International Immunopharmacology 26 (2015) 401–408

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Cordycepin alleviates airway hyperreactivity in a murine model of asthma by attenuating the inflammatory process

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ARTICLE INFO

Article history:
Received 15 September 2014
Received in revised form 4 April 2015
Accepted 8 April 2015
Available online 22 April 2015

Keywords:
Cordycepin (Cor)
Ovalbumin (Ova)
Allergic inflammation
Hyperresponsiveness

ABSTRACT

Cordycepin (Cor), which is a naturally occurring nucleoside derivative isolated from *Cordyceps militaris*, has been shown to exert excellent antiinflammatory activity in a murine model of acute lung injury. Thus, this study aimed to evaluate the antiasthmatic activity of Cor (10, 20, and 40 mg/kg) and to investigate the possible underlying molecular mechanisms. We found that Cor attenuated airway hyperresponsiveness, mucus hypersecretion, and ovalbumin (Ova)-specific immunoglobulin (Ig) E, and alleviated lung inflammation with decreased eosinophils and macrophages in the bronchoalveolar lavage (BAL) fluid. Notably, Cor reduced the upregulation of eotaxin, intercellular cell adhesion molecule-1 (ICAM-1), IL-4, IL-5, and IL-13 in the BAL fluid. Furthermore, Cor markedly blocked p38-MAPK and nuclear factor-kappaB (NF-κB) signalling pathway activation in the Ova-driven asthmatic mice. In conclusion, this study demonstrated that some of the antiasthmatic benefits of Cor attributable to diets and/or tonics may result from reductions in inflammatory processes and that these antiasthmatic properties involve the inhibition of Th2-type responses through the suppression of the p38-MAPK and NF-κB signalling pathways.

1. Introduction

Numerous components of Chinese medicinal herbs exert excellent antiinflammatory effects through the negative regulation of mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B (NF-κB) signalling pathways [1–3]. Pharmaceutical and dietary strategies have targeted these signalling cascades to control asthma, and further many natural products with strong pharmacological properties are good candidates for the alleviation or prevention of asthma [4,5]. *Cordyceps militaris* or Dong-Chong-Xia-Cao (winter worm summer grass) in Chinese, which is a caterpillar-grown traditional medicinal mushroom, has been used as a natural invigorant for longevity, endurance, and vitality for thousands of years in China [6]. Cordycepin (Cor, 3′-deoxyadenosine), which is a nucleoside derivative purified from *C. militaris*, is prescribed for various diseases, such as cancer and chronic inflammation. Multiple biological activities of Cor have been recently elucidated, including antiviral, antifungal, antiinflammatory, antihyperglycemic, and antiatherosclerotic activities [7–9]. Kim and coworkers have previously reported that Cor has antiinflammatory effects in LPS-induced raw 264.7 macrophage cells in association with the suppression of NF-κB activation [10]. Pretreatment with Cor attenuates the inflammatory process, which is an initial step in asthma progression, through antiinflammatory pathways. Nevertheless, no studies have thoroughly explored the possible molecular mechanisms underlying the antiasthmatic activity of Cor in the Ova-driven asthmatic mice.

Cumulative evidence has revealed that the number of individuals with allergic asthma is a rapidly increasing worldwide and that it currently affects approximately 20% of the world's population. It has been described as a global public health problem due to its prevalence, morbidity, and mortality [11,12]. The pathophysiology of allergic asthma is complex and is caused by an aberrant immune response to common allergens, principally coordinated by the Th2-type immune response [13,14]. This allergic reaction is associated with chronic airway inflammation manifested by bronchial hyper-responsiveness, IgE production, mucus hypersecretion, and infiltration of leukocytes, mainly eosinophils, in the airway or lung tissues [13,15]. Several studies have shed light on Th2-dominant eosinophilic inflammation in the airway or lung tissues, aiding in the understanding of the pathogenesis of allergic asthma. Th2 cells participate centrally in all stages of allergic asthma,

