



Effect of Baihe Gujin decoction combined with Shengmai powder on the expression of IL-1 β and IL-1Ra in peripheral blood CD14 $^{+}$ monocytes from patients with pulmonary tuberculosis

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ABSTRACT

This experiment was conducted to investigate the effect of taking Baihe Gujin decoction combined with Shengmai powder on IL-1 β and IL-1Ra expression in peripheral blood CD14 $^{+}$ monocytes from patients with pulmonary tuberculosis. For this purpose, one hundred adult patients with primary pulmonary tuberculosis were selected for the study. The age of the enrolled cases ranged from 18 to 60 years old, with a controlled male to the female sex ratio of about 1:1. The Chinese medical evidence was considered to be a qi-yin deficiency type of pulmonary consumption. The patients were randomly divided into experimental and control groups, 50 cases each. In addition, 50 cases of healthy people were selected as a healthy control group, totaling 150 cases. In the experimental group, patients were given Baihe gujin decoction and Shengmai powder based on conventional western medicine, 1 dose for 1 day, 150mL/time for 2 times. The control group was treated with conventional Western medicine. 2 mL of fasting elbow venous blood from the subjects was taken in the morning. Peripheral blood mononuclear cells were isolated by density centrifugation. Monocytes were obtained after incubation with 5 μ L of CD14 $^{+}$ immune magnetic beads at 4 $^{\circ}$ C. The relative expression of IL1 β and IL1R genes were measured using real-time quantitative PCR (RT-PCR), respectively. Results showed that the relative expression of IL1 β and IL1R genes was significantly lower in the experimental group after 3 months compared with the control group, and the statistical difference was highly significant ($p < 0.01$). It was concluded that the administration of Baihe gujin decoction and Shengmai powder was closely related to the relative expression of IL1 β and IL1R genes in patients' serum, indicating that Baihe gujin decoction and Shengmai powder have an important role in improving the clinical symptoms of pulmonary tuberculosis.

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Introduction

Tuberculosis is an infectious disease of the lungs caused by *Mycobacterium tuberculosis*. At present, it is still one of the priority diseases affecting people's health in China. Pharmacological treatment of pulmonary tuberculosis is mainly based on western drugs, such as rifampin, isoniazid, ethambutol, and pyrazinamide (1-3). However, with the heavy use of drugs, many drug-resistant *Mycobacterium tuberculosis* has emerged. In 2011, there were 310,000 cases of drug-resistant tuberculosis worldwide, and China alone accounted for 30% of the total (4,5), which makes the situation of tuberculosis control in China extremely critical.

Chinese medicine is often used as an adjunct in the

treatment of tuberculosis in combination with traditional Western medicine. Chinese medicine not only stimulates the patient's immune system and slows down tuberculosis symptoms, but is even effective for drug-resistant tuberculosis (6). This can greatly improve the prevalence of tuberculosis in our country. In this study, we applied the traditional Chinese medicine of Baihe gujin decoction and Shengmai powder to intervene in patients with pulmonary tuberculosis to observe the expression of CD14 $^{+}$ monocytes IL-1 β and IL-1Ra in the peripheral blood patients, so as to investigate the mechanism of its effect in preventing and treating pulmonary tuberculosis.

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Materials and methods

Subjects of the study

One hundred adult patients with primary pulmonary tuberculosis treated at Wuhan Jinyintan Hospital between October 2017 and August 2021 were selected for the study. The age of the enrolled cases ranged from 18 to 60 years, with a controlled male to the female gender ratio of about 1:1 and a mean age of 36.3 ± 5.2 . The Chinese medical evidence was considered to be a qi-yin deficiency type of pulmonary consumption. Patients were free of comorbidities such as other infectious diseases, genetic-metabolic diseases, autoimmune diseases, and psychiatric diseases. This study was approved by the Ethics Committee of Wuhan Jinyintan Hospital, and all study subjects signed an informed consent form.

Reagents and instruments

CO₂ cell incubator (Thermo Fisher, USA), Evolution 201 ultra-micro spectrophotometer (Thermo Fisher, USA), DTX880 multi-functional enzyme marker (Beckman, Germany), high-speed centrifuge (Beckman, Germany), ABI 7500 real-time quantitative PCR (ABI, USA), CD14 immunomagnetic beads (Miltenyi, Germany), total RNA extraction kit (HiScript RT SuperMix, Novozymes, China), SYBR Green Master Mix real-time quantitative PCR kit (Vazyme, Novozymes, China).

