

The effect of Qi'ao Decoction on ovalbumin induced and lipopolysaccharide enhanced severe asthma mice and its mechanism

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[ABSTRACT] AIM: To evaluate the effect of Qi'ao Decoction (QAD) on the inflammation and hyperresponsiveness of asthma mice. **METHODS:** 120 Balb/C mice were randomly divided into six groups: normal group, model group, dexamethasone group, high dose QAD group, medium dose QAD group and low dose QAD group. The asthma model was reproduced in Balb/C mice sensitized by ovalbumin, challenged by OVA and LPS. The mice of the normal group were sensitized, challenged and intranasally instilled by PBS. On day 28–34, 6.7, 13.4 and 26.8 g·kg⁻¹ Qi'ao Decoction were administered; 0.0024 g·kg⁻¹ dexamethasone solution was given to the dexamethasone group; normal and model groups were given the same amount of normal saline. Bronchoalveolar lavage fluid, airway hyperresponsiveness, lung histopathology and cytokines were then collected and analyzed. **RESULTS:** Compared with normal group, total cellular score, the number of macrophages, lymphocytes, eosinophils and neutrophils of model group significantly increased ($P < 0.01$). Compared with model group, the administration of dexamethasone induced a significant decrease in eosinophils and neutrophils ($P < 0.05$, $P < 0.01$). The number of eosinophils, which plays an important role in airway inflammatory reaction of asthma, of the three QAD groups all decreased ($P < 0.01$). R_L before and after Ach (5 mg·mL⁻¹) stimulation in the model group both overtook that in the normal group ($P < 0.01$). Compared with model group, dexamethasone group, high dose QAD group, medium dose QAD group and low dose QAD group groups all had significantly lower R_L before and after Ach stimulation ($P < 0.01$). Normal pulmonary histopathology was found in the normal group. In the model group, mice exhibited marked increases in inflammatory cell infiltration, mostly including neutrophils and macrophages, perivascular inflammation and thickened alveolus wall ($P < 0.01$). Dexamethasone application mitigated inflammation around the bronchi ($P < 0.05$). These histopathological changes were ameliorated in the three decoction groups ($P < 0.01$, $P < 0.05$). In addition, alveolus and airway wall lesions of medium dose QAD group and high dose QAD group were reduced, the number of inflammatory cells infiltrated around the walls decreased, no clear degeneration of bronchial epithelial cells was found, and exudates in bronchi declined in different degrees. Compared with normal group, IFN- γ and IL-12 of model group significantly decreased, while IL-4 increased, showing statistic difference ($P < 0.05$). Compared with model group, IFN- γ and IL-12 level of dexamethasone group went up too, but IL-4 declined ($P < 0.05$). The level of IFN- γ of medium dose QAD group and high dose QAD group both increased; IL-4 and IL-12 of medium dose group were found significant differences ($P < 0.05$); but none of the cytokines of low dose QAD group showed statistical significance ($P > 0.05$). **CONCLUSION:** QAD can significantly inhibit airway inflammation and airway hyperresponsiveness of mice with severe asthma induced by ovalbumin and lipopolysaccharide, adjust the balance of cytokines, and improve lung histopathological condition. So, it exhibits great effect on severe asthma.

[KEY WORDS] Qi'ao Decoction; Asthma; Ovalbumin; Lipopolysaccharide

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1 Introduction

Asthma, a difficult chronic disease, has developed into a

severe public health problem in both developed and developing countries^[1]. It is characterized by prolonged inflammation, reversible obstruction and hyperresponsiveness in the airway; and usually infected by microorganism. There is a widely accepted viewpoint that on the early stage of its attack, the exposure in lipopolysaccharide (LPS) environment will induce the response of Th1 of immune system to rival against the reaction of Th2 so that asthma can be prevented deteriorating. While, on the later stage, microorganism infection and LPS exposure both worsen the existing asthma^[2].

San'ao Decoction (SAD), firstly recorded in *Beneficial*

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Formulas from the Taiping Imperial Pharmacy^[3], contains *Heaba Ephedrae* (Mahuang), *Semen Armeniacae Amarum* (Kuxingren), and *Radix et Rhizoma Glycyrrhizae* (Gancao). As a most frequently used decoction, SAD is often accompanied with other herbs to treat asthma of different stages, so the modified SAD, also called Qi'ao Decoction (QAD) is derived by adding *Rhizoma Pinelliae* (Banxia), *Fructus Schisandrae Chinensis* (Wuweizi), *Gypsum Fibrosum* (Shigao) and green tea to SAD. It was firstly recorded in the medical book named *Supplementary to Restoration of Health from the Myriad Diseases*^[4]. In clinical practice, QAD has a good therapeutic effect on asthma patients with severe tracheal inflammation.

