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Original article

Study effect of *Ocimum basilicum* seeds on mucus production and cytokine gene expression in allergic asthma mice model

Effets des graines d'Ocimum basilicum sur la production de mucus et l'expression des gènes de cytokines sur un modèle de souris allergique

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Abstract

Allergic asthma is a complicated disease characterized by coughing, wheezing, airway inflammation, mucus hyper-secretion and increased airway hyperresponsiveness (AHR). Basil seed, or *Ocimum basilicum* (OB), has an anti-inflammatory effect that is utilized in traditional medicine. The aim of this study was to investigate the effects of OB on *IL-4*, *IL-5*, *IL-13* and *MUC5a* gene expression in an asthma model. Eighteen female BALB/c mice were divided into three groups as follows: an untreated asthmatic group, a healthy group and an asthmatic group receiving OB. Broncho-alveolar lavage fluid (BALF) was collected and Real-Time PCR was done for *IL-4*, *IL-5*, *IL-13* and *MUC5a* genes, and lung histopathological sections were prepared. Asthmatic mice treated with OB had suppression of cytokines and mucus gene expression (*IL-4*: 2.90 ± 1.08 , *IL-5*: 1.11 ± 0.37 , *IL-13*: 0.72 ± 0.19 and mucus: 1.83 ± 1.08) in comparison to the untreated asthmatic group (*IL-4*: 8.00 ± 2.54 , *IL-5*: 2.91 ± 0.46 , *IL-13*: 2.00 ± 0.22 and mucus: 12.24 ± 1.88). In the histopathological section, mucus hyper-secretion and goblet cell hyperplasia were reduced in the OB-treated group in comparison to untreated asthmatic group. Th2 cytokines and mucus gene expression were reduced in the OB-treated group in comparison to untreated asthmatic group. Mucus hyper-secretion and goblet cell hyperplasia were regulated by OB treatment in asthmatic mice. OB showed immunomodulatory and anti-inflammatory effects in allergic asthma and could reverse airway obstruction.

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Keywords: Allergy; Gene; Asthma; Herbal Medicine

Résumé

L'asthme allergique est une affection complexe caractérisée par de la toux, des sifflements, une inflammation des voies aériennes, l'hypersécrétion de mucus et une augmentation de l'hyperréactivité bronchique (HRB). Les graines de basilic ou *Ocimum basilicum* (OB) sont pourvues d'un effet anti-inflammatoire utilisé dans les médecines traditionnelles. Le but de ce travail est d'évaluer les effets d'OB sur l'expression des gènes codant pour *IL 4*, *IL 5*, *IL 13* et *MUC 5C* dans un modèle expérimental d'asthme. Dix-huit souris BALB/c ont été réparties en 3 groupes : groupe asthmatique, groupe sain, et groupe asthmatique recevant OB. Le liquide de lavage bronchoalvéolaire (LAB) a été collecté et une PCR en temps réel a été effectuée pour les gènes de l'*IL 4*, *IL 5*, *IL 13* et *MUC 5C* associées à des coupes histopathologiques pulmonaires. Les souris asthmatiques traitées par OB avaient une suppression des gènes codant pour les cytokines et le mucus (*IL 4* : $2,90 \pm 1,08$; *IL 5* : $1,11 \pm 0,37$; *IL 13* : $0,72 \pm 0,19$ et mucus : $1,83 \pm 1,08$) par comparaison au groupe asthmatique (*IL 4* : $8,00 \pm 2,54$; *IL 5* : $2,91 \pm 0,46$; *IL 13* : $2,00 \pm 0,22$ et mucus : $12,24 \pm 1,88$). Sur les coupes histopathologiques, l'hypersécrétion de mucus et l'hyperplasie des

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cellules caliciformes étaient diminuées dans le groupe asthmatique traité par OB par comparaison au groupe asthmatique. L'expression des gènes codant pour les cytokines Th2 et le mucus était réduite dans le groupe asthmatique traité par OB par comparaison au groupe asthmatique. L'hypersécrétion de mucus et l'hyperplasie des cellules caliciformes étaient régulées par le traitement par OB chez les souris asthmatiques. OB a montré des effets immunomodulateurs et anti-inflammatoires chez les souris asthmatiques et pourrait réduire l'obstruction bronchique.

