

Effects of Chinese herbal formula Biminne in regulating endocrine-immune function in mice with airway allergy

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Objective: To investigate the effects of Biminne, a traditional Chinese herbal formula, in regulating the endocrine-immune function in mice with airway allergy.

Methods: Forty BALB/c mice were randomly divided into four groups: control group, untreated group, Biminne group, and dexamethasone (Dex) group. The murine model of airway allergy was established by intraperitoneal injection sensitization and intranasal challenge with ovalbumin (OVA). On day 28, the mice were sacrificed and the sera were collected. The lung tissue of mice was removed for haematoxylin-eosin staining and the pathological changes were observed. The spleen cells of mice were isolated and the ratio of Th2/Th1 was determined by flow cytometry. The isolated spleen cells were also cultured in 24-well plates in the presence of concanavalin A and the supernatants were collected after 72-hour culture. Levels of interleukin-4 (IL-4), IL-5 and interferon-gamma (IFN- γ) in the supernatants and the corticosterone level in sera were analyzed by enzyme-linked immunosorbent assay.

Results: Biminne significantly relieved the inflammatory reaction in the lung tissue of airway allergic mice. Compared with the control group, the Th2 cells in the untreated group increased, while the IFN- γ level decreased and the ratio of Th2/Th1 increased significantly, showing a Th2 response priority. Biminne treatment could down-regulate the Th2 cells and their cytokines IL-4 and IL-5, and up-regulate the Th1 cells and their cytokine IFN- γ . So the ratio of Th2/Th1 decreased significantly, shifting to the control level. In addition, Biminne also inhibited the reduction of the serum corticosterone of sensitized mice.

Conclusion: The Chinese herbal formula Biminne can reverse the functional disorder of the endocrine-immune system, which may explain its therapeutic effects on airway allergic disease.

Keywords: allergy; interleukin-4; interleukin-5; interferon-gamma; corticosterone; mice

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中药复方别敏胶囊对呼吸道变应性小鼠内分泌免疫功能的调节作用

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目的: 观察中药复方别敏胶囊对呼吸道变应性模型小鼠内分泌免疫功能的影响。

方法: 利用卵清蛋白建立呼吸道变应性小鼠模型, 采用苏木精和伊红染色观察肺组织病理变化, 运用流式细胞仪检测脾淋巴细胞中 Th1 和 Th2 细胞变化, 利用酶联免疫吸附测定方法检测脾淋巴细胞培养上清白细胞介素 4(interleukin-4, IL-4)、白细胞介素 5(interleukin-5, IL-5)、干扰素 γ (interferon-gamma, INF- γ) 水平及血清皮质酮浓度。

结果: 别敏能够明显减轻模型小鼠肺组织的炎症反应, 降低模型小鼠 Th2 细胞及其细胞因子 IL-4、IL-5 水

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平, 升高 Th1 细胞及其细胞因子 INF- γ 水平, 显著抑制模型小鼠内源性皮质酮水平的下降。

结论: 别敏对机体内分泌免疫系统功能紊乱的调节作用可能是其治疗呼吸道变应性疾病的重要机制之一。

关键词: 变应性; 白细胞介素 4; 白细胞介素 5; 干扰素 γ ; 皮质酮; 小鼠

Airway allergic diseases including allergic rhinitis and bronchial asthma were airway chronic inflammatory diseases. The recent evidences suggest that the imbalance of Th1/Th2 immune response plays a central role in the pathogenesis of the disease. An impaired adrenal function may also exist in the process of the disease^[1-6]. So the interaction between immune system and endocrine system influences the allergic inflammation. Biminne, a Chinese herbal formula, consists of a number of strengthening-kidney Chinese herbs, and has such therapeutic effects as warming kidney, strengthening qi, and eliminating pathogenic factors. A randomized, double-blind, placebo-controlled clinical trial had been done in Australia to evaluate the effect of Biminne in management of allergic rhinitis. The results showed that the Biminne formula was effective in relieving the symptoms of perennial allergic rhinitis such as nasal itching, sneeze, and nasal obstruction and it also decreased the serum immunoglobulin E (IgE) of allergic rhinitis patients^[7]. Our previous animal studies also indicated that Biminne relieved the nasal vascular permeability of sensitized Brown Norway rats^[8], prolonged latency of asthma attack of sensitized guinea pigs and decreased the eosinophils in the peripheral blood and bronchial lavage fluids (BALF) of the sensitized guinea pigs^[9]. In this study, we aimed to investigate the effects of Biminne in regulating the endocrine-immune function in mice with airway allergy.

