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The Effect of Tibetan Medicine 15-Flavor Gentian Flower Pills Combined with Budesonide on the Expression of Inflammatory Factors in Asthmatic Mice

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Abstract: *Objective:* To investigate the effects of the Tibetan medicine 15-flavor gentian flower pills combined with budesonide on the expression of inflammatory factors in asthmatic mice. *Methods:* 50 healthy female BALB/c mice of 6–8 weeks were selected and randomly divided into five groups, including the control group, the asthma group, the budesonide group, the 15-flavor gentian flower pill group, and the combination group. The budesonide group was fed with budesonide suspension, the 15-flavor gentian flower pill group was fed with 15-flavor gentian flower pill suspension, and the combined group was fed with budesonide suspension and 15-flavor gentian flower pill suspension. Asthma symptoms of mice in the five groups were recorded. Serum ovalbumin (OVA)-specific antibody levels, the proportion of eosinophils in bronchoalveolar lavage fluid (BALF), and the relative expression of inflammatory factors were compared among the five groups of mice. *Results:* Mice in the asthma group, budesonide group, 15-flavor gentian flower pill group, and combination group presented asthma symptoms. The OVA-specific antibody levels, the proportion of eosinophils, and expression of inflammatory factors (IL-4, IL-33, IL-5, and TNF-α) in the budesonide group, the 15-flavor gentian flower pill group, and the combined group were significantly better than those in the asthma group ($P < 0.05$); the OVA-specific antibody levels, the proportion of eosinophils, and expression of inflammatory factors (IL-4, IL-33, IL-5, and TNF-α) in the combined group were significantly lower than those in the budesonide group and the 15-flavor gentian flower pill group ($P < 0.05$). *Conclusion:* The Tibetan medicine 15-flavor gentian flower pills combined with budesonide can effectively inhibit the expression of inflammatory factors in asthmatic mice.

Keywords: 15-flavor gentian flower pills; Budesonide; Asthma; Mice; Inflammatory factors; Tibetan medicine

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1. Introduction

Asthma patients are mainly characterized by recurrent wheezing, coughing, and other symptoms triggered by viral upper respiratory tract infections, weather changes, etc. Surveys have shown that the incidence of asthma in children in China is about 3.02%, of which 50% of children develop the disease before the age of 3 years. There has been an upward trend in recent years, and the lack of timely intervention can significantly increase the burden on society and the family [1]. Budesonide is a commonly used drug for the treatment of asthma, which can effectively improve airway inflammatory response and inhibit airway remodeling, however, the treatment time is long with low safety. It is of great significance to improve the prognosis of asthma patients by finding safer and more effective treatments. 15-flavor gentian flower pill is composed of white-flowered gentian, eugenol, nutmeg, and other medications, belonging to the classic Tibetan medicine prescription, which is currently believed to have a certain degree of therapeutic efficacy in chronic bronchitis, but the effectiveness of its application in asthma treatment still needs to be analyzed in depth [2]. It is currently believed that a variety of inflammatory factors, such as interleukin (IL)-4 and tumor necrosis factor (TNF)-α, are involved in the occurrence and development of asthma [3]. In this study, we analyzed the effect of Tibetan medicine 15-flavor gentian flower pills combined with budesonide on the expression of inflammatory factors in asthmatic mice, in order to provide a theoretical basis for the treatment of asthma.

2. Materials and methods

2.1. Materials and instruments

50 6–8 weeks BALB/c healthy female mice [Kaisheng Biotechnology (Shanghai) Co., Ltd.] were selected, with a body mass of 19–21 g. Ovalbumin (OVA) was purchased from Wuhan Image Technology Co., Ltd, aluminum hydroxide adjuvant was purchased from Xibao Biotechnology (Shanghai) Co., Ltd, and phosphate buffer solution (PBS) was purchased from Shandong West Asia Chemical Co. OVA solution (1 mg/mL) was mixed with aluminum hydroxide adjuvant (50 mg/ml). Solution A: 5 mg/mL of OVA solution. Solution B: Budesonide suspension (State Pharmaceutical License H20213286) or 15-flavored gentian flower pills (State Pharmaceutical License Z63020233). A reverse transcription kit was purchased from Roche, USA, and IL-4, IL-33, IL-5, and TNF-α monoclonal antibodies were provided by Shanghai Lizu Biotechnology Company. The ABI 7500 polymerase chain reaction (PCR) instrument and high-speed low-temperature centrifuge were purchased from Shanghai Anting Scientific Instrument Factory, and ELISA kits were purchased from Sigma, USA.

