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Special Topic for 973 Program

Effect of moxibustion at Feishu (BL 13) on airway inflammation in asthma model rats

艾灸肺俞对哮喘模型大鼠气道炎症的影响

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Abstract

Objective: To observe the effect of moxibustion at Feishu (BL 13) on related inflammatory cells and inflammatory factors in the bronchoalveolar lavage fluid of asthma model rats, and to explore the mechanism of moxibustion in treating asthma.

Methods: A total of 48 Sprague-Dawley (SD) rats were randomly divided into a normal group, a model group, a moxibustion group and a medication group, with 12 rats in each group. Except for the normal group, the rats in the other three groups were subjected to ovalbumin sensitization to stimulate the asthma. At the same time, rats in the moxibustion group received moxibustion at bilateral Feishu (BL 13), and rats in the medication group received dexamethasone by intragastric administration. Rats in the normal and the model groups only received the same fixation and normal saline by intragastric administration. After the interventions, the inspiratory resistance, the expiratory resistance, and the pulmonary compliance were measured for rats in each group; the numbers of the inflammatory cells in the bronchoalveolar lavage fluid were counted; the levels of the involved inflammatory factors in bronchoalveolar lavage fluid were detected; the pathological morphologies of the lung tissues were observed under light microscope.

Results: After modeling, compared with the normal group, the rats in the model group showed obvious asthma attack-like response, significantly increased inspiratory resistance and expiratory resistance (both $P<0.01$), and significantly reduced pulmonary compliance ($P<0.01$); thickened tracheal wall and the narrowed tracheal lumen observed under the light microscope; infiltration of inflammatory cells and increased eosinophils in and around the tracheal wall; significantly increased total number of inflammatory cells and proportion of eosinophils in the bronchoalveolar lavage fluid (all $P<0.01$); significantly reduced levels of interleukin (IL)-10, IL-12 and interferon (IFN)-γ (all $P<0.01$), and significantly increased levels of IL-4, IL-5 and tumor necrosis factor (TNF)-α (all $P<0.01$) in the bronchoalveolar lavage fluid. After intervention, compared with the model group, rats in the moxibustion and the medication groups showed significantly reduced asthma-like reaction, pathological morphological damage of lung tissue, inspiratory resistance and expiratory resistance (all $P<0.01$); significantly increased pulmonary compliance ($P<0.01$); significantly reduced total number of inflammatory cells, proportion of eosinophils, levels of IL-4, IL-5 and TNF-α in the bronchoalveolar lavage fluid ($P<0.05$ or $P<0.01$), while significantly increased IL-12 and IFN-γ levels (all $P<0.01$) in the bronchoalveolar lavage fluid; rats in the medication group also showed a significantly reduced IL-10 level in the bronchoalveolar lavage fluid ($P<0.01$); there was no statistically significant difference between the moxibustion and the medication groups (all $P>0.05$).

Conclusion: Both moxibustion at Feishu (BL 13) and intragastric administration of dexamethasone can improve the asthma attack-like symptoms of ovalbumin-sensitized rats; regulating the inflammatory cell numbers and the inflammatory factor contents in the lung may be one mechanism of moxibustion in treating asthma.

Keywords: Moxibustion Therapy; Moxa Stick Moxibustion; Point, Feishu (BL 13); Asthma; Inflammation; Cytokines; Rats

【摘要】目的：观察艾灸肺俞对哮喘模型大鼠肺泡灌洗液中相关炎性细胞、炎性因子的影响，探讨艾灸治疗哮喘的作用机制。**方法：**将48只Sprague-Dawley (SD)大鼠随机分为正常组、模型组、艾灸组和药物组，每组12只。除正常组外，其余三组大鼠采用卵清蛋白致敏并激发的方法制备哮喘模型大鼠，激发的同时艾灸组接受艾灸双侧肺俞治疗，药物组接受地塞米松灌胃治疗，正常组和模型组仅予以相同的固定和生理盐水灌胃。干预结束后，测定各组大鼠的吸气阻力、呼气阻力和肺通气顺应性，计数肺泡灌洗液中炎性细胞的数量，并检测相关炎性因子的

