Effect of Pingchuan-Guben Decoction on Acute Airway Inflammation in Kuming Mouse Model of Asthma*

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ABSTRACT Objective: To investigate the effect of Pingchuan-Guben Decoction on acute airway inflammation of asthma and relate mechanism in mouse model of asthma. Methods: Forty Kunming mice were randomly divided into five groups averagely: negative control group (A), asthma model group (B), Pingchuan-Guben Decoction group (C), Budesonide group (D), Pingchuan-Guben Decoction + Budesonide group (E). 10 days after therapy on OVA challenged asthma model, total and differential cells were counted from bronchoalveolar lavage fluid(BALF), the levels interleukin-4(IL-4) and Interferon-γ(IFN-γ) in BALF were measured by ELISA. Results: Total cells countings, eosinophils, macrophages and IL-4, IL-4/IFN-γ in BALF of group C, D, E were significantly lower than that in group B ($P<0.05$), IFN-γ was higher than that in group B ($P<0.05$). The level of IL-4, IL-4/IFN-γ had no significant difference between the group C and group D ($P > 0.05$), while the level of IL-4, IL-4/IFN-γ in the group E were significantly lower ($P<0.05$).HE staining showed that compared with those in the treatment groups, there were a large number of inflammatory cells infiltration, heavier smooth muscle hypertrophy and mucous membrane hyperemia in the asthma group. Conclusions: Pingchuan-Guben Decoction may potentially inhibit airway inflammation of asthma by inhibiting of IL-4 expression, inflammatory cells accumulation and promoting the expression of IFN-γ. Pingchuan-Guben composition may have synergistic effect with glucocorticoid hormones.

Key words: Pingchuan-Guben Decoction; Asthma; Airway inflammation; Interleukin-4; Interferon-γ

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Introduction

Bronchial asthma is a common respiratory disorder characterized by recurrent episodes of coughing, breathlessness and wheezing. It can affect people of all ages[1]. The global morbidity of asthma has been increasing in recent years and become an important issue undermining the health of mankind[2]. Despite the availability of effective preventive therapy, costs associated with asthma are increasing, especially for the family with asthma children [3]. Inhaled corticosteroids (ICSs) are the most effective anti-inflammatory medications available for the treatment of asthma and represent the mainstay of therapy for most patients with the disease[4]. However, there are some patients who are not sensitive to the corticosteroids therapy, so exploring new corticosteroids alternative medicine or complementary medicine has become a necessity. The experience from traditional Chinese medicine(TCM) can be helpful for asthma treatment [5]. This study was to determine the effect of Pingchuan-Guben Decoction on acute airway inflammation of asthma and relate mechanism in mouse model of asthma.

1 Materials and methods

1.1 Animals

Female Kunming mice, 3-4 weeks old and weighing 19-22 g, were purchased from the Institute of Animal in the Qingdao Drug Inspection Laboratory Animal Center. During the experiments, mice were kept in specific pathogen-free animal facilities with controlled temperature ((25± 2)℃) and humidity (55%). All animals were acclimated for at least one week before experiments.

1.2 Reagents

Pingchuan-Guben Decoction (Ginseng 8g, Atractylodes 6g, Poria 10g, Licorice 3g, Schisandra 6g, Pastinaca 9g, Asarum 3g, Pinellia 6g, ephedra 9g, all the drugs by soaked, suffered, filtered and concentrated to 100ml, the concentration contains 0.63g crude drug/ml, and concentrated into sterile containers, placed in 4℃ refrigerator); Budesonide Solution (AstraZeneca, USA); Ovalbumin (sigma, USA); ELISA assay kits for IL-4 and IFN-γ (R&D,USA); Ultrasonic nebulizer(PARI, Germany).

1.3 Sensitization, challenge and experimental procedures

Kunming mice were divided into five groups (10 mice per group). Sensitization and challenge were performed according to the previously described technique[6]. In brief, all mice (except for those in Group A) were sensitized with 10 μg of OVA adsorbed to 1 mg of Al $(OH)_3$ adjuvant in 0.1 ml of PBS by intraperitoneal injection on days 0, 7, 14, and then challenged the animal by exposure to a 5% OVA aerosol for 20 min each day for 2 weeks in a plexiglas chamber (30cm× 25cm× 20cm) connected with the ultrasonic nebulizer. Mice in group A (negative control) were sensitized and challenged with 0.1 mL PBS. Mice in group B (asthma

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control) were treated with inhalation of 0.2 mL PBS. Mice in group C (herbal drugs) were pretreated with 0.6ml herbal drugs concentrated solution from the frist day. Mice in group D (budesonide) were treated with budesonide 100 μg aerosol for 30 min each day for 2 weeks. Mice in group E (budesonide+ herbal drugs) were treated with budesonide+ herbal drugs, reference the common methods group C and D group. All mice were sacrificed 24 hours after the last challenge.

