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Therapeutic effect of Chinese medicine formula DSQRL on experimental pulmonary fibrosis

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Abstract

Pulmonary fibrosis (PF) is a restrictive lung disease that may occur idiopathically or as a complication of many diseases. The outcome of the current treatment by glucocorticoids remains very unsatisfactory. This study has tested a new Chinese medicine formula DSQRL for the treatment of experimental PF in comparison with prednisone. Seventy-two rats with PF induced by CCl_4 were randomly divided into four groups to undertake the treatment of either (a) high dose of prednisone; (b) Chinese medicine formula DSQRL; (c) combined treatment of the above two; or (d) tap water of the same volume. At the end of 30 days treatment, the DSQRL treatment achieved a better outcome ($p < 0.05$) than prednisone in terms of histological examination, bronchoalveolar lavage fluid analysis, hydroxyproline assay and complications. The observations support further investigation and clinical trials of this Chinese medicinal formula for the treatment of PF.

Keywords: Pulmonary fibrosis; Prednisone; Chinese medicine; Rat

1. Introduction

Pulmonary fibrosis (PF) is a devastating disease that can be idiopathic or develop as a complication of many respiratory and systemic diseases. Worldwide there are five million people affected by this restrictive lung disease in which ∼65% of the cases are idiopathic. Regardless of the origin, there is an imbalance in PF between inept matrix formation and collagen degradation, and the consequent progressive replacement of the normal lung interstitium by fibrous tissues leading to restriction of lung and gradual loss of function (Razzaque and Taguchi, 2003). As the pathogenesis of the disease remains unclear, the current treatments of PF still often invoke general anti-inflammatory agent glucocorticoids, although anti-fibrotic medications such as pirfenidone, interferon-γ, colchicines and penicillamine are also tried recently (Nathan, 2005). However, the overall outcome of glucocorticoid therapy that remains to be the mainstay of the treatment is by no means satisfactory, with only 10–30% of idiopathic PF patients show some objective improvement even partial and transient (Abdelaziz et al., 2005; Nathan, 2005). Moreover, adverse effects are often seen in the long treatment by glucocorticoids, such as compromised immunity, peptic ulcer, hypertension, osteoporosis, endocrine and metabolic abnormalities, etc.

Traditional Chinese medicine (TCM) treats the PF condition differently by taking a holistic approach. It considers the disease as a weakness of *lung* and stagnation of *blood* and *sputum*, though it must be emphasized that many TCM concepts such as *blood* and *sputum* bear very different meaning to Western medicine. Thus, the common TCM approach for the treatment is to nourish body *Qi* and *Yin*, replenish the *lung*, promote *blood* circulation and to resolve the *sputum*. The commonly used TCM herbs to achieve these purposes include *Radix salviae miltiorrhizae*, *Radix angelicae sinensis* and *Rhizoma chuanxiong*. Previous researches have used various combinations of the above mentioned herbs as the chief medicine to improve PF in animal models. Some recent clinical trials also demonstrated that TCM can improve the lung functions and survival rate of patients with PF, with minimal adverse effects (Dai et al., 2004; Li et al., 2005).

In the current study, we used a new Decoction for Strengthening *Qi* and Replenishing *Lung* (DSQRL) to treat experimental PF induced by carbon tetrachloride (CCl_4) in comparison with glucocorticoid treatment.

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2. Methodology

2.1. PF model

Eighty healthy male Sprague–Dawley rats (200–220 g) were purchased from Chinese University of Hong Kong, housed under controlled conditions of temperature and humidity, and fed with standard rodent chow and water. The experiment was approved by the Hong Kong Baptist University Committee on the Use of Human & Animal Subjects in Teaching & Research, and performed according to the established guidelines.

The rats were injected with CCl_4 (Sigma–Aldrich Chromasolv®, diluted with equal volume of paraffin oil) 0.1 ml per 100 g intra-peritoneally. Four injections were carried out on the 1st, 5th, 9th and 14th days of the experiment, respectively. On the 16th day of the experiment, two rats were sacrificed to validate the pulmonary pathology before any treatment started.

2.2. Treatments

On day 17, 72 rats were randomly divided into four groups with equal number of 18 in each. They were given the following treatments in equal volume (2.5 ml) via a gastric feeding tube twice a day.