1 Materials and methods

1.1 Materials

1.1.1 Animals Forty male BALB/c mice (18 to 22 g) were obtained from Animal Center of Fudan University and kept in the specific pathogen free animal facility. The animal certificate number was SCXK (Hu) 2002-0011.

1.1.2 Drugs and reagents The Biminne formula was composed of the following Chinese herbs: *Radix Rehmanniae* (Dihuang) 4.60 g, *Radix Scutellariae Baicalensis* (Huangqin) 4.60 g, *Rhizoma Polygonati Sibirici* (Huangjing) 3.68 g, *Folium Ginkgo* (Yinxingye) 4.60 g, *Herba Epimedii Brevicornus* (Yinyanghuo) 4.60 g, *Fructus Psoraleae* (Buguzhi) 4.60 g, *Fructus Schisandrae Chinensis* (Wuweizi) 3.68 g, pulp of *Fructus Mume* (Wumei) 1.84 g, *Radix Saposhnikovia* (Fangfeng) 4.60 g, *Radix Angelicae Formosanae* (Baizhi) 3.68 g, and *Radix Astragali Mongolici* (Huangqi) 5.52 g. The extract was prepared from the above dried herbals and packed in 10 capsules (daily dose of a person). Each capsule contained 3.5 mg of baicalin and 1 mg of icariin^[7]. The Bim-

inne capsules were provided by Department of Medicine of Sydney University. Ovalbumin (OVA), aluminum hydroxide gel, dexamethasone, Hanks and RPMI 1640 culture medium were all purchased from Sigma (St. Louis, Mo, USA). Interleukin-4 (IL-4), IL-5 and interferon-gamma (IFN- γ) mouse enzyme-linked immunosorbent assay (ELISA) kits were purchased from Biosource (Camarillo, Calif, USA). Fluorescein isothiocyanate (FITC) anti-mouse CD4 antibody, phycoerythrin (PE) anti-mouse IL-4 antibody and PE anti-mouse IFN- γ antibody were purchased from Bolegend (San Diego, CA, USA). Corticosterone enzyme immunoassay (EIA) kit was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Fetal bovine serum (FBS) was purchased from Gibco Company. Lymphocyte separating medium was purchased from Shanghai Jinghua Biological Limited Company.

1.2 Methods

1.2.1 Animal model establishment Forty BALB/c mice were randomly divided into four groups: control group, untreated group, dexamethasone (Dex) group and Biminne group, with 10 mice in each group. The method to establish animal model was mainly accorded to Matsui *et al*^[10] with a little modification. With the exception of mice in the control group, the mice were sensitized by intraperitoneal injection of 100 μ g OVA suspended in aluminum hydroxide gel on day 0 and day 7. From the day 18 to day 27, the mice were challenged by intranasal instillation of 100 μ g of OVA suspended in 20 μ L normal saline on a daily basis. The mice in the control group were given sham sensitization and challenge with the same volume of normal saline. In the same period (from day 18 to day 27), the mice in the Biminne group were treated with Biminne (1 g/kg) by intragastric administration and the mice in Dex group were treated with Dex (0.5 mg/kg) by intraperitoneal injection. The mice in the control group and the untreated group were given the same volume normal saline by intragastric administration.

