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Abstract
Objective. In this study, we aimed to experimentally investigate the effects of nasal corticosteroids on the levels of secretory immunoglobulin A (sIgA) in nasal mucosa in rats.

Study Design. Prospective, randomized control trial.

Setting. Research laboratory.

Subject and Methods. Twenty-four male Sprague Dawley rats were included in our study. The rats were randomized into 3 groups. In group 1, nasal mometasone furoate was applied to the rats for 30 days. Saline was applied to group 2 for 30 days. Group 3 was the control group and received no treatment throughout the study period. Nasal lavage was conducted on both nasal openings of all rats in the 3 groups at the beginning of the study and on days 15 and 30, and the lavage solution (distilled water) was collected by aspiration.

Results. In group 1, the sIgA value was significantly higher at day 15 than at baseline. No significant difference was found between the sIgA values on day 15 and day 30. In groups 2 and 3, there were no significant differences in sIgA values at baseline, day 15, and day 30. The sIgA value of group 1 on day 15 was significantly higher than the values of groups 2 and 3. The sIgA value of group 1 on day 30 was significantly higher than the values of groups 2 and 3.

Conclusion. Topical corticosteroids (mometasone furoate) applied to the nasal mucosa significantly increase nasal sIgA levels.

Keywords
mometasone furoate, nasal lavage, rat, secretory immunoglobulin A

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Introduction
Allergic rhinitis is a disease that affects all age groups, with its prevalence varying from 5% to 40% in different populations.1,2 Topical steroids have widespread use in otorhinolaryngologic practice. The main objective for corticosteroid use is to alleviate inflammation in nasal and paranasal sinus diseases; however, it is known that topical steroids are highly efficient in the treatment of allergic rhinitis.3 Many patients use topical steroids without interruption for months and even years.4

Topical nasal corticosteroids, which are used frequently in nasal diseases for controlling inflammation and symptoms, exert their clinical effects by preventing the accumulation of inflammatory cells in the airway, selectively suppressing local cytokine production, inhibiting mediator release, and repairing the nasal mucosa.5

The upper respiratory tract mucosa is defended by the secretory immune system. Secretory immunoglobulin A (sIgA) constitutes the major part of the humoral immune system. Its molecular weight is 400,000, and the molecule usually has a dimeric form. Although sIgA is not very effective in systemic humoral immunity, it plays a significant role in mucosal immunity, protecting mucosal surfaces against microorganisms.

The basic functions of sIgA can be summarized as follows: (1) It neutralizes pathogens or exotoxins in the cell or inside the lumen. (2) It is responsible for agglutination of pathogens. (3) It prevents inflammation by hindering attachment of microorganism to the epithelium, thus inhibiting their invasion; (4) Due to the mucophilic properties of sIgA, antigens bound by sIgA are kept inside the mucus, which facilitates their elimination. (5) The fact that sIgA is not an activator of complement results in partial suppression of inflammation.6-8 The major sources of sIgA are the submuco sal glands; sIgA is stored in the periciliary fluid, forming a thin layer on the epithelial surface.9

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Mometasone furoate (MF) is a synthetic steroid that inhibits the synthesis and secretion of proinflammatory mediators more effectively than beclomethasone dipropionate, betamethasone, dexamethasone, and hydrocortisone. In our study, we investigated whether there would be a change in nasal sIgA levels after topical MF application to rats’ noses. The study thus investigated the connection between the effects of nasal topical corticosteroids and sIgA.

Materials and Methods
Our study commenced after gaining approval from the Bezmialem Vakif University Experimental Animals Local Ethics Committee (No. 2012/34). The study included 24 healthy and adult male Sprague Dawley rats, with an average body weight of 301 g (range, 292-310 g). The rats were assigned randomly and were divided into 3 groups. Drug dosages were administered as reported in relevant literature and are specified below.

Study Groups
- Group 1: MF (Nasonex Aqueous Nasal Spray 18 g, 140 doses; Schering-Plough, Kenilworth, New Jersey), 5 µg/kg/d, was administered to both nasal cavities once a day for 30 days with a Genem (Amherst, Massachusetts) adjustable micropipette.
- Group 2: Physiological saline (SA), 5 µg/kg/d, was administered to both nasal cavities once a day for 30 days with a Genem adjustable micropipette.
- Group 3: No treatment (NT) was administered to this group during the 30-day study period.

Nasal samples were collected from all rats at the beginning (day 0), in the middle (day 15), and at the end (day 30) of the study period. The process of acquiring nasal samples was carried out by nasal lavage, administering distilled water (separately) to both nasal cavities with a 2-mL Pasteur pipette. Nasal aspirates were then collected with the Pasteur pipette and were stored at –80°C. The samples were tested for sIgA by a commercial enzyme-linked immunosorbent assay (ELISA) kit at the end of the study period. Levels of sIgA were assessed by a biochemist who was unaware of the purpose of the study and groups.