1.4 Examination of bronchoalveolar lavage fluids (BALF)

Mice were anaesthetized and the trachea was cannulated while gently massaging the thorax. Lungs were lavaged three times with 0.5 mL of 37℃ PBS. The BALF was kept on ice for later use. Cells in BALF were centrifugated (1500 × g, 5 minutes, 4℃), and the supernatant of the BALF was used to measure cytokine levels. Cells were resuspended in 150 μL of PBS. Cells were identified and classified into macrophages cells, lymphocytes and eosinophils based on standard morphology. At least 200 cells were counted and the absolute number of each cell population was calculated. and supernatant stored at -70℃ for cytokine analysis.

1.5 Measurement of cytokines

The levels of cytokines in BALF were determined by enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (R&D, USA). ELISA kits were employed for the measurement of IL-4, interferon-γ (IFN-γ) levels.

1.6 Histological analysis of lung tissues

Twenty four hours after the last challenge, mice were sacrificed and lungs were fixed by an intratracheal infusion of 10% formalin in PBS. Fixed lungs were removed and fixed in formalin for 24 hours followed by being embedded in paraffin. Tissues were sectioned into 3 μm sections and stained with hemotoxylin and eosin (H&E) for histology.

1.7 Statistical analysis

Statistical analyses were performed by using SPSS 17.0 statistical software package. Data are expressed as $\bar{X} \pm S$. One-way analysis of variance (ANOVA), followed by a least-significant difference (LSD) test was performed for comparisons between two groups or Wilcoxon rank sum test used for data not normally distributed. A value of $P < 0.05$ was considered statistically significant.

2 Results and analysis

2.1 Symptoms in each group

Symptoms including behaved restlessness, deep and rapid breath, nod respiration, fur bristling, irritation and spasm, appeared to the mice of group B during challenge stage. The symptoms of mice of group C, D, E were mild compared with that of group B. The negative control group mice generally had few or no symptom.

2.2 Inflammatory cells in BALF

Inflammatory cells in BALF were reduced by pretreatment of Pingchuan-Guben Decoction and budesonide: the total cells, eosinophils, macrophages, lymphocytes in BALF from group B were higher than those in group A($P<0.05$). The cells in group C, group D and group E were lower than that in group B ($P<0.05$). The numbers of eosinophils cells in group E was also markedly improved by Pingchuan-Guben Decoction pretreatment. In addition the difference between them was meaningful ($P<0.05$), but group D and group E showed no significantly difference(Table 1).

Table 1 The counting of inflammatory cells in BALF

Group	Total cells	Eosinophils cells	Lymphocytes cells	Macrophages cells
Group A	0.77± 0.21	0.01 8± 0.01	0.27± 0.07	0.51± 0.10
Group B	8.77± 1.39	0.71 5± 0.31	2.52± 0.79	3.79± 0.57
Group C	4.65± 1.20	0.22 2± 0.09	1.43± 0.36	2.41± 0.68
Group D	3.46± 0.64	0.10 4± 0.02	1.13± 0.45	1.84± 0.38
Group E	1.57± 0.33	0.02 9± 0.01	0.45± 0.11	0.81± 0.15
F	99.60	31.19	32.45	74.81
P	＜0.05	＜0.05	＜0.05	＜0.05

2.3 Cytokine levels in BALF

Th2 cytokines such as IL-4 were important in the initiation and propagation of allergic inflammation responses. Several studies had also demonstrated that IFN-γ played an important role in the pathogenesis of asthma. The BALF was obtained 24 hours after the last airway challenge. The levels of IL-4 and IFN-γ in the BALF were determined by ELISA. The levels of IL-4 and IL-4/IFN-γ in the BALF of group B increased significantly after airway OVA challenge when compared with those of Group A($P<0.05$), group D and group E reduced,significantly compared with that in the group B ($P< 0.05$). In addition, the levels of IL-4 and IL-4/IFN-γ in the BALF reduced markedly by Pingchuan-Guben Decoction pretreatment(group C), Table 2.