1.2.2 Serum and lung tissue collection and examination The mice were sacrificed on day 28, 24 h after the final challenge. The blood samples were obtained and centrifuged at 405 $\times g$ for 10 min. The sera were stored at -80 $^{\circ}$ C until assay. Corticosterone level was determined via a corticosterone EIA kit according to the manufacture's protocol. The left lobe of the lung was surgically removed and fixed in 4% buffered formaldehyde. They were then embedded in paraffin, sectioned and stained with haematoxylin-eosin (HE). Histological findings, including inflammatory cells infiltration, were observed by an independent observer under a light microscope with a focus on inflammatory cells

(mainly eosinophils) and the eosinophils in 5 scopes were quantified.

1.2.3 Flow cytometric analysis The isolated spleen cells were washed with 0.1% bovine serum albumin in phosphate-buffered saline and subsequently incubated in FITC antimouse CD4 antibody for 30 min at room temperature. After staining, they were washed once with 2 mL of cell-wash solution, and then fixed for 15 min. Then permeabilization of the spleen cells was carried out by 1.5 mL penetrant addition and incubated in dark for 15 min. After centrifugation, the cells were incubated with PE antimouse IL-4 antibody or PE antimouse IFN- γ antibody in dark for 30 min; then washed twice with 2 mL cell-wash solution and fixed with 350 μ L of fixative. The flow cytometric assessment with FACSCaliber flow cytometry (Becton Dickinson) was performed within 24 h. Data acquisition and analysis were performed with cell quest software package (Becton Dickinson).

1.2.4 Spleen cell culture and quantification of cytokines Spleen cells were isolated from mice and suspended in complete culture medium (RPMI 1640 containing 10% FBS, 100 U/mL penicillin, 100 U/mL streptomycin and 1% glutamine). Cells (2×10^6 /well) were cultured in 24-well plates in the presence of concanavalin A (10 μ g/mL). Supernatants were collected after 72-hour culture. The levels of IL-4, IL-5 and IFN- γ in spleen culture supernatants were determined by ELISA method according to the manufacturer's protocol.

ing to the manufacturer's protocol.

1.3 Statistic method The data were analyzed by Stata 7.0 software. Results of measurement data were expressed as $\bar{x} \pm s$ if they meet the criteria of analysis of variance. Comparisons among groups were made with one-way analysis of variance. Comparisons between two groups were made with t test. If the variances were not equal, comparisons among groups were made with Kruskal-Wallis test. If the data between two groups were not normal distribution or their variances were not equal, the comparisons were made with Wilcoxon-Mann-Whitney test. Level of a test is 0.05.

2 Results

2.1 Effects of Biminne on pathology of lung tissue in sensitized mice As shown in Figure 1, compared with the control group, the lung tissues in the untreated group showed the following pathological changes such as more eosinophils infiltration, bronchiole smooth muscle thickening, epithelium ablation, and lumens stenosis. Biminne and Dex both relieved the above inflammatory responses in the lung tissues of the sensitized mice. The number of eosinophils in the lung tissues was shown in Table 1. The eosinophil infiltration in the mice of the untreated group was more severe than that in the untreated group. Biminne and Dex reduced the eosinophil number significantly in the sensitized mice.

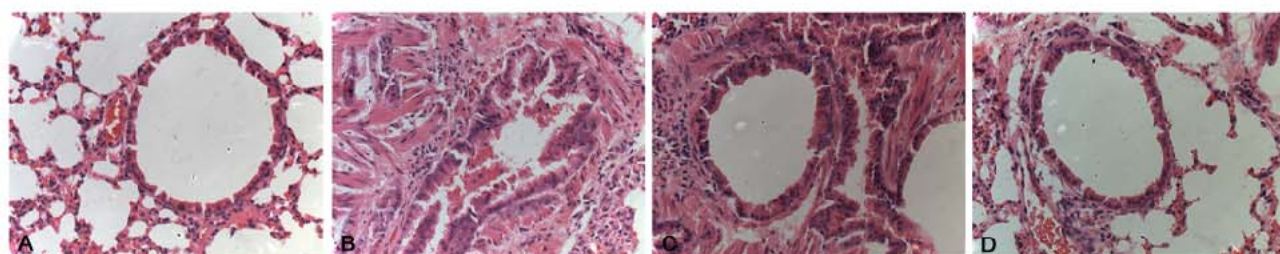


Figure 1 Effects of Biminne on the lung tissues pathology in sensitized mice observed by light microscope (HE staining, $\times 400$)

