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Study on anti-inflammatory effect of peptides-conjugated alumina nanoparticle on allergic rhinitis mice model

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Abstract
Allergic rhinitis (AR) is a common atopic problem in which immune response to the environmental factors leads to clinical symptoms. *Helicobacter pylori neutrophil*-activating protein (HP-NAP) as a peptide attenuates Th2 response and stimulates Th1 activation and mucus adhesion promoting protein (MapA) as a cell-surface protein binds to mucus. This study evaluated the effect of HP-NAP and MapA conjugated with alumina nanoparticle on AR. HP-NAP and HP-NAP with MapA were conjugated to alumina nanoparticle and two separate nanoparticles were produced. The AR mice were treated with these and HP-NAP in peptide form. The AR symptoms, gene expression of mucus, levels of IL-33 and IL-4, and total and ovalbumin (OVA)-specific IgE levels were evaluated. Nasal rubbing, sneezing, gene expression of mucus, and IL-33 and IL-4 levels, and OVA-specific and total IgE were decreased in three treated groups compared to AR, and there was a significant decrease in the symptoms in AR-H-M-A group ($P < 0.05$) when compared to the other treated groups. HP-NAP has a controlling effect on AR, and in nanoparticle-conjugated form it can strongly attach to the airway's mucus via MapA. Therefore, cooperation of HP-NAP-alumina with MapA can produce an effective and applicable treatment for AR.

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Introduction

Allergic rhinitis (AR) is a common atopic disease that is triggered by environmental and genetic factors. There is a genetic predisposition potential for allergic reactions in patients, and immune response to the environmental factors leads to clinical symptoms of AR. Rhinorrhea, wheezing, and dyspnea are the main clinical symptoms of AR, and airway inflammation, mucus production, and hypersensitivity are the main mechanisms of AR pathophysiology.[1,2]

In AR, Th2 cells (type of T-CD4+ lymphocytes) after sensitization releases interleukins (IL) 4, 5, and 13 and other cytokines (such as type-2 cytokines). IL-4 forces the B cells to produce IgE, which binds to the mast cells' receptors and activates mast cells in second contact with the same allergen and release their mediators from granules, and these mediators lead to clinical symptoms of AR. Histamine and other mediators cause itching and swelling, and IL-13 leads to mucus production.[3,4] Anti-atopic medications are the main drivers of direct costs for patients, and the produced medications for AR do not completely cure the symptoms but only control them.[1,3] Therefore, the production of drugs according to efficacy is very important in atopic patients.

Helicobacter pylori neutrophil-activating protein (HP-NAP) is a virulence factor of this bacteria and is also applied as a diagnostic biomarker in *Helicobacter (H.) pylori*-related diseases. It was shown that HP-NAP plays a protective role in allergic asthma that attenuates Th2 response and stimulates Th1 activation.[5,6] *Lactobacillus fermentum* has mucus-binding ability that is mediated by a mucus adhesion promoting protein (MapA), and this strain was reidentified as *Limosilactobacillus reuteri*. MapA is a cell-surface protein and one of the adhesion factors of *L. reuteri*, which can bind to the mucus.[7,8] This study evaluated the the anti-inflammatory effect of HP-NAP conjugated to alumina nanoparticle with MapA and the effect of produced nanoparticle on main type-2 cytokines, gene expression, and clinical symptoms.

Material and Methods

Peptides-nanoparticle preparation

Alpha alumina nanoparticle (α-Al_2O_3) was used as the peptide carrier. HP-NAP and MapA as two separate peptides were used for conjugation to the alumina nanoparticle. Conjugation of two peptides to the alumina was done according to a previous study.[9] In brief, HP-NAP and MapA solutions and alumina suspension were prepared separately. Aldehyde groups on the peptides and hydrazine groups on the nanoparticles were produced. Then, peptides and nanoparticles were placed in citrate buffer and incubated for 2 h. Finally, the conjugated peptides-nanoparticles were separated. Peptides conjugation was approved, and the structural characteristics of the nanoparticles were assessed by scanning electron microscopy.

Allergic rhinitis animal model

Male Balb/c mice were housed under standard conditions. Twenty mice were divided into five groups (N = 4 mice), and AR was induced in four of the five groups according to Athari et al.[10] In brief, for induction of AR, ovalbumin (OVA) and alum adjuvant were injected for 7 days, and the OVA solution was administrated intranasally for 7 days. Three of the four were sensitized, and challenged groups were treated on Days 10 and 12 with HP-NAP (AR-H group), HP-NAP conjugated with alumina (AR-H-A group), HP-NAP and MapA conjugated with alumina (AR-H-M-A group) with nebulizer for 30 min. The fourth group received no treatment (AR group), and the fifth group was sensitized and challenged with only phosphate buffered saline (control group).

