

THE THERAPEUTIC EVALUATION AND MECHANISM ON TREATING BRONCHIAL
HYPER-RESPONSIVENESS COUGH BY ZIYINQINGRE PRESCRIPTION

Yin Zhang[^] Shao dan Li[^] Jun Xiu Zhang, Yi Liu, Yi xin Cui, Min hui Yang^{*}

Department of Traditional Chinese Traditional Medicine, General Hospital of PLA, Beijing 100853, China

***Corresponding authors email address:** ymh9651@sina.com (Yang Minghui)

Abstract

Objective: Discussing the effects of Ziyinqingre prescription on the level of airway resistance (Rrs), airway response threshold (Dmin), airway conductance (sGrs) and the level of inflammatory cytokines interleukin-4 (IL-4) and interferon- γ (IFN- γ) of the bronchial hyper-responsiveness (BHR) cough patients.

Method: 84 subjects diagnosed as BHR were randomly divided into 42 Chinese Traditional medicine group and 42 control group. The Chinese Traditional Medicine group received Ziyinqingre prescription twice a day and the control group received 10mg Montelukast Sodium tablets once a day for two weeks. Observe the clinical symptoms improvement and the changes of the level of the Rrs, Dmin, sGrs and IL-4, IFN- γ .

Results: After receiving the medicine, the symptoms of the Chinese medicine group were obviously alleviated, the outcome was more satisfied than that of the control group. Compared with the control group, the level of Dmin increased and sGrs level decreased more obviously ($P<0.05$); the level of IL-4 decreased and IFN- γ level increased more obviously in the Chinese medicine group ($P<0.05$).

Conclusion: Ziyinqingre prescription can not only improve BHR patients' symptoms, but reduce the level of bronchial responsiveness, which proved a better curative effect of Chinese medicine. The mechanism is probably due to relieving the airway inflammation by keeping the balance between Th1 and Th2 cells.

Key words: Ziyinqingre prescription; cough; bronchial hyper-responsiveness; therapeutic mechanism.

Introduction

Chronic cough is a common respiratory disease in clinic which has one month to several years long course. Patients with chronic cough are always accompanied by Bronchial Hyper Responsiveness (BHR) whose manifestations are excessive contraction of bronchial smooth muscle, an increase in Bronchial secretion, narrowing of lumen, limitations in air flow velocity, etc (Group of Asthma Respiratory Society, 2009; Group of Asthma Respiratory Society, 2008). As diseases caused by Bronchial Hyper Responsiveness (BHR), Cough Variant Asthma, Upper Bronchial Cough Syndrome, Gastroesophageal Reflux Cough, Chronic Bronchitis, Bronchiectasis accounts for 70%-95% chronic cough etiology in respiratory medicine clinic (Ward et al, 2002). Based on many years clinical experience in treatment of chronic cough and persistent cough as well as combined with Chinese Traditional Medicine, the writer adopted Ziyinqingre prescription to treat BHR cough, which proved has significant effects.

Based on the previous study about BHR related inflammatory cytokines, we hypothesize that the mechanism of Ziyinqingre prescription on treating BHR cough is probably related to regulating inflammatory cytokines IL-4, IFN- γ . In order to explore on the therapeutic mechanism of Chinese Traditional Medicine, this paper focuses on clinical observations as follows:

Data and Methods

Clinical Data

We chose 84 patients in General Hospital of PLA from September 2012 to March 2014, who met the diagnostic standard of BHR cough: the result of Air Passage Provocation test was positive (referring to Bronchial Asthma Control Guide established by Respiratory Branch of Chinese Traditional Medicine Association).

Inclusion criteria: (1) the result of Air passage provocation test is positive; (2) having throat discomfort, accompanied by cough, expectoration or not, medical history is more than one month; (3) People who are allergic to cold air, smoke and dust, tend to have symptoms of cough and expectoration; (4) Age from 18 to 65.

Exclusion criteria: (1) People who have asthma attack or medical history of asthma; (2) The result of Air Passage Provocation test is negative; (3) People who are not willing to take Air Passage Provocation test or participate in this research; (4) People who don't fit for this research because of other diseases.

