

Effect of nasal antihistamine on secretory IgA in nasal lavage of rats

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Abstract The humoral IgA is an immunoglobulin which plays a defensive role for organisms on mucosal surfaces. Today, intranasal antihistamines are effectively used in the treatment of allergic rhinitis. In our study, the effect of azelastine hydrochloride—a nasal antihistaminic—on humoral IgA of the nasal mucosa has been reviewed empirically. Twenty-four female Sprague–Dawley rats were included in our study. The rats were divided into three groups randomly. Group 1 (azelastine hydrochloride): rats in this group had nasal azelastine hydrochloride (0.05%) applied for 30 days at 10 µl/nostril dosage. Group 2 (saline): saline (0.09%) was applied to the rats in this group for 30 days at 10 µl/nostril dosage. Group 3 (control): no application was made throughout the study. The chemicals applied in Groups 1 and 2 were applied to both nostrils by mounting a flexible micropipette to the end of an insulin injector. At the beginning of the study, nasal lavage was performed to both nostrils of the rats in every group on the 15th and 30th day to aspirate irrigation solution (distilled water). The aspirated liquids were kept at -80° temperature and reviewed together at the end of study. Within-group comparisons: in Group 1 (azelastine hydrochloride), the humoral IgA value on the 15th day was significantly higher than the basal value ($p = 0.037$). There

is a significant difference between humoral IgA value on the 30th day and humoral IgA value on the 15th day ($p = 0.045$). In Group 2 (saline), no significant difference is available between basal, 15th day and 30th day humoral IgA values ($p = 0.265$). In Group 3 (control), no significant difference is available between basal, 15th day and 30th day humoral IgA values ($p = 0.374$). Between-group comparison: there is no significant difference in between-group humoral IgA basal values ($p = 0.714$). On days 15 and 30, Humoral IgA value of Group 1 was significantly higher than that of Groups 2 and 3 ($p = 0.013$, $p = 0.024$, respectively). According to the results we achieved in our study, nasal antihistaminic (azelastine hydrochloride) significantly increases the level of humoral IgA. Our study is the first one in the literature to reveal a relation between nasal antihistaminic and humoral IgA and there is a further need for clinical, randomized and prospective studies.

Keywords Azelastine hydrochloride · Nasal antihistaminic · Nasal lavage · Secretory Ig

Introduction

Allergic rhinitis is a common disease affecting approximately 30% of adults and 40% of children [1]. It is characterized by nasal congestion, rhinorrhea, sneezing, and nasal itching [1]. Treatment options include intranasal and oral medications, or immunotherapy [2]. Intranasal antihistamines are now being used effectively in the treatment of allergic rhinitis. Azelastine is a second-generation antihistamine that selectively antagonizes H1 receptors [3]. Azelastine nasal spray provides significant improvement in seasonal allergic rhinitis treatment [4].

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SIgA is an immunoglobulin that is released into the mucosal surfaces as the first line of immunologic defense. It is produced in the mucosa of respiratory, gastrointestinal and genitourinary tracts by plasma cells and released into the mucosal secretions as well as saliva, tears and milk. The effects of secretory IgA on the mucosal surface can be summarized as follows: inhibition of adhesion of microorganisms to mucosa, agglutination of antigens, circulatory removal of immune complexes, intravenous virus neutralization, and neutralization of toxins [5, 6]. The SIgA levels in nasal secretions significantly increased following intranasal mometasone furoate application [7].

Despite long-term use, the effects of nasal antihistamines to SIgA in nasal secretions are still unknown. In this study, we investigate the effects of azelastine hydrochloride as a nasal antihistamine to SIgA in nasal secretions.

Materials and methods

Our study commenced after gaining approval (no: 2013/49) from the University Experimental Animals Local Ethics Committee. Twenty-four female Sprague–Dawley rats were included in our study. The rats were randomly divided into three groups.

Study groups

Group 1 (azelastine hydrochloride): nasal azelastine hydrochloride (Allergodil nasal spray 0.14 mg 18 ml, Gen Ltd, Ankara, Turkey) (10 µl/nostril) was administered to rats in this group for 30 days.

Group 2 (saline): intranasal saline (0.09%) (10 µl/nostril) was administered to rats in this group for 30 days.

Group 3 (control): no medication was administered for 30 days.

The chemicals administered in Groups 1 and 2 were given to both nostrils by inserting a flexible micropipette at the end of the insulin injector. Nasal lavage was performed with distilled water and aspirated from both nostrils of all the rats in all groups at the beginning, on the 15th and 30th days of the study. The aspirated liquid samples were stored at -80°C .

At the end of study, the samples were tested for sIgA by a commercial enzyme-linked immunosorbent assay (ELISA) kit. A biochemist who was unaware of the design and purpose of the study and the groups evaluated the levels of SIgA in the samples.

