Free Radical Biology and Medicine 101 (2016) 163–175

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Review article

Caffeic acid phenethyl ester alleviates asthma by regulating the airway microenvironment via the ROS-responsive MAPK/Akt pathway

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ARTICLE INFO

Keywords:
CAPE
Airway microenvironment
ROS
TNF-α
TGF-β1

ABSTRACT

In the pathophysiology of asthma, structural cell dysfunction and concomitant microenvironment changes in airways are crucial to pathological progression, which involves oxidative stress. Caffeic acid phenethyl ester (CAPE) is an active anti-oxidative component obtained from propolis, and has been shown to have beneficial effects on several respiratory disorders, such as chronic obstructive pulmonary disease and lung cancer. However, the impact of CAPE on asthma is not well understood. Therefore, this study investigated the advantages of using CAPE to treat asthma and demonstrated the roles of CAPE in the regulation of airway microenvironments. In ovalbumin (OVA)-sensitized mice, CAPE treatments notably reduced airway hyperresponsiveness, attenuated extensive inflammatory cell infiltration and inhibited goblet cell hyperplasia and collagen deposition and fibrosis. In addition, CAPE improved the airway microenvironment in a dose-dependent manner by inhibiting OVA-induced increases in immunoglobulin E, tumor necrosis factor alpha (TNF-α), transforming growth factor-β1 (TGF-β1), interleukin (IL)-4 and IL-13 and suppressing matrix metalloproteinase-9 and alpha-smooth muscle actin expression as well as malondialdehyde production. To determine the underlying mechanisms responsible for these effects, we used TNF-α-stimulated BECs and TGF-β1-challenged human ASMCs to explore the impacts of CAPE on pro-inflammatory proteins and ASMC proliferation. The results indicated that CAPE significantly limited the secretion of eotaxin-1, monocyte chemoattractant protein-1, IL-8 and intercellular adhesion molecule-1 and dramatically inhibited the proliferation of ASMCs. These effects were shown to be associated with decreased reactive oxidant species (ROS) levels. The phosphorylation of Akt and Mitogen-Activated Protein Kinase (MAPK) caused by increased ROS was significantly decreased by CAPE, which implied a contribution of ROS-MAPK/Akt signaling to the attenuation of asthma. Our findings indicated for the first time that CAPE alleviates airway inflammation and remodeling in chronic asthma by balancing the airway microenvironment, which highlights a novel profile of CAPE as a potent agent for asthma management.

1. Introduction

Asthma is a complex clinical syndrome characterized by airway hyperresponsiveness (AHR), inflammation and remodeling, and it is linked to an increased generation of reactive oxidant species (ROS) and impaired antioxidant defenses [1,2]. Airway structural cells, such as human bronchial epithelial cells (BECs) and airway smooth muscle cells (ASMCs), play important roles in the development of asthma [3,4]. Insults such as infection, allergens or environmental factors can activate BECs, initiate airway inflammation by polarizing T-helper type 2 (Th2) lymphocytes and promote pro-inflammatory protein secretion, including eotaxin-1, monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-8 and intercellular adhesion molecule-1 (ICAM-1) [5–7]. These pro-inflammatory proteins have complex cross-talk signaling and contribute to the change of airway microenvironment, which refers to the intercellular substance and secretion of various cytokines [8,9]. Insults can also alter the profile of ASMCs to produce a range of extracellular matrix (ECM) proteins, such as collagen and matrix metalloproteinases (MMPs), which leads to increases in the ASMC size (hypertrophy) and number (hyperplasia). Such changes contribute to the development of AHR and airway remodeling, which in turn affect airway inflammation and determine the outcome of asthma

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http://dx.doi.org/10.1016/j.freeradbiomed.2016.09.012
Received 16 March 2016; Received in revised form 16 August 2016; Accepted 12 September 2016
Available online 13 October 2016

[10,11].

Furthermore, insults can induce high levels of ROS, which can be generated by activated inflammatory cells and airway structural cells [12–15]. Accumulating data have reported that ROS in airway structural cells exacerbate responses to allergen challenge and cause a number of detrimental effects on airway functions, including airway smooth muscle contraction, AHR induction, mucus hypersecretion, epithelial shedding, and vascular exudation, and these changes produce a vicious cycle [14,16–19]. However, current knowledge of the pathophysiologic roles of ROS in asthmatic airway microenvironment remain incomplete.

ROS has been confirmed to correlate with tumor necrosis factor alpha (TNF-α) and transforming growth factor-β1 (TGF-β1) [20–24]. TNF-α is an important pro-inflammatory molecule that induces ROS generation, elicits pro-inflammatory mediators and evokes the activation of various cells, thereby leading to an amplification of inflammatory responses [23,25]. TGF-β1 primarily contributes to airway smooth muscle dysfunctions in asthma by eliciting ASMC proliferation and hypertrophy, which is probably attributed to the generation of intracellular ROS [26]. Herein, we established an inflammatory model with TNF-α and a proliferation model with TGF-β1 in vitro.