Allergic rhinitis symptoms

Clinical symptoms of AR, including sneezing and nasal itching (nasal rubbing), on Day 14 (after the last challenge), in all mice, were studied.

Gene expression

On Day 15, one day after the last challenge, the mice were anesthetized, and nasal lavage fluids were collected. The cells from lavage were then separated, and total RNA was isolated. After synthesis of cDNA, quantitative real time PCR analysis was performed. The primers for IL-33 were F: TCCAACTCCAAGATTTCCCCG and R: CATGCAGTAGACATGGCAGAA; for mucus were F: CTACTG ACTGCACCAACACAT and R: GTGCAGTCCCCATGTACTGT; and reference gene (GAPDH) were F: TGTTCCTACCCCCAATGTGT and R: GGTCCTCAGTGTAGCCCAAG.

Cytokines levels measurement

After centrifuging of the lavage, the supernatant was used to determine the cytokine level, and IL-4 level was determined using specific ELISA kit.

Immunoglobulins levels

The total and OVA-specific IgE levels in serum were measured by ELISA kit.

Statistical analysis

Results were presented as mean ± standard deviation (SD). The SPSS software was used for analyses, and graphs were drawn by GraphPad Prism. Results were tested by paired t-test and Fisher's LSD for analyzing. A P-value < 0.05 was considered significant.

Result

Peptides nanoparticle

The alumina nanoparticles had spherical morphology with an average size of 130 ± 25 nm (Figure 1). Two types of

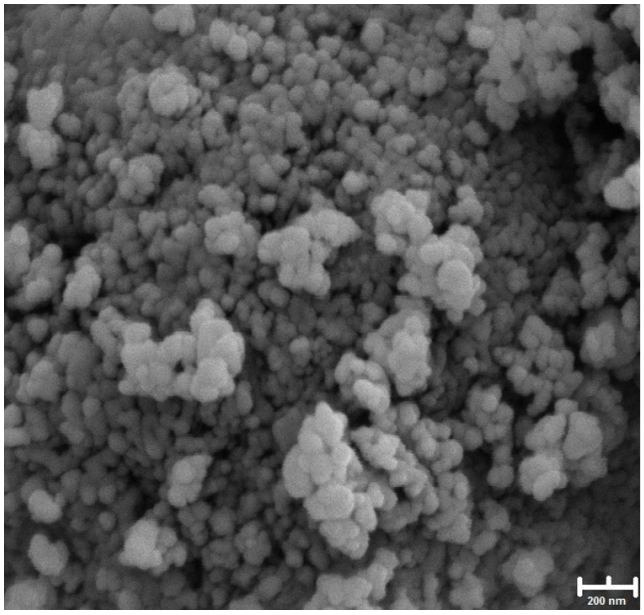

Figure 1 SEM. Alumina nanoparticles were prepared, and picture was taken using Scanning Electron Microscopy.

nanoparticles were produced, the HP-NAP-conjugated alumina and HP-NAP and MapA-conjugated alumina.

Clinical symptoms of allergic rhinitis

Nasal rubbing and sneezing were the main symptoms of AR in all groups (Figure 2), and both symptoms significantly increased ($P < 0.05$) in the AR group (21 ± 2 and 29 ± 1, respectively) compared to the control (2 ± 1 and 1 ± 0, respectively). These symptoms decreased significantly ($P < 0.05$) in AR-H (15 ± 2 and 19 ± 2, respectively), AR-H-A (13 ± 2 and 16 ± 3, respectively), and AR-H-M-A groups compared to the AR group. The control of symptoms in AR-H-M-A (6 ± 2 and 6 ± 1, respectively) was significant ($P < 0.05$) when compared to the AR-H and AR-H-A groups.

Gene expression

Gene expression of mucus as one of the main obstruction factors of airways and IL-33 as upper hand of type-2 cytokines were evaluated. The mRNA expression of IL-33 and mucus was significantly ($P < 0.05$) increased in the AR group (7.98 ± 0.83 and 12.54 ± 1.54, respectively) when compared to the control group (1 ± 0.06 and 1 ± 0.34, respectively). Gene expression of IL-33 and mucus was significantly ($P < 0.05$) controlled in AR-H, AR-H-A, and AR-H-M-A groups when compared to the AR group, and there was a significant difference in the controlling ($P < 0.05$) between AR-H-M-A with AR-H and AR-H-A groups (Figure 3).