2.2. Research methods

50 mice were divided into five groups using the random number method, including the control group, the asthma group, the budesonide group, the 15-flavor gentian flower pill group, and the combination group, with 10 mice in each group. After the mice were acclimatized for 1 week, modeling was carried out, and the mice were sensitized by intraperitoneal injection of solution A at a dose of 100 uL/each on day 1 and day 7, and by nasal drip of 40 uL/dose on day 14 for 10 consecutive days. The mice were fed with the drug 4 hours before the nasal drip, and in the budesonide group, the budesonide suspension (dose of 0.26 mg/kg) was used for the gastric drip. The 15-flavor gentian flower pill group was fed with 200 uL of 15-flavor gentian flower pill suspension (dose of 30 mg/kg), and the combined group was fed with budesonide suspension and 15-flavor gentian flower pill suspension at the same dose. Saline was used to replace solutions A and B in the control group, and PBS was used to replace the drugs in both the control and asthma groups. After starting the modeling, the daily conditions of the mice (fur color, stool, body weight, respiration, etc.) were recorded.

After the last nasal drip, the mice were made to lie supine. After anesthesia, tracheal intubation was carried out, and 0.5 ml of PBS was injected into the lungs of the mice, followed by back suctioning; and the collection of the bronchoalveolar lavage fluid (BALF) was carried out, and the operation was repeated three times. The mice were euthanized within 24 hours of the last nasal drip, blood and lung tissues were preserved, and the lung tissues were placed in liquid nitrogen for rapid freezing and subsequently stored at -80°C for examination.

2.3. Observation indexes

(1) Asthma symptoms of the five groups of mice were recorded.

(2) OVA-specific antibody detection: After centrifugation of blood, serum was taken and the serum OVA-specific antibody levels of the five groups of mice were detected using ELISA.

(3) Cell sorting and counting in BALF: Centrifugation of BALF from five groups of mice was performed for 10 min, with the rotational speed set at 1500 rpm/min, and the total number of cells was recorded under the microscope after taking the precipitate and resuspending it, followed by staining of cell suspensions with smear, and then selecting 200 inflammatory cells under the microscope with oil immersion, and recording the proportion of eosinophils.

(4) Relative expression of inflammatory factors: The expression of IL-4, IL-33, IL-5, and TNF-α mRNA in mouse lung tissue was detected using real-time fluorescence PCR. The extraction of total RNA from mouse lung tissues was carried out using TRIzol reagent, followed by reverse transcription, and PCR was carried out using β-actin as an internal reference to record the relative expression of IL-4, IL-33, IL-5, and TNF-α mRNA.

2.4. Statistical methods

Statistical analysis was performed using SPSS22.0, and the measurement data were analyzed using ANOVA with repeated measurements, expressed as mean ± standard deviation (SD), and intergroup comparisons were performed using the Fisher's Least Significant Difference (LSD) test, with $P < 0.05$ as the difference being significant.

3. Results

3.1. Analysis of asthma symptoms in mice

The mice in the asthma group, budesonide group, 15-flavor gentian flower pill group, and the combined group showed asthma symptoms, including shortness of breath, slowed movement, restlessness, scratching ears and cheeks, sneezing, shortened forelimbs, incontinence, etc. The mice in the control group were in a normal state and did not show the above symptoms.

3.2. Comparison of OVA-specific antibody test results among the five groups

The OVA-specific antibody levels in the asthma group, budesonide group, 15-flavor gentian flower pill group, and combined group were significantly higher than those in the control group ($P < 0.05$). The OVA-specific antibody levels in the budesonide group, the 15-flavor gentian flower pill group, and the combined group were significantly lower than those in the asthma group ($P < 0.05$). The OVA-specific antibody levels in the combination group were significantly lower than those in the budesonide group and the 15-flavor gentian flower pill group ($P < 0.05$). The comparison of OVA-specific antibody levels in the budesonide group and the 15-flavor gentian flower pill group showed no statistically significant difference ($P > 0.05$), as shown in **Table 1**.