Galangin and caffeic acid phenethyl ester (CAPE) (Fig. 1A) are two potent ingredients of propolis. Our previous findings established that galangin could alleviate ovalbumin (OVA)-induced airway inflammation and remolding by inhibiting the NF-κB and MAPK/Akt pathways, respectively [1,27]. Recently we found that the anti-oxidant effect of CAPE is more pronounced than that of galangin. As previous studies have reported, CAPE presents anti-tumor and anti-allergic properties in various disorders. CAPE attenuates cigarette smoke-induced airway inflammation in chronic obstructive pulmonary disease (COPD) through NF-κB inactivation [28]. More recently, CAPE was reported to prevent TGF-β1-induced epithelial-mesenchymal transition in human alveolar epithelial cells via PPARγ activation, suggesting a regulatory potential of CAPE in pulmonary fibrosis and remodeling [29]. Evidence from animal studies suggested that CAPE alleviates OVA-induced airway inflammation while the mechanism has not been determined [30]. And the contribution of CAPE to the pathophysiology of chronic asthma remains incompletely defined. The present study was designed to investigate the effects of CAPE on airway inflammation and remodeling in chronic asthma and to identify the mechanisms in vivo and in vitro.

2. Materials and methods

2.1. Animals

Specific pathogen-free female BALB/c mice (18–22 g) aged 6–8 weeks were obtained from Vital River Laboratories (Beijing, China). The mice were maintained in a temperature-controlled room under a 12 h dark/light cycle and provided with food and water ad libitum. All experiments that involved animal and tissue samples were performed in accordance with the guidelines of the National Institutes of Health and Nanjing Medical University, and all of the procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University (Nanjing, China).

2.2. OVA sensitization and challenge

Fig. 1B schematically depicts the protocols used in this study. In total, 42 specific pathogen-free female BALB/c mice were randomly divided into 6 groups as follows: control, OVA (Grade V, Sigma-Aldrich, Milwaukee, WI, USA), OVA+CL (1 mg/kg CAPE, Sigma-Aldrich), OVA+CH (5 mg/kg CAPE), OVA+dexamethasone (1 mg/kg DEX, Sigma-Aldrich) and OVA+dimethylsulfoxide (DMSO, Sigma-Aldrich). The asthmatic models were established by sensitization to OVA. Specifically, all of the mice in the OVA, OVA+CL, OVA+CH, OVA +DEX and OVA+DMSO groups were sensitized on days 0, 7 and 14 by intraperitoneal injection of 20 μg OVA emulsified in 2 mg aluminum hydroxide gel (Invivo-Gen, San Diego, CA, USA) in a total volume of 200 μl. These sensitized mice were exposed to aerosolized 5% OVA in sterile saline for 30 min three times a week for 8 weeks beginning on the 16th day of the experiment. We placed the mice in 51×31×21 cm chambers that were connected to a jet nebulizer (NE-U11B; OmronCorp, Tokyo, Japan) to create a whole-body inhalation system. CAPE (1 and 5 mg/kg), DEX (1 mg/kg, positive control) and DMSO (0.4 μl in 200 μl saline, solvent control) were administered by intraperitoneal injection 30 min before each OVA challenge. The control subjects were sensitized and challenged using the same protocol with saline alone. The mice were sacrificed at 24 h after the final challenge, and bronchoalveolar lavage fluid (BALF) and lung tissues were collected for analyses.

2.3. Measurement and analysis of airway responsiveness

Lung function was evaluated by direct measurement of lung resistance and dynamic compliance in restrained, tracheostomized, mechanically ventilated mice via the FinePointe RC system (Buxco Research Systems, Wilmington, NC) under general anesthesia as described before [31]. .

2.4. BALF collection and differential cell counts

After the AHR measurements, the tracheae were exposed, and then the BALF were collected by lavage with ice-cold phosphate-buffered saline (PBS, 400 μl ×3; 85–90% of the lavage volume was recovered) via a tracheal catheter. The lavage samples from each mouse were centrifuged at 1000 rpm for 10 min at 4 °C. The total number of inflammatory cells in the BALF was counted using a hemocytometer. Differential cell counts were performed using Wright's staining based on the morphological criteria. The number of cells in the BALF was determined by two independent investigators in a single-blind study, and at least 200 cells each were analyzed from three random locations using a microscope. Then, the supernatant was collected, divided into four equal portions and frozen at −80 °C for enzyme-linked immunosorbent assay (ELISA).

2.5. Lung histology

After the BALF samples were obtained, the right lungs were immersed in 4% paraformaldehyde prior to being embedded in paraffin. A series of microsections (5 μm) were cut with a microtome and stained with hematoxylin and eosin (H & E) to assess inflammatory cell infiltration. The inflammation score was determined as follows: grade 0, no inflammation; grade 1, occasional cuffing with inflammatory cells; and grades 2, 3, and 4, most bronchi or vessels surrounded by a thin layer (1–2 cells: grade 2), a moderate layer (3–5 cells: grade 3), or a thick layer (> 5 cells: grade 4) of inflammatory cells, respectively. The total inflammation score was calculated by the addition of the peribronchial (PB) and perivascular (PV) inflammation scores. Periodic acid-Schiff (PAS) staining was used to quantify the airway goblet cells, and Masson's trichrome staining was used to visualize the collagen deposition and fibrosis. Both staining methods were scored as follows: 0, none; 1, < 25%; 2, 25–50%; 3, 50–75%; and 4, > 75% goblet cells [32–34]. Sections were also immunohistochemically stained for matrix metalloproteinase-9 (MMP-9) and alpha-smooth muscle actin (α-SMA). For the semi-quantitative evaluation of MMP-9 and α-SMA expression, we used the modified scoring method by Sinicrope and Lu [32,33]. The mean percentages of positive epithelial cells in the bronchi were determined in at least five areas at 400× magnification and assigned to one of the following categories: 0, < 5%; 1, 5–25%; 2, 25–50%; 3, 50–75%; and 4, > 75%. The immunostaining intensity of MMP-9 and α-SMA was scored as 1+(weak),