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http://dx.doi.org/10.1016/j.intimp.2015.04.017
1567-5769/

beginning with their secretion of Th2 cytokines, such as IL-4, IL-5, and IL-13, which are considered to be responsible for many of the features of asthma. Furthermore, these Th2 cell-derived cytokines, representing the hallmarks of Th2 immunity in models of allergic asthma, are involved in the production of immunoglobulin (Ig) E by B cells and also in goblet cell hyperplasia with increased mucus secretion [16–18]. Importantly, during the course of asthma, dendritic cells (DCs), serving as sentinels in the airway because of their roles in antigen presentation, are critical for the development of the Th2 immune response and are also linked with the production of proinflammatory cytokines associated with the toll-like receptor 4 ligand and its downstream signal transduction pathways, typically MAPKs and NF-κB [19]. Thus, the blockade of MAPKs and NF-κB activation may be effective in reducing allergic airway inflammation [20,21].

Considering that Cor is a component of the extremely rare medicinal mushroom *C. militaris* and may possess antiallergic functions, as described in the literature. In this study, we investigated whether Cor (10, 20, and 40 mg/kg) ameliorates Ova-induced airway inflammation in addition to its underlying mechanism in a murine model of allergic asthma, attempting to provide evidence of the potential therapeutic values of traditional Chinese medicine for the treatment of asthma.

2. Materials and methods

2.1. Animals and reagents

Male BALB/c mice, 6 weeks old weighing approximately 18 g, were purchased from Shanghai Jingke Industrial Co., Ltd (Certificate: SCXK2003-0003, Shanghai, China). The laboratory temperature was maintained at 24 ± 1 °C, and relative humidity was controlled at approximately 60%. Mice were housed in sterilised microisolator cages, with filtered air and autoclaved bedding, food, and water. The animal experimental protocols were approved by the Ethical Committee on Animal Research of Jilin University. Mice were allowed to acclimatise for 1 week before the onset of the experiments with a 12 h light/dark cycle (light 8 am to 8 pm). All experimental procedures were performed in accordance with the guide for the Care and Use of Laboratory Animals established by the US National Institutes of Health. No mice were dead and no apparent signs of exhaustion were observed during the experimental period.

Cordycepin (Cor, purity: >98%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ova (Grade δ), aluminium hydroxide adjuvant, and dexamethasone (Dex, purity: >99.6%) were provided by Sigma-Aldrich Trading Co., Ltd. (St. Louis, MO, USA). Mouse interleukin (IL)-4, IL-5, IL-13, eotaxin, and intercellular cell adhesion molecule 1 (ICAM-1) ELISA kits were obtained from Biolegend (San Diego, CA, USA). The ELISA kit for immunoglobulin (Ig) E was purchased from R&D (Anniston, AL, USA). The primary antibodies including phosphorylated and non-phosphorylated forms of mitogen-activated protein kinases (MAPKs) family and nuclear factor-kappaB (NF-κB) and the horseradish peroxidase-labelled IgG secondary antibodies were purchased from Cell Signalling Technology Inc (Beverly, MA, USA). Polyclonal rabbit β-actin antibodies were provided by Tianjin Sungene Biotech Co., Ltd (Tianjin, China). Other chemical reagents were obtained from Beijing Dingguo Changsheng Biotech Co., Ltd (Beijing, China). All these reagents were of analytical grade, unless otherwise specified.

2.2. Experimental design

Allergen sensitization/challenge protocol: Male mice were randomly assigned to six groups ($n = 8$) as follows: (1) the Control (Cont) group; (2) the Ova group; (3) the Ova + Cor 10 group; (4) the Ova + Cor 20 group; (5) the Ova + Cor 40 group; and (6) the Ova + Dex group. Hereafter, these group abbreviations are presented to clarify the text. The mice were immunised on days 0, 7, and 14 by intraperitoneal (*i.p.*) injection of 20 μg Ova emulsified in 1 mg aluminium hydroxide adjuvant in a total volume of 0.2 mL [22]. On days 23, 24, 25, and 26 after the initial sensitisation, the mice were anesthetised with an *i.p.* injection of 0.2 mL of a mixture of ketamine (0.44 mg/mL) and xylazine (6.3 mg/mL) in normal saline. The mice were placed on a board in the supine position. Subsequently, they were intranasally challenged with a 2% (*w*/*v*) Ova solution in phosphate-buffered saline (PBS, pH = 7.2), as described previously with minor modifications [4]. The Cont mice received equivalent volumes of PBS without Ova *i.p.* on days 0, 7, and 14 and then were challenged with PBS without Ova (*w*/*v*) each day from days 23 to 26. Airway hyperresponsiveness (AHR) was measured at 24 h after the final Ova challenge on day 27, and then the mice were sacrificed to characterise the protective effects of Cor. The schematic diagram of the treatment schedule is presented in Fig. 1.