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含量，光镜下进行肺组织病理形态学观察。**结果：**造模后，与正常组比较，模型组大鼠表现出明显的哮喘发作样反应，吸气阻力和呼气阻力均显著增高(均P＜0.01)，肺通气顺应性显著降低(P＜0.01)；光镜下可见大鼠气管壁增厚，管腔狭窄，气管壁及其周围可见炎性细胞浸润，嗜酸性粒细胞增多；肺泡灌洗液中炎性细胞总数及嗜酸性粒细胞比例均显著增高(均P＜0.01)；肺泡灌洗液中白细胞介素(IL)-10、IL-12及干扰素(IFN)-γ的含量均显著降低(均P＜0.01)，IL-4、IL-5及肿瘤坏死因子(TNF)-α的含量均显著升高(均P＜0.01)。干预后，与模型组相比，艾灸组和药物组大鼠的哮喘发作样反应和肺组织病理形态学损伤均显著减轻，吸气阻力和呼气阻力均显著降低(均P＜0.01)，肺通气顺应性显著增高(P＜0.01)，肺泡灌洗液中炎性细胞总数及嗜酸性粒细胞比例均显著降低(P＜0.05或P＜0.01)，肺泡灌洗液中IL-12及IFN-γ的含量均显著增加(均P＜0.01)，IL-4、IL-5及TNF-α的含量均显著降低(P＜0.05或P＜0.01)；药物组大鼠肺泡灌洗液中IL-10的含量也显著降低(P＜0.01)；艾灸组与药物组各项目差异均无统计学意义(均P＞0.05)。**结论：**艾灸肺俞及地塞米松灌胃均可改善卵清蛋白致敏大鼠的哮喘发作症状；调节肺部炎性细胞数量及炎性因子的含量可能是作用机制之一。

【关键词】灸法；艾条灸；穴，肺俞；哮喘；炎症；细胞因子；大鼠

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Bronchial asthma (asthma for short) is a chronic airway inflammation involving multiple cells and cellular components, often accompanied by increased airway responsiveness, recurrent wheezing, shortness of breath, chest tightness, and/or cough. It is a common disease of the respiratory system[1]. There are approximately 300 million children with asthma worldwide. The prevalence of asthma is still rising and it tends to affect younger children, especially in developing countries[2]. Disastrous weather such as smog is increasing with the expanding industrialization and urbanization, which results in the rapidly increased incidence of asthma. The repeated asthma attacks not only bring suffering to patients, but also bring a heavy financial burden to their families and the society.

Moxibustion is an external therapy in Chinese medicine. With the thermal stimulation effect on the points, moxibustion causes moderate stress response in the local skin tissues, thus to activate the local skin immune function around the points to produce a preventive effect on the immune-related diseases. Many clinical trials have confirmed that moxibustion not only reduces the airway resistance, increases the effective lung volume, and improves the pulmonary compliance of the asthma patients, but also suppresses the inflammatory response of the respiratory tract by improving various immune indicators of asthma patients, and eventually relieves asthma attack-caused airway spasm[3-7]. By analyzing the clinical literatures on moxibustion treating asthma, Feishu (BL 13) was found to be one of the most commonpoints[8-11]. To reveal the mechanism of moxibustion in treating asthma, in our current work, the effects of moxibustion at Feishu (BL 13) on the airway responsiveness, the inflammatory cells and inflammatory cytokines in the bronchoalveolar lavage fluid of the asthma model rats were investigated, thus to provide an experimental basis for the clinical application and promotion of moxibustion to treat asthma.

1 Materials and Methods

1.1 Experimental animals and groups

A total of 48 SPF grade male Sprague-Dawley (SD) rats, weighing (200±20) g, were provided by Hunan Slake Jingda Experimental Animal Co., Ltd. [License No.: SCXK (Xiang) 2016-0002], and reared in separated cages at the Experimental Animal Center of Hunan University of Chinese Medicine, with the temperature of 20-25 ℃ and the humidity of 50%-70%. After 1 week of adaptive feeding, rats were randomly divided into a normal group, a model group, a moxibustion group and a medication group, with 12 rats in each group.

All treatments of animals throughout the experiment were in accordance with the *Guiding Opinions on the Treatment of Experimental Animals* issued by the Ministry of Science and Technology of the People's Republic of China in 2006[12].