A, Control group; B, Untreated group; C, Biminne group; D, Dex group

Table 1 Effects of Biminne on the eosinophil infiltration in the lung tissues of sensitized mice

($\bar{x} \pm s$, cells/HP)

Group	n	EOS
Control	10	2.0 ± 2.4
Untreated	10	$19.0 \pm 6.4^{**}$
Dex	10	$3.0 \pm 1.4^{\Delta\Delta}$
Biminne	10	$3.1 \pm 2.3^{\Delta\Delta^{**}}$

$^{**} P < 0.01$, vs control group; $\Delta\Delta P < 0.01$, vs untreated group.

2.2 Effects of Biminne on the balance of Th1/Th2 cells As shown in Table 2, the Th2 cells and the ratio of Th2/Th1 in the untreated group increased significantly as compared with the control group. Compared with the untreated group, Biminne

down-regulated the Th2 cells, up-regulated the Th1 cells, and decreased the ratio of Th2/Th1 significantly ($P < 0.05$), making the balance of Th2/Th1 shift to the control level. Both the Th2 and Th1 cells decreased in the Dex group, and the Th2 cells reduced more significantly ($P < 0.05$). The ratio of Th2/Th1 in the Dex group was also lower than that in the untreated group ($P < 0.05$).

2.3 Effects of Biminne on the IL-4, IL-5 and IFN- γ levels in the spleen cell culture supernatants As shown in Table 3, the IFN- γ level in the untreated group decreased significantly and the ratio of IL-4/IFN- γ increased significantly ($P < 0.05$, $P < 0.01$) as compared with the control group. Both Biminne and Dex decreased the IL-4 and IL-5 levels significantly in the sensitized mice ($P < 0.05$). Biminne also increased the IFN- γ level significantly

($P < 0.05$), but Dex did not. The ratio of IL-4/IFN- γ in the Biminne group decreased significantly ($P < 0.05$) as compared with the untreated group.

2.4 Effects of Biminne on the serum corticosterone level As shown in Table 4, the corticosterone level of the mice in the untreated group was significantly

higher than that in the control group ($P < 0.05$). Compared with the untreated group, corticosterone level of the sensitized mice was significantly increased in the Biminne group ($P < 0.05$). However, Dex markedly decreased the corticosterone level of the sensitized mice ($P < 0.01$).

Table 2 Effects of Biminne on the Th1 and Th2 cells of sensitized mice

($\bar{x} \pm s$)

Group	<i>n</i>	Th2 cells (%)	Th1 cells (%)	Th2/Th1
Control	10	0.79 $\pm$ 0.45	0.95 $\pm$ 0.29	1.14 $\pm$ 0.20
Untreated	10	1.47 $\pm$ 0.56*	0.83 $\pm$ 0.41	1.60 $\pm$ 0.60*
Dex	10	0.91 $\pm$ 0.51 Δ	0.68 $\pm$ 0.46	1.29 $\pm$ 0.45 Δ
Biminne	10	1.00 $\pm$ 0.43 Δ	1.24 $\pm$ 0.42 Δ	1.13 $\pm$ 0.41 Δ

* $P < 0.05$, Δ control group; Δ $P < 0.05$, Δ untreated group.

Table 3 Effects of Biminne on the IL-4, IL-5 and INF- γ levels in spleen cell culture supernatants

($\bar{x} \pm s$)

Group	<i>n</i>	IL-4 (ng/L)	IL-5 (ng/L)	INF- γ (ng/L)	IL-4/INF- γ
Control	10	47.93 $\pm$ 17.40	52.87 $\pm$ 24.19	123.50 $\pm$ 88.58	0.51 $\pm$ 0.37
Untreated	10	50.50 $\pm$ 13.49	55.36 $\pm$ 44.00	42.80 $\pm$ 17.49*	1.07 $\pm$ 0.37**
Dex	10	30.90 $\pm$ 13.64 Δ	30.78 $\pm$ 9.82 Δ	46.58 $\pm$ 34.69	0.87 $\pm$ 0.64
Biminne	10	18.25 $\pm$ 5.81 Δ	33.15 $\pm$ 15.34 Δ	126.50 $\pm$ 91.38 Δ	0.24 $\pm$ 0.20 $\Delta\Delta$

* $P < 0.05$, ** $P < 0.01$, Δ control group; Δ $P < 0.05$, $\Delta\Delta$ $P < 0.01$, Δ untreated group.

