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EXPERIMENTAL STUDY

Effect of sodium houttuyfonate on symptom pattern of lung-*Qi* deficiency in rats induced by bacterialbiofilm infection

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Abstract

OBJECTIVE: To investigate the *in vivo* inhibitory effects of sodium houttuyfonate (SH) on symptom pattern of *Qi*-deficiency in rats induced by infection of bacterial biofilm on rat respiratory tract.

METHODS: Symptom pattern is a term used in Traditional Chinese Medicine (TCM) to define a cluster of symptoms in a medical condition. Based on the pattern, TCM therapies are administered. The symptom pattern used in this study was lung-*Qi* deficiency pattern identified in rats, which was induced by nasal intubation drip of Pseudomonas aeruginosa (P. aeruginosa) (two strains) to form bacterial biofilm on airway combined with stimulation of cold and fatigue. We measured the variations of the symptoms of the pattern, weight, spleen and thymus index, blood gas, lung bronchial tissue pathology and cytokine of rat in different treatments and control groups.

RESULTS: The rats of SH-treatment groups had not showed typical symptoms comparing with model group in the early stage of infection. The weight, spleen and thymus index of the SH-treatment groups were significantly higher comparing with untreated model group. The SH-treatment groups also showed higher O_2 partial pressure and lower CO_2 partial pressure than model group. Furthermore, we found that the bronchopulmonary section of SH-treatment groups not showed typical pathogenic variation in model group. The comparison of cytokine concentration in different groups indicated that SH could prevent the over-production of cytokine to reduce the inflammation occurrence.

CONCLUSION: In the early stage of airway infection by biofilm of P. aeruginosa, application of SH can prevent the occurrence of lung-*Qi* deficiency pattern.

Key words: Symptom complex; Lung-*Qi* deficiency; Sodium houttuyfonate; Pseudomonas aeruginosa; Biofilms; *In vivo*

INTRODUCTION

Symptom pattern is a term used in Traditional Chinese Medicine (TCM) to define a cluster of symptoms in a medical condition. Based on the pattern, TCM therapies are administered. The symptoms in *Qi*-deficient pattern may involve a multi-systems and conditiona with the main characteristics of lung dysfunction according to TCM theory.[1] The symptoms of the pattern

includes chronic cough and asthma, white sputum, serious wind-cold superficies, self sweating, low *Qi* and unwilling to talk, losing *Qi* of lung, etc.[2] The symptoms not only exist in every stages of lung-*Qi* deficiency pattern, and become more severe and frequent by the aggravation of the disease. "*Ling Shu Jing, Jiu Zheng Lun*" indicated that "Lung is the shield of *Zang-fu* viscera". Thus, the symptoms of *Qi*-deficient pattern in different stages can present as the heart systems type including heart palpitations, chest, cyanotic lips, insomnia and dreaminess; the spleen and stomach type including nausea and vomiting, epigastric distention, defecate pond drainage, yellow face; kidney types including clear abundant urine, dribble of urine, frequent night urinate, edema of lower extremity; liver types including dizzy, tinnitus, numbness of limb, dry throat etc.[3] The reasons of lung-*Qi* deficiency pattern are exhausting work, chronic cough, summer hot and heavy disease leading to damage of lung *Qi*, or spleen deficient affecting clear *Qi* arising to lung, causing lack of lung *Qi*. Lung-*Qi* deficiency pattern has become one of the most frequent reasons of death of the aged.[4]