Cytokines

IL-4 level was significantly ($P < 0.05$) increased in AR (106.37 ± 4.32 pg/ml) group when compared to the control group

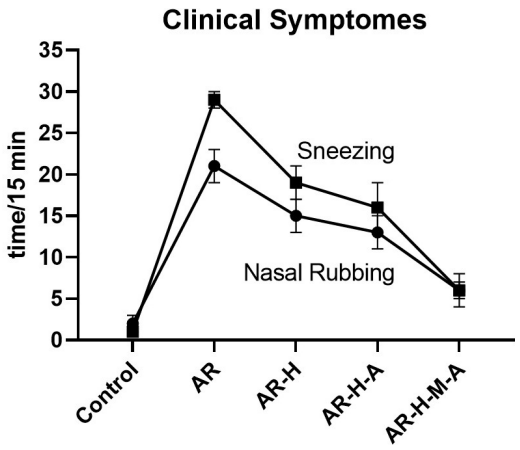

Figure 2 Clinical symptoms. Nasal rubbing and sneezing as the main symptoms of AR were evaluated in all groups.

(49.43 ± 5.01 pg/ml). Treatments led to a significant ($P < 0.05$) decrease in the IL-4 levels in AR-H (83.45 ± 5.21 pg/ml), AR-H-A (84.21 ± 4.82 pg/ml), and AR-H-M-A (52.44 ± 4.11 pg/ml) groups (Figure 4).

Immunoglobulins

Ovalbumin-specific and total IgE levels were significantly ($P < 0.05$) elevated in AR (247 ± 25 and 2785 ± 212 ng/ml, respectively) compared to the control group (0 ± 0 and 109 ± 12 ng/ml, respectively). Total IgE was significantly ($P < 0.05$) decreased in the treated groups compared to the AR group, and this decrease was significant ($P < 0.05$) between AR-H-M-A and other two treated groups. Three treatments decreased OVA-specific IgE levels when compared to the AR group, but this decrease was significant in AR-H-A and AR-H-M-A groups when compared to the AR group. Also, the decrease in OVA-specific IgE level was significant ($P < 0.05$) in AR-H-M-A group when compared to the AR-H-A group (Figure 5).

Discussion

Allergic rhinitis is the most common noncommunicable diseases, and the usage of drugs for the control and treatment of AR is necessary.[1] AR is an increasing problem, and understanding the concepts of AR pathogenesis is important. Allergens induce Th2 cells that release IL-4, IL-5, and IL-13, which promotes IgE and mast cell sensitization. The release of inflammatory mediators and cytokines leads to allergic immune response and AR clinical symptoms.[11] Therefore, our better understanding of the basic AR pathophysiology helps in the development of new medications which may allow more effective modulation of the allergic immune response, allergic diseases process, and their morbidity, and also improves the quality of life in atopic patients.

The exposure to allergens such as pollens, dust mite, animal dander, fungal spores, and other proteins triggers antigens thought processing by antigen-presenting cells (APCs) in the mucosal epithelium of the upper airways. The

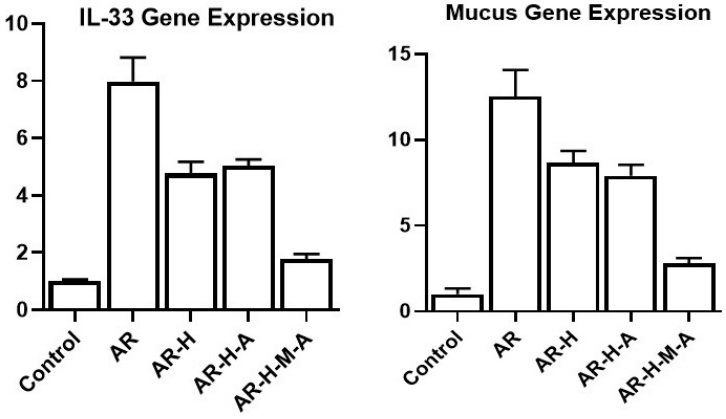

Figure 3 Gene expression. mRNA expression of IL-33 and mucus was evaluated in all study groups.