In our study, ovalbumin (OVA) was applied to sensitize allergic reaction and challenge the mice to establish asthma model, and lipopolysaccharide (LPS) was used to aggravate airway inflammation and hyperresponsiveness of the OVA sensitized mice^[5]. We tried to evaluate the effect of QAD on tracheal inflammation and hyperresponsiveness of this model.

2 Materials and Methods

2.1 Drugs

In QAD, the ratio of *Heaba Ephedrae*, *Semen Armeniacae Amarum*, *Radix et Rhizoma Glycyrrhizae*, *Rhizoma Pinelliae*, *Fructus Schisandrae Chinensis*, *Gypsum Fibrosum* and green tea is 3 : 5 : 2 : 5 : 3 : 10 : 5. All crude herbs were purchased from Nanjing Pharmaceutical Co., Ltd., China. The botanical identities of the herbs are well-defined by Prof. Wu Dekang of Medicinal Plant Department, Nanjing University of Chinese Medicine. The herbs were decocted and concentrated, each millilitre containing 2.68 g of crude drugs. Dexamethasone Acetate Tablets (No.081211, each tablet containing 0.75 mg dexamethasone acetate) were provided by Zhejiang Xianju Pharmaceutical Co., Ltd., China. After grinding, it was added into phosphate buffer solution (PBS) to be 0.24 mg·mL⁻¹ dexamethasone acetate solution.

2.2 Reagents

OVA was purchased from Shanghai Bio Life Science & Technology Co., Ltd. (No. 080814); LPS was purchased from Sigma-Aldrich Inc., USA (No. 127k4048); Acetylcholine (Ach, No. 20070606) and pentobarbital sodium (batch No. 20080405) were purchased from Shanghai Chemical Reagent Company affiliated to China Medicine Group; Formalin solution (No.20080908) was purchased from Nanjing Chemical Reagent Company; Enzyme-linked immunosorbent assay (ELISA) kits for IFN- γ (No. 200904), IL-4 (No. 200904) and IL-12 (No. 200904) were purchased from R&D Systems, USA.

2.3 Apparatus

S-888E ultrasonic atomizers was purchased from Nanjing Dolphin Electronics Co., Ltd., China; MPA-Pulmonary Function Acquisition and Analysis System (MPA-PFS) was supplied by Shanghai Alcott Biotech Co., Ltd., China.

2.4 Animals

120 female Balb/C mice (weighing 18–20 g, 6–8 weeks

of age) were used in this study. All the animals were purchased from Laboratory Animal Centre of the Academy of Military Medical Sciences, Beijing, China, with a certification (No. SCXK (Jun) 2007-004). The mice were housed in microisolator cages and received food and water *ad libitum*. The laboratory temperature was (24 $\pm$ 1) °C, and relative humidity was 40%–80%. The mice were housed for 2–3 days to help them adapt to the environment before experimentation. All experiments were performed in accordance with the National Institutes of Health (NIH) guide for the Care and Use of Laboratory Animals. The experimental procedures were approved by the Ethical Committee for the Experimental Use of Animals at Nanjing University of Chinese Medicine (Nanjing, Jiangsu, China).

2.5 Model establishment and drug treatment

The mice were randomly divided into six groups: (1) normal group; (2) model group; (3) dexamethasone group (DM); (4) low dose QAD group (QAD-L, 6.7 g·kg⁻¹); (5) medium dose QAD group (QAD-M, 13.4 g·kg⁻¹); (6) high dose QAD group (QAD-H, 26.8 g·kg⁻¹) with 20 mice in each. The mice were given subcutaneous and intraperitoneal injection of 0.2 mL OVA for each; and then repeated once on the 8th day.

From the 14th day to the 27th day, the mice were challenged with inhalation of 2.5% OVA solution for 20 min every day. Afterwards, the mice were induced with intranasal instillation of 50 μ g LPS on the 28th, 31st and 34th day. On the 29th, 30th, 32nd and 33rd day, the mice were challenged with inhalation of 2.5% OVA solution once more. Mice of normal group were challenged by PBS and intranasally instilled with 50 μ L PBS at the same days of the previous. From day 28 to 34, 6.7, 13.4 and 26.8 g·kg⁻¹ QAD were respectively administrated to the mice of QAD-L, QAD-M and QAD-H groups 1h before intranasal instillation and inhalation; while DM group were orally given 0.002 4 g·kg⁻¹ DM solution once a day for 7 days. Normal and model groups were given the same amount of normal saline.

