

## The influence of Astragli on the interleukin, IFN- $\gamma$ and Th1Th2 balance in rats with airway inflammation.

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### Abstract

**Objective:** To explore the influence of Astragli on the interleukin, IFN- $\gamma$  and Th1Th2 balance in rats with airway inflammation.

**Methods:** The blank group, model group, Astragli group, Angelica group and compatibility group were relatively set and the method of drug gavage treatment of rats with allergic inflammation was used. Inflammatory model of rats was induced by ovalbumin spray method and interleukin, IFN- $\gamma$  and Th1Th2 balance were observed in serum, lung tissue and nasal mucosa of rats.

**Results:** IFN- $\gamma$  level decreased in the lung tissue, nasal mucosa and serum of rats in the model group, while interleukin increased, and related indicators of lung tissue and nasal mucosa had a certain relevance; single Astragli had the function of reducing IFN- $\gamma$  level of nasal mucosa and serum as well as reducing interleukin in serum and lung tissue; single Angelica had the function of decreasing interleukin in the nasal mucosa, serum and lung tissue. The compatibility of Astragli and Angelica could elevate IFN- $\gamma$  level in the serum and lung tissue as well as reduce interleukin in the nasal mucosa, serum and lung tissues.

**Conclusion:** The compatibility of Astragli and Angelica plays a regulatory role in balancing the two cytokines Th1 and Th2 in the body, functioning in suppressing allergic airway inflammation.

**Keywords:** Angelica, *Astragalus*, Airway inflammation.

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### Introduction

The allergic airway inflammation in the clinic often presents as Bronchial asthma (BR) and Allergic Rhinitis (AR), which is a type I allergic reaction, and means a kind of inflammation reaction due to the allergen inducing [1]. Allergic airway inflammation is formed because of the broken balance of Th1Th2, and Th1Th2 respectively refers to types 1 and 2 of helper T cells [2]. And Huang et al. [3] reported that the TT genotypes of the *IL-4* gene at the -33 and -589 loci were associated with bronchial asthma susceptibility in children. Polymorphisms in *IL-4* were associated with allergic rhinitis and could change the clinical picture of the disease in patients [4]. In addition, a recent study suggested that [5] the serum level of *IL-2* and *IL-4* could be perceived as a marker of severe bronchial asthma. Related research showed that the signal transducing system was involved in the process of PM2.5 promoting airway inflammation in rats which played the key role on the occurrence of the disease [6]. Angelica and Astragli are two drugs frequently used in allergic diseases in clinic. *Astragalus* polysaccharide (APS) the main active ingredient in Astragli, which could effect on the Toll-like receptor 4 (TLR4) of B cell and macrophage membrane, and has many functions such as immunoregulation [7]. Shao et al. [7] reported for the first time that APS directly interacted with TLR4 molecule. But the influence of Astragli on the interleukin, IFN- $\gamma$  and

Th1Th2 balance in airway inflammation is not clear. This paper studied the influence of Astragli on the interleukin, IFN- $\gamma$  and Th1Th2 balance in rats with airway inflammation, and conducted corresponding reports.

### Materials and Methods

#### *Experimental grouping*

50 clean grade and healthy Sprague-Dawley (SD) rats were chosen with body weight  $120 \pm 10$  g and gender of male. They were randomly divided into 5 groups, respectively as the blank group, model group, Astragli group, Angelica group and the compatibility group. This research was approved by the Animal Ethical Committee of medical college of Qinghai university according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985).

#### *Experimental pharmacy*

Aluminium Hydroxide Gel (sigma Co.), OVA (V level, sigma Co.), EnVision reagent (Dako, Denmark), interleukin-4 Kit (Dr. De biological engineering company in Wuhan), IFN- $\gamma$  kit (Invitrogen), a resistance (America, Cruz, Santa), Astragli, Leguminosae Mongolia, Huangqi, batch number: 091211,

origin, Gansu, angelica, Umbelliferae, batch number: 091128, origin: Gansu.

### Main instrument

Microplate reader: BIO-TEK ELX80. IMS cell image analysis software (Shen Teng (Shanghai) Information Technology Co., Ltd.). 402A ultrasonic atomizer, Yuyue medical equipment (Jiangsu) Co., Ltd.

### Modeling method

**(1) Sensitization:** Except the blank group, the other groups were injected with Aluminium Hydroxide Gel (fresh configuration, concentration of 1 mg/ml). 10 points were chosen from each rat for injection, respectively as: both sides of the groin, plantar, neck, waist and back. The injection amount of each point was 0.05 ml, in addition, intraperitoneal injection was 0.5 ml, and injection time was d 1 and d 8 of the experiment.

