Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation

Title： Comprehensive Research on Jing-Si-Herbal-Tea in Respiratory System Diseases

Keywords： Jing-Si-Herbal-Tea, Chronic Obstructive Pulmonary Disease

The study protocol was approved by the Ethics Committee of Taipei Tzu Chi Hospital (IRB number 10-XD-132).

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1. Purpose of study

Jing-Si-Herbal-Tea (JSHT) is currently sold in Jingsi Hall. It contains eight kinds of traditional Chinese medicine ingredients, namely Ophiopogon japonicus, Houttuynia cordata, Platycodon grandiflorum, fish needle grass, licorice, mugwort leaf, perilla leaf, and chrysanthemum. Ophiopogon japonicus has the effect of moistening the lungs and relieving coughs, mainly targeting coughs, dry coughs, hemoptysis and other symptoms caused by yin deficiency and dryness of the lungs caused by insufficient lung yin. Houttuynia cordata has a significant inhibitory effect on a variety of pathogenic microorganisms, strengthens immune function, relaxes bronchial smooth muscle, and achieves antitussive and asthmatic effects. Platycodon has the effect of draining pus, relieving coughs and removing phlegm. It is often used for various coughs and phlegm syndromes. It is also very effective for those with sore throat and hoarseness. Fish needle grass is a commonly used medicinal herb, mainly used to treat colds and fever, vomiting and abdominal pain, gastric pain, skin eczema, etc. Licorice is a traditional Chinese medicinal material that has pharmacological effects such as relieving coughs and moisturizing the lungs. Mugwort can treat cold cough and asthma, and has the functions of relieving cough, eliminating phlegm and relieving asthma. Perilla is often used to treat colds, colds and coughs, and has a certain effect in treating allergies. Chrysanthemum has the effect of clearing away heat and detoxifying, which can help relieve the discomfort of throat inflammation and oral ulcers. It can also improve body fever, headache, thirst, cough with yellow phlegm, sore throat, yellow and thick nasal discharge, etc. These Chinese medicinal materials have anti-inflammatory effects. For example, Houttuynia Cordata can enhance the phagocytosis function of macrophages and has also been proven to have anti-inflammatory effects. Some studies have found that it can inhibit tumor

necrosis factor-α (TNF-α) in sepsis, interleukin-1β (IL-1β) production and toll-like receptor 4 (TLR4) expression. In addition, Houttuynia cordata also has antioxidant effects and can relieve peroxidative stress.

Research purposes:

COPD is a disease caused by long-term inflammation of the respiratory tract or interstitium of the lungs, resulting in respiratory obstruction, which prevents gas from flowing in and out of the respiratory tract smoothly, or interstitial pulmonary fibrosis that prevents gas exchange. These patients suffer from poor pulmonary gas exchange function, symptoms of chest tightness, wheezing and coughing often occur. In addition, these patients are more likely to be accompanied by other comorbidities, such as cardiovascular disease, osteoporosis, diabetes, lung cancer, etc. These diseases have a huge impact on the patient's quality of life and life safety. Research is very important for the control of respiratory diseases. Although some drugs are currently used to treat these diseases, the therapeutic effect is limited. JSHT has anti-inflammatory and antioxidant effects, which can inhibit the inflammatory response of respiratory diseases. We aim to study the effect of JSHT in COPD.

2. Study Design

The main inclusion and exclusion conditions of the study:

Inclusion criteria:

1. Outpatients: patients with chronic stable respiratory diseases (COPD, asthma, bronchiectasis, pulmonary fibrosis, interstitial pulmonary disease, sarcoidosis)
2. Hospitalized patients: patients hospitalized due to acute attacks of respiratory diseases (COPD, asthma, bronchiectasis, pulmonary fibrosis, interstitial pulmonary disease, sarcoidosis)
3. Severe patients: Respiratory failure due to acute attack of respiratory diseases (COPD, asthma, bronchiectasis, pulmonary fibrosis, interstitial lung disease, sarcoidosis) using invasive or non-invasive respirators and admitted to intensive care patients in ward

Exclusion criteria:

1. Under legal age
2. Those who are unwilling to join
3. Those with severe liver function abnormalities

4. People with severe renal dysfunction

5. Have a history of allergies to JSHT

Research Methods and Procedures:

This study comprises two parts, focusing on patients with COPD acute exacerbation (COPDAE). In the COPDAE part, the control group received standard treatment including intravenous steroids, inhaled butanyl and ipratropium, and parenteral antibiotics for secondary infections. Placebo mimics of JSHT were administered to the control group. The JSHT group received standard COPDAE treatment plus JSHT for one week. Baseline and post-treatment HRQL were assessed using the COPD assessment test (CAT), along with blood tests including white blood cells (WBCs), percentages of different types of WBCs (neutrophils, lymphocytes, monocytes, eosinophils, basophils), hemoglobin (Hb), hematocrit (Hct), platelets (PLT), blood urea nitrogen (BUN), creatinine (Cr), uric acid (UA), liver enzymes (aspartate aminotransferase, alanine aminotransferase), electrolytes (sodium, potassium), C-reactive protein (CRP), and pro-brain natriuretic peptide (pPro-BNP). For patients with stable COPD, the control group received standard inhaled medications according to the GOLD guidelines. Placebo mimics of JSHT were administered to the control group. The JSHT group additionally received JSHT daily for three months. Baseline and post-treatment HRQL assessments, blood tests, and pulmonary function tests (PFT). The study protocol was approved by the Ethics Committee of Taipei Tzu Chi Hospital (IRB number 10-XD-132). Informed consent was obtained from all participants.

HRQL

The Taiwan Society of Pulmonary and Critical Care Medicine offers the Chinese version of the COPD Assessment Test (CAT) on the website. This test consists of eight items designed to evaluate COPD symptoms. These symptoms include cough, phlegm production, chest tightness, breathlessness, limitations in daily activities, confidence in leaving the house, sleep disturbances, and energy levels. Each symptom is rated on a scale from 0 to 5, culminating in a total CAT score ranging from 0 to 40. A higher score reflects more severe COPD symptoms. A score of 10 or higher is indicative of a significant symptom burden. The Modified Medical Research Council (mMRC) scale was used to evaluate dyspnea. This scale, comprising a 5-point grading system ranging from 0 to 4, measures dyspnea severity. A score of 0 indicates dyspnea only during intense exercise, while a sore of 4 represents breathlessness at rest.

The 5-item Brief Symptom Rating Scale (BSRS-5) was used to assess psychological

distress. It consists of five items: feeling tense, being easily angered, feeling depressed, feeling inferior to others, difficulty with sleep, and suicidal thoughts. The scale is a 5-point scale ranging from 0 (not at all) to 4 (extremely), with higher scores indicating more severe symptoms.

Pulmonary function tests

PFT were conducted using a spirometer following the guidelines set by the American Thoracic Society.

Cellular study for assessing the effects of JSHT on Inflammation

In the cellular study conducted on A549 cells, five groups were analyzed to evaluate the effects of JSHT. The groups were: Control group: did not receive treatment; lipopolysaccharide (LPS) group: treated with LPS for 16 hours to induce inflammation; JSHT group: treated with JSHT for 12 hours; Pre-JSHT+LPS group: cells were pre-treated with JSHT for 1 hour, then LPS treatment for 16 hours; Post-JSHT+LPS group: cells were exposed to LPS for 16 hours followed by JSHT for 12 hours. Measurements were performed for DAMPs such as high mobility group box 1 (HMGB1), formyl peptide receptor 1 (FPR1), and extracellular adenosine triphosphate (ATP); transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); phosphorylated mitogen-activated protein kinase (p-MAPK) and c-Jun N-terminal kinase (p-JNK); apoptotic marker cleaved Caspase 3 (cCaspase 3); and pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α).

Measurement of DAMPs

A549 cells were cultured in a 6-well plate and incubated at 37℃ for 24 h in a humidified incubator. Afterward, the cells were cultured under the conditions of the five experimental groups; the medium was collected and centrifuged, and then the supernatant was stored at -80℃. Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to measure the expression levels of DAMP, including HMGB1, FPR1, and extracellular ATP, in the medium, following the manufacturer's instructions. The absorbance was measured at a specified wavelength using an Infinite 200 PRO microplate reader.

Measurement of cCaspase-3, NF-kB, p-MAPK, and p-JNK

The cells were collected and lysed in cold Radioimmunoprecipitation Assay buffer containing protein and phosphatase inhibitors. Proteins were dispensed into each well and subjected to electrophoresis on a Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis at 140 volts for one hour, followed by the transfer of proteins onto

Polyvinylidene Difluoride (PVDF) membranes at 200 mA for two hours. The membranes were blocked with TOOLSpeed Blocking Reagent and incubated with cCaspase-3, NF-kB, p-MAPK, and p-JNK primary antibodies overnight at 4℃, then with secondary antibodies for an additional hour. The protein bands were visualized using enhanced chemiluminescence reagents and radiographic films. The intensities of the reactive bands were analyzed using the Bio-Rad ChemiDoc XRS+ system.

Cytokines ELISA

A549 cells were cultured in a 6-well plate in a humidified incubator for 24 hours at 37℃. Following culture, the cells were subjected to the conditions of the five experimental groups. The culture medium of each experimental treatment group was collected and stored at -80℃. The cytokines were quantified utilizing ELISA kits, following the protocols provided by the manufacturer.