Biochemical Evaluation
Rat nasal IgA levels were determined in duplicate by the ELISA using commercial kits (Life Diagnostic, Inc, Catalog No. 5016-2; West Chester, Pennsylvania) with a DV-990-BV ELISA Microplate Reader (NT laboratory, Rome, Italy). IgA is present in rat serum at concentrations of 0.1 to 1 mg/mL depending on age and nutritional status. To obtain values within range of the standard curve, we diluted the samples 20,000-fold. We dispensed 297.5 mL and 497.5 mL of 1:10 diluent into 2 tubes per sample to be tested. We pipetted and mixed 2.75 mL of the serum/plasma sample into the first tube containing 297.5 mL of diluent, which provided a 100-fold diluted sample. We mixed 2.5 mL of the 100-fold diluted sample with the 497.5 mL of diluent in the second tube, which provided a 20,000-fold dilution of the sample. Diluted test samples were then incubated in the microtiter wells for 45 minutes alongside rat IgA standards. The assay used goat anti-rat IgA for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated goat anti-rat IgA antibodies for detection. The microtiter wells were subsequently washed, and HRP conjugate was added and incubated for 45 minutes. IgA molecules were thus sandwiched between the immobilization and detection antibodies. The wells were then washed to remove unbound HRP-labeled antibodies, and tetramethylbenzidine (TMB) reagent was added and incubated for 20 minutes at room temperature. This resulted in the development of a blue color. Color development was stopped by the addition of Stop Solution, changing the color to yellow, and optical density was measured spectrophotometrically at 450 nm. The concentration of IgA was proportional to the optical density of the test sample and was derived from a standard curve. The inter- and intra-assay variation coefficients were <12% and 10%, respectively, as assayed in the duplicate. The assay range was 2.34 to 75 ng/mL.

Statistical Analysis
Statistical analysis was carried out using the Statistical Package for the Social Sciences version 16.0 for Windows (SPSS Inc, Chicago, Illinois). All quantitative variables were estimated using measures of central location (ie, mean and median) and measures of dispersion (ie, standard deviation). Data normality was checked using the Kolmogorov-Smirnov tests of normality. The Friedman test was used for in-group comparisons. The Wilcoxon test was used to determine on which days group values differed.

The Kruskal-Wallis test was used to compare differences in biochemical values on different days between groups (P < .05 was accepted as statistically significant). The Mann-Whitney U test was used to determine intergroup differences (P < .05 was accepted as statistically significant).

Results
Between-Group Comparisons
There were no significant differences between the baseline sIgA values of the 3 groups (P = .633) (Figure 1).

Significant differences were found between sIgA values for the 3 groups on day 15 (P = .006). The sIgA value for group 1 (MF) on day 15 (21.82 ± 9.7 ng/mL) was significantly higher than sIgA values for group 2 (SA) (15.07 ± 2.0 ng/mL) and group 3 (NT) (15.95 ± 5.27 ng/mL) on day 15 (P = .003 and P = .011, respectively) (Figure 1).

There were significant differences between sIgA values for the groups on day 30 as well (P = .010). The sIgA value for group 1 (MF) on day 30 (22.00 ± 9.4 ng/mL) was significantly higher than day 30 sIgA values for group 2 (SA) (16.60 ± 4.1 ng/mL) and group 3 (NT) (15.35 ± 5.15 ng/mL) (P = .012 and P = .008, respectively) (Figure 1).
Within-Group Comparisons

In group 1 (MF), the sIgA value for day 15 (21.82 ± 9.7 ng/mL) was significantly higher than the baseline value (13.20 ± 1.4 ng/mL) ($P = .021$). No significant difference was found between sIgA values on day 30 (22.00 ± 9.4 ng/mL) and day 15 (21.82 ± 9.7 ng/mL) ($P = .374$) (Figure 1).

In group 2 (SA), no significant differences were found between the baseline (14.05 ± 4.9 ng/mL) sIgA value and the sIgA values on day 15 (15.07 ± 2.0 ng/mL) and day 30 (16.60 ± 4.1 ng/mL) ($P = .196$) (Figure 1).

In group 3 (NT), there were no significant differences between the baseline (13.94 ± 2.84 ng/mL) sIgA value and the sIgA values on day 15 (15.95 ± 5.27 ng/mL) and day 30 (15.35 ± 5.15 ng/mL) ($P = .459$) (Figure 1).

Discussion

Topical nasal corticosteroids are currently being used quite effectively for numerous nasal diseases. Among the many different topical nasal corticosteroids, MF has the lowest rate of systemic bioavailability (<0.1%). MF is used in the European Union for both the treatment and prophylaxis of seasonal and perennial allergic rhinitis, for adults as well as for children over 6 years old. It is also used in the United States for the treatment of similar indications after 2 years of age and is administered for prophylaxis after 12 years of age. Apart from the above indications, being a topical steroid, MF is used in dermatological diseases, in asthma, and for nasal polyps. It has been reported that MF is also effective by itself in uncomplicated rhinosinusitis cases.