1.2 Main reagents and instruments

1.2.1 Main reagents

Ovalbumin (Batch No.: A5253, Sigma, USA); 10% aluminum hydroxide gel (Batch No.: BF040, Xi'an Hutt Biotechnology Co., Ltd., China); dexamethasone tablets (Batch No.: 131220, Zhejiang Xianju Pharmaceutical Co., Ltd., China); hematoxylin-eosin (HE) staining solution (Batch No.: 20160607, Nanjing Jiancheng Technology Co., Ltd., China); interleukin (IL)-12 enzyme-linked immunosorbent assay (ELISA) kit (Batch No.: 201404, Beijing Chenglin Biotechnology Co., Ltd., China); IL-4 ELISA kit (Batch No.: 20140331, Nanjing Jiancheng Bioengineering Research Institute, China); IL-5 ELISA kit (Batch No.: P03032893, Wuhan Huamei Biological Engineering Co., Ltd., China); IL-10 ELISA kit (Batch No.: 12/2016, Nanjing Jiancheng Bioengineering Research Institute, China); tumor necrosis factor (TNF)-α ELISA kit (Batch No.: P26032895, Wuhan Huamei Biological Engineering Co., Ltd., China); interferon (IFN)-γ ELISA kit (Batch No.: 20140321, Nanjing Jiancheng Bioengineering Research Institute, China).

1.2.2 Main instruments

SPECTRAMAX M5 multifunctional microplate reader (Molecular Devices, USA); PFT Pulmonary Maneuvers animal lung function tester (Buxco, USA); YP10002 small animal electronic scale (Shanghai Youke Instrument Co., Ltd., China); EG1160 histopathology embedding instrument and RM2135 histopathology slicer (Leica, Germany); 2 mm diameter moxa stick (Li Shizhen Qiai Group Hubei Co., Ltd., China); DYY-6C thermostat (Beijing Liuyi Biotechnology Co., Ltd., China); −20 ℃/4 ℃ refrigerator (Panasonic Corporation, Japan).

1.3 Preparation of asthma model

According to the relevant literatures, rats in the model, moxibustion and medication groups were used to prepare the asthma models by sensitization and stimulation with ovalbumin[13-14]. Preparation of ovalbumin antigen solution: 5 g ovalbumin and 50 g aluminum hydroxide gel were dissolved in normal saline to a final volume of 500 mL. On the first day of the experiment, ovalbumin antigen solution was injected subcutaneously into the back and groin (0.5 mL) on both sides, and intraperitoneally (1 mL) for each rat. On the 8th day of the experiment, sensitization was repeated with the same dose and method. Starting on the 15th day of the experiment, rats were challenged daily with nasal drop of 1% ovalbumin, 40 μL for each nasal cavity and 80 μL for both sides, once a day for 14 d. The restlessness, shortness of breath, nodding, abdominal muscle twitching, wheezing, and cyanosis of the lips or extremities of rats indicated the successful modeling.

1.4 Intervention methods

1.4.1 Normal group

According to the method of preparing asthma rat model, rats in the normal group were given the same dose of normal saline on the first day and the 8th day of the experiment. Beginning on the 14th day of the experiment, after nasally dripping the same dose of normal saline, rats were fixed in a prone position on the rat board for 15 min every day followed by intragastric administration of normal saline at 10 mL/(kg·bw), once a day, for 14 d.

1.4.2 Model group

To replicate the asthma rat models, from the 14th day of the experiment, after daily stimulation with ovalbumin, rats were fixed in a prone position on the rat plate for 15 min followed by intragastric administration of normal saline at 10 mL/(kg·bw), once a day for 14 d.

1.4.3 Moxibustion Group

Points: Bilateral Feishu (BL 13).

Methods: Points were positioned according to the *Experimental Acupuncture Science*[15] and the anthropomorphic comparison method. Feishu (BL 13) is located beneath the spinous process of the 3rd thoracic vertebra, laterally away 1.5 cun. From the 14th day of the experiment, the replicated asthma model rats were subjected to daily nasal challenge with ovalbumin, followed by moxibustion at bilateral Feishu (BL 13) and intragastric administration of normal saline at 10 mL/(kg·bw), once a day, for 14 consecutive days. When receiving moxibustion, rats were fixed on the rat board in a prone position to expose the bilateral Feishu (BL 13); the customized fine moxa sticks (2 mm in diameter) were then lighted and placed at 3-5 cm above the bilateral Feishu (BL 13) for 15 min (a self-made body surface temperature sensor was fixed to the moxibustion site for determination of the moxibustion distance to keep the skin temperature between 45 ℃ and 55 ℃ during moxibustion for the first time).