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Mots clés : Allergie ; Gènes ; Asthme ; Herbes médicinales

1. Introduction

Allergic Asthma is a complicated disease of the respiratory system characterized by coughing, wheezing and dyspnea, with eosinophilia believed to have the main role in airway inflammation. Bronchial spasm, mucus hyper-secretion and increased AHR are the key features in asthma pathophysiology [1,2]. Interleukin (IL)-4, IL-5 and IL-13 as well as Th2 cytokines play an important role in asthma progression. IL-4 is involved in overproduction of IgE, IL-5 activates eosinophils, and IL-13 leads to mucus hyper-secretion [3,4]. In asthmatic patients, controlling eosinophilic inflammation, mucus hyper-secretion and bronchial muscle spasm can prevent asthma attacks [4,5].

OB, which exerts anti-inflammatory, antioxidant and antibacterial effects, has been used in traditional medicine for centuries. In Iranian traditional medicine, basil seed has long been used to treat common cold, cough and asthma. Because of its anti-inflammatory and antispasmodic effects which help reduce swelling and edema, it can be used as an anti-asthmatic agent [6–8].

The aim of this study was to investigate the effects of OB on gene expression of asthmatic factors in ovalbumin (OVA)-induced allergic asthma in mice (*IL-4*, *IL-5*, *IL-13* and *MUC5a* genes).

2. Methods

2.1. Animals

Eighteen 6–8 week-old female BALB/c mice were purchased from the Pasteur Institute of Iran (Tehran, Iran) and kept in an allergen-free environment (free access to food and water, 18–24 °C temperature, 30–60% relative humidity, 12-hour light/dark cycle).

2.2. Antigen Sensitization, challenge and treatment

The mice were immunized and challenged with ovalbumin (OVA) in the same way as in previous studies [9] to form an animal model of allergic asthma. In short, the mice were sensitized by intra-peritoneal injection of 20 µg of ovalbumin with 50 µL of aluminum hydroxide (alum adjuvant) and 50 µL of PBS on day 1, and this was repeated at day 14. The mice were challenged with inhaled 1% ovalbumin solution aerosolized by an ultrasonic nebulizer for 30 min per day on days 24, 26, 28 and 30 (Fig. 1).

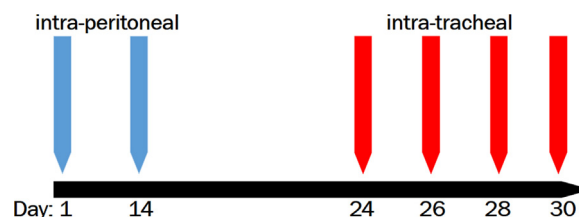


Fig. 1. Allergic asthma animal model producing protocol. Sensitization and challenge of mice were done with ovalbumin.

The mice were divided into three groups as follows: an untreated asthmatic group (positive control group), healthy mice (negative control group) and an asthmatic group receiving OB (orally on days 25, 27 and 29). After day 31, the tests were done.

2.3. Real-Time PCR

BALF was collected, total RNA was extracted, cDNA was synthesized, and real-time quantitative PCR was done for *IL-4*, *IL-5*, *IL-13* and mucus (*MUC5a*) genes [1]. The results of *IL-4*, *IL-5*, *IL-13* and mucus gene expression were analyzed as fold-increase compared to *GAPDH* gene expression (housekeeping gene). Primer sequences were used in accordance with Athari et al., 2016 [1].

2.4. Histopathology

Histopathological sections were prepared and stained with hematoxylin and eosin (H&E). Eosinophilic infiltration and mucus-secreting goblet cells were investigated. The mucus stain in the histopathology sections was scored and attributed 5 levels/scores as follows; score 1: $0 \leq$ bronchial obstruction $< 5\%$, score 2: $5\% \leq$ bronchial obstruction $< 25\%$, score 3: $25\% \leq$ bronchial obstruction $< 50\%$, score 4: $50\% \leq$ bronchial obstruction $< 75\%$, score 5: $75\% \leq$ bronchial obstruction $\leq 100\%$.

2.5. Statistical analysis

Gene expression changes were shown in graphs on logarithmic scales. The Kolmogorov–Smirnov test (for normal distribution), one-way analysis of variance (ANOVA) and Student's *t*-test (comparing results from different groups) and the Mann–Whitney U test (comparing differences between positive and negative groups) were used. A *P* value less than 0.05 was considered to be significant.

