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Lavender essential oil inhalation suppresses allergic airway inflammation and mucous cell hyperplasia in a murine model of asthma

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ABSTRACT

Aims: Lavender essential oil (Lvn) has been reported to have anti-inflammatory effects. Bronchial asthma is characterized by bronchial allergic inflammation with airway remodeling. Therefore, we evaluated the anti-inflammatory effect of Lvn on experimentally induced bronchial asthma in a murine model.
Main methods: BALB/c mice were sensitized by an intraperitoneal injection of ovalbumin (OVA) at days 0 and 14, and subsequently challenged with nebulized OVA on days 28–30 (Control-Asthma group). Mice in the treatment group inhaled Lvn on days 14–31 (Lvn-Asthma group). The allergic inflammatory response was determined on days 32 and 33.
Key findings: An increase in airway resistance was inhibited in the Lvn-Asthma group than in the Control-Asthma group. The Lvn-Asthma group showed lower total cell numbers and eosinophils in bronchoalveolar lavage (BAL) fluids and peribronchial and perivascular tissues when compared with the Control-Asthma group. The Lvn-Asthma group also had less mucin hyperplasia than the Control-Asthma group. Furthermore, the Lvn-Asthma group showed lower interleukin (IL)-5 and IL-13 cytokine levels in BAL fluids, as well as reduced IL-4 and IL-5 mRNA expression in lung tissue, compared with the Control-Asthma group and determined by FlowCytomix and reverse transcriptase-polymerase chain reaction (RT-PCR), respectively. In addition, Lvn inhalation reduced *Muc5b* mRNA expression in the lungs without significantly changing the expression of *Muc5ac* mRNA.
Significance: Lvn inhibits allergic inflammation and mucous cell hyperplasia with suppression of T-helper-2 cell cytokines and *Muc5b* expression in a murine model of asthma. Consequently, Lvn may be useful as an alternative medicine for bronchial asthma.

Introduction

Atopic asthma is the most prevalent form of asthma which is initiated by the exposure to allergens, and resultant of allergen-specific immune responses, such as airflow obstruction, airway hyperresponsiveness (AHR), and airway inflammation (Busse and Lemanske, 2001). The airway dysfunction by inflammation is the major feature in murine asthma model, and causes development of AHR (Takeda and Gelfand, 2009). The development of AHR and airway eosinophilic inflammation depends on T-helper-2 (Th2) cells producing several cytokines, including interleukin (IL)-4, IL-5 and IL-13 (Foster et al., 1996; Perkins et al., 2006).

Hypersecretion of mucus contributes to mucous plugging and airway obstruction in bronchial asthma. MUC5AC and MUC5B are the most prominent gel-forming mucins secreted in the respiratory tract (Curran and Cohn, 2010). Muc5ac and Muc5b are produced by mucous cells, such as goblet cells, submucosal glands, and Clara cells (Evans et al., 2004; Zhu et al., 2008). Muc5ac and Muc5b are upregulated in mucous cells during asthmatic inflammation in murine models (Yu et al., 2006; Zuhdi Alimam et al., 2000). Th2 cytokines are also known to induce mucus production (Whittaker et al., 2002).

Aromatherapy uses volatile aromatic compounds found in essential oils extracted from many types of plants. Essential oils help to improve health and alleviate symptoms, including anxiety, pain, insomnia, and bacterial infections. Lavender essential oil (Lvn) has been reported to have sedative and antibacterial effects (Cavanagh and Wilkinson, 2002). Several biological studies using animal models have clarified that Lvn inhibited the mast cell-dependent ear swelling response induced by injection of compound 48/80 in mice. Lvn also inhibited passive cutaneous anaphylaxis induced by anti-dinitrophenyl (DNP) IgE

Abbreviations: Lvn, lavender essential oil; BAL, bronchoalveolar lavage; AHR, airway hyperresponsiveness; RL, lung resistance; OVA, ovalbumin.

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in rats (Kim and Cho, 1999). Degranulating mast cells secrete several chemical mediators and accelerate the accumulation of inflammatory cells in the bronchial wall, causing multiple features of asthma such as increased AHR, inflammatory cell accumulation in the lungs, and mucous cell hyperplasia (Amin, 2012; Yu et al., 2006). Based on these considerations, we hypothesized that Lvn could attenuate allergic inflammation through suppression of inflammatory cell accumulation and mucous cell hyperplasia. There are no reports evaluating Lvn's effect on allergic inflammation in asthma. Using a murine model of acute asthma, we present the previously unreported effect of Lvn on allergic inflammation and mucous cell hyperplasia.

Materials and methods

Animals

BALB/c mice (females, 6–8 weeks of age) were purchased from Charles River Japan, Inc. (Yokohama, Japan). The mice were maintained on diets free of ovalbumin (OVA). All experimental animals used in this study were housed under constant temperature and light cycles and under a protocol approved by the Institutional Animal Care and Use Committee of Okayama University.

Sensitization and airway challenge

Mice were sensitized and challenged with OVA (Control-Asthma group and Lvn-Asthma group). Sensitization was carried out by intraperitoneal injection of 20 μg OVA (albumin from chicken egg, Grade V; Sigma Chemical Co., St. Louis, IL, USA) emulsified in 2.25 mg of aluminum hydroxide (Imject Alum, Thermo Fisher Scientific Inc., Rockford, IL, USA) in a total volume of 0.1 mL on days 0 and 14. Mice were challenged via the airways with aerosolized 1% OVA administered by ultrasonic nebulizer (Omron Healthcare, Kyoto, Japan) for 20 min on days 28, 29, and 30 (Fig. 1). Mice were divided into 4 groups: Non-Asthma group, Control-Asthma group, and 5 μL or 20 μL of Lvn-treated asthma group (Lvn-Asthma group). For mice in the Non-Asthma group, saline (0.1 mL) was intraperitoneally administered on days 0 and 14. Lvn treatment in the Lvn-Asthma group was performed as indicated below. AHR was assessed from 48 to 72 h after the last challenge, and the lungs and bronchoalveolar lavage (BAL) fluids were obtained for further assays.

