

# Study on the Effects of Radix Bupleuri Dampness-Percolating Decoction on Serum IL-5 & IL-13 in Rat Asthma Model

YAN Yun-xia<sup>1</sup>, WANG Yan-qing<sup>2△</sup>, ZHOU Zhao-shan<sup>2</sup>, GUO Gui-ming<sup>2</sup>  
(1. Qingdao University Medical College Qingdao, shandong, 266021, China,  
2. Qingdao Haici Medical Group, Qingdao ,Shandong, 266033, China)

**ABSTRACT Objective:** To investigate the effects of radix bupleuri dampness-percolating decoction on the IL-5 and IL-13 in the rat asthma model serum and its function mechanism of asthma treatment. **Methods:** Eighty-four healthy male Wistar rats were divided by random number method into normal control group, model control group, dexamethasone group, dingchuan decoction group, low dosage radix bupleuri dampness-percolating decoction group, medium dosage radix bupleuri dampness-percolating decoction group and high dosage radix bupleuri dampness-percolating decoction group, with 12 in each group. Then the model of rat asthma was made with ovalbumin and intervened by relevant medicine. The IL-5 and IL-13 in rat serum from each group were tested by enzyme-linked immunosorbent assay (ELISA); experimental data were analyzed by SPSS11.5 statistical software. **Results:** Mortality ratio of each group after experiment had no difference,  $P>0.05$ . There existed significant difference between the model control group and the normal control group in terms of serum IL-5 and IL-13 content, which indicated a pathological state that serum IL-5 and IL-13 in rat asthma model abnormally increased. Compared with model control group, IL-5 and IL-13 in treatment groups' serum obviously decreased, especially to the dexamethasone group, medium dosage radix bupleuri dampness-percolating decoction group and high dosage radix bupleuri dampness-percolating decoction group. **Conclusion:** The function mechanism of radix bupleuri dampness-percolating decoction for asthma treatment is related to reduction of the IL-5 and IL-13 level. A certain dose-response relationship existed in the said effect, but the effect does not become stronger with higher dosage.

**Key word:** Asthma; Radix bupleuri dampness-percolating decoction; Rat; IL-5; IL-13

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## Introduction

Bronchial asthma (asthma for short) is a chronic airway anaphylactic inflammatory disease. It is estimated that there are 300,000,000 asthma sufferers in the world. Prevalence rate and mortality rate of asthma is increasing year by year <sup>[1]</sup>. In addition, the economical burden caused by this disease in the world exceeds that brought by tuberculosis, HIV infection and Aids in total <sup>[2]</sup>, which has made asthma a globally serious public hygiene issue. Professor Zhou ZS, based on years of clinic experience, concluded that lesser yang, stagnation, fire-transmission and internal stagnation of fluid-dampness were the important pathogenesis of hot-type asthma, which should be treated through reconciliation and diuresis promotion. The radix bupleuri dampness-percolating decoction, invented under the direction of reconciliation and diuresis promotion method, has quick and high therapeutic effects on hot-type asthma. This study investigates the possible mechanism of radix bupleuri dampness-percolating decoction for preventing and curing asthma from cellular elements, which provides modern scientific grounds for further promotion and application of this

therapy.

## 1 Materials and Methods

### 1.1 Experimental animals and grouping

Eight-four clean and healthy male Wistar rats of 2-3 months, weighing  $200\pm 20$ g, were bought from Shandong Lukang Pharmaceutical Group, Ltd and had a license number as SCXK Lu 20110017. After keeping them, six in each cage, for a week with normal feed in natural light at room temperature of  $18\sim 22^{\circ}\text{C}$  and humidity of 28%, 84 male Wistar rats were divided by random number method into normal control group, model control group, dexamethasone group, dingchuan decoction group, low dosage radix bupleuri dampness-percolating decoction group, medium dosage radix bupleuri dampness-percolating decoction group and high dosage radix bupleuri dampness-percolating decoction group, with 12 in each group.