2.4 Pathological changes in lung tissue

Group B (asthma control): There was significant inflammation in OVA-sensitized and challenged mice. Inflammatory cell infiltration was noted between epithelial cells, and in the lamina propria. There was Goblet-cell hyperplasia in the airway. Additional-

Table 2 IL-4 , IFN-γ and IL-4/IFN-γ cytokine levels in BALF

Group	n	IL-4	IFN-γ	IL-4/IFN-γ
Group A	8	61.69± 3.53	633.73± 24.75	0.09± 0.01
Group B	8	103.02± 5.59	390.21± 21.79	0.26± 0.01
Group C	8	90.27± 4.82	444.73± 26.32	0.20± 0.02
Group D	8	83.71± 4.95	509.96± 26.29	0.16± 0.01
Group E	8	71.49± 4.39	573.69± 19.69	0.12± 0.01
F		96.44	143.28	159.62
P		＜0.05	＜0.05	＜0.05

ly, and airway epithelial cells were disrupted. Group A (negative control): there were few monocytes in the lung. Group C: Base membrane was thickened accompanied by remodeling. Group D: Inflammatory cell infiltration was alleviated compared with that in Group B; Group E: After herbal drugs pretreatment and budesonide, inflammatory cell infiltration reduced significantly compared with that in Group B(Fig. 1).

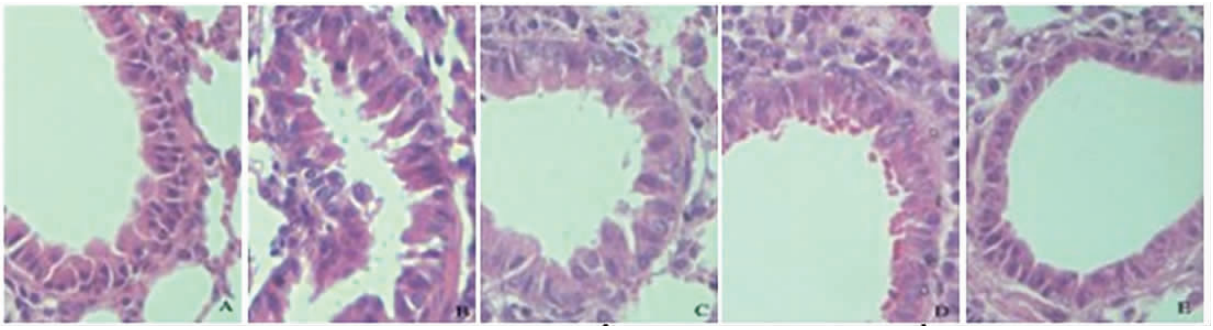

Fig.1 Histology of lung tissues (H&E staining, original magnification × 200).

3 Discussion

Asthma is defined as a chronic inflammatory disease of the airways. The chronic inflammation is associated with airway hyperresponsiveness (an exaggerated airway narrowing response to triggers, such as allergens and exercise), which leads to recurrent symptoms such as wheezing, dyspnea (shortness of breath), chest tightness and coughing [7]. It is characterized by intense infiltration of eosinophils and CD_4^+ T cells into the submucosal tissue of airways.

It is now generally believed that asthma arises as a result of dysregulated immune responses in which Th1/Th2 lymphocyte imbalance play a central role in disease pathogenesis and pathology[8,9]. Elevated levels of Th2 cells in the airways release specific cytokines, including interleukin (IL)-4, IL-5, IL-9 and IL-13, that promote eosinophilic inflammation and immunoglobulin E (IgE) production by mast cells. IgE production, in turn, triggers the release of inflammatory mediators, such as histamine and cysteinyl leukotrienes, that cause bronchospasm, edema and increased mucous secretion, which lead to the characteristic symptoms of asthma [7,10]. IL-4 is a critical regulator of Th2 development. Studies using IL-4-deficient mice clearly showed that IL-4 was required for the development of allergic inflammation, as antigen-induced allergic inflammation decreased significantly in IL-4-deficient mice as compared with wild-type mice[11]. An essential biological activity of IL-4 in the development of allergic inflammation is to drive the differentiation of naive Th0 cells into Th2 cells[12,13]. Otherwise ,accumulating evidence indicated that IL-4 can suppress interferon-γ (IFN-γ) secretion, inhibit apoptosis of eosinophils, promote infiltration of eosinophil and induce mucin gene expression and hypersecretion of mucus, all of which led to the airway obstruction stenosis. The Th1 cells produced IFN-γ can inhibit the inflammatory response by promoting the differentiation of Th0 cells to Th1 cells, suppressing Th2 clones, the expression of IL-4mRNA system and the IL-4-mediated IgE synthesis. A research showed that IFN-γ support its regulatory role in airway inflammation, through augmenting IL-17F-induced Interferon-gamma-inducible protein 10 (IP-10) expression [14].

