpium Citri Reticulatae*), Fangfeng (*Radix Saposhnikoviae*), Xinyi (*Flos Magnoliae Biondii Immaturus*), Wuweizi (*Fructus Schisandrae Chinensis*) and stir-frying with liquid adjuvant Gancao (*Radix Glycyrrhizae*). The ratio of mass in grams used in the formula was 5∶3.3∶3.3∶3.3∶5∶2∶2∶1∶2∶2∶1 and the granules were supplied by Jiangsu Province Hospital of Traditional Chinese Medicine. The decoction was produced using the granules and double distilled water at a concentration of 2.25 g/mL. Montelukast sodium tablets (20070058, Hangzhou MSD pharmaceutical Co., Ltd., Hangzhou, China) were used to prepare a solution of approximately 0.16 mg/mL, in sterile distilled and stored stored at 4 ℃ Ovalbumin (OVA) was supplied by Sigma (St. Louis, MO, USA). Respiratory syncytial virus (RSV) was obtained from the Institute of Pediatrics of Nanjing University of Chinese Medicine (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kits for IL-13 and lL-6 were supplied by eBioscience (San Diego, CA, USA). Real-time PCR master mix Plus (SYBR Green) and RNA extraction kits were supplied by Takara Biotechnology (Shanghai, China). Monoclonal antibodies against ORMDL3 were supplied by Abcam (Massachusetts, MA, USA) and against β-actin by Santa Cruz (California, CA, USA). Secondary antibodies were also supplied by Abcam. Primers for IL-13, ORMDL3 and β-actin were designed and synthesized by Takara Biotechnology.

Animals and asthma model establishment

Seventy-two healthy, female, 6-week-old BALB/c mice [certificate of quality No. SCXK (Hu) 2012-0002], weighing [(18 ± 2) g] were supplied by the Laboratory Animal Center of Shanghai Slack Co., Ltd., (Shanghai, China). Mice received food and water ad libitum for 1 week before experiments [temperature at (24 ± 1) ℃ and humidity at 50% ± 5%]. Mice were maintained in the Laboratory Animal Center in the Nanjing University of Traditional Chinese Medicine. All animal studies were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Nanjing University of Traditional Chinese Medicine.

Mice were randomly divided into six groups: normal, model, GBFXD high dose, GBFXD moderate dose, GBFXD low dose and montelukast. Asthma was induced in all mice, except those in the normal group. A sensitization and challenge protocol was employed, as described previously,[8,9] with small modifications. Mice were immunized *via* intraperitoneal injection of 100 μg OVA, conjugated to 1 mg aluminum potassium sulfate, in a total volume of 0.2 mL on the 1st and 8th day. Mice inhaled aeroallergens as 2.5% OVA, every day from the 15th to the 28th day, for 30 min. Asthma was induced using intranasal instillation of 50 μL RSV on the 29th, 42nd, and 55th day. From the 29th to the 55th day, mice were challenged with inhalation of 2.5% OVA solution for 30 min, once every 3 days.[10]

GBFXD treatment

The standard dose of GBFXD was based on individual animal body weight. The formula used was Db = Da × Rab, which was derived from the formula used to determine the standard dose for humans.[11] The formula used for humans was based on a ratio of 24 g crude medicine to 1 kg of murine body weight. GBFXD high-dose (GBFXD- H) was 36 g/kg, the moderate dose (GBFXD-M) was 24 g/kg and the low dose (GBFXD-L) was 12 g/kg. GBFXD and montelukast acetate (2.6 mg/kg) treatments were intragastrically administered, once a day from the 2nd day of model establishment. The normal and model groups were given an equal volume of distilled water, intragastrically at the same time points. All mice were euthanized after 28 days of treatment.

RSV

RSV supernatants were prepared according to the method described previously.[12] The 50% tissue culture infective dose (TCID50) of RSV was measured as $1.0\times 10^{6.5}$ TCID50/mL.

Bronchoalveolar lavage fluid collection

Mice were sacrificed 24 h after the final treatment. BALF was acquired as described previously.[7] Three aliquots of 0.5 mL PBS were injected and withdrawn through a tracheal cannula. Following centrifugation, the supernatant was stored at − 70 ℃, for measurement of IL-6 and TGF-β concentrations, using ELISA.