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Effect of Biospray Dressings on Eosinophil Infiltration in the Nasal Mucosa and Serum IgE Levels After Nasal Provocation in Experimental Allergic Rhinitis

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Abstract

Purpose: To investigate the effect of biospray dressing on the extent of eosinophil infiltration in the nasal mucosa and the level of serum IgE in experimental allergic rhinitis with nasal provocation.

Method: Twenty-four BALB/c mice were randomly divided into the normal control group, allergic rhinitis (AR) group, dexamethasone (DEX) treatment group, and biospray dressing (BD) group. The mice in the latter 3 groups were prepared for animal models of AR according to standard protocols. Mice in the BD group were administered a nasal spray before the nasal provocation, and those in the DEX group were administered an intraperitoneal injection of DEX. The nasal mucosa and serum were collected from each group. Nasal mucosa eosinophil infiltration was evaluated using hematoxylin and eosin staining, and enzyme-linked immunosorbent assay (ELISA) was performed to analyze the serum IgE expression.

Results: Eosinophil infiltration (AR vs BD $P = .009$) in the nasal mucosa and serum IgE expression (AR vs BD $P = .001$) were significantly lower in the BD group than in the AR group. There were no significant differences in the extent of eosinophil infiltration in the nasal mucosa or serum IgE expression between the BD and DEX groups.

Conclusion: Biospray dressings can significantly reduce allergen provocation in the nasal cavity and have a protective effect on the nasal mucosa. They can be used for the prevention and treatment of AR.

Keywords

biospray dressing, allergic rhinitis, eosinophils, serum IgE

Introduction

Allergic rhinitis (AR) is one kind of nasal allergic diseases involving IgE-mediated release of inflammatory mediators, immunocompetent cells, and cytokines after exposure to allergens.[1] The prevalence of AR has increased annually.[2] IgE-mediated allergic responses are the most widely recognized form of allergy and are characterized by the rapid onset of symptoms.[3] During initial sensitization, allergen stimulates production of IgE antibodies which then bind to tissue basophils and mast cells.[4] Allergens further bind to their specific IgE antibodies and trigger the release of mediators, such as histamine, prostaglandins, and leukotrienes, causing "clinical reactivity" (allergic symptoms).[5,6] At present, there is still no effective prevention and therapeutic measures available, and AR can interfere with the life, work, and study of patients, resulting in physical, mental health, and social problems.

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According to the Clinical Practice Guideline: Allergic Rhinitis in China (2015, Tianjin), the current recommended treatments for AR include the following: avoidance of contact with the allergen, drug treatment (oral and nasal antihistamines, nasal glucocorticoids, antileukotrienes, chromones, intranasal decongestants, intranasal anticholinergics, and traditional Chinese medicine), immunotherapy (subcutaneous and sublingual immunotherapy), and surgical treatment (posterior nasal neurectomy and vidian neurectomy).[7] However, studies have shown that up to 40% of patients are still dissatisfied with the efficacy of the above treatments. Thus, new methods for the prevention and treatment of AR are urgently needed. In this study, a biospray dressing (BD) was applied to the nasal mucosa of an AR animal model to try to prevent the onset of AR by blocking allergen provocation and to explore the potential of BDs to prevent and treat AR.

Materials and Methods

Experimental Animals

Experimental mouse model of AR was established as previously reported.[8] Twenty-four BALB/c male mice aged 6 to 8 weeks were purchased from the SLACCAS Experimental Animal Company (Shanghai, China) and randomly divided into 4 groups (A–D) with 6 mice in each group. Group A was the normal control group, group B was the AR group, group C was the dexamethasone (DEX) treatment group, and group D was the BD group (in the experiment, the double-blind principle was followed, and group D was named the unknown drug group). All animal experiments in this study followed the guidelines for Institutional Animal Care at The First People's Hospital of Zhangjiagang City, Suzhou, China. The animal protocol was approved (#20170818).