### 1.2 Experimental pharmaceuticals

Ovalbumin (OVA) freeze drying powder, 10g per ampoule, produced by Sigma Corporation, Grade II ); Dexamethasone tablets, produced by Tianjin Tian Yao Pharmaceutical Co., Ltd, was grounded and dissolved in normal saline to make 0.033mg/ml sterilizing liquid; Dingchuang decoction contained 9g ginkgo seed, 10g ephedra, 10g tussilago, 12g pinellia ternate, 10g white mulberry rootbark, 6g perilla fruit, 6g scutellaria baicalensis, 3g liquorice and 10g almond; Radix bupleuri dampness-percolating decoction contained 12g radix bupleduri, 15g scutellaria baicalensis, 12g

Author introduction: YAN Yun-xia (1981-), female, master, Mainly engaged in Respiratory Medicine research

E-mail: yanyunxia2009@126.com

△Corresponding author: WANG Yan-qing, Tel:13370857961,

E-mail: yanyx2009@126.com

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pinellia ternate, 15g dangshen, 10g ginger, 10g Chinese-date, 6g liquorice, 30g plaster, 30g coix seed, 15g pyrrosia lingua, 15g plantain, 10g poria cocos, and 15g thunberg fritillary bulb. Traditional Chinese prescription was made into sealed concentrated sterilizing preparation by TCM preparation room of Qingdao Haici Medical Group. Crude drug content of the dingchuang decoction was 0.5g/ml while that of the low, medium and high dosage radix bupleuri dampness-percolating decoction control groups were respectively 0.66g/ml, 1.320g /ml, 2.64g /ml.

### 1.3 Reagents and instruments

Rat IL-5 ELISA kit, produced by Wuhan Boster Company; Rat IL-13 ELISA kit, also produced by Wuhan Boster Company; Bsl10 electronic balance from Sartorius Company; Atomizer made by respiratory department of Qingdao Haici Medical Group; Ultra-sonic atomizer, produced by Jiangsu Yuyue Medical Equipment Co., Ltd while distributed by Shanghai Yuyue Medical Equipment Co., Ltd, with the product standard code Q / 3211812YQ007-2000; Low temperature refrigerator, made in Qingdao, Shandong, China; Water-proof electro-heating standing-temperature cultivator of PYX-DHS-X model, produced by Shanghai Yuejin Medical Instrument Factory; ELIASA of Multi skan Ascent V 1.24 345-0063T model, produced by Zhejiang Analysis Instrument Factory.

### 1.4 Animal model preparation

Sensitization stage: inject 1mL 10% ovalbumin normal saline into the abdominal cavity of asthma model group and the treatment groups twice on the first, eighth and fifteenth day of the experiment respectively. Activation stage: one week after the third sensitization, make the model group and the treatment groups to inhale 1% ovalbumin normal saline for about 20 minutes every day. As a result, asthma exacerbation was induced to happen until there appeared in rats the syndromes of dysphoria, sneezing, coughing, atemnot and asthma. What is even worse, the rats would suffer from gatism, scratching their ears and cyanosis, etc. Only after the appearance of all the syndromes could we say the rat model was successfully prepared. 21 days were needed to activate them. As for the normal group animals, inhale normal saline instead of antigen for sensitization and activation.

### 1.5 Medication method and administration

Each group was given the medicine from the 15th day of the activation stage and the dosage was calculated according to the conversion factor of per kilogram rats to per kilogram human body weight. Dexamethasone group were lavaged with 0.5mg dexamethasone tablets per kilogram body weight per day for 7 consecutive days. Dingchuang decoction group were lavaged with 1.27g crude drug per kilogram body weight per day for 7 consecutive days. Low, medium and high dosage radix bupleuri dampness-percolating decoction groups were lavaged with 10.15g, 20.31g and 40.62g crude drug respectively per kilogram body weight per day

for 7 consecutive days. The normal control group and model control group were lavaged with normal saline for 7 consecutive days.

### 1.6 Specimen collection and testing method

Inject the abdominal cavity of anesthetized animal with 10% chloral hydrate (10ml/kg) after 24 hours since the last activation. Then follow the procedures of fixing, dissecting the thoracic cavity, taking 4ml blood sample and keeping it at temperature of 20 degrees below zero. Use ELISA to measure the IL-5 and IL-13 contents respectively in the serum in accordance to the instructions.

### 1.7 Statistical analysis

Statistical analyses were conducted using SPSS 11. 5. Results, expressed as average  $\pm$  standard deviation ( $\bar{x} \pm s$ ), was taken for normality test and homogeneity of variance test first. Data accorded with normal distribution and homogeneity of variance was analyzed using one-way ANOVA. LSD method was adopted for multiple comparisons; while data that was not normally distributed was subjected to repeated rank sum test.  $P < 0.05$  was taken as significant difference.

## 2 Results

### 2.1 Comparison of rat livability after experiment

Mortality of animals in the experiment: two died in the activation process in model control group; one died of atemnot in the activation process in low dosage radix bupleuri dampness-percolating decoction group and the same was to the high dosage group; five rats died in the high dosage radix bupleuri dampness-percolating decoction group, one of which died of atemnot in the activation process and the others died in administration process (excluding lavage period). Livability ratio of the normal control group, dexamethasone group and dingchuang decoction group was 100%, while that of the low dosage radix bupleuri dampness-percolating decoction group, medium dosage radix bupleuri dampness-percolating decoction group, model control group and high dosage radix bupleuri dampness-percolating decoction group was respectively 91.67%, 91.67%, 83.33% and 58.33% in descending order. After statistical analysis, mortality of each group has no difference,  $p > 0.05$ , as shown in table 1.

