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Effect of treatment with geraniol on ovalbumin-induced allergic asthma in mice

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ABSTRACT

Background: Asthma, a complex highly prevalent airway disease, is a major public health problem for which current treatment options are inadequate.
Objective: To evaluate the antiasthma activity of geraniol and investigate its underlying molecular mechanisms.
Methods: In a standard experimental asthma model, Balb/c mice were sensitized with ovalbumin, treated with geraniol (100 or 200 mg/kg) or a vehicle control, during ovalbumin challenge.
Results: Treatment of ovalbumin-sensitized/challenged mice with geraniol significantly decreased airway hyperresponsiveness to inhaled methacholine. Geraniol treatment reduced eotaxin levels in bronchoalveolar lavage fluid and attenuated infiltration of eosinophils induced by ovalbumin. Geraniol treatment reduced T_H2 cytokines (including interleukins 4, 5, and 13), increased T_H1 cytokine interferon γ in bronchoalveolar lavage fluid, and reduced ovalbumin-specific IgE in serum. In addition, treatment of ovalbumin-sensitized/challenged mice with geraniol enhanced T-bet (T_H1 response) messenger RNA expression and reduced GATA-3 (T_H2 response) messenger RNA expression in lungs. Furthermore, treatment of ovalbumin -sensitized/challenged mice with geraniol further enhanced Nrf2 protein expression and activated Nrf2-directed antioxidant pathways, such as glutamate-cysteine ligase, superoxide dismutase, and glutathione S-transferase, and enhanced formation of reduced glutathione and reduced formation of malondialdehyde in lungs.
Conclusion: Geraniol attenuated important features of allergic asthma in mice, possibly through the modulation of T_H1/T_H2 balance and activation the of Nrf2/antioxidant response element pathway.

Introduction

Asthma, characterized by intermittent episodes of wheezing and coughing, is a complex inflammatory disorder that involves inflammation of the pulmonary airways, intermittent reversible airway obstruction, airway hyperreactivity, and excessive mucous production.[1,2] Asthma affects more than 300 million patients worldwide. Of these, 10% to 15% have severe asthma that is refractory to commonly available drugs, such as inhaled glucocorticoid.[3] New therapies for asthma are urgently required.

Natural traditional herbal medicines[4,5] and plant-derived metabolites, such as alkaloids, terpenes, flavanoids, isoflavones, and saponins,[6,7] have potential in the treatment of asthma. Geraniol (GE; 3,7-dimethyl-2,6 octadien-1-ol) is a monoterpene alcohol that naturally occurs in small quantities in geranium, lemon, and other essential oils from medical plants and is the aromatic component in many cosmetic products.[8] Geraniol could be rapidly absorbed to reach peak plasma concentration after approximately half an hour in rodents,[9] Geraniol has exhibited antioxidative,[10,11] antimicrobial,[12] antitumor,[13,14] and anti-inflammatory activities[15,16] in several previous studies. In this work, we sought to delineate the effects of treatment with geraniol in an ovalbumin mouse model of asthma and possible molecular mechanisms of its action.

Methods

Experimental Protocol

Female BALB/c mice aged 6 to 8 weeks and free of murine-specific pathogens were obtained from the Sino-British SIPPR/BK Lab Animal Ltd (Shanghai, China). All the mice used in this work received humane care in adherence with institutional animal care

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guidelines, and the studies were approved by the Animal Ethics Committee. All protocols were conducted in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, formulated by the Ministry of Science and Technology of China. The mice were immunized intraperitoneally with 50 μg of ovalbumin (Sigma-Aldrich, St Louis, Missouri) emulsified in 1 mg of aluminum hydroxide (Pierce Chemical Co, Rockford, Illinois) on days 0, 7, and 14 or just aluminum hydroxide alone. The mice were then challenged with ovalbumin (inhalation of 5% ovalbumin) for 30 minutes each day from day 21 to day 32. The control mice were injected with phosphate-buffered saline and challenged with ovalbumin. Geraniol (Sigma-Aldrich) was dissolved in corn oil (0.25 mL/100 g body weight). Animals receiving only corn oil (0.25 mL/100 g body weight) were used as controls. All drug treatments (100 or 200 mg/kg)[11,17] were conducted by gavage, daily from day 21 to day 32, 1 hour before challenge.

Airway Hyperresponsiveness

The protocol of invasive airway hyperresponsiveness (AHR) measurement was performed as previously described.[18] Lung resistance of inspiration (RL) was used to evaluate the airway responsiveness. Mice were administered 1% (wt/vol) pentobarbital sodium (90 mg/kg) for anesthesia. The neck skin was sterilized with 75% ethanol and incised and the trachea exposed. A tracheal tube was inserted into the trachea and sutured in place. Mice were placed in the body plethysmograph chamber and the tracheal tube connected to the ventilator. Mice received ventilatory support with a tidal volume of 6 mL/kg and a respiratory rate of 90 strokes/min (Bestlab, Beijing, China). Increasing methacholine (0, 0.025, 0.05, 0.1, and 0.2 mg/kg) was injected intravenously at intervals of 5 minutes. Lung function was assessed by the AniRes 2005 lung function system (AniRes 2005, version 3.0; Bestlab).

