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Research paper

Oligomeric proanthocyanidins attenuate airway inflammation in asthma by inhibiting dendritic cells maturation

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ABSTRACT

To date, although a promising anti-inflammatory activity of oligomeric proanthocyanidins (OPCs) has been observed in asthma, the mechanism responsible for these immunomodulatory properties remains obscure. Dendritic cells (DCs) that reside in the airway have been widely perceived as an important contributor to asthma. Our study was to demonstrate OPCs' effects on maturation and immunoregulation of pulmonary $CD11c^+$ dendritic cells (DCs). BALB/c mice were exposed to ovalbumin (OVA) to induce murine model of asthma. In addition, pulmonary DCs and bone marrow-derived DCs (BMDCs) cultures were used to evaluate impacts of OPCs on DCs function. The results obtained here indicated that OPCs treatment dramatically reduced airway inflammation, such as the infiltration of inflammatory cells and the levels of allergen-specific serum IgE and Th2 cytokines. The expression of co-stimulatory molecules especially CD86 distributed on pulmonary DCs and bone marrow-derived DCs (BMDCs) also markedly declined. The phosphorylation of cAMP responsive element-binding protein (CREB) was significantly inhibited while no changes were observed in the expression of cAMP responsive element modulator (CREM). By transferring BMDCs into the airways of naïve mice, we found that OPCs-treated DCs (DC + OVA + OPC) were much less potent in promoting $CD4^+$ T cells proliferation than OVA-pulsed DCs (DC + OVA), followed by the ameliorated eosinophilic inflammation in airway. Our findings tailor a novel profile of OPCs in the regulation of DCs function, shedding new light on the therapeutic potential of OPCs in asthma management.

1. Introduction

Allergic asthma is an atopic airway disease characterized by airway inflammation, hyperresponsiveness (AHR), remodeling and mucus hypersecretion. Although Th2 cells have been widely confirmed to contribute most to the cytokines production in asthma (Cho et al., 2016; Leigh et al., 2004), the vital role of dendritic cells (DCs) in the initiation and extension of allergic inflammation might not be underestimated (van Helden and Lambrecht, 2013; Hammad et al., 2010; Phythian-Adams et al., 2010). Serving as a sentinel unit to incoming antigens, DCs are the most efficient antigen-presenting cells. Upon exposure, DCs undergo a maturation process involving the secretion of assorted cytokines as well as the transformation of co-stimulatory molecules (Lombardi et al., 2010). Actually, DCs maturation is so pivotal for uncommitted Th0 to differentiate to specific effector T subsets, promoting the development of consequent airway inflammation (Cochand et al., 1999; Vermaelen et al., 2001). Thus, pulmonary DCs have taken the center stage as cellular therapeutic targets in asthma.

Comprising the mainstay of asthma therapy, corticosteroids are known as non-specific agents and have presented eminent systemic and topical side effects. Since the poor performance of corticosteroids in curing asthma radically or even retarding the disease progression, recent studies have paid sufficient attention to natural plant products as a complementary or an alternative treatment.

Oligomeric proanthocyanidins (OPCs) are polyphenolic compounds found in vegetables, flowers, fruits and grape seeds. OPCs bind less

Abbreviations: OPCs, oligomeric proanthocyanidins; DCs, dendritic cells; OVA, ovalbumin; BALF, bronchiolar lavage fluid; AHR, airway hyperresponsiveness; CREB, cAMP responsive element-binding protein; CREM, cAMP responsive element modulator; IL, interleukin; Mch, methacholine; MFI, median fluorescence intensity; RL, resistance of lung; CD, cluster of differentiation; BMDCs, bone marrow derived dendritic cells

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strongly to proteins, and are more soluble and biologically active (Fine, 2000). More specific pharmacological actions of OPCs have been recently reported, such as scavenging free radical, anti-oxidative damage and regulating immune response. Previous studies have shown that OPCs exhibited an anti-inflammatory activity in some disorders such as inflammatory bowel disease (Wang et al., 2011) and arthritis (Park et al., 2011). It has also been demonstrated that OPCs effectively suppressed airway inflammation, AHR and remodeling in asthma (Lee et al., 2012). However, the underlying mechanism still remains obscure.

Our preliminary study has revealed that OPCs exhibited a potent potential in asthma management (data not published). As the major components of inflammatory network, DCs are involved in the pathogenesis of asthma. In the present study, by using pulmonary DCs and bone marrow-derived DCs (BMDCs), we investigated the impacts of OPCs on DCs function both *in vivo* and *in vitro*.