### 2.2 Comparison of IL-5 content in the serum of each group

The following experimental results of IL-5 could be concluded from Table 2. First, there existed difference between normal group and asthma model control group in terms of IL-5 content, with the latter obviously higher than that of the former, which indicated a pathological state that IL-5 in the asthma model group serum abnormally increased. The difference had statistical significance. Second, in comparison with model control group, IL-5 content in every treatment group serum all decreased and the difference had statistical significance. Third, IL-5 content of low dosage radix bupleuri dampness-percolating decoction group was higher

than that in dexamethasone group, medium dosage radix bupleuri dampness-percolating decoction group and high dosage radix bupleuri dampness-percolating decoction group. The difference had statistic significance. However, IL-5 content showed no difference between low dosage radix bupleuri dampness-percolating decoc-

tion and dingchuang decoction group. Fourth, difference between medium dosage radix bupleuri dampness-percolating decoction group, high dosage radix bupleuri dampness-percolating decoction group and dingchuang decoction group had no statistical significance.

Table 1 Comparison of rat livability

Group	Animal number before experiment	Animal number after experiment	Llivability(%)
Normal control group	12	12	100%
Model control group	12	10	83.33%
Dexamethasone group	12	12	100%
Dingchuang decoction group	12	12	100%
Low dosage radix bupleuri dampness-percolating decoction group	12	11	91.67%
Medium dosage radix bupleuri dampness-percolating decoction group	12	11	91.67%
High dosage radix bupleuri dampness-percolating decoction group	12	7	58.33%

Note:After non-parametric test of several independent samples, X2=6.000 and P=0.423 was concluded, which meant mortality of every group had no significant difference. ◆stands for the existence of significant difference compared with normal control group; ● for the existence of significant difference compared with asthma model control group; ■ for the existence of significant difference compared with dexamethasone group; ▲ for the existence of significant difference compared with dingchuang decoction group; ★ for the existence of significant difference compared with low dosage radix bupleuri dampness-percolating decoction group; ▼ for the existence of significant difference compared with medium dosage radix bupleuri dampness-percolating decoction group; ○ for the existence of significant difference compared with high dosage radix bupleuri dampness-percolating decoction group.

Table 2 Comparison of IL-5 content in the serum of each group

Group	IL-5(pg/ml)
Normal control group	20.45± 1.58◆ ■ ▲★▼○
Model control group	47.80± 1.79◆■ ▲★▼○
Dexamethasone group	24.45± 1.66◆● ▲★
Dingchuang decoction group	38.19± 0.95◆● ■ ▼○
Low dosage radix bupleuri dampness-percolating decoction group	38.12± 1.65◆● ■ ▼○
Medium dosage radix bupleuri dampness-percolating decoction group	24.64± 1.95◆● ▲★
High dosage radix bupleuri dampness-percolating decoction group	24.55± 1.25◆● ▲★▼

Note: ◆stands for the existence of significant difference compared with normal control group; ● for the existence of significant difference compared with asthma model control group; ■ for the existence of significant difference compared with dexamethasone group; ▲ for the existence of significant difference compared with dingchuang decoction group; ★ for the existence of significant difference compared with low dosage radix bupleuri dampness-percolating decoction group; ▼ for the existence of significant difference compared with medium dosage radix bupleuri dampness-percolating decoction group; ○ for the existence of significant difference compared with high dosage radix bupleuri dampness-percolating decoction group.

2.3 Comparison of IL-13 content in the serum of each group

The following experimental results of IL-13 could be concluded from Table 3. First, there existed difference between normal group and asthma model control group in terms of IL-13 content, with the latter obviously higher than that of the former. The difference had statistical significance and indicated the existence of pathological state that IL-13 in the asthma model group serum abnormally increased. Second, in comparison with model control

group, IL-13 content in every treatment group serum all decreased and the difference had statistical significance. Third, IL-13 content of low dosage radix bupleuri dampness-percolating decoction group was higher than that in dexamethasone group, medium dosage radix bupleuri dampness-percolating decoction group and high dosage radix bupleuri dampness-percolating decoction group. The difference had statistical significance. However, IL-5 content showed no difference between low dosage radix bupleuri dampness-percolating decoction and dingchuang decoction group.