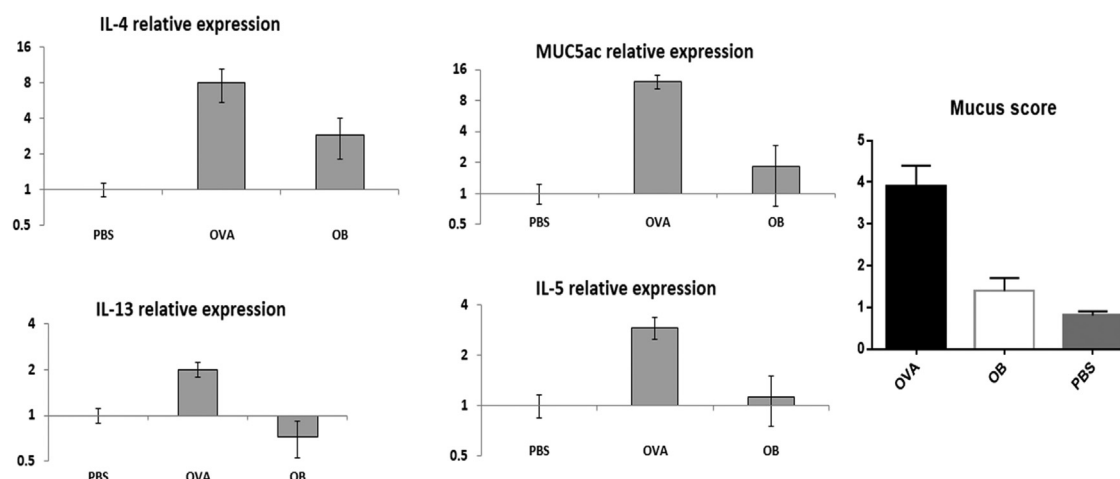


Fig. 2. Effect of OB on IL-4, IL-5, IL-13 and mucin mRNA expression. After total RNA extraction, Real Time-PCR to assess *IL-4*, *IL-5*, *IL-13*, and *MUC5ac* gene expression was done. The results of genes expression were analyzed as fold-increase compared to GAPDH gene expression. Data is shown as mean $\pm$ SD ($P \leq 0.05$). A significant increase in the mRNA expression for three cytokines and mucus were noted in OVA-sensitized mice (OVA-group) as compared to PBS-treated controls. OB treatment significantly reduced mRNA expression of all genes in mice. Mucus secretion (mucus score in histopathology section) was decreased significantly in OB group in comparison to OVA group.

2.6. Ethical considerations

Animal investigations were carried out to a high ethical standard and all ethical quid lines were applied in this study in accordance with the method approved by the animal ethics committee.

3. Results

3.1. Mouse model of allergic asthma

To confirm the allergic asthma model, the positive and negative controls were compared in terms of differences between inflammatory cell infiltration in airways, the presence of eosinophils in lavage fluid, mucus hyper-secretion and goblet-cell hyperplasia.

3.2. Gene expression

Asthmatic mice treated with OB were able to suppress Th2 cytokine expression in BAL fluid cells (IL-4: 2.90 ± 1.08 , IL-5: 1.11 ± 0.37 and IL-13 0.72 ± 0.19) in comparison to the untreated asthmatic group (IL-4: 8.00 ± 2.54 , IL-5: 2.91 ± 0.46 and IL-13 2.00 ± 0.22). Mucus gene was decreased in the OB-treated group (1.83 ± 1.08) in comparison to the asthmatic control group (12.24 ± 1.88) (Fig. 2).

3.3. Histopathology

In the histopathological sections, mucus hyper-secretion and goblet cell hyperplasia were controlled in the OB-treated group in comparison to the asthmatic control group. The OB-treated group was similar to the healthy group as it exhibited decreased mucus secretion and goblet cells showed no hyperplasia (Fig. 3). Eosinophils in lavage fluid were decreased in the OB group in comparison to the asthmatic control group (OVA: 62 ± 4 , PBS:

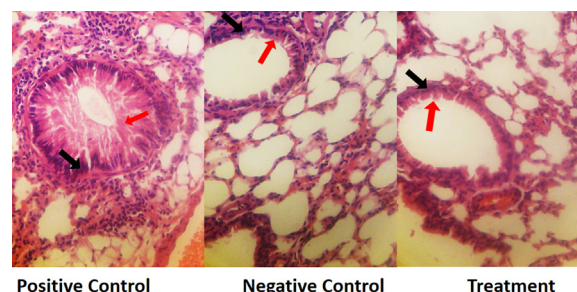


Fig. 3. Histopathology of lung sections in treated and control groups animals ($\times 400$). Tissues were stained with Hematoxylin-Eosine peribronchial infiltration, goblet cell hyperplasia within the airway epithelium and mucus hyper-secretion in lung sections were investigated. Goblet cell is shown with black arrow and Mucus is shown with red arrow in sections. Asthmatic group showed goblet cells hyperplasia and mucus hyper secretion and healthy group (negative control group) had no airway obstruction, mucus hyper-secretion and no goblet cells hyperplasia. In the OB treated group goblet cells hyperplasia and mucus hyper-secretion were decreased in comparison to positive control group. The results in the mentioned group was similar to that of negative control group.