1.4.4 Medication group

From the 14th day of the experiment, the replicated asthma model rats were subjected to daily nasal challenge with ovalbumin, followed by being fixed on the rat board for 15 min in a prone position, and then intragastric administration of dexamethasone, once a day, for 14 consecutive days. Dexamethasone was prepared as a solution with distilled water (the final concentration of 0.2 mg/mL) for intragastric administration at 10 mL/(kg·bw).

1.5 Test items and methods

1.5.1 General behavior

The demeanor, activity, breathing status, urinary status, and other general behaviors of rats were observed, and special attention should be paid to the secretions of the mouth, nose and other parts. Possible abnormal changes mainly included restlessness or curling up, sneezing, coughing, shortness of breath, abdominal muscle twitching, wheezing, cyanosis of lips or extremities, increased secretions in the mouth and nose.

1.5.2 Airway responsiveness

After the last challenge with ovalbumin, the rats were fasted for 24 h, and then anesthetized with 10% urethane by intraperitoneal injection. The rats were fixed in a supine position on the rat board to expose the trachea and perform tracheal intubation. Then the inspiratory resistance, expiratory resistance, and pulmonary compliance of the rats were determined using a small animal lung function tester.

1.5.3 Classification and counting of inflammatory cells in bronchoalveolar lavage fluid

The lung tissues were quickly separated, the right main bronchus was ligated, and 2.5 mL of normal saline was slowly injected into the left lung through the tracheal intubation when the airway responsiveness measurement was completed. After a short while, the normal saline was slowly drawn back and then slowly injected into the left lung again. The first bronchoalveolar lavage fluid was obtained after this

procedure was repeated three times. By the same way, the second and the third bronchoalveolar lavage fluids were obtained. The bronchoalveolar lavage fluids from 3 procedures were mixed, and centrifuged at 2 000 r/min for 10 min to collect the supernatant for detecting the cytokines to be tested; the cell sediment for sorting and counting the inflammatory cells after Wright staining.

1.5.4 Cytokine level in bronchoalveolar lavage fluid

The supernatant of the bronchoalveolar lavage fluid was collected by centrifuging. The levels of IL-12, IL-4, IL-5, IL-10, TNF-α and IFN-γ in the bronchoalveolar lavage fluid were detected by ELISA strictly following the instruction of the kit.

1.5.5 Pulmonary histopathology

The right upper lobes of the rat lung tissues in each group were fixed with 4% paraformaldehyde, embedded in paraffin, stained with HE staining, and the pathological and the morphological changes of lung tissues were observed under the light microscope.

1.6 Statistical analysis

All data were analyzed using SPSS version 20.0 statistical software. The measurement data were first tested for normal distribution and homogeneity of variance. The data meeting the normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$). One-Way ANOVA was used for comparison between groups. The least significant difference (LSD) method was used for comparing the data with homogeneity of variance. Dunnett T3 method was used for comparing the data with heterogeneity of variance. The rank-sum test was used when the normal distribution was not satisfied. $P<0.05$ indicated statistical significance.

2 Results

2.1 General behavioral observations

Throughout the experiment, the rats in the normal group were in good mental state and responsive, with neat and shiny hair, strong body, regular and uniform breathing rhythm, normal defecation and urine, without abnormal secretions in the mouth, nose and other parts. After the nasal dripping stimulation with the ovalbumin, rats in the model group gradually started to scratch the nose and mouth, frequently nod, together with breathlessness, sneezing and other reactions; white viscous secretions in the mouth and nose, and occasional wheezing. With the increased challenge time, the rats showed lassitude or restlessness, and dull hair; rats in the moxibustion and the medication groups also showed the above-mentioned reactions after nasal challenge with ovalbumin, but the overall symptoms were milder than in the model group.