Reagents and Materials

1. Reagents: ovalbumin (OVA, Sigma, USA); aluminum hydroxide powder [AL(OH)3] (Chemically Pure, Nanjing Chemical Reagent Co., Ltd.); dexamethasone (Sigma); and 0.9% saline (Sigma).
2. Instrument: ultrasonic nebulizer (Jiangsu Yuyue Medical Equipment & Supply Co., Ltd., Model: 402A).
3. IgE Immunoassay Kit: SinoBest Bio Co., Ltd., Shanghai, China).

AR Mouse Model Preparation and Intervention Treatment

1. OVA was used as an allergen to sensitize the mice in the B, C, and D groups. A total of 1 mL of a saline suspension containing 25 μg of OVA and 0.43% aluminum hydroxide gel was intraperitoneally injected into the mice twice a week for a total of 3 weeks to induce sensitization.
2. The B, C, and D groups were administered a 20-mL saline ultrasonic nebulization containing 500 μg of OVA for 30 minutes each time, twice/week for 2 weeks.
3. Thirty minutes before each ultrasonic nebulization, BD was applied to the nasal cavity of the mice in group D; the treatments were administered twice a day for 2 weeks, with each treatment consisting of 1 spray per side of the nasal cavity.
4. DEX was prepared at a concentration of 10 mg/kg. The mice were intraperitoneally injected with 1 mL of the DEX solution once daily for 2 weeks.

Sample Collection

1. Serum collection: After the intervention experiment, the whiskers of the mice in all groups were cut, and blood samples were collected from the orbital sinus into centrifuge tubes. The samples were centrifuged at high speed for 1 minute, and the serum was then stored at 4°C until further examination.
2. Collection of nasal mucosa specimens: The mice were sacrificed by exsanguination, and the middle section of the nasal cavity was then removed in the apical coronal position and sectioned. The sections were frozen, and hematoxylin and eosin (H&E) staining was performed.
3. Nasal mucosal eosinophil count and serum IgE detection: (i) To count the number of eosinophils in the nasal mucosa, each nasal mucosa sample was observed under a microscope at 400× magnification. The number of eosinophils in 5 fields was counted, and the average number of cells per field was calculated. (ii) The concentration of serum IgE was evaluated using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. Briefly, 96-well immuno plates were coated overnight at 4°C. Serum samples were tested at the diluted concentration of 1:250 and 1:500. Detection was performed with biotinylated rat anti-mouse IgE and then streptavidin-horseradish peroxidase and developed using tetramethylbenzidine substrate and read at 450 nm. Reagents and ELISA kits were purchased from IgE Immunoassay Kit: SinoBest Bio Co., Ltd.).

Data Analysis

The experimental data were analyzed using SPSS software. Results are shown as the mean ± standard error of the mean. For comparison between 2 groups, Student's 2-tailed t test was used. $P < .05$ was deemed to indicate statistical significance.

Results

Significant reduction of AR symptoms and signs after biospray dressings in experimental allergic rhinitis was noted. Histological analysis demonstrated reduced AR lesions in the submucosa of BD group compared to AR group (Figure 1). There was no difference of AR lesions between BD and DEX ($P > .05$). We further compared the eosinophils number in the submucosal area of the whole nasal septum. The eosinophil count in each group was shown in Figure 2. There was a significant reduction in eosinophils in BD treatment of OVA-sensitized mice ($n = 6$) compared to AR group ($n = 6$) (AR: 17.13 ± 1.59 vs BD: 9.33 ± 0.96, $P = .009$, Figure 2). In addition, we found a significantly lower total IgE levels in BD treatment of OVA-sensitized mice ($P = .001$) compared to AR group (Figure 3). The serum IgE concentration and comparisons among the groups are presented in Figure 3.