Treatment methods

The control group was given conventional western medicine basic treatment (isoniazid, rifampin, pyrazinamide and other conventional treatment TB drugs). The patients were also given certain symptomatic treatments according to their specific symptoms and observed for 3 months. In addition to the basic treatment of western medicine, maintenance of body homeostasis and prevention and control of infection, the experimental group was also treated with Baihe gujin decoction and Shengmai powder (lily, rehmannia glutinosa, radix rehmanniae, angelica, peony, etc.), 1 dose for 1 day, 150mL/time for 2 times, and observed for 3 months.

Sorting of peripheral blood CD14+ monocytes

2 mL of fasting elbow venous blood was collected from the subject in the morning using a lithium heparin anticoagulation tube. Peripheral blood

mononuclear cells (PBMCs) were isolated by density centrifugation. 1×10^7 PBMCs were incubated with 5 μ L of CD14-immune magnetic beads at 4°C for 20 min, and then CD14+ monocytes were obtained using a MACS automated bead sorter.

RNA extraction and RT-PCR assay

Total RNA was extracted from CD14+ monocytes according to the steps in the HiScript RT SuperMix instructions. The quality and concentration of RNA were measured by Evolution 201 ultramicro spectrophotometer. 1 μ L of RNA was taken, and genomic DNA was removed using 4 x gDNA wiper Mix, then 5 x qRT SuperMix II 4 μ L was added at 50°C for 15 min to generate cDNA. The assay was performed using SYBR Green Master Mix real-time quantitative PCR kit.

The gene IL1B primers were (5'-TTCTTCGACACATGGGATAACG-3'; 5'-TGGAGAACACCACTTGTTGCT-3'), The gene IL1RN primers were (5'-GGAAGATGTGCCTGTCCTGT-3'; 5'-TCTCGCTCAGGTCAGTGATG-3'), and the internal reference gene GAPDH primers were (5'-GCACCGTCAAGGCTGAGAAC-3'; 5'-TGGTGAAGACGCCAGTGGA-3'). The reaction system was 2 x AceQ qPCR SYBR Green Master Mix 10 μ L, upstream and downstream primers 0.4 μ L each, 50 x ROX Reference Dye1 0.4 μ L, cDNA template 2.8 μ L, add double distilled water to 20 μ L.

Statistical processing methods

IBM SPSS 17.0 statistical software was applied for statistical analysis. Statistical data were expressed as rates (%) or relative number composition ratios (%), using the χ^2 test. Normally distributed measures were expressed as mean \pm standard deviation ($\bar{x} \pm s$). Two independent samples t-test was used for comparison between two groups, one-way ANOVA was used for comparison between multiple groups, and LSD-t test was performed for two comparisons. Non-normally distributed measures were expressed as median and interquartile spacing [M(P25, P75)], using the Mann-Whitney U test. Pearson correlation analysis was used for correlation analysis. The difference was considered statistically significant at $P < 0.05$.

Results and discussion

Application of RT-PCR technique to detect IL1 β and IL1RN gene expression

The qPCR amplification curves of IL1 β , IL1RN and GAPDH genes (Figure 1) showed that they could be amplified efficiently, while the melting curve plots showed that different genes had specific melting curves. This indicates that the primers have good specificity and can amplify the genes specifically.

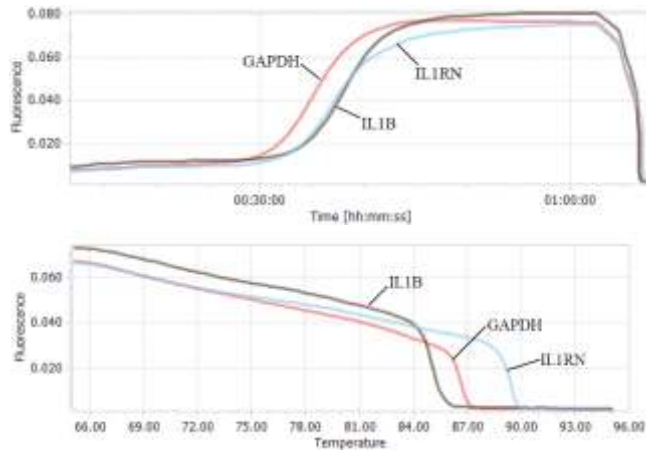


Figure 1. Detection of IL1B and IL1RN gene expression by RT-PCR

Differences in IL-1 β expression levels of CD14+ monocytes among the experimental group, control group and healthy control group

The level of IL-1 β secretion in the experimental group was found to be significantly higher than that in the control group (taking only conventional western medicine) after 3 months of observation, as shown in Figure 2. In contrast, there was no significant difference compared with the healthy group, indicating that Baihe gujin decoction and Shengmai powder accelerate the healing of the patient's symptoms, which in turn reduces the ability of the body's immune system to secrete IL-1 β .

