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Chrysin alleviates allergic inflammation and airway remodeling in a murine model of chronic asthma

Jing Yao [a], Mingzi Jiang [b], Yunshi Zhang [a], Xing Liu [a], Qiang Du [a,*,1], Ganzhu Feng [a,*,1]

[a] Department of Respiratory Medicine, the Second Affiliated Hospital of Nanjing Medical University, 121 Jiangjiayuan Road, Nanjing, Jiangsu 210000, PR China
[b] Department of Respiratory Medicine, The First People's Hospital of Kunshan, Qianjinxi Road No. 91, Kunshan, Jiangsu 215300, PR China

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ABSTRACT

Asthma is a chronic airway inflammatory disorder and progresses mainly due to airway remodeling. Chrysin, a natural flavonoid, has been reported to possess multiple biologic activities, including anti-inflammation, anti-oxidation and anti-proliferation. The present study aimed to investigate whether chrysin could relieve allergic airway inflammation and remodeling in a murine model of chronic asthma and the mechanism involved. The female BALB/c mice sensitized and challenged with ovalbumin (OVA) successfully developed airway hyperresponsiveness (AHR), inflammation and remodeling. The experimental data showed that chrysin could alleviate OVA-induced AHR. Chrysin could also reduce OVA-induced increases in the number of inflammatory cells, especially eosinophils, interleukin (IL) -4, and IL-13 in bronchoalveolar lavage fluid (BALF) and total IgE in serum. The decreased interferon-γ (IFN-γ) level in BALF was also upregulated by chrysin. In addition, inflammatory cell infiltration, goblet cell hyperplasia and the expression of α-smooth muscle actin (α-SMA) around bronchioles were suppressed by chrysin. Furthermore, the phosphorylation levels of Akt and extracellular signal-regulated kinase (ERK) could be decreased by chrysin, which are associated with airway smooth muscle cell (ASMC) proliferation. These results indicate the promising therapeutic effect of chrysin on chronic asthma, especially the progression of airway remodeling.

1. Introduction

Asthma is a chronic airway inflammatory disease associated with reversible airway obstruction, airway hyperresponsiveness (AHR) and airway remodeling [1]. T-helper type 1 (Th1)/T-helper type 2 (Th2) imbalance is classically considered to lead to asthma, related to upregulated Th2 cytokine production, decreased Th1 cytokine production, increased IgE and eosinophilic inflammation in the airway [2].Airway inflammation and remodeling are believed to interact with each other, resulting in the occurrence and progress of asthma. Among these, airway remodeling manifests a range of structural alterations, including increased deposition of extracellular matrix proteins (ECMs), goblet cell metaplasia, angiogenesis and increased airway smooth muscle mass, which has been proposed to contribute to persistent airflow limitation and lower baseline of lung function [3]. Airway smooth muscle cells (ASMCs), as a pivotal element involved in asthma, not only participates in airway inflammation and AHR, but also play a key role in remodeling mainly due to its increased mass. Several signaling molecules are involved in ASMC proliferation, such as extracellular signal-regulated kinase (ERK) and Akt [4]. Nowadays, bronchodilatation and anti-inflammation are the main therapies for asthma [5], which can't stop the progression of structural changes effectively [6]. Corticosteroids still play a crucial role in asthma control, but with lots of side effects [7]. New effective drugs are urgently needed for asthma therapy.

An increasing number of researches indicate that natural products and traditional medicines may be promising in asthma treatment. Flavonoids are commonly present in vegetables, nuts, fruits, beverages or herbal remedies, described as a health-promoting and disease-preventing dietary supplement [8]. Chrysin (5, 7-dihydroxyflavone) is a natural flavonoid, widely distributed in medicinal herbs [9,10]. It has been confirmed that chrysin exerts multiple biological activities, such as anti-inflammation, anti-proliferation and anti-oxidation [11–13]. However, the impacts of chrysin on chronic asthma are still unknown. In our previous study, we have demonstrated that chrysin could significantly inhibit the proliferation of human airway smooth muscle cells (HASMCs) through ERK1/2 pathway [14]. Therefore, we assumed that chrysin might play a therapeutic role in chronic asthma. In the present study, we aimed to define the impacts of chrysin on the ovalbumin (OVA)-sensitized and challenged chronic asthma model and the mechanism involved. We also used dexamethasone (DEX) as a positive treatment control [15].