2.6 Bronchoalveolar lavage fluid collection

Ten mice of each group were anaesthetized and then inserted with a cannula into the trachea. Bronchoalveolar lavage was performed with 2 aliquots of 0.5 mL PBS (pH 7.2) instilled up to a total volume of 1 mL, and withdrawn twice each, the fluid recovery rate was 75%. Bronchoalveolar lavage fluid (BALF) samples were centrifuged (1 200 r·min⁻¹) for 5 min. The sediment cells were resuspended in 0.5 mL PBS. The total BALF cells were counted double-blindly using a hemocytometer followed by the differential counting of leukocytes (HE staining; two counts per slide, 300 cells per count). The supernatants of BALF were subjected to ELISA assays with kits specific for IFN- γ , IL-4 and IL-12.

2.7 Airway hyperresponsiveness

Airway hyperresponsiveness was analyzed 24 h after the last LPS induction with Ach stimulation to restrained mice by MPA-PFS. Briefly, mice were anesthetized and fixed on the board. Their trachea was isolated by blunt

dissection. A small caliber “Y” figure tube was inserted and secured in the airway. According to the instructions, three tubes were connected to detect esophageal pressure, airway pressure and respiratory flow respectively. Afterwards, negative chest pressure, airway pressure, transpulmonary pressure, respiratory flow and tidal volume were recorded. Total lung resistance (RL) representing airway responsiveness was calculated by the analysis system. The mice were stimulated by 50 μ L Ach (5 $\text{g}\cdot\text{L}^{-1}$), and then changes of RL were recorded.

2.8 Lung histopathology

The mice were sacrificed one day after airway hyperresponsiveness assay. The lungs were fixed in 10% formalin and then embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin. Histopathological changes in the lung tissues were observed under a light microscope. The scores of lung injury for each animal were histopathologically evaluated using a semi-quantitative scoring method. Lung injury was graded from 0 (normal) to 4 (severe) in two categories: inflammatory cell infiltration, thickened alveolar wall. The total lung injury score (TLIS) was calculated by adding the individual scores for each category.

2.9 Statistical analysis

Values were presented as the $\bar{x} \pm s$. Statistical analysis was performed using SPSS11.5 software. Data were evaluated using one-way analysis of variance (ANOVA) followed by LSD or Dunnett's T3 post hoc analysis. $P < 0.05$ was considered to be significant.

3 Results

3.1 Inflammatory cell count in BALF

As can be seen from Table 1, compared with normal group, total cellular score (TCS), macrophages, lymphocytes, eosinophils and neutrophils of model group were significantly higher ($P < 0.01$). Compared with model group, the administration of DM induced a significant decrease in eosinophils and neutrophils ($P < 0.05$, $P < 0.01$). The number of eosinophil, which plays an important role in airway inflammatory reaction of asthma, of the three QAD groups all decreased ($P < 0.01$). QAD-L group exhibited macrophages, raised but lymphocytes fell ($P < 0.01$). In addition, TCS,

macrophages and neutrophils of QAD-M were both reduced ($P < 0.05$), lymphocytes remarkably declined in comparison to model group ($P < 0.01$).

3.2 Change of airway responsiveness

Table 2 showed the result of the changes of airway responsiveness. RL before and after Ach (5 $\text{mg}\cdot\text{mL}^{-1}$) stimulation in the model group both overtook that in the normal group ($P < 0.01$). Compared with model group, DM, QAD-L, QAD-M and QAD-H groups all had significantly lower RL before and after Ach stimulation ($P < 0.01$). Importantly, QAD-M group and QAD-H group exhibited much slighter RL before stimulation, while DM and QAD-M showed much slighter RL after stimulation.