Differences in IL-1Ra expression levels of CD14+ monocytes among the experimental group, control group and healthy control group

As shown in Figure 2, CD14 + monocyte IL-1Ra expression level was significantly decreased in the experimental group, while the administration of western medicine in the control group was not able to restore IL-1Ra expression to normal levels, and IL-1Ra expression inhibited the effect of IL-1 β on

resistance to tuberculosis bacteria (7). This suggests that Baihe gujin decoction and Shengmai powder San also has a reducing effect on IL-1Ra expression, which in turn has a positive effect on curing tuberculosis in patients.

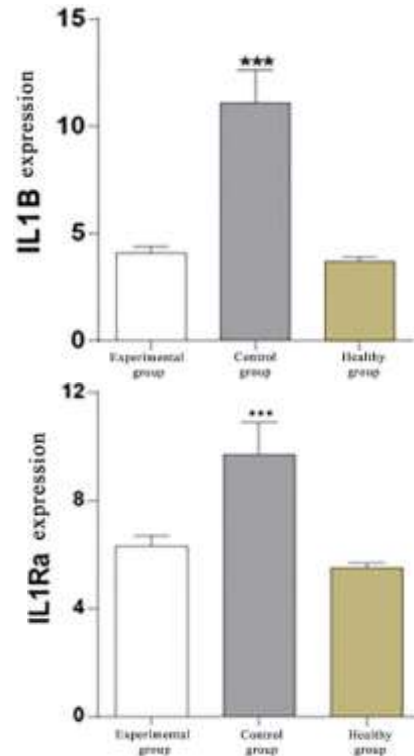


Figure 2. Differences in the relative expression of IL-1 β and IL1Ra among the three groups

Tuberculosis is a disease caused by infection of the lungs by *Mycobacterium tuberculosis* branching bacteria, which in turn causes an immune and inflammatory response in the body. When the lung is infected, macrophage receptors are stimulated by *Mycobacterium tuberculosis* antigens, prompting them to secrete a variety of cytokines such as IL-17, IL-6, IL-10, IL-1 β , and IL1Ra (8,9). These inflammatory factors mediate immune and inflammatory responses, causing immune damage and tissue destruction. Therefore, the changes in the expression levels of IL-17, IL-6, IL-10, IL-1 β and IL1Ra are often used as indicators of tuberculosis attacks (10).

Chinese medicine has a long history of understanding and treating pulmonary tuberculosis, which belongs to the category of "consumption" in Chinese medicine. Baihe gujin decoction and Shengmai powder have been used for a long time in

the treatment of pulmonary tuberculosis for their ability to moisten the lung, produce fluid, clear heat, resolve phlegm, stop coughing, and stop sweating. When the lung is infected or injured, the body undergoes a systemic inflammatory response and simultaneously releases inflammatory mediators, such as tumor necrosis factor α , IL-6, IL-10, and IL-1 β (11-14), to resist the infection of the lung. In contrast, IL-1Ra expression competitively inhibits the anti-tuberculosis immune response mediated by IL-6, IL-10, and IL-1 β , leading to a prolonged course and exacerbation of TB disease (15,16). The expression levels of IL-1 β and IL1Ra were reduced in the treatment of pulmonary tuberculosis. This indicates that the administration of Baihe gujin decoction and Shengmai powder on top of western medicine can reduce the inflammatory response of the body, alleviate the burden on the body, improve the attack of tuberculosis on the body, lessen the overall disease process, and lower the time consumed to cure tuberculosis.

In conclusion, this study confirms that the use of Baihe gujin decoction and Shengmai powder in conjunction with Western medicine can accelerate the cure of tuberculosis, improve the prognosis of patients, and reduce their suffering during the course of the disease. It also illustrates the increasingly prominent role played by Chinese medicine in the prevention and cure of tuberculosis.

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Conflict interest

The authors declare no conflict of interest.

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