* Corresponding authors at: Department of Respiratory Medicine, The Second Affiliated Hospital of Nanjing Medical University, 121 Jiangjiayuan Road, Nanjing, Jiangsu 210011, PR China.
E-mail addresses: jingshuyue@163.com (Q. Du), fenggz@njmu.edu.cn (G. Feng).
[1] The two corresponding authors contributed equally to this article.

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2. Method

2.1. Antigen sensitization, challenge and treatment

Fotry-eight female specific pathogen-free (SPF) BALB/c mice (6 weeks old) were purchased from Cavens Laboratory Animal Company (Changzhou, China) and housed in the SPF facility. The mice were acclimatized for a week before experimental study. All experimental procedures were approved by the institutional animal ethics committee. Animal experiments were carried out in strict accordance with the Nanjing Medical University's guidelines for their care and use. Mice were randomly divided into four groups: (a) control group; (b) OVA group; (c) DEX group and (d) chrysin group. On Days 1 and 14, the mice in the OVA, DEX and chrysin groups were immunized by intraperitoneal injection of 20 μg OVA (Grade V; Sigma, St Louis, MO, USA) emulsified in 2.25 mg aluminum hydroxide (InvivoGen, San Diego, CA, USA) in a total volume of 0.2 ml. For aerosolized challenges, mice were placed in a Plexiglas box (29 × 22 × 18 cm) and exposed to aerosolized OVA or phosphate buffer solution (PBS) using an ultrasonic nebulizer (PAPI TurboBOY N, Germany). From Day 21, the mice of OVA, DEX and chrysin groups were exposed to aerosolized 1% OVA for 30 min per day, 3 days per week for 8 weeks. The mice in the DEX group received an intraperitoneal injection of DEX (2 mg/kg body weight) 30 min before every OVA challenge. Chrysin (purity 97%; Sigma, St. Louis, MO, USA) was suspended in a 1% carboxymethylcellulose (CMC) solution. From Day 21, the mice in chrysin group were administered with chrysin (100 mg/kg body weight) intragastrically and daily for 8 consecutive weeks. The mice in the control group received the same schedule for sensitization and challenge with PBS. After sensitization and challenge, six animals of each group were used for detection of airway resistance, the other six were used for collection of bronchoalveolar lavage fluid (BALF) and lung tissues (as shown in Fig. 1).

2.2. Evaluation of AHR

Airway responsiveness to acetylcholine chloride (Ach; Sigma, St. Louis, MO, USA) was measured with a whole-body and invasive plethysmography (Buxco Electronics Inc., NY, USA). Twenty-four hours after the last OVA challenge, mice were anesthetized by intraperitoneal injection of pentobarbital sodium (75 mg/kg body weight). A tracheal tube was inserted into the trachea via a tracheotomy. Then, mice were placed in the whole-body plethysmography chamber and the tracheal tube was connected to the ventilator for mechanical ventilation with a tidal volume of 0.2 ml and a frequency of 140 breaths/min. After 3 min of equilibration on the ventilator, PBS and increasing doses of Ach (3.125, 6.25, 12.5 and 25 mg/ml) were administered through the ventilator with an ultra-sonic nebulizer. Lung resistance (R_L) was measured to express the change of AHR.