2.2 Airway responsiveness comparison

Compared with the normal group, the inspiratory resistance and the expiratory resistance of the model group were significantly increased (both $P<0.01$), and the pulmonary compliance was significantly decreased ($P<0.01$); compared with the model group, the inspiratory resistance and the expiratory resistance of the moxibustion and the medication groups were significantly reduced (all $P<0.01$), and the pulmonary compliance was significantly increased (both $P<0.01$); there was no statistically significant difference between the moxibustion and the medication groups (all $P>0.05$). These results indicated that ovalbumin stimulation increased the inspiratory resistance and expiratory resistance, and reduced the pulmonary compliance of rats; moxibustion at Feishu (BL 13) and dexamethasone administered by gavage showed a certain preventive effect on this process (Table 1).

2.3 Effects on the pathological morphology

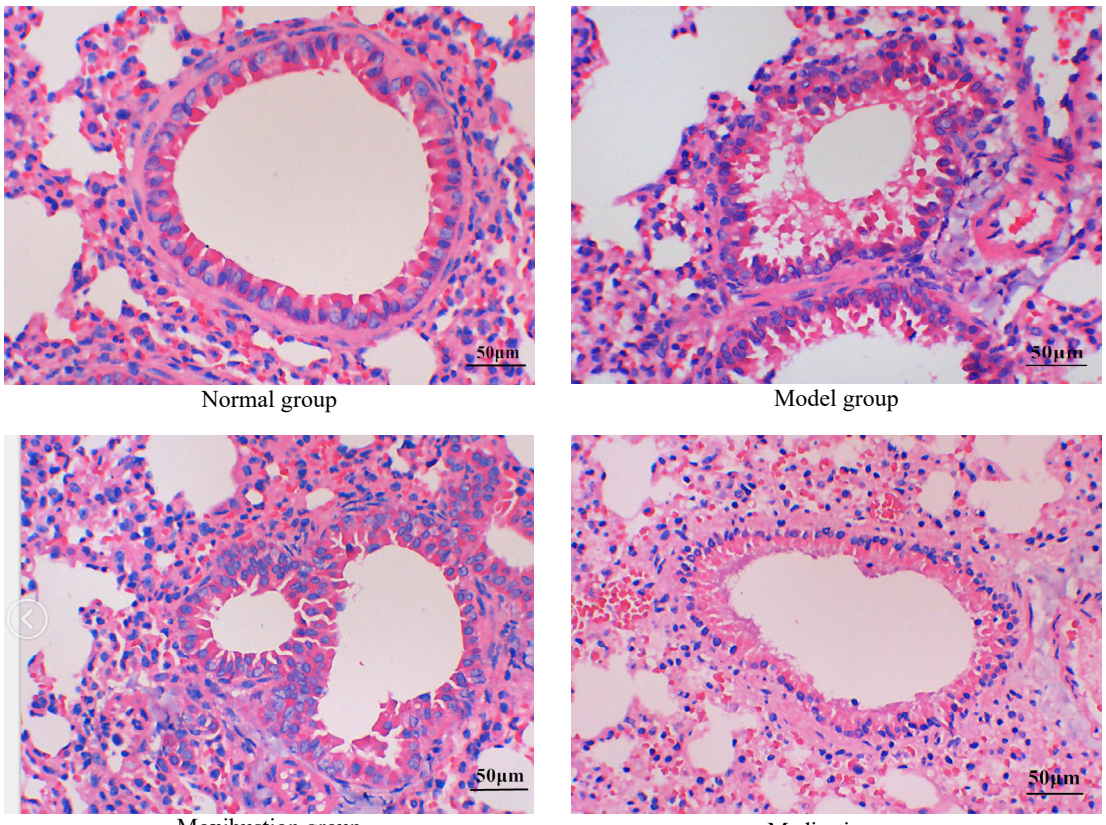
The alveoli and bronchioles were normal in the shape and structure; the bronchial lumen was smooth and regular, and the cilia were arranged neatly; there was no inflammatory secretion in the vascular cavity, bronchoalveolar cavity, and interstitial lung of rats in the normal group.

Rats in the model group showed thickened tracheal wall, narrowed lumen, inflammatory cell infiltration and increased eosinophils in and around the tracheal wall; compared with the model group, the bronchoalveolar wall structure was complete, the bronchiole morphology was regular, and there was no mucus plug formation and epithelial cell shedding in the lumen of rats in the moxibustion and the medication groups; there was no significant difference between the moxibustion and the medication groups (Figure 1).