2. Materials and methods

2.1. Animals

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

2.2. Ovalbumin (OVA) sensitization and challenge

Fig. 1B schematically depicts the protocols used in this study. In total, 49 specific pathogen-free female BABL/c mice were randomly divided into 7 groups (n = 7 per group) as follows: control; ovalbumin (OVA) (Grade V, Sigma-Aldrich, St. Louis, MO, USA), OVA + OL (OPC 7.5 mg/kg OPC, Sigma-Aldrich); OVA + OM (OPC 15 mg/kg OPC); OVA + OH (OPC 30 mg/kg OPC) (OL, OM and OH represent low, medium and high dose of OPCs, respectively); OVA + dexamethasone (DEX, 1 mg/kg); and OVA+ dimethylsulfoxide (DMSO). Dexamethasone is known for its potent anti-inflammatory effect and used as the positive-control. DMSO is the solvent for OPCs and used as the vehicle-control. The acute asthma model was established by OVA sensitization. Specifically, all of the mice in the OVA, OVA + OL, OVA + OM, OVA + OH, OVA + DEX and OVA + DMSO groups were sensitized on days 0, 7 and 14 by the intraperitoneal injection of 50 μg of OVA emulsified in 2 mg of aluminum hydroxide gel (Invivo-Gen, San Diego, CA, USA) in a total volume of 200 μL. Mice were anesthetized using inhaled sevoflurane and instilled with an OVA solution (4 mg/mL) alone by administering 12.5 μL per nostril on days 20, 21 and 22. OPCs and DMSO (0.4 μL in a total of 200 μL of saline) were administered by intraperitoneal injection at 30 min prior to each OVA challenge (Daubeuf and Frossard, 2013). The control subjects were sensitized and challenged using the same protocol with saline alone. All mice were sacrificed 24 h after the last OVA challenge. Bronchoalveolar lavage fluid (BALF) and lung tissues were collected for analysis.

2.3. Measurement and analysis of airway responsiveness

Lung function was assessed by direct measurement of lung resistance. Mice underwent tracheostomies and were mechanically ventilated using the FinePointe RC system (Buxco Research Systems, Wilmington, NC, USA) under general anesthesia as previously described (Kerzerho et al., 2013).

2.4. BALF collection and differential cell counts

After measurement of AHR, the tracheae were exposed and BALF was collected by lavage with ice-cold phosphate-buffered saline via a tracheal catheter (PBS, 400 μL × 3; 85%-90% of the lavage volume was recovered). The lavage samples from each mouse were centrifuged at 1000 rpm for 10 min at 4 °C. The total number of cells in the BALF was counted on a hemocytometer. Differential cell counts were performed using Wright's staining on the basis of morphological criteria by two independent investigators in a single-blind study and at least 200 cells each were analyzed from three different random locations using a microscope. Then, the supernatant was collected, divided into 5 equal portions and frozen at −80 °C for use in an enzyme-linked immunosorbent assay (ELISA).

2.5. Lung histologic analysis

Lungs were inflated with 4% paraformaldehyde at a standard pressure of 20 cm H_2O. Tissues were then paraffin embedded and cut at a thickness of 5 μm. Sections were stained with hematoxylin and eosin

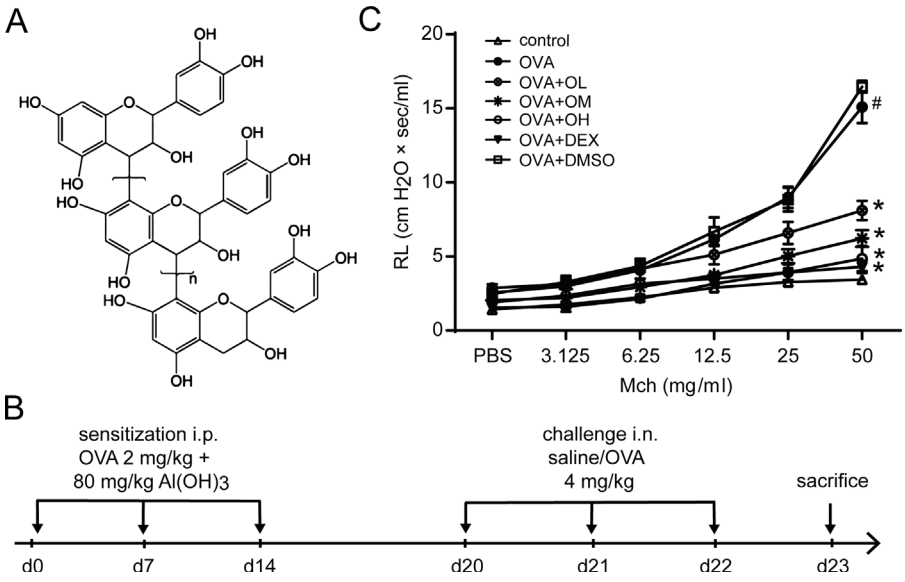

Fig. 1. Chemical structure of OPCs and experimental protocol for the acute asthma model. Treatment with OPCs decreased AHR. (A) General chemical structure of OPCs. (B) Brief flowchart of animal sensitization and challenge. (C) Mice inhaled increasing doses of methacholine (3.125–50 mg/mL). AHR was measured and shown as RL (cm H_2O × s/mL). Data represent the mean + SEM. (n = 7 per group). $^{\#}P < 0.05$ compared to the control group, and $^{*}P < 0.05$ compared to the OVA group.