84 patients with BHR cough were divided into two groups. There are 25 males and 17 females in Chinese Traditional Medicine group whose age from 18 to 62, average age is (46.2±9.3), the course of the disease is from 1 month to 1 year, average course is (0.7±0.3) year. There are 26 males and 16 females in control group whose age from 19 to 64, average age is (48.5±10.2), the course of the disease is from 1 month to 1.5 years, average course is (0.8±0.5) years. The two groups of patients with gender, age, course of disease, as there was no significant statistical difference ($P > 0.05$), are comparable.

Treatment Methods

Chinese Traditional Medicine group took Ziyinqingre prescription which composes of Radix Glehniae, Radix Ophiopogonis, Rhizoma Anemarrhenae, Huang Qin, forsythia and reed root each 15 grams; 15 grams Quanguaiou and 10 grams almond were added for patients with much phlegm; 15 grams Arctium lappa and 3 grams Senmen Oroxyli were added for patients with symptom of throat itching. (all Chinese Traditional Medicine was bought from Bei Jing Tong Ren Tang Technology Development Co. Ltd). One dose a day, decoction, twice a day. The control group took Montelukast Sodium Tablets (bought from Hang Zhou Mo Sha Dong Pharmaceutical Co. Ltd., State Approve No. J20110076) for treatment. For oral use, 10mg, once a day. The treatment course of two groups is two weeks.

Outcome Measures

1. **Clinical symptoms:** the symptom integral changes of cough, difficult expectoration and throat itching were observed before and after the treatment. Standards of classification were referred to Chinese Traditional Medicine Quantization Diagnosis(1997 First Edition) edited by Xu Dihua.

Table 1: Symptoms Standards Classification of Traditional Chinese Medicine

Symptoms	Grades	Scores	Symptom
Cough	None	0	
	Slightly cough	1	slightly cough, two or three coughs at one time; only cough two or three times a day.
	Heavily cough	2	heavily cough, six or seven coughs at one time; cough four to nine times a day.
	Severe cough	3	severe cough, frequent coughs; or cough frequently, cough ten times a day even an hour
Difficult Expectoration	None	0	
	Slightly difficult expectoration	1	slightly difficult expectoration, cough up one or two times for phlegm.
	Difficult expectoration	2	difficult expectoration, cough up three or four times for phlegm.
	Very difficult expectoration	3	very difficult expectoration, cough up five or six times for phlegm. Symptoms of vomiting, dull pain in stomach, dark-purple complexion.

Throat itching	None	0	
	Slightly throat itching	1	slight throat itching, just starts or happens occasionally. Symptoms of coughing or expectoration.
	Heavily throat itching	2	rather obvious throat itching, upsetting. Symptoms of coughing and expectoration.
	Severe throat itching	3	couldn't bear throat itching which causes frequent coughing and anxiety. Desire for medical care.

2). **Indicators of Bronchial Responsiveness:** We adopted Bronchial Responsiveness testing machine (made by Japanese Chest company, Astograph-21) to test the improvement of Rrs, Dmin and sGrS after treatment.

PB IL-4, IFN— γ : Subjects, on empty stomach for 12 hours, were drawn 3ml vein blood to separate plasma for testing. IFN— γ was tested by enzyme-linked immunoassay. The kits were supplied by Beijing Kang Yuan Rui De Bio-Tech Co. , Ltd. Testing machine was supplied by Finland WellsCan automatic ELISA instrument. IL-4 was tested by radioimmunoassay (RIA), The kits were supplied by Beijing Pu Rui Kang Bio-Tech Co. , Ltd. Testing machine was supplied by Shanghai He Suo Ri Huan Photoelectric Instruments Sn-695B immunology counter. All testing steps were strictly followed by instructions.

Efficacy Evaluation Criteria: According to Chinese Traditional medical symptomatology curative assessment in Chinese Traditional medicine new drug clinical research guiding principle (issued by State Drug Administration, May 2002, first edition) : (1) clinical cure: clinical symptoms and signs disappear or largely disappear; score of TCM symptoms decreases $\geq 95\%$.(2) Significantly effective: clinical symptoms and signs are improved significantly, score of TCM symptoms decreases $\geq 70\%$. (3) Effective: clinical symptoms and signs are improved , score of TCM symptoms decreases $\geq 30\%$.(4) Not effective: clinical symptoms and signs aren't improved or worse, score of TCM symptoms decreases less than 30%.

Curative effect = (scores before treatment – scores after treatment) \div scores before treatment $\times 100\%$.