Table 1. Comparison of airway responsiveness among groups ($\bar{x} \pm s$)

Group	n	Inspiratory resistance [kPa/(s·mL)]	Expiratory resistance [kPa/(s·mL)]	Pulmonary compliance (mL/kPa)
Normal	12	0.174±0.012	0.181±0.011	3.17±0.11
Model	12	0.342±0.032[1)]	0.326±0.037[1)]	0.22±0.09[1)]
Moxibustion	12	0.213±0.023[2)]	0.206±0.024[2)]	1.61±0.27[2)]
Medication	12	0.213±0.210[2)]	0.201±0.020[2)]	1.49±0.16[2)]

Note: Compared with the normal group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$



Normal group　　Model group

Moxibustion group　　Medication group

Figure 1. Comparing the pathological morphology of rat lung tissues among groups (HE staining, ×200)

2.4 Effects on the inflammatory cells in the bronchoalveolar lavage fluid

Compared with the normal group, the total number of inflammatory cells and the proportion of eosinophils in the bronchoalveolar lavage fluid of the model group were significantly increased (both $P<0.01$). Compared with the model group, the total number of inflammatory cells and the proportion of eosinophils in the bronchoalveolar lavage fluid of the moxibustion and the medication groups were significantly reduced ($P<0.05$ or $P<0.01$); there was no significant difference between the moxibustion and the medication groups (both $P>0.05$). These results indicated that ovalbumin challenge stimulated the inflammatory response in rat lung tissues, while moxibustion at Feishu (BL 13) and intragastric administration of dexamethasone showed a certain anti-inflammatory effect (Table 2).

2.5 Effect on the inflammatory cytokines in the bronchoalveolar lavage fluid

Compared with the normal group, the levels of IL-10, IL-12 and IFN-γ were significantly reduced (all $P<0.01$), and the levels of IL-4, IL-5 and TNF-α were significantly increased in the bronchoalveolar lavage fluid of the model group (all $P<0.01$). Compared with the model group, the IL-12 and IFN-γ levels were significantly increased, while the IL-4, IL-5 and TNF-α levels were significantly reduced in the rat bronchoalveolar lavage fluid of both the moxibustion and the medication groups ($P<0.05$ or $P<0.01$); and the IL-10 level was significantly decreased in the medication group ($P<0.01$); there were no significant differences between the moxibustion and the medication groups (all $P>0.05$). These results indicated that ovalbumin challenge reduced the levels of IL-10, IL-12 and IFN-γ, and increased the levels of IL-4, IL-5 and TNF-α in rat lung tissues. Moxibustion at Feishu (BL 13) or intragastric administration of dexamethasone increased the levels of IL-12 and IFN-γ, and reduced the levels of IL-4, IL-5 and TNF-α in the lung tissues of the experimental rats (Table 3).

Table 2. Comparing the inflammatory cell counts in the bronchoalveolar lavage fluid of rats among groups ($\overline{x}\pm s$)

Group	n	Total number of inflammatory cells ($\times10^5$/mL)	Eosinophil ratio (%)
Normal	12	11.9±1.19	1.05±0.12
Model	12	27.5±3.85[1)]	6.95±1.11[1)]
Moxibustion	12	24.7±2.54[3)]	5.86±1.21[3)]
Medication	12	23.7±2.21[2)]	5.55±1.15[2)]

Note: Compared with the normal group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$, 3) $P<0.05$

Table 3. Comparing IL-10, IL-12, IFN-γ, IL-4, IL-5 and TNF-α levels in the rat bronchoalveolar lavage fluid among groups ($\bar{x}$ ±s, ng/L)

Group	*n*	IL-10	IL-12	IFN-γ	IL-4	IL-5	TNF-α
Normal	12	4.10±0.38	222.00±9.59	33.3±3.05	11.70±1.25	18.90±1.68	40.10±6.19
Model	12	2.71±0.44[1)]	42.20±6.28[1)]	21.5±1.38[1)]	30.50±2.35[1)]	38.60±7.57[1)]	97.30±9.61[1)]
Moxibustion	12	2.37±0.52	130.00±13.70[2)]	28.2±2.59[2)]	24.50±2.33[2)]	31.70±8.79[3)]	84.90±15.10[3)]
Medication	12	1.94±0.81[2)]	146.00±14.00[2)]	27.9±2.05[2)]	22.50±1.82[2)]	30.40±5.83[2)]	72.20±16.60[2)]

