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ORIGINAL ARTICLE

Efficacy of parthenolide on lung histopathology in a murine model of asthma

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Abstract
Background: Parthenolide is the active constituent of the plant '*Tanacetum parthenium*' (Feverfew) which has been used for centuries as a folk remedy for inflammatory conditions.
Aim of the study: In this study we aimed to investigate the effects of parthenolide in a murine model of chronic asthma.
Materials and methods: Thirty-five BALB/c mice were divided into five groups; I (control), II (placebo), III (dexamethasone), IV (parthenolide) and V (dexamethasone and parthenolide combination). Lung histology was evaluated after treatment with the study drugs. Levels of interleukin (IL)-4 and IL-5 were determined by ELISA.
Results: Histologic parameters except the number of mast and goblet cells improved in the parthenolide group when compared with placebo. All parameters except basal membrane thickness and number of mast cells were improved significantly better in the group receiving dexamethasone when compared with the parthenolide group. Improvement of most of the histologic parameters was similar in Groups III and V. Interleukin-4 levels were significantly reduced in the parthenolide group when compared to the placebo group.
Conclusion: We demonstrated that parthenolide administration alleviated some of the pathological changes in asthma. But parthenolide alone is not efficient as dexamethasone therapy and the parthenolide and dexamethasone combination also did not add any beneficial effect to the dexamethasone treatment.

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Introduction

Asthma is a chronic inflammatory disease of the airways characterised by airway hyperresponsiveness, airflow obstruction and remodelling. Airway inflammation is the principal factor in the pathogenesis of asthma in which numerous cell types and mediators released from these cells have been demonstrated to play significant roles. The transcription factor, nuclear factor κB (NF-κB) is known to be a critical modulator of inflammation in the pathogenesis of asthma. It is expressed in many cell types, which plays a key role in the expression of many pro-inflammatory genes, leading to synthesis of cytokines, adhesion molecules, chemokines, growth factors and enzymes.[1] Nuclear factor κB pathway is known to be active in asthma. In previous studies it has been demonstrated that levels of NF-κB p65 and NF-κB p50 were increased in asthmatic patients, while in mice lacking NF-κB p50, diminished eosinophilic infiltration was observed in response to an aerolised allergen in a model of allergic asthma.[2–5]

Current strategies for the management of asthma focus mainly on suppressing airway inflammation, and steroids are the most effective anti-inflammatory drugs in asthma.[6] Inhaled steroids are currently the mainstay of asthma therapy but they also have several systemic and local side effects when used at high doses for a long time. Research for the use of alternative and complementary treatments that reverse histopathological changes and have fewer side effects is continuously increasing.

Sesquiterpene lactones are the active constituents of medicinal plants from the Asteraceae family which have been used for centuries as a folk remedy in inflammatory conditions such as migraine, inflammation and arthritis.[7,8] Parthenolide is one of the important sesquiterpene lactones found in plant *Tanacetum Parthenium (feverfew)* and the active constituent of this plant is responsible for its inflammatory effects.[7,8] Previous studies suggest that anti-inflammatory effects of parthenolide are related to inhibition of NF-κB pathway. As this pathway is also known to be active in asthma pathogenesis, it may be speculated that parthenolide may be beneficial in the treatment of asthma. To our knowledge there is no study in the medical literature evaluating the efficacy of parthenolide on chronic airway changes of asthma. The aim of the present study was to investigate the effects of parthenolide on chronic changes of lung in a murine model of asthma for the first time.

Materials and methods

Animals

Conventionally raised, 6- to 8-week-old, 35 female BALB/c mice weighing 20–25 g were used in the experiment. The animals were fed a commercial diet ad libitum and housed in an air-conditioned facility on a 12-h light/12-h dark cycle. The studies adhered to the National Institutes of Health guidelines for the experimental use of animals. The Local Animal Care and Use Committee granted approval for the study.

Experimental protocol

Mice were sensitised by an intraperitoneal (ip) injection of ovalbumin (10 μg/0.1 ml, two weeks apart, i.e. on day 0 and day 14) consisting of a chicken egg albumin (ovalbumin, grade V, ≥98 pure, Sigma, St. Louis, MO, USA) with alum adjuvant as described by Temelkovski et al.[9]. Mice in study groups II, III, IV, V and VI were then challenged with an aerosol of 5 ml 2.5% ovalbumin (OVA) in saline for 30 min per day on three days of the week for eight weeks beginning from the 21st day of the study. The mice in the control group (group I) received normal saline with alum intraperitoneally on days 0 and 14 of the experiment and aerosolised saline without alum for 30 min per day on three days of the week for eight weeks beginning from the 21st day of the study. Exposures were carried out in a whole body inhalation exposure system in a plexiglass chamber with 40 × 60 × 120 diameters designed for placement of cages. Temperature and relative humidity were maintained at 20–25 °C and 40–60%, respectively. A solution of 2.5% ovalbumin in normal saline was aerosolised by delivery of compressed air to a sidestream jet nebuliser with a flow rate of 6 L/min (Medicair, UK) and injected into a chamber. The aerosol generated by this nebuliser comprised >80% particles with a diameter of <4 μm. Particle concentration was maintained in the range of 10–20 mg/mm^3 in the chamber.[10]

Study drugs

Mice in group III received dexamethasone 1 mg/kg;[11] group IV received parthenolide 3 μg/g;[12] group V received both dexamethasone 1 mg/kg and parthenolide 3 μg/g; and group II received DMSO (solvent of parthenolide) 50 μL[13] once a day in the last seven days of the challenge period. Parthenolide was obtained from Calbiochem (Merck Millipore, Darmstadt, Germany). Animals were sacrificed by an overdose of ketamin 24 h after the last drug administration. Experimental protocol and study drugs are presented in Table 1.

Preparations of lung homogenates

Animals were sacrificed by an overdose of ketamin 24 h after the last drug administration. Lungs were removed and washed with cold PBS three times in order to remove blood. Lung tissues were taken into 2 ml microcentrifuge tubes and stored at −80 °C until analysis. On the study day, frozen lungs were thawed, weighed (60–80 mg), transferred into different tubes on ice containing 5 ml of stainless beads, 0.1% SDS, protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO, USA) and 0.1 mg/ml phenylmethanesulfonyl fluoride (PMSF) in PBS. Microcentrifuge tubes were transferred to precooled Tissuelyser LT racks and placed into TissueLyser (Qiagen, Germany) homogenisator. Frequency and time were set to 50 and 5 min, respectively. The homogenates were then centrifugated at 15,000 × *g* for 1 h at 4 °C. The supernatants were stored at −80 °C.

Measurement of cytokines

Levels of IL-4 and IL-5 were quantified in the supernatants of the lung tissue by standard ELISA protocols using

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