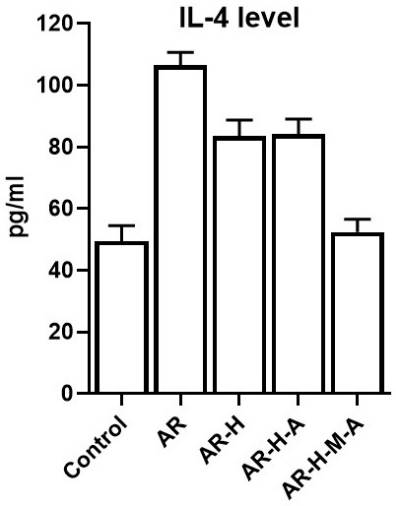

Figure 4 Cytokine level. The level of IL-4 was measured in all groups.

cleaved antigens bind to the antigen recognition sites of the Class II major histocompatibility complex (MHC). The antigen-specific receptors of Th2 cells recognize the antigen (allergen) on Class II MHC of the APCs, and Th2 cells release type-2 cytokines (IL-4, IL-5, IL-13, and possibly others), which stimulate IgE production by B cells, and inhibit Thl responses development. Produced IgEs bind to high-affinity Fce receptors (FceRI) on surfaces of the mast cells and basophils, and reexposure to allergen leads to IgE cross-linking on the mast cells, tyrosine kinases activity, and mast cell degranulation (releasing of histamine, prostaglandins [PGs], tryptase, chymase, and bradykinin are released rapidly) that are the critical initiating events of acute allergic symptoms (sneezing, resistance of nasal airways, itching, rhinorrhea, congestion, nasal discharge, and rubbing). On the other hand, phospholipase A2 activation releases arachidonic acid from membrane phospholipids that is metabolized by cyclooxygenase to PGs (D2, E2, and I2) and thromboxane A2. Also, via 5-1ipoxygenase, leukotrienes are produced (A4, B4, C4, D4, and E4) and late phase of allergy will be started.[11,12] TGF-ß and platelet-derived growth factor induce collagen production by fibroblast, which contributes to the thickening of collagen deposits beneath and fibrosis with "stiffening" of nasal polyp.[11,13] IL-33 can promote Th2 cells, basophils, mast cells, and eosinophils to produce Th2 cytokines, which participate in the promotion of allergic reactions, such as AR and asthma.[14-17] Also, IL-33 can promote the production of chemokines such as IL-8 and directly acts on the inflammatory reaction of nasal mucosa through its receptor, ST2. Expression of IL-33 and ST2 is increased in AR patients, and treatment with fluticasone propionate reduces IL-33 and ST2 expressions, thereby playing a therapeutic role. Moreover, IL-33 and ST2 play important roles in eosinophil-mediated inflammation and can provide new therapeutic targets for AR controlling.[14,16,17] Mucus hypersecretion leads to obstruction of upper airways in AR, and IL-13 acts as the main cause of goblet cell activity and mucus secretion. IL-33 is upper hand of IL-13 (one of the type-2 cytokines). The mRNA expression of IL-33 and mucus is increased in AR, and treatment with produced nanocomponents could control gene expression of IL-33 and mucus. IL-4 is one of the main allergic cytokines that can force B cells to produce IgE and lead to degranulation of mast cells. This cytokine is increased in AR, and in this study, treatments lead to decrease in the IL-4 level in treated AR groups.

Epithelial cells play an important role in AR as physical barriers that prevent the entry of allergens.[18,19] Epithelial barriers are the first line of defense that play vital roles in both innate and adaptive mucosal immunity regulation.[18,20] Mucociliary clearance is conducted by ciliated epithelial cells that involves trapping of allergens in mucus layer secreted by goblet cells.[18,21] The alumina nanoparticle is one of the safe nanoparticles and may have adjuvant effect on the immune response and may also enhance Th1 response. The produced nanoparticles have a suitable shape and size that can be used in inhalation form. In produced nanoparticles, HP-NAP has strong therapeutic effect, and it can attach suitably to the mucus of airway by MapA.

Allergic rhinitis affects about 10–20% of the world population, and as a global health concern is one of the most common chronic forms of diseases with an incidence rate of 11.8–46.0% in Western countries. AR has no high mortality rate, but it reduces the quality of life in patient populations worldwide.[22] AR is the main cause of nasal obstruction