2 ± 1 , OB: $24 \pm 2\%$). Mucus hypersecretion was decreased in the OB group (1.5 ± 0.5) in comparison to the asthmatic group (3.5 ± 0.5) but secretion was increased in comparison to the PBS group (0.5 ± 0.5). Perivascular inflammation was decreased in the OB group in comparison to the asthmatic control group (OVA: 3.5 ± 0.5 , PBS: 0.5 ± 0.5 , OB: 1 ± 0.5). Prebronchial inflammation was decreased in the OB group (0.5 ± 0.5) in comparison to the asthmatic control group (3 ± 0.5), and pre-bronchial inflammation in the OB group was similar to the PBS group (0.5 ± 0.5).

4. Discussion

In this study, the asthmatic group treated with OB showed dramatic suppression in gene expression for the main asthmatic Th2 cytokines (IL-4, 5 and 13). Airway obstruction with mucus hyper-secretion was eliminated by OB in the asthmatic lung

sections. The histopathological sections showed that the OB-treated group had normal airways similar to those seen in the healthy group.

Previous studies have shown that OB has anti-inflammatory effect and can reduce swelling [6–8]. In this study, OB reduced the pathological signs of asthma in the airways and may be considered an effective agent for the treatment of allergic airway inflammation and asthma. Sensitized and challenged mice with OVA showed significant inflammatory and pathophysiological changes (increased IL4, IL5 and IL13 expression, mucus hypersecretion and goblet-cell hyperplasia).

Increased IL-4, IL-5 and IL-13 expression in lung play key roles in the severity of asthma. IL4 increases allergic response in lung and induces proliferation of Th2 cells, while IL-5 leads to eosinophil activation and migration in bronchi and IL-13 is a trigger for mucus secretion and airway hyper-responsiveness (AHR) in the airways [10–13]. Thus, the modulation of Th1/Th2 cytokine equilibrium in lung could decrease airway inflammation, allergic reaction and AHR. While decreasing Th2 cytokine gene expression, OB may increase Th1 cytokines gene expression and modulate Th1/Th2 cytokine balance. This balance is necessary for the control and cure of asthma. In the present study, decreased Th2 cytokine expression in the OB-treated group compared to the asthmatic control group may be interpreted as the exertion by OB of a significant effect on opening of airways and inflammation reduction. Current examination has demonstrated that OB has the potential ability to inhibit cytokine-induced allergy in lung as well as mucus production. Therefore, OB acts mainly by suppressing asthmatic cytokines and mucus secretion in the airways.

In Iranian traditional medicine, a wide range of herbal therapy is used in the treatment of asthma and allergy and hundreds of plants are used in this field. There are many studies about anti-asthmatic herbs and the effects of some of these on cytokines gene expression. Black seed is one of traditional medicinal plants used and the suppressive effect of black seed on Th2 cytokine gene expression has been studied [14,15]. The anti-inflammatory effect of black seed in the lung tissue has also been confirmed [16]. *Ocimum basilicum* essential oil and its major component, estragole, are the chemical constituents having anti-inflammatory activity [17]. OB components may also control inflammation through inhibition of arachidonic acid metabolism (cyclooxygenase and lipoxygenase pathway inhibitors) and of the leukotriene-induced pathways [18]. However, studies of OB as anti-asthma plant and of the effects of this plant on cytokine gene expression and mucus hyper-secretion are not adequate. Moreover, we found significantly increased mucus secretion and Th2 cytokine expression in the sensitized, non-treated group compared with the OB-treated group and non-asthmatic airways.

In this study, mucus gene expression was surveyed by real time-PCR localization using the *MUC5a* gene in the OB-treated asthmatic group that had a notable effect in asthma treatment. We surveyed the immunomodulatory effects of OB in a mouse model of allergic asthma by controlling cytokine expression and mucus secretion in the airways. This study suggested that OB possesses immunomodulatory and anti-inflammatory effects and could reduce mucus hyper-secretion on OVA-induced

allergic asthma in mice. Thus, OB may be recommended as a therapeutic herb for the control of allergic asthma.

We would point out some limitations of this study. It would have been perfect to investigate the effects of OB on Th1, Th17 and T regulatory cytokines and other inflammatory molecules, but we were unable to do so in the current project.

Disclosure of interest

The authors declare that they have no competing interest.

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