3.3 Histopathologic evaluation

To evaluate the histological changes after QAD treatment for OVA and LPS induced asthmatic mice, lung sections were subjected to hematoxylin and eosin staining. The results were showed in Table 3 and Fig. 1. Normal pulmonary histopathology was found in the control group (Fig. 1-1). In the model group (Fig. 1-2), mice exhibited marked increases in inflammatory cell infiltration, mostly including eosinophils, neutrophils, perivascular inflammation and thickened alveolus wall ($P < 0.01$). Dexamethasone (Fig. 1-3) application mitigated inflammation around the bronchi ($P < 0.05$). These histopathological changes were ameliorated in the three decoction groups with statistically significant differences ($P < 0.01$, $P < 0.05$). In addition, alveolus and airway wall lesions of QAD-M and QAD-H groups were reduced, the number of inflammatory cells infiltrated around the walls decreased, no clear degeneration of bronchial epithelial cells was found, and exudates in bronchi declined in different degree (Fig. 1-5, 1-6).

3.4 Cytokines assay

Cytokines assay results were shown in Table 4. It can be seen that compared with normal group, IFN- γ and IL-12 of model group significantly decreased, while IL-4 increased, showing statistic difference ($P < 0.05$). Compared with model group, IFN- γ and IL-12 level of DM group went up too, but IL-4 declined ($P < 0.05$). The level of IFN- γ of QAD-M and QAD-H groups both increased; QAD-M's IL-4 and IL-12

Table 1 Inflammatory cells in BALF ($\bar{x} \pm s$, $n = 10$)

Group	Dose ($\text{g}\cdot\text{kg}^{-1}$)	TCS ($\times 10^5/\text{mL}$)	Macrophages ($\times 10^5/\text{mL}$)	Lymphocytes ($\times 10^5/\text{mL}$)	Eosinophils ($\times 10^5/\text{mL}$)	Neutrophils ($\times 10^5/\text{mL}$)
Normal	—	2.08 ± 0.98	1.26 ± 0.60	0.52 ± 0.24	0.02 ± 0.01	0.28 ± 0.13
Model	—	$46.84 \pm 16.09^{**}$	$4.43 \pm 1.52^{**}$	$16.37 \pm 5.62^{**}$	$2.14 \pm 0.74^{**}$	$23.90 \pm 8.21^{**}$
DM	0.0024	$31.16 \pm 14.63^{\Delta}$	5.37 ± 2.52	12.12 ± 5.69	$0.29 \pm 0.14^{\Delta\Delta}$	$13.38 \pm 6.28^{\Delta}$
QAD-L	6.7	45.03 ± 17.65	$7.93 \pm 3.11^{\Delta\Delta}$	$10.08 \pm 3.95^{\Delta\Delta}$	$0.11 \pm 0.04^{\Delta\Delta}$	26.91 ± 10.55
QAD-M	13.4	$24.38 \pm 14.52^{\Delta\Delta}$	$2.78 \pm 1.66^{\Delta}$	$7.33 \pm 4.37^{\Delta\Delta}$	$0.04 \pm 0.02^{\Delta\Delta}$	$14.23 \pm 8.47^{\Delta}$
QAD-H	26.8	41.92 ± 15.63	4.41 ± 1.64	13.92 ± 5.19	$0.16 \pm 0.06^{\Delta\Delta}$	23.43 ± 8.73

Notes: $^{*}P < 0.05$, $^{**}P < 0.01$ vs normal group; $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ vs model group

Table 2 Airway responsiveness (R_L) ($\bar{x} \pm s$, $n = 10$)

Group	Dose (g·kg ⁻¹)	R_L (cmH ₂ O·mL ⁻¹ ·s ⁻¹)	
		Before Stimulation	After Stimulation
Normal	—	1.02 ± 0.44	2.47 ± 0.75
Model	—	1.80 ± 0.28**	9.86 ± 1.84**
DM	0.0024	1.20 ± 0.13 ^{△△}	3.74 ± 1.53 ^{△△}
QAD-L	6.7	1.20 ± 0.24 ^{△△}	4.14 ± 1.67 ^{△△}
QAD-M	13.4	1.11 ± 0.28 ^{△△}	2.41 ± 1.74 ^{△△}
QAD-H	26.8	1.11 ± 0.15 ^{△△}	4.17 ± 2.27 ^{△△}

Notes: * $P < 0.05$, ** $P < 0.01$ vs normal group; [△] $P < 0.05$, ^{△△} $P < 0.01$ vs model group

Table 3 Lung histopathological score ($\bar{x} \pm s$, $n = 10$)

Group	Dose (g·kg ⁻¹)	Score
Normal	—	1.10 ± 0.66
Model	—	4.92 ± 2.20**
DM	0.0024	2.33 ± 0.52 [△]
QAD-L	6.7	2.08 ± 0.66 [△]
QAD-M	13.4	1.58 ± 0.66 ^{△△}
QAD-H	26.8	1.92 ± 0.49 ^{△△}