**(2) Excitation:** The rats were placed in the atomizer with atomization quantity of 0.8 ml/min, 1 times daily, and atomization time of 15 min. Moreover, the atomization of blank group was normal saline, and the other group was 1% OVA excitation liquid.

### Method of administration

The water decoction of Angelica and Astragali were dosed and the compatibility ratio of the two drugs was 1:1 in compatibility group with 1 times a day according to the dosage of every 10 g (crude drug)/Kg (weight), and gavage for medication method. Saline water was used in blank group.

### Material handling

After the last excitation, the rats were anesthetized (25% urethane), and respectively collected aorta blood 10 ml, lung tissue and nasal mucosa which were put and saved in 10% formaldehyde solution.

### Immunohistochemical method

Refer to DoKa (Denmark) and operating instructions of Envision system (immunohistochemical Kit). Specific methods referred to the literature [8].

### Statistical analysis

EXCEL software and SPSS18.0 were selected for survey data arrangement and statistical analysis, and t test and  $\chi^2$  test for comparative analysis of measurement data and count data. The method for data expression was based on the convenience and when  $P < 0.05$ , a statistically significant difference was believed.

## Results

### Comparison analysis of different drugs' influence on interleukin and IFN- $\gamma$ in nasal mucosa

Comparing the model group and other groups, there was no significant difference in the expression of IFN- $\gamma$  in nasal mucosa except Astragali group with no statistical significance ( $P > 0.05$ ); compared to model group, the expression of interleukin in nasal mucosa of blank group was significantly reduced with statistical significance ( $P < 0.05$ ); comparing with the model group, ratio of IFN- $\gamma$ /IL-4 and the expression in blank group increased significantly with statistical significance ( $P < 0.05$ , Table 1).

**Table 1.** Comparison analysis of different drugs' influence on interleukin and IFN- $\gamma$  in nasal mucosa.

Group	IFN- $\gamma$	IL-4	IFN- $\gamma$ /IL-4
Blank group	828.38 $\pm$ 93.20	973.13 $\pm$ 81.94	0.85 $\pm$ 0.12
Model group	709.28 $\pm$ 124.61	1424.38 $\pm$ 248.21	0.50 $\pm$ 0.07
Astragali group	838.87 $\pm$ 86.38*	1333.40 $\pm$ 159.30	0.63 $\pm$ 0.10
Angelica group	808.56 $\pm$ 158.41	1022.28 $\pm$ 130.89*	0.80 $\pm$ 0.21*
Compatibility group	785.85 $\pm$ 100.45	906.06 $\pm$ 59.01*	0.86 $\pm$ 0.15*

Note: Comparing with blank group  $P < 0.05$ , comparing with control group  $P < 0.05$ , and the same below.

### Comparison analysis of different drugs' influence on interleukin and IFN- $\gamma$ in lung tissue

Comparing with model group, the expression of IFN- $\gamma$  in blank group increased with statistically significant ( $P < 0.05$ ); there was significant difference in the expression of IFN- $\gamma$  between compatibility group and model group with statistically significant ( $P < 0.05$ ); comparing with model group, the expression of interleukin in lung tissue of other groups was significantly decreased with statistical significance ( $P < 0.05$ ); ratio of IFN- $\gamma$  and interleukin in lung tissue of the blank group was significantly higher than that of control group with statistical significance ( $P < 0.05$ , Table 2).

**Table 2.** Comparison analysis of different drugs' influence on interleukin and IFN- $\gamma$  in lung tissue.

Group	INF- $\gamma$	IL-4	INF- $\gamma$ /IL-4
Blank group	980.67 $\pm$ 132.08	1124.62 $\pm$ 68.66	0.87 $\pm$ 0.13
Model group	801.41 $\pm$ 84.88	2306.76 $\pm$ 456.74	0.35 $\pm$ 0.09
Astragali group	905.66 $\pm$ 149.15	1914.80 $\pm$ 310.04*	0.49 $\pm$ 0.06*
Angelica group	795.13 $\pm$ 126.30	1939.07 $\pm$ 547.59*	0.43 $\pm$ 0.13*

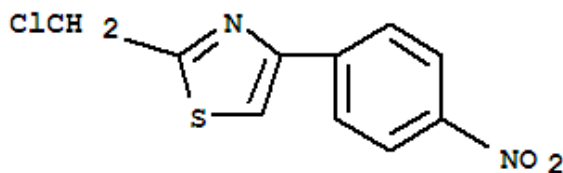
Compatibility group	925.78 $\pm$ 69.80*	1314.87 $\pm$ 74.53*	0.70 $\pm$ 0.06*
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**Comparison analysis of different drugs' influence on interleukin and IFN- $\gamma$  in serum**

Comparing with model group, the expression of IFN- $\gamma$  in serum of blank group increased with statistically significant ( $P < 0.05$ ); there was significant difference in the expressions of IFN- $\gamma$  between compatibility group and model group with statistically significant ( $P < 0.05$ ); comparing with model group, the expression of interleukin in serum of other groups was significantly decreased with statistical significance ( $P < 0.05$ ); ratio of IFN- $\gamma$  and interleukin in serum of the blank group was significantly higher than that of control group with statistical significance ( $P < 0.05$ , Table 3).