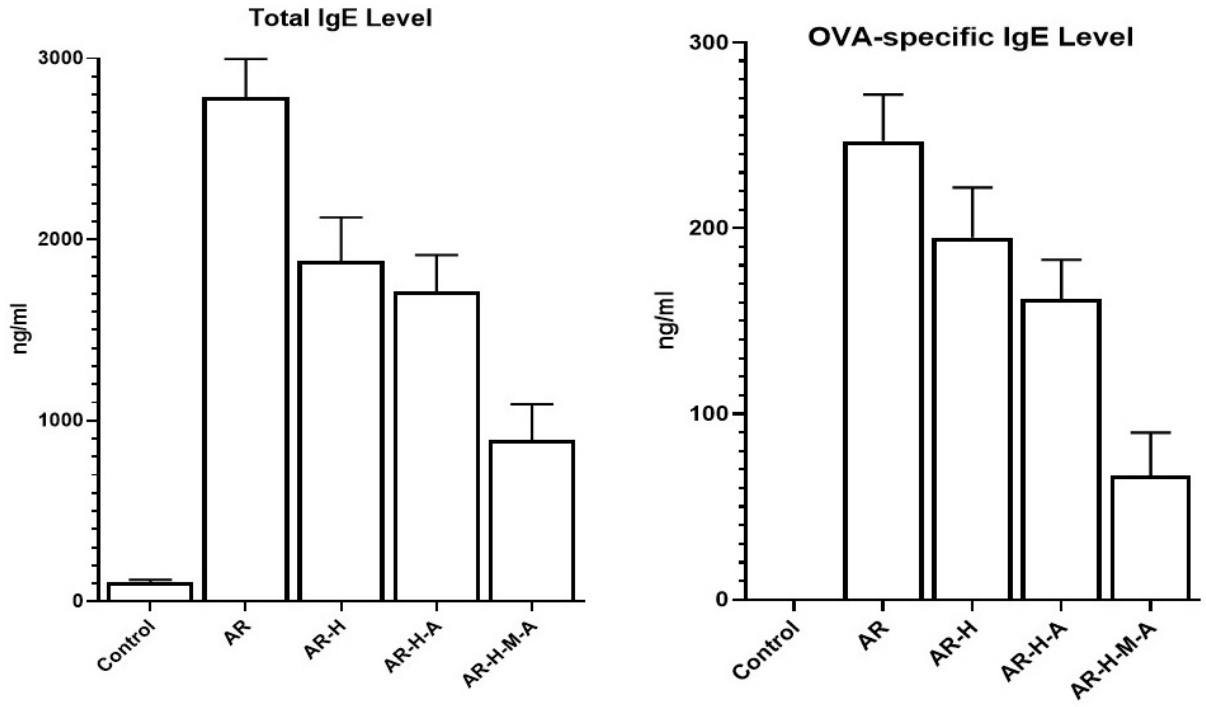

Figure 5 Immunoglobulins. Levels of total and OVA-specific IgE were measured in all groups.

in children, and as a common chronic disease, is not fatal, but is associated with many comorbidities such as allergic diseases. Moreover, in some patients with AR, persistent upper airway obstruction is related to pulmonary arterial hypertension.[23] On the other hand, in AR patients, there is a relationship between AR and dental-facial abnormalities. The face shape differences were present in AR children compared to that of healthy ones. Therefore, AR factors can influence craniofacial development in children.[24,25] Nasal rubbing and sneezing, the main allergic symptoms in AR group, were harnessed by treatment with the two produced nanoparticles (conjugated alumina nanoparticles with HP-NAP and MapA), and this harnessing was significant in the treated group with alumina nanoparticles containing both HP-NAP and MapA.

It was proposed that peptide therapy for AR greatly enhances the safety and improves management of this disease. Peptide therapy approaches are effective and will greatly control AR.[26] IgE is the main atopic immunoglobulin, which leads to mast cells and basophils sensitization and degranulation. Levels of specific and total IgE are increased in AR, and clinical symptoms appear. In our study, OVA-specific and total IgE levels were elevated in AR, and treatment with produced nanoparticles containing the mentioned components could decrease OVA-specific and total IgE levels. HP-NAP has anti-inflammatory effect and can control AR pathophysiology, and when used with alumina nanoparticles, the mentioned effect was strong and alumina could delivery HP-NAP to the upper airways. MapA has no effect on control of AR, but when HP-NAP-alumina nanoparticle was used with MapA, its effect was notable and MapA could keep HP-NAP-alumina nanoparticle in airway by attaching to the mucus of airways, which led to the dominant effect of produced nanoparticle.

Ethics Approval and Consent to Participate

The study methods and mice study were approved by the Ethical Committee of Animal House of ix.med.vet.dep, 2022 (No. IX.MED.VET.DEP.REC.2022.5100038.4).

Consent for Publication

Not applicable.

Availability of Data and Materials

Data are available upon request from the corresponding author.

Conflict of Interest

The authors declare that there are no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

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Authors' Contributions

LL, JW, SSA, and CWJ contributed to the *in vitro* and *in vivo* examinations, result analysis, scientific manuscript writing, and revising. All authors confirmed the final manuscript before submission.

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