Notes: * $P < 0.05$, ** $P < 0.01$ vs normal group; [△] $P < 0.05$, ^{△△} $P < 0.01$ vs model group

Table 4 Level of IFN- γ , IL-4 and IL-12 in BALF supernatant of each group ($\bar{x} \pm s$, $n = 10$)

Group	Dose (g·kg ⁻¹)	IFN- γ (ng·L ⁻¹)	IL-4 (ng·L ⁻¹)	IL-12 (ng·L ⁻¹)
Normal	—	143.58 ± 84.17	15.58 ± 2.16	1.31 ± 0.15
Model	—	41.75 ± 47.04*	24.49 ± 4.83**	0.68 ± 0.17**
DM	0.0024	349.08 ± 175.50 ^{△△}	14.46 ± 2.52 ^{△△}	0.94 ± 0.21 [△]
QAD-L	6.7	121.73 ± 71.47	20.59 ± 5.80	0.78 ± 0.13
QAD-M	13.4	364.66 ± 73.71 ^{△△}	19.67 ± 0.71 [△]	0.88 ± 0.13 [△]
QAD-H	26.8	271.02 ± 192.71 [△]	20.35 ± 5.09	0.77 ± 0.07

Notes: * $P < 0.05$, ** $P < 0.01$ vs normal group; [△] $P < 0.05$, ^{△△} $P < 0.01$ vs model group

were found significant differences ($P < 0.05$); but none of the cytokines of QAD-L showed statistical significance ($P > 0.05$).

4 Discussion

SAD which is considered as the primarily classic TCM formula for asthma has been found great therapeutic effect on allergic asthma model induced by OVA. Previous research has proved that SAD is effective on mice model of OVA induced asthma with successive respiratory syncytial virus (RSV) infection, and the derived prescriptions of SAD exhibited better effect than SAD itself according to the results of airway responsiveness and histopathological evaluation^[6-7]. So, it is necessary to investigate QAD, the derived formula of SAD, to determine its effect on asthma.

The present study successfully established the asthma model with severe inflammation of bronchi and more obvious airway hyperresponsiveness by OVA sensitization and challenge and LPS induction. LPS works with other tracheal inflammatory agents like allergen on the development of asthma,

even though its exposure on the early stage of asthma helps to prevent disease from deteriorating^[8]. Asthmatic patients are more likely to inhale LPS, thus developing bronchial obstruction and pre-inflammation reaction. Animal experiments have proved LPS induction in the stimulating process of OVA challenge aggravates airway infection of the asthma mice^[9-10], and the dose and time of LPS induction is the key to the success of the model^[11]. Therefore, we duplicated the severe asthma model through subcutaneous and intraperitoneal injection of 0.2 mL OVA and inhalation of 2.5% OVA solution and intranasal instillation of 50 μ g LPS in turn. Based on this severe asthma model, QAD's effect on inflammation and hyperresponsiveness of asthma was evaluated.

Bronchial asthma is chronic airway inflammation caused by a variety of inflammatory cells, mediators and cytokines, characterized by airway hyperresponsiveness, reversible airway obstruction and airway allergic inflammation. This disease is closely linked to the activation of Th which secretes a variety of cytokines. Recent studies have

revealed that the imbalance between Th1 and Th2, the two subsets of Th, is of very close relationship with asthma^[12]. Under normal circumstances, Th1 generated IL-2, IFN- γ , tumor necrosis factor (TNF- α , TNF- β), etc. to activate macrophages; while Th2 produces cytokines like IL-4, IL-5, IL-6 and IL-10 to induced eosinophils and immunoglobulin subtype transformation. The expression of these two types

of cell factors is decided by the mutual balance of Th1/Th2. However, this balance is destroyed in the process of asthma, because of the irregular secretion of these cytokines, among which IL-4 and IFN- γ are considered as a significant pair of mutual antagonism and often used to represent the balance of Th1/Th2. Asthma raises the amount of IL-4, leading to the increase of Th2; mean while, IL-4/IFN- γ goes up as well