Nasal IgA levels of the rats were assessed in duplicate by the ELISA using commercial kits (Life Diagnostic, Inc, Catalog no. 5016-2; West Chester, PA, USA) and a DV-990-BV ELISA Microplate Reader (NT laboratory, Rome, Italy).

Statistical analysis

Statistical analysis was carried out using the Statistical Package of the Social Sciences version 16.0 for Windows (SPSS Inc, Chicago, IL, USA). All quantitative variables were estimated using measures of central location (i.e., mean and median) and measures of dispersion (i.e., standard deviation). Data normality was checked using the Kolmogorov–Smirnov tests of normality.

The Friedman test was used for in-group comparisons and $p < 0.05$ was accepted as statistically significant. The Wilcoxon test was used to determine on which days group values differed. Bonferroni correction was made ($p < 0.016$ was accepted as statistically significant).

The Kruskal–Wallis test was used to compare differences in biochemical values on different days between groups ($p < 0.05$ was accepted as statistically significant). The Mann–Whitney U test was used to determine intergroup differences. Bonferroni correction was made ($p < 0.016$ was accepted as statistically significant).

Results

Within-group comparisons

In Group 1 (azelastine hydrochloride), the SIgA value on the 15th day (18.95 ± 4.6 mg/ml) was significantly higher than the baseline value (12.05 ± 2.4 mg/dl) ($p = 0.013$). There was a significant difference between the SIgA values on the 15th day (18.95 ± 4.6 mg/ml) and the 30th day (24.37 ± 3.8 mg/ml) of the study ($p = 0.014$) (Fig. 1).

In Group 2 (saline) there was no significant difference between SIgA values in basal (13.27 ± 2.8 mg/ml), 15th day (14.65 ± 3.2 mg/ml) and 30th day (15.53 ± 4.3 mg/ml) measurements ($p = 0.265$) (Fig. 1).

In Group 3 (control), there was no significant difference between SIgA values in basal (15.08 ± 3.27 mg/ml), 15th day (16.57 ± 6.38 mg/ml) and 30th day (14.92 ± 6.24 mg/ml) measurements ($p = 0.374$) (Fig. 1).

Between-group comparisons

There was no significant difference between SIgA baseline values of all groups ($p = 0.714$) (Fig. 2).

On day 15, the SIgA level of Group 1 (18.95 ± 4.6 mg/ml) was significantly higher than Group 2 (14.65 ± 3.2 mg/ml) and Group 3 (16.57 ± 6.38 mg/ml) levels ($p = 0.013$) (Fig. 2).

On day 30, the SIgA level of Group 1 (24.37 ± 3.8 mg/ml) was significantly higher than Group 2 (15.53 ± 4.3 mg/ml) and Group 3 (14.92 ± 6.24 mg/ml) levels ($p = 0.014$) (Fig. 2).