Discussion

The pathogenesis of AR is complicated. At present, in addition to the atopic individuals theory and the hygiene hypothesis, the T helper types 1 (Th1) and 2 (Th2) balance hypothesis and the effects of gene regulation and neuromodulation, IgE and its receptor are also thought to have an important contribution to the pathogenesis of AR.[9–12] IgE is a key cytokine mediating the pathogenesis of AR.[4] It is mainly produced by plasma cells in the lamina propria of the respiratory tract or digestive tract. After first contact with an allergen, the body produces specific IgE antibodies during the basic sensitization stage.[4,5] After the body comes in contact with the allergen again, the specific IgE antibodies bind to and crosslink with the antigen, which activates mast cells and eosinophils to release histamine and leukotrienes, causing a series of nasal-based clinical symptoms.[10,11]

Polyquaternium and hydroxyethyl cellulose, which are cellulose derivatives modified from natural cellulose, can be used as BDs. The spatial structure of polyquaternium salt and hydroxyethyl cellulose, which is similar to

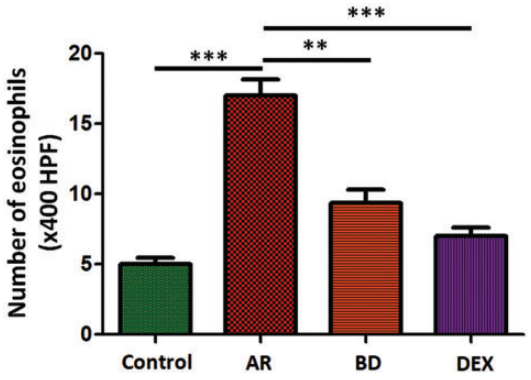

Figure 2. The count of Eosinophil in nasal membrane between AR, BD and control group are all significant different. AR, allergic rhinitis; BD, biospray dressing; DEX, dexamethasone.

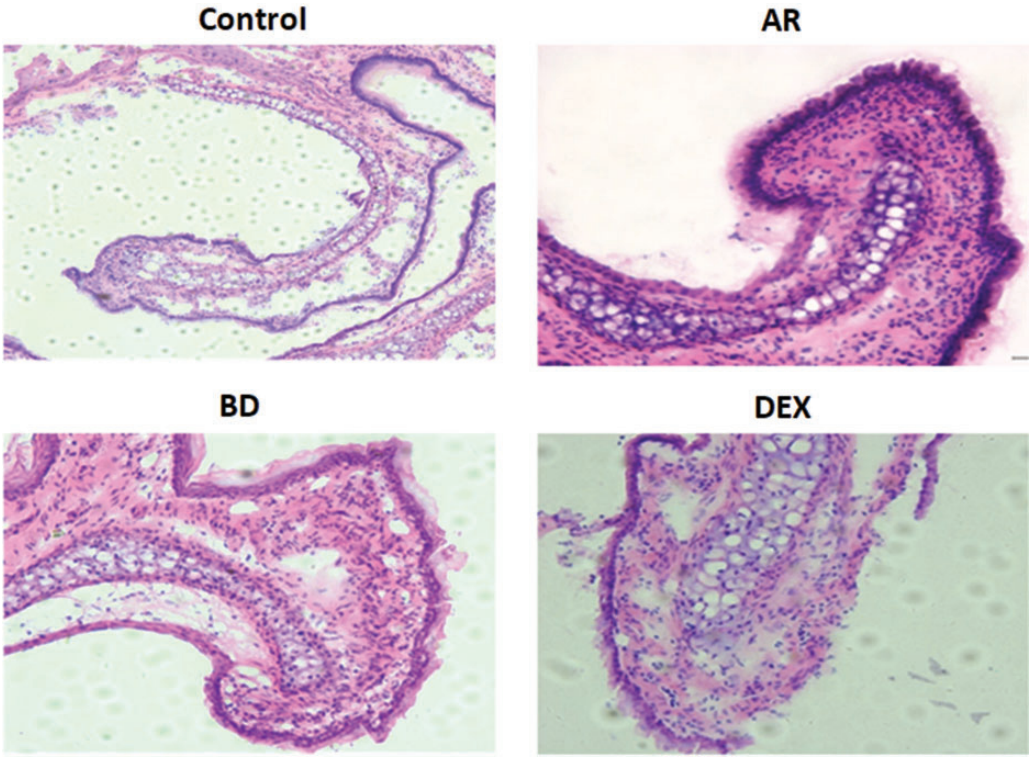

Figure 1. The pathological section with hematoxylin and eosin stain shows the difference in the membrane of infraturbinal among 4 groups. AR, allergic rhinitis; BD, biospray dressing; DEX, dexamethasone.