Table 1. Comparison of OVA-specific antibody test results among the five groups

Groups	Number of cases	OVA-specific antibody
Control group	10	0.02 ± 0.01
Asthma group	10	0.84 ± 0.24[a]
Budesonide group	10	0.63 ± 0.07[ab]
15-flavor gentian flower pill group	10	0.56 ± 0.17[ab]
Combined group	10	0.29 ± 0.12[abcd]
F	-	37.656
P	-	< 0.001

Note: Compared with the control group, [a]$P < 0.05$; compared with the asthma group, [b]$P < 0.05$; compared with the budesonide group, [c]$P < 0.05$; compared with the 15-flavor gentian flower pill group, [d]$P < 0.05$.

3.3. Comparison of cell counts in BALF of five groups

The comparison of the total number of cells in the five groups showed no statistically significant difference ($P > 0.05$). The proportion of eosinophils in the asthma group, budesonide group, and 15-flavor gentian flower pill group was significantly higher than that in the control group ($P < 0.05$). The proportion of eosinophils in the combined group was significantly lower than that in the asthma group, budesonide group, and 15-flavor gentian flower pill group ($P < 0.05$), and the proportion of eosinophils was not statistically significant when comparing the level of the proportion of eosinophils in the combined group and the control group ($P < 0.05$), as presented in **Table 2**.

Table 2. Comparison of cell counts in BALF of the five groups

Groups	Number of cases	Total number of cells ($\times 10^7$/L)	Proportion of eosinophils (%)
Control group	10	99.75 ± 9.53	4.36 ± 2.21
Asthma group	10	111.22 ± 37.30	10.98 ± 2.73[a]
Budesonide group	10	104.29 ± 12.96	7.21 ± 1.92[ab]
15-flavor gentian flower pill group	10	103.10 ± 11.29	7.03 ± 1.40[ab]
Combined group	10	101.75 ± 12.49	5.05 ± 1.57[bcd]
F	-	0.408	18.126
P	-	0.802	< 0.001

Note: Compared with the control group, [a]$P < 0.05$; compared with the asthma group, [b]$P < 0.05$; compared with the budesonide group, [c]$P < 0.05$; compared with the 15-flavor gentian flower pill group, [d]$P < 0.05$.

3.4. Comparison of the relative expression of IL-4, IL-33, IL-5, and TNF-α in the five groups

The relative expressions of IL-4, IL-33, IL-5, and TNF-α in the control group were significantly lower than those in the asthma group, budesonide group, 15-flavor gentian flower pill group, and combined group ($P < 0.05$). The relative expressions of IL-4, IL-33, IL-5, and TNF-α in the asthma group were significantly higher than those in the budesonide group, the 15-flavor gentian flower pill group, and the combined group ($P < 0.05$). The relative expression of IL-4, IL-33, IL-5, and TNF-α in the combined group was significantly lower than that in the budesonide group and the 15-flavor gentian flower pill group ($P < 0.05$), as shown in **Table 3**.

Table 3. Comparison of relative expression of IL-4, IL-33, IL-5, and TNF-α among five groups

Group	Number of cases	IL-4	IL-33	IL-5	TNF-α
Control group	10	1.96 ± 0.17	0.46 ± 0.13	0.39 ± 0.14	1.04 ± 0.24
Asthma group	10	10.85 ± 2.05[a]	2.35 ± 0.68[a]	1.46 ± 0.32[a]	2.49 ± 0.75[a]
Budesonide group	10	6.54 ± 1.84[ab]	1.47 ± 0.37[ab]	0.87 ± 0.22[ab]	1.97 ± 0.66[ab]
15-flavor gentian flower pill group	10	6.25 ± 1.73[ab]	1.38 ± 0.40[ab]	0.93 ± 0.24[ab]	1.86 ± 0.57[ab]
Combined group	10	3.89 ± 1.05[abcd]	0.73 ± 0.19[abcd]	0.65 ± 0.16[abcd]	1.23 ± 0.36[abcd]
F	-	11.341	6.348	5.259	4.369
P	-	< 0.001	< 0.001	< 0.001	0.001

Note: Compared with the control group, [a]$P < 0.05$; compared with the asthma group, [b]$P < 0.05$; compared with the budesonide group, [c]$P < 0.05$; compared with the 15-flavor gentian flower pill group, [d]$P < 0.05$.

