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Original article

Cordycepin inhibits airway remodeling in a rat model of chronic asthma

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ABSTRACT

The potential suppression role of cordycepin (Cor) on airway remodeling in a rat model of chronic asthma was investigated in this paper. We evaluated the anti-remodeling of Cor (50 mg/kg) combined with or without budesonide (BUD) and investigated the possible underlying molecular mechanisms. We found that Cor attenuated immunoglobulin (Ig) E, alleviated the airway wall thickness, and decreased eosinophils and neutrophils in the bronchoalveolar lavage fluid (BALF). Notably, Cor reduced the up-regulation of IL-5, IL-13 and TNF-α in the BALF. Cor also regulated the increase of A_{2A}ARmRNA and the decrease of TGF-β_1 expression. Furthermore, Cor markedly blocked p38MAPK signaling pathway activation in the OVA-driven asthmatic mice. The combination treatment of Cor and BUD showed profound efficacy in regulating the levels of inflammatory cells and the expression of IL-13, TGF-β_1 and A_{2A}ARmRNA. Collectively, this study demonstrated that Cor combined with glucocorticoids treatment shows synergistically profound efficacy in inhibiting airway remodeling, and some benefits of Cor may result from the increased A_{2A}ARmRNA expression, the reduced TGF-β_1 levels and the inhibition of Th2-cytokines through the suppression of the p38MAPK signaling pathways.

1. Introduction

Cumulative evidence has revealed that allergic asthma is a major global epidemic and a public health problem due to its rapidly rising prevalence, morbidity, and mortality, and the number of individuals with allergic asthma is rapidly increasing worldwide which currently affects approximately 20% of the world's population [1–3]. Although asthma is mostly well controlled by conventional therapies including inhaled corticosteroids, about 5–10% of asthma patients have a severe phenotype described as "steroid-insensitive asthma" or "difficult to control asthma" [4–6], that is to say, glucocorticoids insensitivity, and a number of chronic asthmatic patients develop irreversible alterations of pulmonary function. These alterations result from structural changes of the airways, known as airway remodeling [7,8], which is a prominent pathologic feature of asthma. The extent of airway wall thickening is associated with disease progression, and this thickening is the most important cause in the reduction of lung function [9]. The severity of asthma is related to the degree of airway remodeling, which is mostly marked in cases of fatal asthma. However, despite advances in understanding the inflammatory features of asthma, the cellular and molecular mechanisms underlying the remodeling process in asthma remain poorly understood.

TGF-β, with important immunomodulatory and fibrogenic characteristics, has been implicated in inflammation and remodeling processes. Gene polymorphisms in the TGF-β_1 promoter have been associated with asthma development [10]. TGF-β_1 has direct effects on remodeling in mouse models; instillation into mouse lungs and TGF-β_1 transgenic overexpression or adenoviral expression in the airway epithelium can induce airway collagen mRNA and protein deposition [11]. Consequently, anti-TGF-β_1 antibody was effective in inhibiting pulmonary fibrosis and significantly reduced collagen deposition, smooth muscle cell proliferation, and goblet cell mucus production in an asthma model [12]. In OVA-sensitized asthmatic mice, significantly reduced TGF-β_1 mRNA levels in pulmonary tissue and protein

Abbreviations: Cor, cordycepin; OVA, ovalubumin; AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; PBS, phosphate-buffered saline; Ig, immunoglobulin; IL, interleukin; mRNA, messenger ribonucleic acid; RT-PCR, reverse transcription–polymerase chain reaction; TNF-α, tumour necrosis factor-α; MAPK, mitogen-activated protein kinase; ERK, extracellular regulating kinase.

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levels in BALF inhibited airway remodeling [13]. These studies suggest that the expression of TGF-β_1 is an important contributor to airway remodeling in asthma.

Cordyceps militaris, is a caterpillar-grown traditional medicinal mushroom. Cordycepin (Cor) (3′-deoxyadenosine), a major bioactive component isolated from cordyceps militaris, has been reported to possess multiple pharmacological activities, including anti-anoxia, anti-inflammatory immunological modulation and anti-hepatic fibrosis, but the molecular mechanisms of Cor on airway remodeling of asthma have not been clearly elucidated yet.

From previous literature, we know adenosine is a key endogenous molecule that regulates tissue function by activating four G-protein coupled adenosine receptors: A_1, A_{2A}, A_{2B} and A_3. Of the four receptors, the adenosine A_{2A} receptor has been strongly linked to controlling inflammation, so we hypothesize that the target of Cor regulating the airway remodeling might be on the A_{2A} receptor. However, there have been no data from respiratory disease models in animals that would support the concept. In addition, in regard to the high risk of adverse effects of glucocorticoids including diabetes, osteoporosis, muscle wasting, skin thinning and weight gain [14,15], and the corticosteroid insensitivity of asthma therapy, more effective therapeutic approaches are needed to explore. So we report here the effects of Cor, a potent and selective adenosine A_{2A} receptor agonist, on the airway remodeling of asthma models of the ovalubumin (OVA)-sensitized Wistar rats, explore its underlying mechanism, observe the synergistic effects of combination therapy of Cor and glucocorticoids, and provide evidence of the potential therapeutic values of Cor.