Fourth, difference between medium dosage radix bupleuri dampness-percolating decoction group, high dosage radix bupleuri dampness-percolating decoction group and dingchuang decoction group had no statistical significance.

Table 3 Comparison of IL-13 content in the serum of each group

Group	IL-13(pg/ml)
Normal control group	13.42± 1.69● ■ ▲★○
Model control group	41.19± 2.00◆■ ▲★○
Dexamethasone group	24.32± 1.48◆● ▲★
Dingchuang decoction group	34.05± 2.25◆● ■ ○
Low dosage radix bupleuri dampness-percolating decoction group	32.57± 2.03◆● ■ ○
Medium dosage radix bupleuri dampness-percolating decoction group	24.89± 2.30◆● ▲★
High dosage radix bupleuri dampness-percolating decoction group	22.95± 1.14◆● ▲★

Note: ◆stands for the existence of significant difference compared with normal control group; ● for the existence of significant difference compared with asthma model control group; ■ for the existence of significant difference compared with dexamethasone group; ▲ for the existence of significant difference compared with dingchuang decoction group; ★ for the existence of significant difference compared with low dosage radix bupleuri dampness-percolating decoction group; ▼ for the existence of significant difference compared with medium dosage radix bupleuri dampness-percolating decoction group; ○ for the existence of significant difference compared with high dosage radix bupleuri dampness-percolating decoction group.

3 Discussion

Asthma is a paroxysmal disease and has time rhythm. It can attack in spring, summer, autumn, winter or the season change period and this regulation can be referred to as season rhythm. Asthma attack process also has circadian rhythm: it attacks more seriously in the night than in the daytime and even only in the night. As stated in Lishi <sup>[3]</sup> investigation report, the occurrence and aggravation of asthma are regular, which tend to be more serious in the night than in the day, in the first half night than the second half night. This regulation is in accordance with the description in Treaties on Febrile Damage that less Yang is likely to be alleviated from Yin (which means 3 to 5 a.m.) to Chen (which means 7 to 9 a.m.)<sup>[4]</sup>. Chen Xiuyuan explained that less Yang energy accumulates during Yin and Mao (which means from 3 to 7 a.m.) and dissipates till Chen when Yang Qi is extremely strong and can surpass pathogenic factors. The period from Yin to Chen means that from 3 to 9 in the morning, during which less Yang energy is at its most intense. Chole, the organ of clean and healthy energy, when offended by the pathogenic factors, will fight against the pathogenic factors, thus causing the hot-type asthma sufferers to cough and feel chest distress. That's the reason why attach of asthma occurs more from Yin to Chen, especially from 3 in the early morning to 9 in the morning<sup>[5]</sup>.

Season and circadian rhythms of asthma prove that this disease occurs and disappears rather regularly, which is absolutely in accordance to the explanation of less Yang pathogenesis mechanism in Treaties on Febrile Damage that the pathogenic factors invades and struggles with the healthy energy when flood pulse becomes weak and Yang qi less and the grain of the skin and the texture of the subcutaneous flesh opens; this happens regularly and can be cured by decoction of minor bupleurum.

Therapeutic method should be based on the overall analysis of the illness and the patient's condition, while the prescription and treatment should be made following the therapeutic method. Radix Bupleuri dampness-percolating decoction selects medicine strictly under the guidance of this rule of prescription. This medicine mainly contains minor bupleuri decoction which can remove pressed heat of lessYang, recuperate liver and gall qi, reset the cardinal function of less Yang so as to eliminate the original cause of hot-type asthma. It also contains dampness-percolating medicine of which the function is to promote urine and clear away heat. The application of poria cocos, semen coicis, plantain and herba pyrosiae can induce diuresis, clear away the lung-heat and thus disperse the heat pathogenic factors with urine. The prescription of radix bupleuri dampness-percolating decoction, which absorbed the ancestors' achievements of asthma control, used the plaster and liliaceae to clear away the lung-heat and reduce phlegm. The plaster, with acrid-sweet flavor and cold character, can remove the external skin heat and internal lung channel heat. The liliaceae, bitter and cold, can clear away heat, reduce phlegm and activate the lung qi, which has proven to be very effective to cure hot-type asthma. The prescription has characteristics of combination of cold and warm contents, internal heat clearance and external heat removal, reinforcement and elimination, as well as interdependence of ascending and descending, which can not only alleviate the symptoms of asthma but eliminate the root permanently. The hot-type asthma will be successfully cured when dispersion and descending of lung qi becomes normal the liquid metabolism recovers.