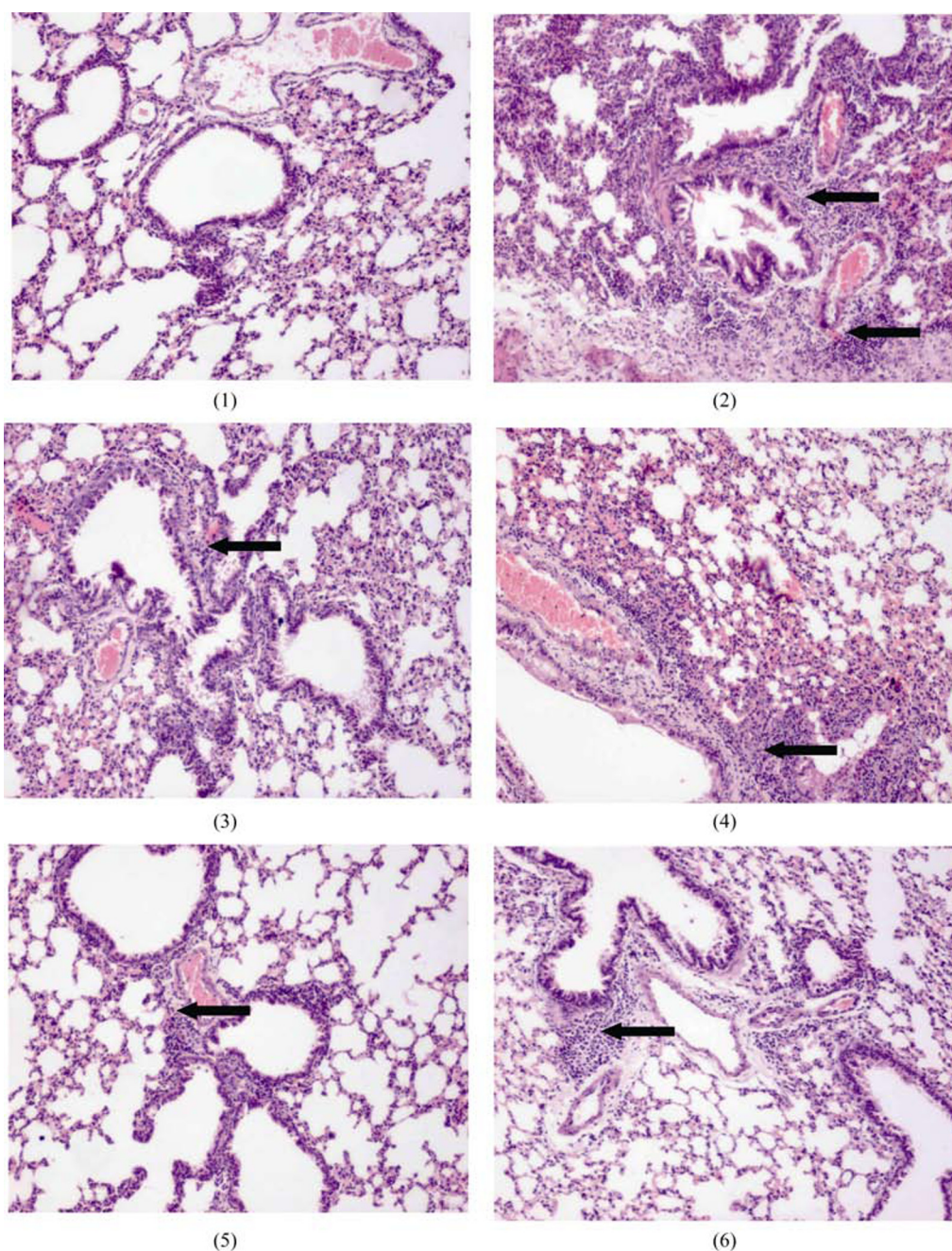


Fig. 1 Pulmonary histopathological changes (HE $\times$ 100)

Note: (1) The lung section from normal group; (2) The lung section from model group; (3) The lung section from DM group ($0.0024 \text{ g}\cdot\text{kg}^{-1}$); (4) The lung section from QAD-L group ($6.7 \text{ g}\cdot\text{kg}^{-1}$); (5) The lung section from QAD-M group ($13.4 \text{ g}\cdot\text{kg}^{-1}$); (6) The lung section from QAD-H group ($26.8 \text{ g}\cdot\text{kg}^{-1}$)

according to the increasing severity of the disease^[13].