Table 4 Effects of Biminne on the serum corticosterone level of sensitized mice

($\bar{x} \pm s$, ng/L)

Group	<i>n</i>	Corticosterone
Control	10	898.04 $\pm$ 645.58
Untreated	10	506.13 $\pm$ 459.33*
Dex	10	20.64 $\pm$ 8.07 $\Delta\Delta$
Biminne	10	1 144.27 $\pm$ 786.15 Δ

* $P < 0.05$, Δ control group; Δ $P < 0.05$, $\Delta\Delta$ $P < 0.01$, Δ untreated group.

3 Discussion

Airway allergic disease is a chronic inflammatory disease characterized by the eosinophil infiltration. In this study, the lung tissue in the untreated group showed such pathological changes as more eosinophil infiltration, bronchiole smooth muscle thickening, epithelium ablation, and lumens stenosis. Biminne could reduce such inflammatory response in the lungs. It was consistent with the previous results that Biminne could decrease the eosinophils in the peripheral blood and BALF of the sensitized guinea pigs^[9] and relieve the nasal vascular permeability of the sensitized BN rats^[8]. These results suggested that Biminne reduced the airway inflammatory response which played an important role in treating the airway allergic disease.

It is presumed that airway allergic disease is associated with the functional disorder of the immune system, which is characterized as Th1/Th2

response imbalance. The Th2 cell response enhances, while the Th1 cell response lessens. So the ratio of their cytokines changes, especially the ratio of IL-4/INF- γ , which is closely related to the progress of the disease^[11]. The imbalance of Th1/T2 response plays an important role in the whole progress of the airway allergic disease^[12]. In our study we found that Th2 response enhanced and Th1 response lessened in the sensitized mice, which showed a Th2 response priority. After the treatment of Biminne, the Th2 cells and their cytokines such as IL-4 and IL-5 decreased, while the Th1 cells and their cytokine INF- γ increased significantly. So the balance of Th1/Th2 shifted to the normal level. The mechanism of Biminne in regulating the immune response was not like the glucocorticoid drugs, which made general immunosuppression. So it could also avoid the side effects of the overall immunosuppression.

It is well known that glucocorticoids can regulate the immune response and the inflammatory response. It has been suggested in some clinical studies that the asthma patients may show impairment of adrenal function^[1-6]. In the present study we found that the corticosterone level in the untreated group decreased significantly as compared with the control group. It suggested that impairment of adrenal function might be important pathological changes in the process of the airway allergic disease. Glucocorticoids are most effective anti-inflammatory drugs and play an important role

in treating the airway allergic disease. The inhaled glucocorticoids are important in the control of the local inflammatory response, but it cannot modulate the systemic disorder of the endocrine-immune system. In addition, chronic local or systemic use of glucocorticoids may cause many side effects such as the suppression of hypothalamus-pituitary-adrenal axis. The present study showed that the corticosterone level in the Dex group decreased significantly as compared with the control group and the untreated group, suggesting that the adrenal function was seriously suppressed in the Dex group mice. However, the serum corticosterone level in the Biminne group increased significantly as compared with the untreated group. It indicated that Biminne could improve the adrenal function and reversed the disorder of the endocrine-immune system, which may explain the results obtained in the clinical trail that Biminne therapeutic effects might be prolonged as some benefits could still be demonstrated at 12 months.

In conclusion, addition to airway local inflammatory response, the endocrine-immune system disorder also existed in the airway allergic disease. Biminne was an effective alternative approach in treating airway allergic disease. It not only relieved the airway local inflammatory response, but also reversed the imbalance of Th1/Th2 and improved the adrenal function.

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