Administration of drugs: Mice were administered an intraperitoneal injection of normal saline or Cor (10, 20, and 40 mg/kg, dissolved in normal saline) at 1 h prior to each corresponding Ova challenge on days 23, 24, 25 and 26. Dex, which is a steroid hormone drug of the glucocorticoid class, is a potent inhibitor of airway inflammation and remodelling [23,24]. Thus, in our present study, Dex (2 mg/kg, dissolved in normal saline, *i.p.*) served as a positive control.

2.3. Collection of blood and bronchoalveolar lavage (BAL) fluid and lung tissue separation

Twenty-four hours after the last challenge, the mice were anesthetised and bled via the brachial plexus for the collection of blood samples, which were used to estimate IgE production. The collection of BAL fluid was performed three times through a tracheal cannula with 0.5 mL of autoclaved PBS (pH = 7.2) to yield a total volume of 1.3 mL. The recovery rate of the fluid was approximately 87%. The collected BAL fluid samples were centrifuged at 700 *x*g for 10 min at 4 °C, and the supernatants were frozen at −80 °C immediately for further enzyme-linked immunosorbent assays (ELISAs). The cell pellets were then treated and used as samples for inflammatory cell analysis. Lung tissues were harvested simultaneously from mice not subjected to BAL fluid collection and then were treated or stored at −80 °C for the other experiments.

2.4. Mouse anti-Ova IgE measurement

The levels of Ova-specific IgE in the serum were measured by an ELISA as described previously [25]. Briefly, microtitre plates were coated with 1% Ova in coating buffer (0.05 M sodium carbonate–bicarbonate, pH = 9.6) overnight at 4 °C. After blocking and washing, diluted serum samples (1/20) were added at room temperature for 2 h and

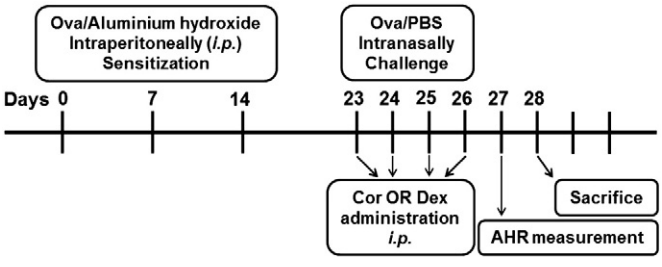

Fig. 1. Experimental protocol for development of allergic asthma and treatment with Cor (10, 20, and 40 mg/kg) or Dex (2 mg/kg) using a murine model. Mice were divided into four groups ($n = 8$) and sensitised via an intraperitoneal injection of 20 μg Ova emulsified in 1 mg aluminium hydroxide in 200 μL PBS on days 0, 7, and 14, respectively. Subsequently, mice were given an intranasal instillation with 2% (*w*/*v*) Ova solution in PBS on days 23, 24, 25, and 26 after the initial sensitization. Mice were given an intraperitoneal injection of Cor (10, 20, and 40 mg/kg, dissolved in normal saline) or Dex (2 mg/kg, diluted in normal saline) each day from days 23 to 26 consecutively, 1 h prior to each corresponding Ova challenge. Control mice were sensitised and challenged with equivalent volumes of PBS without drug administration. AHR assay was performed 24 h after the last Ova challenge. Then all mice were sacrificed for further experiments.