In present study, all the three different doses of QAD were found to reduce IL-4 and IL-4/IFN- γ , especially QAD-M with statistic significance ($P < 0.05$), indicating QAD's inhibition of Th2 dominant responses and regulation of immune balance. IL-12 can prevent Th cells differentiating into Th2 cells; thus enhance Th1 pathway. Furthermore, the ability of IL-12 to induce the production of Th1 is enhanced by IFN- γ ; at the same time, IL-12 also induces the generation of IFN- γ , thus to form a positive feedback regulation to enhance Th1 response and inhibit Th2^[14]. This study also exhibited that IL-12 of all the three QAD groups increased, of which QAD-M was of highest result. So, QAD greatly improves Th1 to restore the balance of Th1/Th2. In this way, QAD proved to recover bronchial hyperresponsiveness and immune reaction of the patients with asthma.

The fact that QAD significantly inhibited the airway inflammation of mice with severe asthma induced by LPS is probably caused by the addition of *Gypsum Fibrosum* which can greatly cure lung inflammation and green tea containing an anti-oxidant of acacia catechuic acid that can activate metabolism and cure inflammation. The reason for the failure of QAD-L's inhibition of airway inflammation of mice with severe asthma induced by LPS may be due to its small dose. However, QAD-H did not show more therapeutic effect than QAD-M. There is a possibility that too much *Gypsum Fibrosum* prevented the absorption of decoction. In QAD, the active components of *Rhizoma Pinelliae* are alkaloids for arresting vomiting, stopping coughing, eliminating sputum, lowering blood pressure and lipid; and organic acids of *Fructus Schisandrae Chinensis* also can release cough and asthma. The two drugs, thus, are combined together to effectively reduce sputum of asthmatic mice, and even QAD-L also significantly inhibited hyperresponsiveness in the airway of the model mice. Since different ingredients of *Fructus Schisandrae Chinensis* are of different effects on the immune system, they often counteract each other. So, *Radix et Rhizoma Glycyrrhizae* is actually the main drug for immune regulation.

Previous study into the effect of SAD on the OVA and LPS induced asthma of mice found that SAD exhibited extreme effect on airway hyperresponsiveness and made a little influence on airway inflammation. The low dose SAD could not inhibit airway inflammation or hyperresponsiveness in the LPS enhanced asthma. The medium dose and high dose SAD significantly inhibited airway hyperresponsiveness, but hardly stopped airway inflammation^[5]. In present study into QAD, it was found that QAD-L still works on airway hyperresponsiveness, QAD-H is effective on both airway hyperresponsiveness and inflammation, and QAD-M is of the best effect on the OVA and LPS induced severe asthma model among three different doses. Therefore, it is clear that QAD is

more effective on the severe asthma than SAD. In summary, QAD, especially its medium dose, can significantly inhibit airway inflammation and hyperresponsiveness of mice with severe asthma challenged by OVA and induced by LPS, adjust the balance of Th1/Th2, improve lung histopathological condition, thus achieving much better effect than SAD. This result is corresponding to the theory of TCM.