4. Discussion

Asthma is mainly characterized by airway hyperresponsiveness and its mechanism of occurrence is more complex. It is mostly considered to be a chronic airway inflammatory disease caused by immunity, genetics, environment, and other factors [4]. It is currently believed that repeated acute asthma attacks can aggravate lung function damage, and early and reasonable intervention can help to control the patient's condition and reduce recurrence [5]. Hormonal drugs such as budesonide are mostly used in the clinic to reduce airway hyperresponsiveness, relieve bronchospasm, and improve lung function, but the efficacy is more limited. 15-flavor gentian flower pill belongs to the commonly used Tibetan medicines, which have the effects of strengthening the body's foundation, clearing heat, and moisturizing the lungs. Clarifying the effects of the 15-flavor gentian flower pill combined with budesonide on the expression of inflammatory factors in asthmatic mice helps to provide more theoretical support for the clinical application of the 15-flavor gentian flower pills.

Asthma is a disease mediated by specific antibodies, and the high level of specific antibodies suggests that Th2 activity is enhanced, Th1 is weakened, and the immune response is in a hyperactive state, which is the key to the development of asthma [6]. Eosinophils can contribute to airway mucus secretion and aggravate airway epithelial damage by participating in processes such as the synthesis of inflammatory factors, and it is currently believed that eosinophils are significantly correlated with the severity of airway inflammation in the body [7]. IL-4, IL-33, IL-5, and TNF-α are all significantly correlated with the onset and progression of asthma, of which IL-4 can participate in the proliferation and transformation of B cells and impede eosinophil apoptosis [8]; IL-5 can promote the aggregation of eosinophils [9]; IL-33 is closely related to the development of airway inflammation and can promote airway remodeling in asthmatic mice [10]; and TNF-α can be expressed in eosinophils, macrophages, and smooth muscle cells, and is significantly correlated with the severity of airway inflammation [11].

In this study, we found that the level of OVA-specific antibodies, the proportion of eosinophils, and the expression of inflammatory factors were significantly increased in the BALF of mice in the asthma group compared with the control group, suggesting that there was a significant inflammatory response in asthmatic mice. Among them, the OVA-specific antibody level, eosinophil proportion, IL-4, IL-33, IL-5, and TNF-α levels in the combined group were significantly better than those in the budesonide group and the 15-flavor gentian flower pill group. It is suggested that the combination may further contribute to the decrease of inflammatory response in asthmatic mice compared with budesonide and 15-flavor gentian flower pill treatment alone. This may be due to the fact that budesonide may reduce the release of inflammatory factors and inhibit collagen

deposition in the airways by improving vascular permeability, which in turn promotes the improvement of patients' conditions [12]. In 15-flavor Gentian Flower Pills, white-flowered gentian and yuganzi have the effects of moistening the lungs, generating fluids, benefiting the throat, and resolving phlegm. Mullein, sedum, and broad-skinned vine have the effects of relieving pain, reducing adverse reactions, dispersing cold, calming asthma, dispelling wind, and regulating qi. Additionally, other ingredients such as broad jujube help to clear away heat, activate the blood, and clear the lungs while nourishing the blood [13,14]. Modern pharmacological studies have shown that 15-flavor gentian flower pills can inhibit the development of lung lesions, enhance phagocytic activity, improve body immunity, remove toxins, and reduce airway inflammatory damage [15]. The combination of the two can achieve a complementary effect from different mechanisms, which in turn leads to a further decrease in the expression of inflammatory factors in asthmatic mice.

5. Conclusion

In conclusion, the combination of 15-flavor gentian flower pills and budesonide can effectively inhibit the expression of inflammatory factors in asthmatic mice, but its clinical application still needs to be analyzed in depth.

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Disclosure statement

The authors declare no conflict of interest.

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