**Table 3.** Comparison analysis of different drugs' influence on interleukin and IFN- $\gamma$  in serum.

Group	IFN- $\gamma$	IL-4	IFN- $\gamma$ /IL-4
Blank group	81.74 $\pm$ 15.85	122.16 $\pm$ 46.54	0.75 $\pm$ 0.33
Model group	64.45 $\pm$ 6.16	284.44 $\pm$ 54.72	0.23 $\pm$ 0.05
Astragli group	87.07 $\pm$ 22.25	159.50 $\pm$ 48.30*	0.60 $\pm$ 0.28*
Angelica group	72.37 $\pm$ 12.42	229.53 $\pm$ 26.27*	0.31 $\pm$ 0.04*
Compatibility group	86.47 $\pm$ 17.43*	144.30 $\pm$ 75.65*	0.72 $\pm$ 0.30*



**Figure 1.** Chemical structure of Astragalus polysaccharides.

**Discussion**

APS is the main active ingredient in Astragli and has been widely used in the clinical treatment of diseases of the allergic airway inflammation because of its extensive biological activities, such as anti-inflammatory [9], anti-carcinogenic [10], anti-virus [11] and immunomodulatory [12]. And the chemical structure of it was shown in Figure 1. Banche et al. [13] found that APS could effectively correct the imbalance of Th1/Th2 cells and reduce inflammatory infiltration in animal models of asthma. Recent study showed that intraperitoneal injection of APS could reduce the over expression of tumor necrosis factor (TNF)- $\alpha$  and IL-1 $\beta$  during experimental colitis induced by haptens [14]. And APS could enhance T lymphocyte transformation and activation of B lymphocytes and dendritic cells [15,16].

Th1 and Th2 are two kinds of cells playing different immune roles [17]. Th1 cells mainly reflects the cell immune, and when

Th1 cells of the body have advantage, the body mainly presents proinflammatory, and clears various pathogens, which has obvious effect on the inhibition of infection in the body. Thus, it can build up chronic infection or improve the development of disease [18,19]. Th2 cells mainly reflect the humoral immunity, and when Th1 cells have advantage in the body, the body mainly presents anti-inflammatory. Thus, it can produce a variety of pathological product, as well as generally lead to various pathological changes [20,21].

In allergic airway inflammation, the balance of two kinds of cells as Th1 and Th2 moves toward the direction of the latter [22]. Only when the two kinds of cells as Th1 and Th2 are in a dynamic equilibrium state, can the body is able to show a healthy state. In addition, balance regulation on the two kinds of cells as Th1 and Th2 is also seen as an important aspect of body's Yin and Yang balance regulation [23,24].

China's traditional Chinese medicine believes that blood is the mother of Qi, and Qi is the commander of blood [25]. The principle for compatibility of Astragli and Angelica is blood and Qi simultaneous treatment, which is often used in clinic [26]. Not only can Astragli and Angelica be used as a prescription, but they also can be used as a kind of basic couplet medicines for combination use with other drugs, so the couplet medicines have received much attention from many doctors [27]. In addition, Astragli and Angelica are often used in the treatment of allergic airway diseases [28]. Relative research showed that Astragli could activate the body's T cells and significantly activate and enhance the activation of lymphokine killer cell, the possible reason of which might be it could associate with the function of body related tonifying Qi [29]. Astragli can regulate the balance between the two kinds of cell factors as Th1 and Th2, improving the ventilation function of patients with asthma, and further reducing airway hyperresponsiveness.

This study showed that the IFN- $\gamma$  in lung tissue, nasal mucosa and serum of rats in model group decreased, while interleukin increased, and related indicators of lung tissue and nasal mucosa had a certain relevance; single Astragli could reduce IFN- $\gamma$  in nasal mucosa and serum as well as interleukin in lung tissue and serum; single Angelica could reduce interleukin in nasal mucosa, serum and lung tissues; Astragli and Angelica compatibility had the function of elevating IFN- $\gamma$  in lung tissue and serum as well as reducing interleukin in nasal mucosa, serum and lung tissues.

In summary, Astragli and Angelica compatibility can play a role in regulating the balance on the two kinds of cell factors as Th1 and Th2 in the body, so that the balance moves toward the direction of Th1, then functioning in the inhibition of airway allergic inflammation.

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