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Protective roles of Cordyceps on lung fibrosis in cellular and rat models

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ABSTRACT

Ethnopharmacological relevance: *Cordyceps sinensis* is a fungus used in traditional Chinese medicine as a tonic to soothe the lung for the treatment of fatigue and respiratory diseases.

Idiopathic pulmonary fibrosis is a chronic, irreversible and debilitating lung disease showing fibroblast/myofibroblast expansion and excessive deposition of extracellular matrix in the interstitium leading to breathing difficulty. Our previous observation revealed a partial relief of lung fibrosis in patients suffering from severe acute respiratory syndrome (SARS). We hypothesize that Cordyceps has beneficial effects on lung fibrosis and the objective of this study is to explore the target(s) of Cordyceps in the relief of lung fibrosis in animal and cell models and to gain insight into its underlying mechanisms.

Material and methods: A rat model of bleomycin (BLM)-induced lung fibrosis and a fibrotic cell model with transforming growth factor beta-1 induction were employed in the studies.

Results: Reduction of infiltration of inflammatory cells, deposition of fibroblastic loci and collagen, formation of reactive oxygen species, and production of cytokines, as well as recovery from imbalance of MMP-9/TIMP-1, were observed in fibrotic rats after treatment with Cordyceps in preventive (from the day of BLM administration) and therapeutic (from 14 days after BLM) regimens. In a fibrotic cell model with transforming growth factor beta-1 induction, the human lung epithelial A549 acquired a mesenchymal phenotype and an increase of vimentin expression with a concomitant decrease of E-cadherin. This epithelial–mesenchymal transition could be partially reverted by cordycepin, a major component of Cordyceps.

Conclusion: The findings provide an insight into the preventive and therapeutic potentials of Cordyceps for the treatment of lung fibrosis.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, irreversible and debilitating lung disease showing progressive scarring of the alveolar tissue and leading to difficulty in breathing. The etiology of this disease is largely unknown but it is generally considered to be a result of exposure to occupational and/or environmental risk factors acting in concert with genetic susceptibility to fibrosis (Antoniou et al., 2007). There are more than 5 million people worldwide suffering from this disease, and there is no effective treatment or a cure. Most patients die from this lung disorder within 5 years of diagnosis (Verma and Slutsky, 2007).

IPF has a poor prognosis (Kinnula and Myllärniemi, 2008), and it normally initiates inflammation and oxidant production in the site of injury at early stage. It is characterized by transdifferentiation of

Abbreviations: BALF, bronchoalveolar lavage fluid; BLM, bleomycin; CE, Cordyceps powder extract in culture medium; CS, Cordyceps sinensis; CS1, low-dose CS powder suspension treatment; CS2, high-dose CS powder suspension treatment; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; IPF, idiopathic pulmonary fibrosis; MMP, matrix metalloproteinases; ROS, reactive oxygen species; TGF-β1, transforming growth factor beta-1; TIMP, tissue inhibitors of metalloproteinases

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the epithelial cells, accumulation of fibroblasts/myofibroblasts, formation of fibroblastic foci, and distortion of the lung architecture (Antoniou et al., 2007). The change of epithelial cobblestone-like morphology to myofibroblast phenotype through epithelial–mesenchymal transition (EMT), and the expression of mesenchymal marker vimentin with a concomitant disruption of E-cadherin junction are the hallmarks for IPF. Many patients, however, show little evidence of inflammation, and anti-inflammatory treatments have little impact on the disease (Willis et al., 2006).

The development and progression of IPF is associated with an activation of transforming growth factor beta-1 (TGF-β1), which induces excessive production and deposition of collagen and extracellular matrix (ECM) by the myofibroblasts, as well as disruption of the basement membrane by an imbalance of gelatinases (matrix metalloproteinases) and tissue inhibitors of metalloproteinases (MMP/TIMP) (Coward et al., 2010). The coupling of mitochondrial dysfunction with reactive oxygen species (ROS) production also aggravates the epithelial cell damage in idiopathic interstitial disorder (Kabuyama et al., 2010).

Cordyceps sinensis (Dong-Chong-Xia-Cao, abbreviated as CS) is an entomogenous fungus whose mycelia grow inside the pupae or larvae of Lepidoptera moth in the autumn, with its fruiting body protruding from the dead insect body during the summer. A renowned Chinese medicine to soothe the lung, CS is now often used for the treatment of respiratory diseases and strengthening immune responses. It has also been reported to be an antioxidant and an inhibitor of angiogenesis. Cordyceps extracts have been shown to relieve fibrosis in the liver (Das et al., 2010; Zhu et al., 1998a, 1998b), kidney (Chai et al., 2009), and lung (Wang et al., 2007; Xu et al., 2011) through an inhibition of TGF-β1 expression (Wang et al., 2007; Xu et al., 2011) and promotion of collagen degradation (Li et al., 2006a). The dried form of CS contains ergosterol, polysaccharides, glycoprotein and peptides. Adenosine and cordycepin (3′-deoxyadenosine) are the major components (Li et al., 2006b; Tsai et al., 2010) and they have been demonstrated to modulate cell activation and alter cell morphology (Shin et al., 2009). Several therapeutic strategies targeting various stages of fibrogenesis are being investigated (Selman et al., 2011), and it is the objective of the present study to explore the target(s) of CS in the relief of lung fibrosis and to gain insight into its underlying mechanisms.