2. Materials and methods

2.1. Experimental animals

8-week-old female Wistar rats, weighing 200 ± 30 g, were purchased from Shanghai Laboratory Animal Co. Ltd., (Shanghai, China). The rats were divided randomly into each experimental groups and housed five rats in each laminar flow cabinet under specific pathogen free conditions and maintained on 12-h light-dark cycle, with food and water ad libitum. All the animal experiments were strictly conducted in accordance with the protocols approved by the Ethics Committee for Animal Studies at Shanghai General Hospital, China. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Antigen sensitization, challenge and treatment protocol

50 rats were randomly divided into five groups: control group (sensitized and challenged with saline), OVA-sensitized asthma group (sensitized and challenged with OVA), Cor treatment group (50 mg/kg), budesonide (BUD) (10 mg/L) treatment group, and combination treatment group (BUD + Cor). Rats in the last four groups were sensitized on days 0 and 8 by intraperitoneal injection of 1 mg OVA (Grade V; Sigma, St. Louis, MO, USA) together with 200 mg of $Al(OH)_3$ in 1 mL of saline. Simultaneously, 1 mL of Bordetella pertussis vaccine, containing 6×10^9 heat killed organisms, was administered intraperitoneally as an adjuvant. Then they were challenged to OVA (1% in saline) aerosolized for 30 min via airways thrice weekly since day 15. The aerosol was generated with a nebulizer (PARIBOY N037; PARI, Starnberg, Germany) and was drawn into the transparent plastic challenged chamber (approximate volume of 4L) containing the awake animals. Rats in Cor treatment group were administered intragastrically with Cor at a dose of 50 mg/kg daily since day 58 for 8 weeks. Rats in BUD treatment group were treated with aerosolized budesonide (AstraZeneca AB) 10 mg/L for 30 min and intragastrically with saline at the same time before challenged, rats in combination treatment group received the same schedule for aerosolized drug as BUD treatment group and intragastrically with Cor 50 mg/kg instead of saline, the duration of treatment is 8 weeks. Animals were killed 24 h after the last intragastric medicine or intranasal inhalation.

2.3. BALF collection and determination of cell numbers

After the rats were killed with sodium pentobarbital, the lungs were lavaged through the tracheal cannula with PBS, airway lumina were washed three times with 0.4 mL sterile saline, then BALF were collected. The cell suspension was concentrated by centrifugation (1000 rpm, 10 min, and 4 °C). The supernants were stored at −80 °C until use. The cell pellet was resuspended in 1 mL of saline. To perform the differential leukocyte cell count, 0.1 mL of the cell suspension was smeared on a glass slide and stained with Wright-Giemsa. Two hundred nucleated cells were then counted under × 400 magnification by the same inspector in a blinded manner. Cells were identified by standard morphology and classified as eosinophils, lymphocytes, neutrophils, and macrophages.

2.4. Analysis of tumour necrosis factor (TNF)-α and Th2 associated cytokines in BALF and total IgE in serum

TNF-α, IL-5 and IL-13 of BALF and the level of total IgE in serum was examined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's recommendations (R&D Systems, Minneapolis, MN, USA, and BioSource International, Camarillo, CA, USA). The limit of detection was 0.2 ng/ml. Data were expressed as ELISA units, which was the product of the dilution and the optical density.

2.5. Histological analysis of lung tissue and measurement of bronchial wall thickness

After BALF, the right lobe of the lung from each animal was removed for histological examination. The lungs were fully inflated by intratracheal perfusion with 4% paraformaldehyde. The remains were micro-dissected and placed into liquid nitrogen and kept in −80 °C refreeze till further process for Western blotting. Lungs were then dissected and placed in fresh paraformaldehyde for 48 h. Routine histological techniques were used to paraffin-embed the tissue, and 3-μm sections of lung tissue were stained with hematoxylin–eosin (HE). Digital image analysis was performed on histological sections, using Image-Pro Plus software version 6.0 (Media/cybernetics, Silver Springs, MD, USA), the bronchial wall thickness was analyzed by applying the Axiovision Rel 4.8 software (Carl Zeiss). In general, 3 bronchi with a diameter of 150–200 Fm while the lumen shortest diameter/longest diameter ≥0.6 from each lung tissue sections stained with HE were selected under the Axioplan microscope (Carl Zeiss) under the magnification of 400 × and quantitative analysis was performed. The thickness of bronchial wall was calculated according to the formula: Wat/Pi = (The outer area of the airway wall (Ao)-The inner area of the airway wall (Ai))/The perimeter of the inner airway wall (Pi).

2.6. Immunohistochemistry of TGF-β_1

Sections (3 μm thickness) cut from right lung tissues embedded in paraffin blocks were deparaffinized with xylene and hydrated in ethanol. The presence of TGF-β_1 were confirmed by immunohistochemistry. For immunohistochemical detection of TGF-β_1, the lung section were incubated overnight at 4 °C with either primary