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Triptolide suppresses airway goblet cell hyperplasia and Muc5ac expression *via* NF-κB in a murine model of asthma

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ABSTRACT

Background: We have reported that triptolide inhibited pulmonary inflammation in patients with steroid-resistant asthma. In the present study, we investigated whether suppresses airway remodeling and goblet cell hyperplasia, studied the mechanism of triptolide on mucin5ac (Muc5ac) expression in a murine model of asthma.
Methods: BALB/c mice were sensitized to intraperitoneal ovalbumin (OVA) followed by repetitive ovalbumin challenge for 6 weeks. Treatments included triptolide (40 μg/kg) and dexamethasone (2 mg/kg). The area of bronchial airway (WAt/Pbm), smooth muscle (WAm/Pbm) and mucus index were assessed 24 h after the final OVA challenge. Levels of Muc5ac were assessed by ELISA, immunohistology and real-time PCR. Western blot was performed to analyze the phosphorylation of NF-κB p65.
Results: Triptolide and dexamethasone significantly reduced allergen-induced increases in the thickness of bronchial airway, smooth muscle and goblet cell hyperplasia. Levels of lung Muc5ac and Muc5ac mRNA were significantly reduced in mice treated with triptolide and dexamethasone. Phosphorylation of NF-κB p65 was significantly reduced in mice treated with triptolide and dexamethasone.
Conclusion: Triptolide may inhibit airway goblet cell hyperplasia and Muc5ac expression in asthmatic mice *via* NF-κB. It may be a potential drug for the treatment of patients with severe asthma.

1. Introduction

Airway mucus hypersecretion is one of the most important pathophysiological changes of asthmatic airway, that also contributes to the high incidence and mortality of asthmatic patients (Lai and Rogers, 2010). Goblet cell is the main producer and secretor of mucus in airway epithelium. Its hyperplasia is not only a part of the airway remodeling, but also an important mechanism leading to deterioration of asthma when degranulation happens severely (Rogers, 2004).

The main ingredient of mucus is mucin. Twenty-one different mucin genes have been identified. Of these, mucin5ac (Muc5ac) is the major respiratory mucin produced by bronchial epithelium and submucosal glands. Muc5ac is the predominant mucin in mild-to-moderate asthma and increases in severe asthma (Kraft et al., 2008; Ueno-Iio et al., 2014; Johnson et al., 2013).

Severe asthma has a distinct pathophysiology including airway hypersecretion that contributes to the decreased effectiveness of standard therapy (Hirota and Martin, 2013). The treatment strategy for severe asthma consists mainly of the use of bronchodilators (such as β-agonists, theophylline, anticholinergics, corticosteroids, H_1-anti-histamine and anti-leukotrienes). However, approximately 5% of patients do not respond to this therapy. For these reasons, effective therapies that are targeted at severe asthma and can inhibit asthma airway hypersecretion are needed.

Triptolide which is a diterpenoid triepoxide purified from a Chinese herb *Tripterygium Wilfordii Hook F* (TWHF) is the major

Abbreviations: WAt, area of airway wall; WAm, area of airway smooth muscle; Pbm, basement membrane perimeter; DEX, dexamethasone; HE, hematoxylin and eosin; IOD, integrated optical density; MMLV, Moloney murine leukemia virus; Muc5ac, mucin5ac; NF-κB, nuclea factor-kappaB; OVA, ovalbumin; PAS, periodic acid-Schiff; TGF-β1, transforming growth factor-β1; TWHF, Tripterygium Wilfordii Hook F; TRI, triptolide.

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component responsible for the immunosuppressive and anti-inflammatory effects of TWHF. Besides, triptolide has the effects of inhibiting proliferation and inducing apoptosis (Li et al., 2014a; Phillips et al., 2007). A lot of clinical and basic studies were performed to investigate the usefulness of triptolide in the treatment of asthma (Zhang and Ma, 2010; Mao et al., 2008). We previously showed that triptolide inhibited pulmonary inflammation in patients with steroid-resistant asthma and some studies indicate that triptolide possesses the benefits of relieving pulmonary pathology and controlling the progress of asthma (Jiang et al., 2006). However, the effects of triptolide on airway goblet cell hyperplasia remain unknown.

NF-κB plays an important role in the signal transduction and modulate a variety of gene expression in the nucleus (Hinz and Scheidereit, 2014). It is known that NF-κB mediates Muc5ac gene expression (Chen et al., 2014; Sikder et al., 2014; Nie et al., 2012). That means under certain conditions, activated NF-κB translocates into the nucleus and triggers the expression of its target genes Muc5ac, which may further cause airway mucus hypersecretion. A few years ago, Chang et al. reported that that triptolide can inhibit the activity of NF-κB (Chang et al., 2007). We therefore propose that triptolide treatment may inhibit airway goblet cell hyperplasia *via* NF-κB signaling pathway.

In the present study, our goal was to investigate the effect of triptolide on airway Muc5ac expression and goblet cell hyperplasia in a murine model of asthma, and if so, whether it works by inactivating NF-κB.