2. Materials and methods

2.1. Preparation of Cordyceps

Dried *Cordyceps sinensis* (Berk.) Sacc. (containing the mycelia-filled insect body and the fungal fruiting body) originated from Tibet was donated by the Eu Yan Sang (Hong Kong) Ltd. The herbal materials were authenticated by Prof. Songming Liang of the School of Chinese Medicine (SCM), the Chinese University of Hong Kong, and voucher specimens have been deposited in the herbarium of the SCM. The herbal drug was ground to powder, passed through a sieve of aperture no. 6, and stored at 4° C until use. To mimic the traditional route of administration, the powder was suspended in distilled water and given to the animals intragastrically using a feeding tube.

2.2. Rat model for pulmonary fibrosis induced by bleomycin

All experiments were performed in accordance with the Hong Kong Guidelines for Animal Welfare and the protocols were approved by the Animal Research Ethics Committee of the Chinese University of Hong Kong (Ref. nos. 08/026/ERG and 461908).

Male Sprague-Dawley rats of 8–12 weeks old were housed in normal laboratory conditions at 21 ± 2° C under a 12/12 light–dark cycle. Pulmonary fibrosis was induced by intra-tracheal administration of a single dose of 2.5 mg/kg of bleomycin (BLM, Nippon Kayaku Co. Ltd., Japan) dissolved in 0.25 mL of physiological saline (Bonniaud et al., 2005) (Day 1). Pulmonary fibrosis developed in 2 weeks (Day 14), confirmed by morphological changes in the lungs and an accumulation of excessive interstitial collagen (Chaudhary et al., 2006). Intragastric administration of CS during the course of fibrosis development (Days 1–14 after BLM administration) was considered prophylactic/preventive, whereas administration of CS after fibrosis had already developed (Days 14–28) was considered therapeutic in the present protocol.

In order to determine an optimal dose of CS, Experiment 1 was performed in which 32 rats were equally and randomly divided into four groups, namely (a) bleomycin-induced fibrosis rats (BLM group), (b) low-dose CS treatment (CS1) (0.54 g/kg body weight, equivalent to 2-fold human dose of 0.27 g/kg) (Hui and Chan, 2006), (c) high-dose CS treatment (CS2) (1.35 g/kg body weight, 5-fold human dose), and (d) normal animals receiving vehicle (CTL) as sham controls. The CS1 and CS2 groups of animals received daily intragastric administration of CS1 or CS2 (each 0.5 mL) for 14 days before the lung tissues were collected for morphological and biochemical analyses.

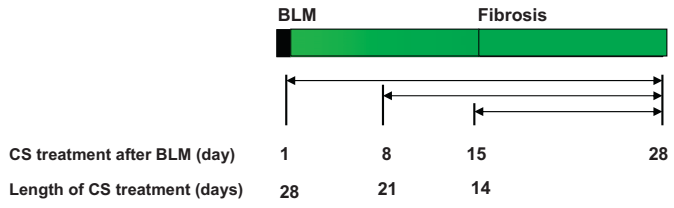
All rats survived BLM treatment. The results of Experiment 1 showed more potent antifibrotic activity in the CS2 group. A dose of CS at 1.35 g/kg was therefore used in the subsequent time course study in Experiment 2 (see Scheme 1). Thus, forty rats were divided into five groups: four groups received intra-tracheal administration of BLM, and the fifth group only received water as sham control (CTL). Among the four groups of BLM-treated rats, three groups received daily CS2 dose (1.35 g/kg) starting from Day 1, Day 8 and Day 15, respectively, and they were all sacrificed on Day 28. One group of fibrotic rats received water to serve as positive lung fibrosis control.

2.2.1. Microscopy

Rats were anaesthetized by 3.5% chloral hydrate (1 mL/100 g body weight) and the lungs were removed and divided into two halves: the right lung was used for differential alveolar cell counts by May-Grunwald–Giemsa stain, and the left lung was further separated into two portions: The upper portion fixed in 3% paraformaldehyde for histopathological and immunohistochemical analyses, and the lower portion frozen in liquid nitrogen for RNA analysis and histochemical analysis of ROS production (oxidative stress) in alveolar tissues.

2.2.2. Differential cell count

Alveolar cells were collected from right lungs of treated and untreated rats by bronchoalveolar lavage with physiological saline. Cells in the bronchoalveolar lavage fluid (BALF) were spread on clean glass slides using a Thermo Shandon Cytospin 2 centrifuge (Southern Products Ltd., Cheshire, UK), air-dried, fixed with ethanol, and stained with May Grünwald–Giemsa. Total cell number and percentages of macrophages, lymphocytes,



Scheme 1. Schematic diagram showing CS treatment schedules in Experiment 2.