(a) Prednisone acetate (Southern China Pharmaceutical Co. Ltd., Guangdong, China) 1.269 mg per 200 g/day was given, equivalent to high dosage (1 mg/kg day) in adult human (Nathan, 2005).
(b) *DSQRL*. Normal prescription of DSQRL for an adult human is: *Radix ginseng* 15 g, *Radix astragali* 30 g, *Radix ophiopogonis* 15 g, *Radix angelicae sinensis* 10 g, *Radix salviae miltiorrhizae* 15 g and *Rhizoma chuanxiong* 15 g. They were boiled in a water extraction system and concentrated by a rotatory evaporator such that each ml contains 1 g raw herbs. Each rat received 1.8 g raw herbs per 200 g body weight diluted in 2.5 ml water.
(c) Combined treatment of both of the above.
(d) Control group, with sham water feeding only.

2.3. Observations and analyses

The animals' general behavior and body weights were recorded everyday. After 30 days treatment, the rats were sacrificed. The lungs were taken out together with the heart and trachea, photographed, inflated with 10% formalin via the trachea, fixed in cold formalin for 10 days, and then cut into 5 μm sections. For histology examination, 20 sections were randomly chosen from upper, middle and lower lobes, respectively, from each specimen, and stained with Masson's Trichrome method to highlight fibrotic changes.

Since most (99%) of the hydroxyproline in vertebrates is found in collagen fiber, the assay of this compound was used as a direct measure to quantify the severity of fibrosis. The removed lung was homogenized in 2 ml of PBS solution (pH 7.4), digested in 6N HCl for 8 h at 120 °C. Five microliters of sample were mixed with 5 μl citrate/acetate buffer, then 100 μl of Ehrlich's solution, and then incubated for 15 min at 65 °C. The hydroxyproline concentration was analyzed by spectrophotometer at 550 nm, and cross-compared with a standard curve constructed by 0–500 μg/ml hydroxyproline.

To quantifying the degree of pulmonary injuries, bronchoalveolar lavage (BAL) fluid was collected from the lungs. The freshly collected lungs were lavaged with a total volume of 5 ml warm sterile PBS solution. The BAL fluid was centrifuged at 1000 rpm. After removal of the supernatant, the cell pallets were collected and re-suspended in 2 ml PBS solution. After staining with trypan blue, the cell numbers were counted by means of hemocytometer.

Mean and S.D. were derived from each group, and statistical analysis was carried out by using Statistics Package for Social Science (SPSS) 12.0 for Windows. Differences among groups were assessed by one-way analysis of variance (ANOVA) and the Tukey test where $p < 0.05$ was considered significant.

3. Results and discussion

3.1. PF induction

After four doses of CCl_4 injection, the autopsy at day 16 demonstrated that the animals' lung had diffuse hemorrhagic interstitial pneumoconitis, with apparently thickened interstitium and mononuclear cell infiltration. Collagen fiber was seen increased mainly around vasculature, and the lesion appeared more severe in the lower lobes.

CCl_4 induced pulmonary fibrosis model was adopted in this experiment instead of commonly used bleomycin induction, chiefly because it has great consistency in making the lesion, as has been demonstrated in a previous report (Pääkkö et al., 1996) and our pilot study. In order to assess the treatment effect reliably, a highly reliable and reproducible model is apparently crucial. Furthermore, the model is relatively easy to make by an i.p. injection that favors a large scale study. In contrast, bleomycin instillation is more operator-dependent and the tracheotomy is sometimes needed for small animals. From our pilot study to compare the two models, we found that the PF induced by CCl_4 was diffusely distributed, particularly at the periphery of the lower lobes. Consistent with a previous report (Borzone et al., 2001), the bleomycin-induced lesion was found inconsistent and mainly focused around bronchi where the drug reached depending on the operator's manipulation and liquid surface tension.

3.2. Treatment grouping and general observations

The rats treated with prednisone had noticeably worse general behavior and hair condition, and they had significantly less body weight gain during the treatment period than the DSQRL and control groups ($p < 0.005$).

3.3. Histology examination

At the termination of the experiment after the treatments for 30 days, the lungs in the control group that received sham treat-