In our previous research, based on the guidelines of "achieving the symptom of disease animal model" and "chronic disease causing deficiency" in TCM treatment, we adopted the chronic air way infection induced by Pseudomonas aeruginosa (P. aeruginosa) biofilm formation to achieve the rat model of lung-*Qi* deficiency pattern.[5] The results showed that the symptoms, physical and pathological indexes of rat of model set were close to the phenotype of lung-*Qi* deficiency pattern (data not shown). Sodium houttuyfonate [SH, $CH_3(CH_2)_8COCH_2CHOHSO_3Na$] is the addition compound of sodium bisulfite and houttuynin, which is the major constituent of the volatile oil of the Yuxingcao (*Herba Houttyniae Cordatae*), a wild perennial herb which was widely used in TCM.[6,7] Sodium houttuyfonate (SH) was traditionally used in clinical application of prevention and cure of external evil invasion and lung-*Qi* deficiency pattern.[8] Previously, we found that SH can effectively prevent *in vitro* biofilm and planktonic formation of P. aeruginosa, Staphycoccus epidermidis and Candida albicans,[9,10] and in vitro synergistic effects with levofloxacin against biofilm formation of P. aeruginosa.[11] SH also showed alone and synergistic high inhibitory effects on multi-drug resistance S. aureus strains,[12] and activity of inducing autolysis of S. aureus.[13] Therefore, based on the mature animal model, we selected the sodium houttuyfonateto suppress the biofilm formation of P. aeruginosa in early infected rats, and observe inhibitory effect of sodium houttuyfonate on lung-*Qi* deficiency pattern *in vivo*.

MATERIALS AND METHODS

Strains and drugs

The clinical P. aeruginosa strain AH16[14] isolated from a chronic pneumonia patient in the First Affiliated Hospital of Anhui University of Chinese Medicine, China. The model strain ATCC27853 (PA) was stored in our laboratory. The SH and azithromycin (AZM) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China). Healthy female Sprague-Dawley (SD) rats of clean grade, weighing (250 ± 10) g, six-month-old were purchased from experiment animal center in Jiangsu University (Zhenjiang, China) [Certificate of quality No. SCXK (Su) 2009-0002]. After one week of normal feeding, the rats were randomly divided by random number table method into 11 groups including blank control (not infected), negative control (not drug intervention after infected), positive control (AZM intervention, which is a typical antibiotics applied in curing infection of P. aeruginosa biofilm in clinic[15]), and high, medium, low SH treatment groups infected by strains AH16 and ATCC27853. Every group contained 15 animals. All surgery was performed under sodium pentobarbital anesthesia, and every effort was made to minimize suffering. All of the animal experiment procedures conformed to the guidelines of our institution for the care and use of laboratory animals in Anhui University of Chinese Medicine (Hefei, China), and conformed to the National Institute of Health Guide for Care and Use of Laboratory Animals. P. aeruginosa biofilm formation and determine of 50% lethal dose (LD50).

P. aeruginosa was inoculated in Trypticase Soy Broth (TSB) broth (Sangon, Shanghai, China), cultivated in a constant-temperature shaker GLY (Fuma, Shanghai, China) of 90.7 ×*g* for 48 h at 37 ℃. Then, the bacteria were harvested by GL-20G-II high-speed refrigerated centrifuge (Fuma, Shanghai, China) at 670.8 × *g* for 10 min. The supernate was discarded, and the precipitate was resuspended with pH 7.2 phosphate-buffered saline (PBS), centrifuged at 670.8 ×*g* for 10 min. The collection was mixed with fresh culture, and adjusted to 1×10^9 CFU (colony forming units) /mL. The 200 μL prepared culture was added to 2 mL TSB in each well of six well culture plates which contained a sterile cover glass for 7 day at 37 ℃. Then the glasses were washed by PBS, and stained by silver (Sangon, Shanghai, China) to observe morphological structure of biofilm by scanning electron microscope (SEM) (Sirion200, FEI Company, Hillsboro, OR, USA). The prepared culture was also infected the SD rat to determine the LD50. Afterwards, the bacteria in infected animals were collected, and detected the biofilm formation activity as the method mentioned above.

Bacterial infection and intervention

Animals in negative control group were normal fed. Other groups were treated as our previous building method of lung-*Qi* deficiency pattern model.[5] The method in detail was as follows: (a) Bacterial infection. 0.2 mL culture contained 2 fold LD50 bacteria were