Note: Compared with the normal group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$, 3) $P<0.05$

3 Discussion

3.1 Effects of moxibustion at Feishu (BL 13) on the asthmatic symptoms in the experimental rats

Moxibustion produces warm stimulation to patients through the heat generated by moxa burning, which in turn can warm meridians, remove cold, activate blood, warm yang and prevent collapse. It is a common method to treat asthma[16]. Feishu (BL 13) is located where the qi and blood of the lung transfuses on the back. This point can relieve cough and asthma, is an important point for the treatment of lung diseases and often used to treat respiratory diseases such as cough, asthma, hemoptysis, pulmonary dystrophy, tidal fever due to lung atrophy, and allergic rhinitis. Wen X, *et al*[17] found that ginger cake-partitioned moxibustion at Feishu (BL 13) combined with acupuncture had a significant effect in treating acute exacerbation of bronchial asthma. Chen JS, *et al*[18] found that Qi Ai Tian Jiu Gao plus suspended moxibustion at Feishu (BL 13) could prevent and control asthma in kids, reduce and control the asthma exacerbation.

The results of this study showed that after the nasal dripping challenge with ovalbumin, the experimental rats gradually appeared asthma-like reactions such as scratching the nose and mouth, frequent nodding, breathlessness, and sneezing; significantly increased inspiratory resistance and expiratory resistance, along with significantly decreased pulmonary compliance. These findings are basically consistent with what has been reported[13-14]. This study also found that moxibustion at Feishu (BL 13) significantly alleviated ovalbumin-sensitized asthma-like symptoms.

3.2 Effects of moxibustion at Feishu (BL 13) on the inflammatory cells in the bronchoalveolar lavage fluid of asthma model rats

Bronchial asthma is a chronic airway inflammatory reaction involving multiple cells and cellular components. It is a common chronic respiratory diseases characterized by airway inflammation, hyperresponsiveness and remodeling. The airway inflammation is the basis of airway hyperresponsiveness and airway remodeling. Infiltration and increase of inflammatory cells in the airway are the important features of asthma, and the degree is significantly positively correlated with the severity of bronchial asthma[19]. The immune response during the asthma exacerbation activates the inflammatory cells such as neutrophils, macrophages, lymphocytes, and eosinophils, and the activated inflammatory cells are chemotactically and aggregated to the inflammation site, and release the inflammatory factors, which indirectly or directly damage the epithelial cells in the airway, causing airway inflammation[20]. Therefore, eosinophils, neutrophils, macrophages, and lymphocytes are considered to be the main inflammatory effector cells in asthma exacerbation, and the most important indicators for the clinical diagnosis of asthma. It is also an important index to evaluate the therapeutic effect in treating of asthma. Acute asthma exacerbation and chronic airway inflammation are accompanied by substantial infiltration and accumulation of inflammatory cells[21-23]. Effective control of airway inflammatory response is the key to treating asthma. Therefore, the current treatment of asthma is mainly to regulate the immune function and control the airway inflammatory response.

Many experiments have confirmed that the inflammatory cell levels such as eosinophils, neutrophils, macrophages and lymphocytes in the bronchoalveolar lavage fluid of asthma model animals are significantly increased[24-25]. The results of this study also confirmed that sensitization and stimulation with ovalbumin increased the total inflammatory cell numbers and the eosinophil level in the bronchoalveolar lavage fluid of experimental rats; moxibustion at Feishu (BL 13) reduced the total numbers of inflammatory cells and eosinophil level in bronchoalveolar lavage fluid, indicating that moxibustion at Feishu (BL 13) inhibited the activation, prevented the chemotaxis and accumulation of the inflammatory cells to lung in the asthma model rats, and reduced the release of inflammatory mediators in the lungs, thereby protecting airway epithelial cells, reducing airway inflammation, and ultimately relieving or inhibiting asthma exacerbation.

3.3 Effects of moxibustion at Feishu (BL 13) on the inflammatory cytokines in the bronchoalveolar lavage fluid of the asthmatic model rats

At present, it is generally believed that immune dysfunction is an important mechanism of asthma. T cells, as the most important effector cells in the

immune system, plays central role in the cellular immune response. Previous studies have suggested that the ratio and function imbalances of T helper (Th) 1/Th2 cells are the key factors in asthma exacerbation. In recent years, the important role of Th17/Treg imbalance in the pathogenesis of asthma has also received increasing attention[26-31]. Activated T cells mainly release specific cytokines to regulate the immune function, and the functions of different cytokines are antagonistic to each other, thereby ensuring the immune homeostasis of the body[32].