Research in recent years has shown that among different fac-



tors, unbalance of T lymphocyte subsets (Th1/Th2) is the vital cause of asthma. Under normal circumstances, the Th1 cell produces interleukin-2, interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , tumor necrosis factor- $\beta$ , which can activate macrophage and cause delayed type hypersensitivity; the Th2 cell produces cell factors including IL-4, IL-5, IL-6, IL-10, IL-13, which will induce generation and accumulation of EOS, cause transformation of immunoglobulin subtype and produce IgE and so on<sup>[6-10]</sup>. EOS is the key effector cell to cause airway allergic inflammation<sup>[11-12]</sup>.

The chief function of IL-5, which has strong chemotaxis effect on EOS, is to enhance the adhesion capability of eosinophils (EOS) to blood vessel endothelial cells. IL-5 can cause EOS to differentiate and extend its survival time. Besides, IL-5 has distinct invigorating effect on the EOS toxic proteins release and the cytotoxic function of itself, thus reinforcing the effect of EOS on asthma airway inflammation<sup>[13-16]</sup>.

IL-13 is excreted by TH2 subsets of the CD4T cell and belongs to TH2 excretory cell factors<sup>[17-20]</sup> like IL-13 与 IL-4, IL-5, IL-9 and IL-10. Some research believed IL-13 caused EOS to selectively accumulate on mucosa of asthma bronchus through adding VCAM-1 to the endothelial cell of blood vessel. Research by Kumar certified<sup>[21]</sup> that IL-13 played a very important part in occurrence and development of bronchus asthma through inducing inflammatory response of chemotactic factor, regulating transforming growth factor to make lung tissue fibrosis, leading airway mucoprotein gene expression and inter-coordination between cell factors, and stimulating hyperplasia of vascular endothelial growth factors, actions of immune cells and coupling of adenosine monophosphate and IL-13.

This study explores the function mechanism of radix bupleuri dampness-percolating decoction for prevention and treatment of asthma from cell factors and has proven that this decoction can reduce the density of IL-5 and IL-13 in the asthma rat model so as to effectively restrain the animal's airway inflammation. The following can be concluded from the experiment results: first, no significant difference exists between the low dosage radix bupleuri dampness-percolating decoction group and dingchuang decoction group; second, no significant difference exists between the medium dosage radix bupleuri dampness-percolating decoction group, the high dosage radix bupleuri dampness-percolating decoction group and the dexamethasone group; third, among the experimental groups of radix bupleuri dampness-percolating decoction, the effect of the medium radix bupleuri dampness-percolating decoction group resembles that of the high dosage radix bupleuri dampness-percolating decoction group and the therapeutic effect does not appear more remarkable when the dosage is increased.

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# 柴胡渗湿汤对哮喘大鼠血清中 IL-5 及 IL-13 影响的实验研究

闫云霞<sup>1</sup> 王燕青<sup>2△</sup> 周兆山<sup>2</sup> 郭桂明<sup>1</sup>

(1 青岛大学医学院 山东 青岛 266021 2 青岛市海慈医疗集团 山东 青岛 266033)

**摘要** 目的 观察柴胡渗湿汤对哮喘大鼠血清中 IL-5 及 IL-13 含量的影响,探讨柴胡渗湿汤治疗哮喘的作用机制。方法 将 84 只雄性 Wi star 大鼠按随机数字表法分为正常对照组、模型对照组、地塞米松组、定喘汤组、柴胡渗湿汤低剂量组、柴胡渗湿汤中剂量组、柴胡渗湿汤高剂量组,每组 12 只,采用卵蛋白制作大鼠哮喘模型,予相应药物干预。用酶联免疫吸附试验(ELISA)法检测各组大鼠血清中 IL-5 及 IL-13 水平。实验数据采用 SPSS11.5 统计软件进行分析。结果 实验后各组死亡率比较均无差异,  $P>0.05$ 。模型对照组与正常对照组血清中 IL-5 及 IL-13 含量水平有显著差异,表明大鼠哮喘模型存在着血清 IL-5 及 IL-13 含量异常增高的病理状态;与模型对照组相比较,各治疗组血清中 IL-5 及 IL-13 的含量明显降低,其中尤以地塞米松组、柴胡渗湿汤中剂量组和柴胡渗湿汤高剂量组的作用更明显。结论 柴胡渗湿汤治疗哮喘的作用机制与降低 IL-5 及 IL-13 的水平有关;上述作用具有一定的量效关系,但并不因给药剂量的增加而增加。

**关键词** 哮喘;柴胡渗湿汤;大鼠;IL-5;IL-13

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作者简介:闫云霞(1981-),女,硕士,主要从事呼吸病的研究,  
E-mail:yanyunxia@126.com

△通讯作者:王燕青, Tel:13370857961

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