Pingchuan-Guben Decoction is composed of Ginseng-Schisandrae Decoction, Little Dragon Decoction prescription. The research showed that Ginseng-Schisandrae Decoction and Little Dragon Decoction prescription could effectively improve the airway inflammation and airway remodeling of asthma by regulating the balance between Th1 and Th2 on the mouse model of asthma, and had the similar curative effect on the treatment of inhaled budesonide [15,16]. Glycyrrhizic Acid, the triterpenoids seperated

from Licorice, may potentially decrease the expression of interleukin-4(IL-4) in the peripheral blood and lung tissue of the mouse with asthma, promoting the expression of Interferon-γ(IFN-γ), inhibiting the production and infiltration of neutrophilic granulocyte, lymphocyte and eosinophil, significantly relieving the degree of asthma attacks on mouse [17]. Ephedra, as drugs relieving asthma, composing of Ephedrine, D-ephedrine and Ephedroxane, can excite the β2 receptor on bronchial smooth muscle cells, aslo increasing cAMP in cells by exciting K channel, relaxing bronchial smooth muscle. Xiong found that ephedra abstraction could obviously regulate airway inflammation on guinea pig of asthma, decrease cellular amount and EOS in bronchoalveolar lavage fluid of guinea pig , ease the injury and fall of airway epithelial as well as the infiltration of inflammatory cells [18]. Baicalein, the major ingredient of Scutellaria, treats the bronchial asthma by inhibiting the production of related eosinophil chemotactic factor which is collected by eosinophils[19].

To investigate the effect of Pingchuan-Guben Decoction on airway inflammation of asthma, This experiment used sensitized asthma model via OVA as the research object, to imitate the acute phase of the asthma attack. The results showed that, in Kunming mice's bronchoalveolar lavage fluid (BALF) which are treated by Pingchuan-Guben Decoction group and Budesonide group, the total cells countings, eosinophils, macrophages reduced significantly ($P<0.05$) than that in the asthma groups , between the first two group, the budesonide group was better than the traditional Chinese medicine group, but there was no statistical difference between the two groups. It was found that the damaged and the fall of the airway mucosal epithelium and inflammatory cells infiltration were obviously relieved. The IL-4 and IFN-γ content and proportion in the bronchoalveolar lavage fluid (BALF) which reflect Th1 and Th2 cell 's balance were similar in Pingchuan-Guben Decoction group and Budesonide group, both had no significant diversity. What's more, the curative effect of Pingchuan-Guben Decoction combined with the Budesonide was obviously better than the single treatment group, the result has significant difference. The findings suggest that the two drugs may have a synergistic action.

Pingchuan-Guben Decoction can apparently control the airway inflammation of the mouse with asthma, and the mechanism may be related with inhibiting the expression of IL-4, the accumulating of inflammatory cells and promoting the expression of IFN-γ, while that which signal Pingchuan-Guben Decoction translate though, it needs further study.

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平喘固本合剂对昆系小鼠急性哮喘模型气道炎症作用影响*

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摘要 目的:探讨平喘固本合剂对昆系小鼠急性哮喘模型气道炎症作用影响及相关机制。方法:昆明系小鼠40只,随机分为对照组(A),哮喘模型组(B),喘固本合剂治疗组(C),布地奈德雾化治疗组(D)及平喘固本合剂联合布地奈德雾化治疗组(E)。用鸡卵蛋白(OVA)致敏建立昆系小鼠哮喘急性气道炎症模型,并给与药物治疗10天。对各组支气管肺泡灌洗液(BALF)中各种细胞进行分类并计数,观察肺组织的病理变化,同时应用ELISA法测定灌洗液中炎症相关因子的水平变化。结果:与B组比较,C组、D组、E组BALF中细胞总数、嗜酸性粒细胞数、巨噬细胞数及IL-4、IL-4/IFN-γ明显降低($P<0.05$),IFN-γ明显升高($P<0.05$)。对于IL-4、IL-4/IFN-γ水平的比较C组与D组无明显统计学差异,E组与两者具有明显统计学意义($P<0.05$)。HE染色显示C组、D组、E组较单纯模型组炎症细胞浸润,平滑肌肥厚及黏膜肺组织水肿等炎症表现明显减轻。结论:平喘固本合剂对哮喘昆系小鼠气道炎症有明显的抑制作用,其作用机制可能与抑制IL-4的表达、炎性细胞聚积及促进IFN-γ的表达有关,并且可能与糖皮质激素有一定协同作用。

关键词:平喘固本合剂;哮喘;气道炎症;IL-4;IFN-γ

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