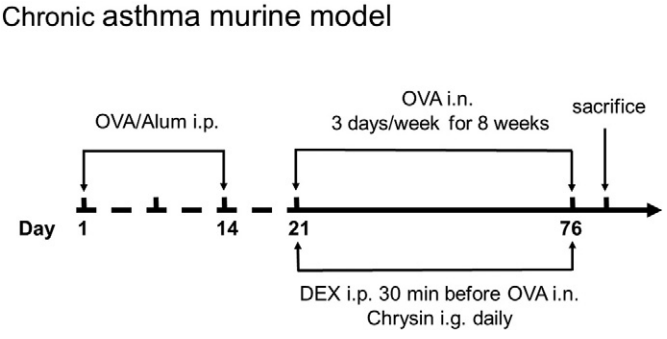

Fig. 1. Experimental protocol for chronic asthma murine model. BALB/c mice were sensitized with OVA and aluminum hydroxide by intraperitoneal injection on Days 1 and 14 and then challenged with aerosolized 1% OVA three times per week for 8 weeks from Day 21. The control mice were sensitized and challenged with PBS using the same protocol. DEX was administered via intraperitoneal injection 30 min before the challenge. Chrysin was administered intragastrically and daily from Day 21. Mice were sacrificed within 24 h after the last OVA challenge and treatment. OVA, ovalbumin; Alum, aluminum hydroxide; i.p., intraperitoneal injection; i.g., intragastric administration; i.n., inhalation; DEX, dexamethasone.

2.3. Analysis of BALF and serum

The mice were anesthetized and bled from the retro-orbital venous plexus 24 h after the last OVA challenge. Blood samples were centrifuged (20 min, 4 °C, 1000 rpm), and the plasma was stored at −80 °C until use. After the removal of blood, the lungs were washed two times by intratracheal instillation of 0.5 ml ice-cold PBS via a tracheotomy to collect BALF. The BALF was centrifuged (10 min, 4 °C, 1000 rpm) and the supernatants were transferred and stored at −80 °C before use for the cytokine detections. Cell pellets were resuspended in ice-cold PBS with a final volume of 30 μl. The numbers of total and different inflammatory cells in BALF were determined with a hemocytometer. The levels of interleukin (IL)-4, interferon-γ (IFN-γ) (Biotechnology Company, Wuhan, China) and IL-13 (eBioscience, San Diego, Calif., USA) in BALF and total serum IgE (CUSABIO Biotechnology Company, Wuhan, China) were determined by ELISA according to the manufacturer's protocol.

2.4. Histological examination

The right lower lungs obtained from sacrificed mice were immersed in 4% paraformaldehyde overnight, and then embedded in paraffin and sectioned. The sections (5 μm thick) were stained with hematoxylin–eosin (HE), periodic acid–Schiff (PAS) and Masson's trichrome stain [16]. Histological analysis was performed by pathologists blinded to grouping. To quantify the level of mucus production in airways, numbers of PAS-negative and PAS-positive epithelial cells (goblet cells) in individual bronchioles were counted. Results were expressed as the percentage of PAS-positive cells per bronchiole, which was calculated from the number of PAS-positive epithelial cells per bronchiole divided by the total number of epithelial cells in each bronchiole. The area of peribronchial trichrome staining in lung tissues was quantified with an image-analysis system (Image-Pro Plus 6.0; Media Cybernetics, Inc., Georgia, USA). Results are expressed as the area of trichrome staining per μm length of basement membrane of bronchioles with an internal diameter of 150–200 μm by counting at least 10 bronchioles in each slide.

2.5. Immunohistochemistry analysis

Sections of lung tissues were deparaffinized in xylene and rehydrated in graduated ethanol solutions. Endogenous peroxidase activity was quenched by 3% H_2O_2. To avoid non-specific absorption of immunoglobulin, specimens were blocked with 5% non-fat milk. Thereafter, specimens were incubated with mouse monoclonal antibody against α-smooth muscle actin (α-SMA; DAKO, Glostrup, Denmark) at a dilution of 1:50 at 4 °C overnight. Substitution of the primary antibody with normal mouse IgG was used as a negative control. Sections were then incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (Bioworld Technology, Shanghai, China) for 30 min at room temperature, followed by staining with diaminobenzidine (DAB; Santa Cruz, CA, USA) [17,18]. Sections were counter-stained with hematoxylin and observed under a light microscope. Data were expressed as the area of α-SMA immunostaining per μm length of basement membrane of bronchioles with an internal diameter of 150–200 μm by counting at least 10 bronchioles in each slide, which was examined using an image-analysis system (Image-Pro Plus 6.0; Media Cybernetics, Inc., Georgia, USA).