Histochemistry

Lungs were fixed in 10% buffered formalin solution, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin for routine histopathologic analysis. A standard quantitative scoring system was used to grade the extent of lung infiltrates.[19] In brief, the degree of peribronchial inflammation was evaluated by a single pathologist (X.-g.Z.) who was unaware of the experimental groups on a scale of 0 to 4: 0 indicating no inflammation detectable; 1, occasional cuffing with inflammatory cells; 2, most bronchi were surrounded by a ring of inflammatory cells that were 1 cell layer deep; 3, most bronchi were surrounded by a ring of inflammatory cells that were 2 to 4 cells deep; and 4, most bronchi were surrounded by a thick layer of inflammatory cells (>4 cells deep).

Bronchoalveolar Lavage Fluid

Bronchoalveolar lavage fluid (BALF) was collected at 24 hours after the last challenge. Mice were euthanized, the trachea was isolated by blunt dissection, and a small-caliber tube was inserted into the airway and secured. Two volumes of 1 mL of phosphate-buffered saline with 0.1% bovine serum albumin were instilled, gently aspirated and pooled (BALF), and maintained at 4°C. A mean (SD) of 0.59 (0.04) mL of BALF was regained after the lung lavage. BALF was then centrifuged at 1250*g* for 5 minutes, and the supernatants were collected and stored at −70°C for analysis. BALF cell distribution was quantified in Cytospin preparations (Perbio Science, Lausanne, Switzerland).

The levels of interleukin (IL) 4, IL-5, IL-13, interferon (IFN) γ, and eotaxin were quantified in the supernatant of BALF with enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, Minnesota).

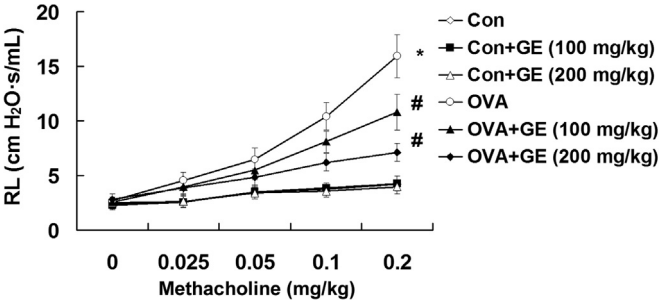

Figure 1. Effect of treatment with geraniol on airway hyperresponsiveness (AHR) to methacholine in ovalbumin (OVA)-sensitized/challenged mice. Con indicates control; RL, lung resistance of inspiration.

Serum IgE

Blood samples were collected at 24 hours after the last challenge, and the serum samples were obtained by centrifugation at 800*g* for 15 minutes at 4°C. The serum IgE levels were quantified by using an enzyme-linked immunosorbent assay kit (Elabscience, Wuhan, China).

Reverse Transcriptase–Polymerase Chain Reaction

Total RNA samples isolated from lungs or cultured cells with TRIzol reagent (Invitrogen, Carlsbad, California) were used for reverse transcription with a SuperScript II reverse transcription kit (Invitrogen) according to the manufacturer's instructions. Real-time polymerase chain reaction analysis was performed with a QuantiTectTM SYBR Green PCR (Qiagen, Shanghai, China) according to the manufacturer's instructions. Amplification was programmed as 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The highly specific measurement of messenger RNA (mRNA) was performed for T-bet (5'-TCAACCAGCACCAGACAGAG-3'; 5'-AACATCCTGTAATGGCTTGTG-3'), GATA-3 (5'-CTTATCAAGCCCAAGCGAAG-3'; 5'-CCCATTAGCG TTCCTCCTC-3'), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (5'-TGTGGGCATCAATGGATTTGG-3'; 5'-ACACCATG-TATTCCGGGTCAAT-3') using the LightCycler system (Bio-Rad, Carlsbad, California). A negative control (distilled water) was included in the test. Each sample was run and analyzed in duplicate. T-bet or GATA-3 mRNA levels were adjusted per the values relative to GAPDH, which was used as the endogenous control to ensure equal starting amounts of complementary DNA. The fold-change relative to control values was calculated as the experimental change in gene expression.

In Vitro Study

$CD4^+$ T cells were purified from a single-cell suspension procured from spleens of mice by means of negative selection with the EasySep kit from Stem Cell Technologies (Vancouver, British Columbia). All $CD4^+$ T cells were stimulated on coated plates (1 mg/mL of anti-CD3 and 0.5 mg/mL of anti-CD28). $CD4^+$ subsets were generated by means of culture under the following conditions: T_H1, IL-12 (10 ng/mL; R&D Systems, Minneapolis, Minnesota), and anti–IL-4 (10 mg/mL; clone 11B11) and T_H2, IL-4 (100 U/mL; R&D Systems), and anti–IL-12 (10 mg/mL; clone C17.8). $CD4^+$ T cells were stimulated with anti-CD3/CD28 antibodies and skewed toward a T_H1 or T_H2 phenotype in the presence or absence of geraniol (100 μM). On day 3 after stimulation, cells were replated with IL-2 alone for an additional 2 days. Then the cells were collected for measurements.