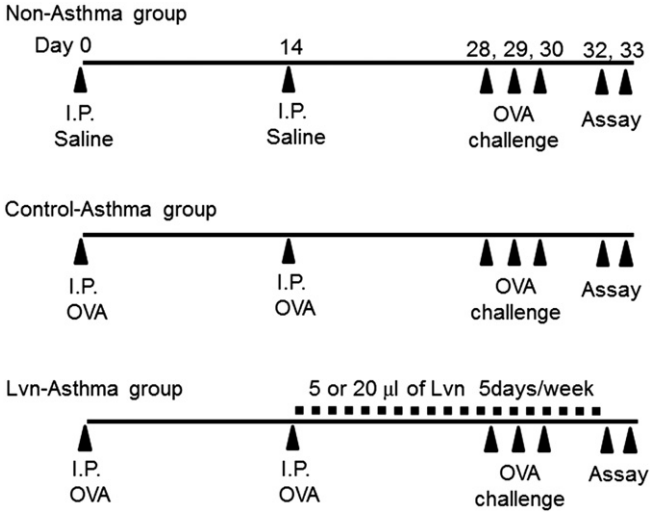

Fig. 1. Experimental protocols. Mice were divided into 4 groups: non-sensitized and ovalbumin (OVA)-challenged mice (Non-Asthma group); OVA-sensitized and OVA-challenged mice (Control-Asthma group); 5 or 20 μL of lavender essential oil (Lvn)-treated, OVA-sensitized, and OVA-challenged mice (Lvn-Asthma group). In the Control-Asthma group, mice were sensitized by two intraperitoneal injections of OVA/alum and subsequently received 3 consecutive days of an aerosolized OVA challenge on days 28–30. In the Lvn-Asthma group, OVA-sensitized and OVA-challenged mice inhaled 5 or 20 μL of Lvn for 20 min, 5 consecutive days per week, from day 14 to day 31.

Inhalation of lavender essential oil

Lavandula angustifolia (lavender) essential oil was purchased from ROHTO Pharmaceutical Co., Ltd (Osaka, Japan). The analysis sheet of essential oil was supplied by ROHTO and analyzed by gas chromatography mass spectrometry. The main component of essential oil was as follows; linalyl acetate 31.78%, linalool 25.56%, cis-β-ocimene 4.89%, β-caryophyllene 4.78%, lavandulyl acetate 4.7%, terpinene-4-ol 4.03%, cis-β-farnesene 3.7%, trans-β-ocimene 2.77%, and other component were less than 2%. The specific gravity is 0.882 and the refractive index is 1.459. Mice were transported into inhalation cages (28.5 cm × 21.5 cm ×10.0 cm) according to group assignment. Lavender essential oil (5 μL or 20 μL) was applied to a piece of 10 cm × 10 cm filter paper and placed on the upper side of the inhalation cage. The Lvn-Asthma group was treated by inhalation of Lvn 5 days per week for 20 min from day 14 to day 31 (Fig. 1). The Non-Asthma group and Control-Asthma group were treated by water in the same manner.

Determination of lung resistance

A flexiVent small animal ventilator (SCIREQ, Montreal, PQ, Canada) was used to assess pulmonary function (SnapShot) in anesthetized (intraperitoneal injection of sodium pentobarbital, 70 mg/kg), mechanically ventilated mice, measuring changes in lung resistance (RL) in response to increasing doses of inhaled methacholine (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as previously described (Koga et al., 2013). Briefly, RL was assessed (150 breaths/min, tidal volume: 10 mL/kg) as a change in airway resistance after a challenge with aerosolized methacholine administered for 10 s (60 breaths/min, tidal volume: 20 mL/kg) in increasing concentrations (0, 3.125, 6.25, 12.5, and 25 mg/mL). Maximum values of RL were recorded and expressed as percent change from baseline after saline aerosol.

Bronchoalveolar lavage fluid and blood collection and preparation of lung tissue

After the assessment of airway responsiveness, the lungs were lavaged via the tracheal tube with saline (1 mL, 37 °C). The volume of collected BAL fluid was measured in each sample, and the number of cells in the BAL fluid was counted. BAL fluid was centrifuged at 1500 rpm for 10 min at 4 °C. After centrifugation, the supernatant was stored at −80 °C. The sediment was resuspended in 1.0 mL of phosphate buffered saline (PBS), and smear preparations were prepared by an automatic cytocentrifuge system (1000 rpm, 1 min). Preparations were stained with Giemsa, and cell differentiation was performed in a blinded fashion by counting at least 200 cells under light microscopy. Blood samples were collected from the abdominal space by excising the vena cava. Blood was centrifuged at 2500 rpm for 15 min. After centrifugation, serum was stored at −80 °C. The lungs were removed and fixed in 10% formalin for histological studies.

Histological studies

Formalin-fixed blocks of lung tissue were cut around the main bronchus and embedded in paraffin blocks. Tissue sections (4 μm thick) were affixed to microscope slides and deparaffinized. After staining with hematoxylin–eosin (HE) and periodic acid-Schiff (PAS) for the identification of mucus-containing cells, the slides were examined under light microscopy. In HE-stained lung sections, the number of inflammatory cells (eosinophils, lymphocytes, and neutrophils) was counted in areas of peribronchial and perivascular tissues using the NIH Image Analysis system (National Institutes of Health, Bethesda, MD, USA). More than 10 bronchioles in a minimum of 10 high-power fields per lung tissue