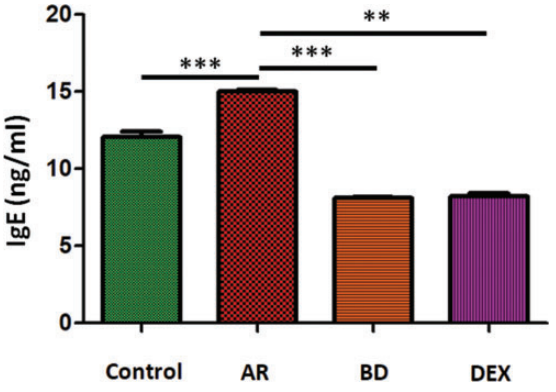

Figure 3. The level of IgE in serum between AR, BD and control group are all significant different. AR, allergic rhinitis; BD, biospray dressing; DEX, dexamethasone.

that of natural cellulose under an electron microscope, is a 3-dimensional mesh scaffold with a large specific surface area and good permeability.[13] Its pore diameter is less than or equal to the size of red blood cells; therefore, it can effectively block objects with a volume larger than the structural pores, including catkins, pollen, and dust mites, from making contact with the nasal mucosa. In addition, it can effectively stop bleeding, seal wounds, promote wound healing, and maintain wound moisture and breathability.[13] Due to the biological properties of the materials, a large amount of polyquaternium cations can effectively kill and inhibit bacterial growth. Because BDs are patented products, there are no related reports on the clinical application of nasal cavity isolation and protection.

This study utilized OVA and aluminum hydroxide gel as a sensitizer. The AR model was generated through basic sensitization and provocation steps. To study whether the biological fluid membrane could prevent the provocation and symptoms of AR by preventing contact between the allergen and the nasal target organs, after basic allergen-induced sensitization in BALB/c mice, the nasal cavity was sprayed with the BD, followed by routine OVA nebulization. The BD was able to block contact between the OVA and the target organ of the nasal mucosa following nasal nebulization, thus avoiding an AR episode caused by subsequent OVA stimulation. Thus, a BD containing polyquaternium and hydroxyethyl cellulose as the main components has protective effects on the nasal mucosa. There was a significant difference in the serum IgE levels between the experimental group and the nonintervention group, but there was no significant difference between the experimental group and the DEX group, indicating that protection via the BD was indeed effective. Studies will be further detailed on controls, AR, BD, and DEX at the onset of the study (baseline) and then serially once or 2 times during the whole study. The serum levels of total IgE were lower in our study as compared to those from previous studies,[14] probably due to the animals we chose and different OVA administration routes. We observed reduced AR lesions in the submucosa of BD group compared to AR group. Serum total IgE level was tested as an AR disease marker, shown lower IgE level in BD group compared to AR group.

Although no significant increase was observed in the serum IgE or rhinitis levels in the BD and DEX groups compared with the rhinitis group, the degree of eosinophil infiltration in the nasal mucosa was not as significant as that in the AR group, suggesting that the pathways and mechanisms of the intervention were different among the groups. The BD nasal spray prevents the stimulatory effects of the allergen by blocking contact between the allergen and the nasal mucosa. DEX is a commonly used immunosuppressive agent in clinical practice, and intraperitoneal injection of DEX exerted therapeutic effects through multiple immunosuppressive and anti-inflammatory actions in the animals of the experimental group. For those animals that have already developed AR, whether the BD exerts the same protective effects remains to be further studied.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: S. H. was employed at Yifatu Biotechnology (Wuhan) Co. Ltd., Wuhan, China. Other authors (G. W., X. G., H. Z., X. M., Y. Y., Y. L., D. L., and L. Z.) declare that they have no conflict of interest.

Ethical Approval

The study was approved by the research committee of The affiliated Zhangjiagang hospital of Soochow University, Suzhou, China.

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Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

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