2. Materials and methods

2.1. Animals

BALB/c mice (females) were obtained and maintained in a pathogen-free environment in the facility of Center of Experiment Animal of Sun Yat-sen University (Certificate of Conformity: Guangdong Experimental Animal Testing by certificate No. 2006A059). All the experiments were performed in accordance with the regulations of the Centre of Animal Experiments of Sun Yat-sen University. Ethical approval for this investigation was obtained from the Research Ethics Committee, Sun Yat-sen University. The mice were housed in a temperature controlled room with 12-h dark/light cycles, and allowed food and water *ad libitum*. All the experiments described below were performed in accordance with the Center of Experiment Animal of Sun Yat-sen University regulations.

2.2. Reagents

The following drugs and chemicals were purchased commercially and used: chicken egg ovalbumin (OVA) (grade V, A5503, Sigma, USA); aluminum hydroxide (alum) (Guangzhou Chemical Reagent Factory, China); crystalline triptolide (PG490, molecular weight 360.40, purity 98%) was purchased from Sigma (St. Louis, MO, USA). Triptolide was dissolved in dimethyl sulfoxide (DMSO) and stock solutions (1 mg/ml) stored at −20 °C. Triptolide was freshly diluted to the indicated concentration with culture medium before use in experiments. DMSO concentration in test conditions did not exceed 0.001% (*w*/*v*). Anti-MUC5ac antibody (Abcam Company, Cambridge, UK); Histostain-Plus Kits (ZSGB-BIO, Beijing, China); Trizol (Invitrogen Corporation, USA); PCR kit (promega company, USA); reverse transcriptase kit (Fermentas Inc, USA); antibodies against NF-κB p65, Phospho-NF-κB p65 (Ser536) (Cell Signaling Technology, USA) were purchased. Antibodies against α-actin (Thermo Scientific IHC, Fremont, CA, USA) were also obtained. All other chemicals used were of the highest grade available commercially.

2.3. Sensitization and antigen challenge

Forty female BABL/c mice were randomly divided into four groups with 10 mice in each group: (1) control group: mice were treated with saline; (2) asthma group: mice were sensitized and challenged with OVA mice were sensitized on days 0 and 14 by intraperitoneal injection of 10 μg OVA emulsified in 1 mg of aluminum hydroxide in a total volume of 200 μl. Seven days after the last sensitization, mice were exposed to ovalbumin aerosol (2.5% w/v diluted in sterile physiological saline). Mice were exposed for up to 30 min three times per week for 6 weeks. The aerosol (particle size; 2.0–6.0 μm) was generated by a nebulizer (Ultrasonic nebulizer boy037G6000, Pari, Germen) driven by filling a perspex cylinder chamber (Diameter 50 cm, Height 50 cm) with a nebulized solution; (3) triptolide-treated group (TRI group): mice were sensitized and challenged as asthmatic group, and treated with 40 μg triptolide by intraperitoneal injection before challenged (Chen et al., 2014; Ordonez et al., 2001); (4) dexamethasone-treated group (DEX group): mice were sensitized and challenged as above, and were given 2 mg dexamethasone by intraperitoneal injection before challenged (Ueno-Iio et al., 2014; Johnson et al., 2013).

2.4. Measurement of Muc5ac level in bronchoalveolar lavage fluid

Twenty-four hours after the last challenge, bronchoalveolar lavage fluid (BALF) was obtained from the mice under anesthesia using 1 ml sterile isotonic saline. Lavage was performed four times each mouse and the total volume was collected separately. The volume of fluid collected in each mouse ranged from 3.0 to 3.5 ml. The lavage fluid was centrifuged 3000 rpm at 4 °C for 10 min. The Muc5ac concentrations in the BALF were measured with an ELISA-kit (R&D Systems) following the protocol offered by manufacturer's instruction.

2.5. Lung histology

At 24 h after the last challenge, lungs were removed from the mice after sacrifice. The left lung tissues were fixed with 10% neutral buffered formalin. The specimens were dehydrated and embedded in paraffin. For histological examination, 5 μm sections of fixed embedded tissues were cut on a rotary microtome, placed on glass slides, deparaffinized, and stained sequentially with hematoxylin and eosin (H&E) to assess the airway remodeling. Mucus production and goblet cell hyperplasia were assessed from lung sections stained with periodic acid Schiff (PAS).

2.6. Morphometric analysis

The histological analyses were preformed by observers who were not aware of the groups of mice from which the samples originated. Images were captured with a digital camera. For each sample, the entire length of basement membrane was considered. At least 10 bronchioles with 150–200 μm of inner diameter were selected and counted in each slide. For measurement of tracheal basement membrane thickness, three measures were taken, and the average basement membrane thickness was calculated. The area of airway wall (WAt) and smooth muscle (WAm) were determined by morphometric analysis (image-pro plus6.0; MediaCybernetics Co., USA) on transverse sections after HE staining. Basement membrane perimeter (Pbm) was measured for normalization of WAt and WAm. Then we used WAt/Pbm, WAm/Pbm to evaluate airway remodeling.