Statistics: SPSS13.0 statistics software was adopted. Rank sum test and analysis of variance was adopted. $P < 0.05$ was considered to be statistically significant.

Results

1). Symptom score comparisons between two groups after treatment

Patients' symptoms of the two groups are all alleviate in different degrees. Treatment group are better than control group in alleviating cough, difficult expectoration and throat itching. There are significant differences between the two groups. ($P < 0.05$) See Table 2.

Table 2: Symptom score comparisons between two groups after treatment ($\bar{x} \pm s$)

Group		Cough	Difficult expectoration	Throat itching
Control group (n=42)	before	2.59 \pm 0.20	2.37 \pm 0.28	2.48 \pm 0.25
	after	1.68 \pm 0.18*	1.57 \pm 0.15*	1.62 \pm 0.16*
Treatment group (n=42)	before	2.65 \pm 0.23	2.42 \pm 0.30	2.54 \pm 0.27
	after	1.06 \pm 0.14* Δ	1.08 \pm 0.13* Δ	1.11 \pm 0.15* Δ

Note: comparisons between groups, * $P < 0.05$, compared with control group, $\Delta P < 0.05$.

2). Effect comparisons

There are 14 cases significantly effective, 23 cases effective, total effective rate reaches 88.1% in treatment group; there are 6

cases significantly effective; 24 cases effective in control group, total effective rate is 71.4%. There are significant differences in two groups. ($P < 0.05$) See Table 3.

Table 3: Effect Comparisons [(%)]

Group	Significantly Effective	Effective	Not Effective	Total Effective Rate
Control Group (n=42)	6 (14.3%)	24 (57.1%)	12 (28.6%)	71.4%
Treatment Group (n=42)	14 (33.3%)	23 (54.8%)	5 (11.9%)	88.1%*

Note: compared with control group, * $P < 0.05$

3). Comparisons in indicators of Bronchial responsiveness between two groups

The level of Dmin increased and Rrs level decreased significantly in both groups ($P < 0.05$), but more significantly in treatment group. See Table 4.

Table 4: Comparisons in indicators of Bronchial responsiveness ($\bar{x} \pm s$)

Group		Rrs (cmH2O·L ⁻¹ ·s ⁻¹)	Dmin (u)	sGrS [L·s/ (cmH2O·min)]
Control Group (n=42)	Before	4.63±1.28	2.83±0.48	0.06±0.03
	After	3.27±0.82*	6.84±1.18*	0.08±0.04 [‡]
Treatment Group (n=42)	Before	4.60±1.37	2.97±0.56	0.04±0.02
	After	3.00±0.79* ^Δ	8.52±1.27* ^Δ	0.08±0.05 ^{‡Δ}

Note: comparisons between groups, * $P < 0.05$, compared with control group, $\Delta P < 0.05$.

4). Comparisons in Peripheral Blood IL-4、IFN- γ

The level of IL-4 decreased and the level of IFN- γ increased in two groups significantly ($P < 0.05$), but more significantly in treatment group. See Table 5.

Table 5: Comparisons in Peripheral Blood IL-4、IFN- γ ($\bar{x} \pm s$)

Group	Cases	Time	IL-4(ng/ml)	IFN- γ (pg/ml)
Control Group	42	Before	1.643±0.652	150.369±34.146
		After	1.336±0.433*	164.736±33.088*
Treatment Group	42	Before	1.634±0.853	142.651±42.292
		After	1.072±0.555* ^Δ	181.977±41.937* ^Δ

Note: comparison before and after treatment, * $P < 0.05$, compared with control group, $\Delta P < 0.05$

Discussions

Bronchial Hyper Responsiveness (BHR) cough is a common respiratory disease in clinic. According to modern medicine, bronchial inflammation is one of the important mechanisms to cause Bronchial Hyper Responsiveness. When airway stimulated by allergens or other substances, the level of Bronchial Responsiveness is increased by participation of various inflammatory cells, inflammatory mediator, cytokines and damage of bronchial epithelium and superior nerves (Zhu, 2003; Ricarda, 2004). Until now there is no effective treatment for Bronchial Hyper Responsiveness. It's reported that leukotriene receptor antagonists can alleviate inflammation of airways and relieve Bronchial Hyper Responsiveness. (Minoguchi et al, 2002).