References

- [1] Virchow, J Christian. Asthma [J]. *Semin Respir Crit Care Med*, 2012, **33** (6): 8-577.
- [2] Murakami D, Yamada H, Yajima T, et al. Lipopolysaccharide inhalation exacerbates allergic airway inflammation by activating mast cells and promoting Th2 responses [J]. *Clin Exp Allergy*, 2007, **37**: 339-347.
- [3] Imperial Medical Bureau. *Beneficial Formulas from the Taiping Imperial Pharmacy* [M]. Beijing: People's Medical Press, 2007: 36.
- [4] Gong TX. *Supplementary to Restoration of Health from the Myriad Diseases* [M]. Shanghai: Jiangdong Publishing House, 1912: 21.
- [5] Gu PC, Fan XS, Jiang CX, et al. Effect of San'ao Decoction on the airway inflammation and hyperresponsiveness in a murine model of lipopolysaccharide enhanced asthma [J]. *Chin J Integr Med*, 2011, **17** (7): 537-541.
- [6] Xu HQ, Gu PC, Fan XS. San'ao Decoction and its analogous formulas for tracheal hyperresponsiveness of asthmatic model mice induced by RSV [J]. *World Sci Technol Mod Tradit Chin Med*, 2009, **11** (5): 702-706.
- [7] Zhang Y, Tong HJ, Yu JH, et al. Effects of San'ao Decoction and its analogous prescriptions on airway inflammation in mice with respiratory syncytial virus and OVA induced asthma [J]. *Chin J Integr Med*, 2009, **7** (4): 354-359.
- [8] Boehlecke B, Hazucha M, Alexis NE. Low-dose airborne endotoxin exposure enhances bronchial responsiveness to inhaled allergen in atopic asthmatics [J]. *J Allergy Clin Immunol*, 2003, **112**: 1241-1243.
- [9] Michel O, Duchateau J, Sergysels R. Effect of inhaled endotoxin on bronchial reactivity in asthmatic and normal subject [J]. *J Appl Physiol*, 1989, **66** (3): 1059-1064.
- [10] Eldridge MW, Peden DB. Allergen provocation augments endotoxin-induced nasal inflammation in subjects with atopic asthma [J]. *J Allergy Clin Immunol*, 2000, **105** (3): 475-481.
- [11] Gu PC, Xu HQ, Fan XS, et al. Reproduction of asthma model in ovalbumin sensitized mice by ovalbumin challenge and lipopolysaccharide induction [J]. *Chin J Tuberc Respir Dis*, 2010, **33** (1): 56-59.
- [12] Movahedi M, Mahdavian SA, Rezaei N. IL-10, TGF- β , IL-2, IL-12, and IFN- γ cytokine gene polymorphism in asthma [J]. *J Asthma*, 2008, **45** (9): 790-794.
- [13] Jan S, Tomasz Z, Olga J. Evaluation of Th1/Th2 lymphocyte balance and lipopolysaccharide receptor expression in asthma patients [J]. *Pneumonol Alergol Pol*, 2009, **77** (2): 123-30.
- [14] Rajiv Pant. The mechanism of asthma and Th1/Th2[J]. *J Tianjin Med Univ*, 2002, **8** (3): 403-406.

七拗汤对内毒素诱导加重型哮喘小鼠的作用和机理研究

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【摘要】 目的: 评价七拗汤对哮喘小鼠气道炎症和高反应性的影响。**方法:** 120只 Balb/C 小鼠随机分为 6 组: 空白组、模型组、地塞米松组、七拗汤高剂量组、七拗汤中剂量组、七拗汤低剂量组。除空白组外, 各组小鼠以皮下注射、腹腔注射 OVA 致敏, 雾化吸入 OVA 激发, 内毒素鼻腔滴入诱导。空白组小鼠用 PBS 进行致敏、激发并在气道内滴入 PBS。七拗汤高、中、低剂量组分别给予 26.8、13.4、6.7 g·kg⁻¹ 的七拗汤灌胃; 地塞米松组予 0.002 4 g·kg⁻¹ 的醋酸地塞米松溶液, 1 次/天, 连续 7 天; 末次滴鼻激发 24 h 后测定相关指标。**结果:** 模型组中嗜酸性粒细胞和中性细胞数显著上升 ($P < 0.01$); 与模型组相比, 地塞米松组嗜酸性粒细胞、中性细胞数量降低 ($P < 0.05$, $P < 0.01$)。七拗汤高、中、低剂量组嗜酸性粒细胞水平均有下降 ($P < 0.01$)。模型组气道反应性升高, 与模型组相比, 地塞米松组、七拗汤高、中、低剂量组均有所降低 ($P < 0.01$)。病理显示, 模型组小鼠肺组织炎细胞浸润, 主要是中性粒细胞和嗜酸性粒细胞, 并见气管壁增厚。与模型组相比, 地塞米松组病变明显减轻, 仅见轻度的支气管周围炎; 七拗汤高、中、低剂量组肺泡壁、气道壁病变程度均较模型组减轻, 管壁及其周围浸润的炎细胞数量减少, 支气管上皮细胞变性不明显, 支气管腔内渗出物不同程度地减少。模型组 IFN- γ 和 IL-12 水平显著降低, IL-4 上升 ($P < 0.05$); 与模型组相比, 地塞米松组 IFN- γ 和 IL-12 水平升高, IL-4 降低 ($P < 0.05$)。七拗汤高、中剂量组中 IFN- γ 水平升高, 中剂量组中 IL-4 和 IL-12 水平与模型组相比具有统计学意义 ($P < 0.05$); 七拗汤低剂量组细胞因子水平改变与模型组相比无意义。**结论:** 七拗汤能够显著抑制卵蛋白致敏激发结合内毒素诱导的加重型哮喘小鼠的气道炎症和气道高反应性, 通过调整其细胞因子水平, 改善肺组织病理结构等发挥治疗效果。

【关键词】 七拗汤; 哮喘; 卵蛋白; 内毒素

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