IL-10 is an important cytokine with immune-regulatory effects and significantly reduced when asthma occurs to limit the inflammatory response and autoimmune response, and inhibit airway hyperresponsiveness, thus to play complex biological role in asthma effector cells[33]. Studies have shown that the deficiency of IL-10 and IL-12 leads to asthma aggravation; IL-10 and IL-12 are positively correlated in the course of asthma; treatment with IL-10 and IL-12 alone or together significantly reduces the incidence of asthma[34]. IFN-γ is mainly derived from $CD8^+$ cells to antagonize IL-4 by inhibiting the transcription level of IL-4 mRNA, thereby inhibiting the production of immunoglobulin E (IgE) *in vivo*[35]. Ma L, *et al*[36] found that vaccine can be used to treat allergic asthma by increasing the IFN-γ level, thus to reduce the serum IgE level. Mitchell C, *et al*[37] found that IFN-γ significantly inhibited IL-4-induced proliferation of B lymphocytes, and reduced airway inflammatory response, thus to reduce the degree of airway obstruction.

The development of asthma is mediated by proinflammatory cytokines, mainly including IL-4 and IL-5 secreted by Th2 cells, and TNF-α secreted by Th1 cells[27]. It was reported that sensitization with ovalbumin increased IL-4, IL-5 and TNF-α in the bronchoalveolar lavage fluid of the experimental rats[25]. IL-4 is an inflammation promoting factor secreted by Th1/Th2, which can not only switch Th0 to Th2, but also promote the secretion of IL-5 and IL-12 by Th2, and indirectly inhibit the secretion of inflammatory inhibitory factors of IFN-γ and IL-12[31]. Clinical studies by Gao ZG, *et al*[38] suggested that the high level of serum IL-4 promoted the formation of airway hyperresponsiveness in children with asthma. During the onset of asthma, the Th1/Th2 balance is broken, and the immune response tends to Th2, which reduces the Th1-type cytokine IFN-γ and increases the Th2-type cytokine IL-4[30].

Study by Zhao YZ, *et al*[23] found that the levels of IL-4, IL-5 and IL-13 were significantly increased, and the IFN-γ level was significantly reduced in the bronchoalveolar lavage fluid of asthma model rats, and imperatorin regulated the above factors. The study by Zhu YT, *et al*[25] found that the levels of IL-4, IL-5 and TNF-α in the bronchoalveolar lavage fluid of asthma model rats were significantly increased, and verbenaside regulated these factors. Ke LQ, *et al*[39] found that the IL-10 concentration in the bronchoalveolar lavage fluid of asthma model rats was significantly lower than that of normal rats, and vasoactive intestinal peptide had a certain regulatory effect on it. The study by Li Y, *et al*[40] found that the IL-12 expression levels in the plasma and the bronchoalveolar lavage fluid of the asthma model rats were lower than those of the normal rats, and smoke exposure further reduced the IL-12 levels in the plasma and the bronchoalveolar lavage fluid of asthmatic rats. The results of this study also showed that the levels of IL-10, IL-12 and IFN-γ were significantly reduced, and the levels of IL-4, IL-5 and TNF-α were significantly increased in the bronchoalveolar lavage fluid of asthma model rats. Moxibustion at Feishu (BL 13) significantly regulated IL-12, IFN-γ, IL-4, IL-5 and TNF-α, but had no significant regulatory effect on IL-10.

In summary, the results of this study suggested that moxibustion at Feishu (BL 13) significantly improved the asthma-like symptoms, reduced the airway resistance, improved the lung ventilation function, and reduced the pathological and morphological damage of lung tissues in the experimental asthma rats; at the same time, moxibustion at Feishu (BL 13) had a beneficial regulatory effect on the total number of inflammatory cells, eosinophil level, IL-12, IFN-γ, IL-4, IL-5, TNF-α and other inflammatory cytokines, indicating that moxibustion at Feishu (BL 13) may regulate the immune response of the lungs in asthmatic rats, thereby preventing asthma.

Conflict of Interest

There is no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria.

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