In Chinese Traditional Medicine, the cough is caused by disorder of dispersive and descending function of lung and reverse flow of gas from lung. There was an appropriate metaphor in Medicine Thoughts to describe the cause of cough: “the lung is like a bell needs to be knocked. Wind, coldness, hotness, humidity, dryness knock it from outside; tiredness, bad mood and disordered diet knock it from inside”. Diseased lung is the basic cause of cough. From many years’ clinic experience, the writer sums up that the reason of Bronchial Hyper Responsiveness cough attacks frequently, lasts long and difficult to cure is Yin deficiency. Yin deficiency, disorder of throat and dispersive and descending cause cough; deficiency of Qi and Yin caused by pathogenic Qi, which leads to disfunction of zang-fu organs. Ziyinqingre treatment is significantly effective to Bronchial Hyper Responsiveness cough. The research proves that Ziyinqingre treatment can not only improve symptoms of cough, difficult expectoration and throat itching, but also increase Dmin and decrease sGr_s, thus decrease bronchial responsiveness.

It has been widely accepted that ratio imbalance between Th1 and Th2 is the cause of bronchial inflammation. IL-4、IFN- γ , which represent functions of Th1 and Th2 , is a couple of antagonism cytokines. As Th2 size cytokines’ representative, IL-4 is an indicator to reflect bronchial inflammation. Through promoting expression of IgE’s low affinity receptors, IL-4 further promotes the synthesis and release of IgE. Besides, it plays an important role in collecting, chemotax and infiltration of eosinophiles. As Th1 size cytokines’ representative, IFN- γ can inhibit the generating of Th2 size cytokines IL-4 and block the synthesis of IgE, which inhabits allergenic-induced bronchial chronic inflammation and Bronchial Hyper Responsiveness in an effective way (Schmidt et al, 2012; Ito et al, 2012; Mao et al, 2003). In this research, Ziyinqingre treatment decrease the level of patients’ IL-4 in peripheral blood, increase the level of IFN- γ , remains the balance of Th1/Th2 cells, as a result, it decreased the synthesis of IgE, alleviated bronchial inflammation, reduced bronchial responsiveness and therefore alleviate cough.

Acknowledgement

Grant: Scientific Research Supporting Fund of General Hospital of PLA (2012FC-TSYS-3038); Major project of military logistics scientific research plan (BWS12J052)

Conflict of interest: No conflict of interest exists in the submission of this manuscript, and this manuscript is approved by all authors for publication.

References

1. Group of Asthma Respiratory Society (2009). Chinese Medical Association. Diagnosis and Treatment for Cough. Chinese TB and Respiratory, 32(6): 408-409.
2. Group of Asthma Respiratory Society (2008). Chinese Medical Association. Guide for Bronchial Asthma Prevention (definition, diagnosis, treatment and management of Bronchial Asthma). Chinese Asthma, 2(1): 3-13.
3. Ito Y, Shigemori S, Sato T, Shimazu T, Hatano K, Otani H, Kitazawa H, Shimosato T (2012). Class I/II hybrid inhibitory oligodeoxynucleotide exerts Th1 and Th2 double immunosuppression. *FEBS Open Bio*, 12(3): 41-45.
4. Mao Xiaohui, Ni Diantao (2003). Interferon γ 's function in bronchial asthma [J]. *Cells and molecular immunology*, 19(4): 415-416. Minoguchi K, Kohno Y, Minoguchi H, Kihara N, Sano Y, Yasuhara H, Adachi M (2002). Reduction of eosinophilic inflammation in the airways of patients with asthma using montelukast. *Chest*, 121:732-738.
5. Ricarda JA (2004). Neurokinin-1 Receptor Mediates Stress-Exacerbated Allergic Bronchial Inflammation and Bronchial Hyper responsiveness in Mice. *Psychosomatic Medicine*, 66(4):564.
6. Schmidt S, Hoving JC, Horsnell WG, Mearns H, Cutler AJ, Brombacher TM, Brombacher F (2012). Nippostrongylus-induced intestinal hypercontractility requires IL-4 receptor alpha-responsiveness by T cells in mice. *PloS One*, 7(12):e52211.
7. Ward C, Pais M, Bish R (2002). Bronchial inflammation, basement membrane thickening and bronchial hyper responsiveness in asthma. *Thorax*, 57(4): 309.
8. Zhu Minli (2003). Research on relationship between bronchial hyper responsiveness and cytokines. *Foreign Medical respiratory system*, 23(1): 34.