Mometasone furoate binds to cytosolic glucocorticoid receptors, forming a complex, and enters the cell nucleus where it regulates the expression of various proinflammatory and anti-inflammatory genes. Mometasone furoate inhibits the early and late phases of the allergic reaction, prevents inflammatory cells from entering the nasal mucosa, and hinders the expression of soluble mediators, such as histamine, interleukin (IL)-1, IL-4, IL-5, IL-6, IL-8, interferon (IFN), leukotrienes, and tumor necrosis factor (TFN). Although the above effects of MF are well studied, the entire range of its pathophysiological effects are not yet fully understood, and while MF has been used on nasal mucosa for quite a long while, there is yet no clear information in medical literature about how MF affects sIgA, which has the most important role in the mucosal defense system.

Secretion of sIgA occurs in the mucosal cells of the gastrointestinal and respiratory tracts. It has 2 subtypes: sIgA, which is found in serum and secreted by the B cells of bone marrow, and sIgA2, which is secreted by the B cells of mucosal membranes and is found in mucous secretions like colostrum, saliva, tears, and breast milk. Two IgA molecules that are bound together by the secretory component and the J-protein are responsible for the formation of sIgA. After sIgA binds to an antigen, it is transported to the luminal surface of the cell and is secreted by exocytosis.

The transport of sIgA to the mucosal surface is carried out by binding to the polymeric immunoglobulin receptor secreted at the basolateral membrane of mucosal epithelial cells. In the body’s defense system against microorganisms, sIgA is the first protective barrier. It prevents the adhesion of antigens to epithelial cells, thus inhibiting their entrance into the cell, while facilitating their clearance with peristaltic and mucociliary movements. By reducing the motility and invasive capacity of bacteria, sIgA affects bacterial virulence. A decrease in the slgA concentration of the secretions responsible for mucosal immunity causes an increase in the frequency of upper respiratory tract infection. Also, sIgA has a key function in the immunological response against the allergens and microorganisms of the upper respiratory tract.

In our study, based on the above information, we have investigated the role of slgA on the effects of MF at nasal mucosa. In group 1 (MF), the slgA value was significantly higher on day 15 than at baseline. That finding demonstrates that slgA levels increased in the nasal mucosae of rats that received MF. Such a result suggests that an increase in slgA may play a role in MF’s anti-inflammatory and antiallergic effects.

The importance of the immune system in controlling allergic symptoms has been demonstrated in numerous animal and human studies. Neutralization of allergens is carried out by slgA, which is found abundantly in mucous secretions; slgA thus facilitates the entrance of allergens to lamina propria and contributes to the decrease in the inflammatory response. Passive application of antigen-specific or nonspecific slgA causes a decrease in airway response and pulmonary eosinophilia or allergic rhinitis. It has also been demonstrated that slgA decreased allergic asthma attacks by inhibiting mast cell activation. The above information is compatible with the results of our study and supports the connection between MF and slgA.

The maximal therapeutic effect of MF is seen 1 to 2 weeks after the start of therapy. In our study, the slgA level of rats that received nasal MF was increased between day 0 and day 15. The slgA levels of group 1 (MF) showed no significant difference between days 15 and 30. That finding is compatible with the time period of MF’s maximal therapeutic effect reported in relevant literature. Our study showed that MF application could provide a certain amount

Figure 1. Intergroup comparison of secretory immunoglobulin A levels. MF, mometasone furoate; NT, no treatment; SA, saline.
of increase in the sIgA content of the nasal mucosa. Such an effect may be due to the immunological makeup of the nasal mucosa, and further experimental and clinical studies are needed.

In our study, sIgA levels in the group of rats that received saline did not show any significant difference. Saline is used in routine otorhinolaryngologic practice for its mild decongestive effect and for clearing debris. The sIgA levels of our no-treatment group likewise did not show any significant changes.

The strong points of our study are the appropriate application period, the presence of saline and no-treatment control groups, and sensitive biochemical evaluation.

Conclusion
Our experimental study demonstrated that a topical corticosteroid (MF), when applied for 30 days to rats’ nasal mucosa, significantly increased nasal sIgA levels. While the sIgA level was increased between days 0 and 15 in the rats, no significant change took place from days 15 to 30. The relationship between nasal corticosteroids and sIgA has been demonstrated for the first time in the medical literature and needs to be developed by further clinical randomized prospective studies.

Author Contributions
Fadullah Aksoy, designed study, revised article; Remzi Dogan, designed study, drafted article; Ilker Kocak, analyzed data, drafted article; Bayram Veysseler, interpreted data, revised article; Orhan Ozturan, collected data, revised article; Said Incir, interpreted data, drafted article (biochemical section).

Disclosures
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