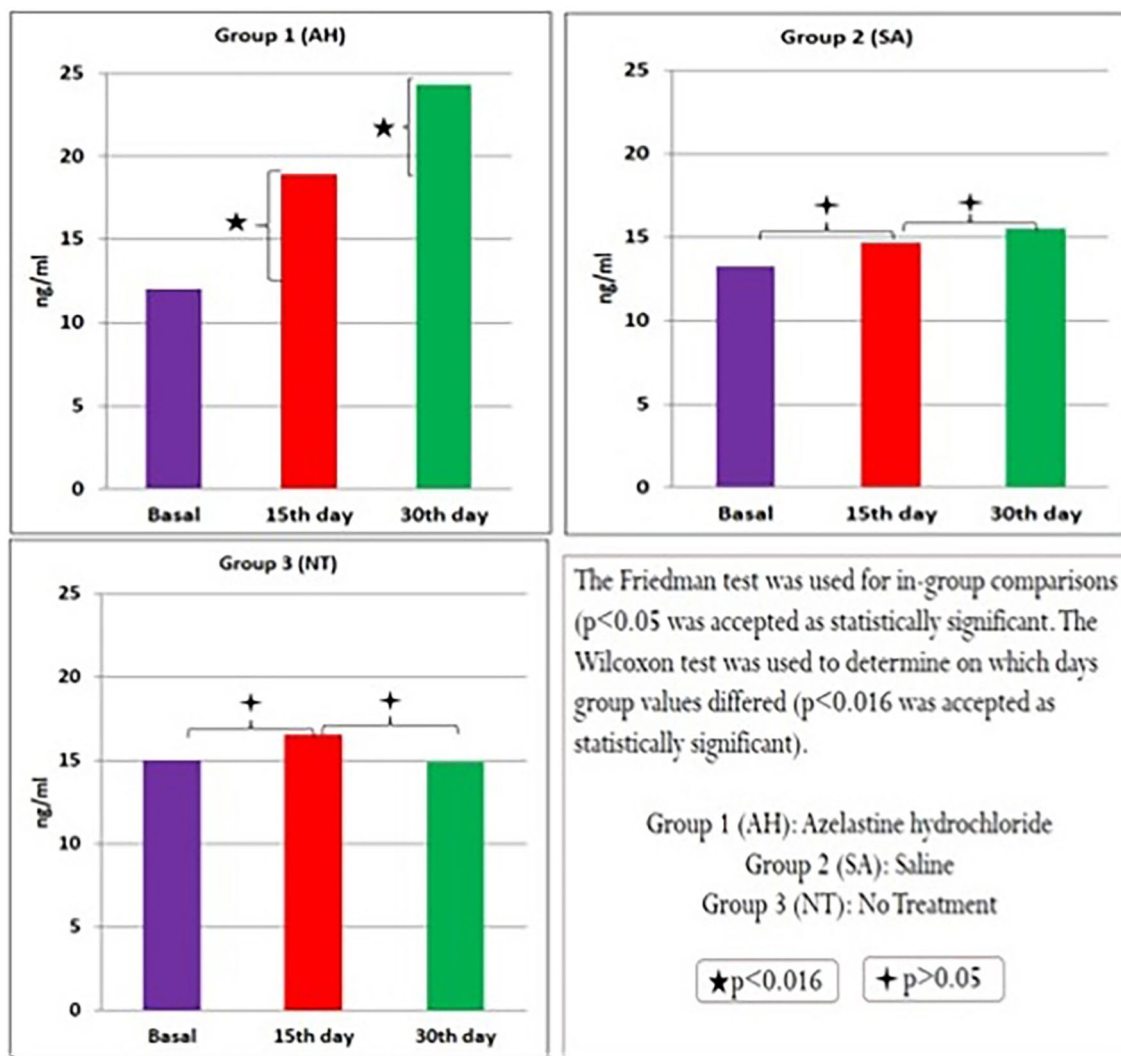


Fig. 1 In-group comparison of secretory immunoglobulin A levels

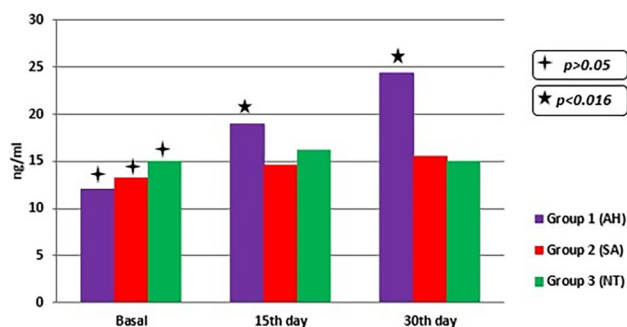


Fig. 2 Intergroup comparison of secretory immunoglobulin A levels

Discussion

For the treatment of nasal symptoms of AR patients, intranasal antihistamines have shown to be effective in

well-designed randomized, controlled, and blinded studies [8]. The advantages of topical intranasal application include the rapid onset of action (only 15–30 min after administration) [9], the ability to affect the inflammation area, and the low incidence of systemic side effects [10]. This drug has a half-life of about 22 h for oral use, and in its intranasal spray form it has been shown that the duration of action is about 12–14 h in recent studies. Due to its long half-life, a single dose or two doses can be used daily.

Secretory IgA (SIgA) is the predominant Ig in milk, colostrum, tears, saliva, and mucosal secretions that are on the mucosal surfaces of the respiratory, gastrointestinal, and genitourinary tracts. It is synthesized locally and is mainly in a dimeric form, although low levels of some larger polymers, particularly tetramers, are also present [11]. Secretory IgA is found in two different forms: IgA1 and IgA2. In serum, IgA1 is present predominantly and is produced by the B cells in

bone marrow. IgA2 is produced by the B cells in the mucosa and found in colostrum, milk, tears and saliva [12].

SIgA directly protects mucosal surfaces by cross-linking microorganisms or macromolecules, thus preventing epithelial cells from contact with the surface [13]. It blocks adhesions of microorganisms to the epithelial cell surface and inhibits their motility [11].

SIgA also prevents infections by pathogen neutralization [14]. It has been defined that sIgA plays a protective role in mucosal immunity. It has also been determined that sIgA is a potential healer in inflammation associated with allergic diseases. There is a large amount of IgA content in mucus secretions in allergic inflammation sections and IgAs in this mucosa are formed by sIgA [15]. In asthmatic patients, IgA is available at a higher rate than normal controls [16]. This finding made us think that the body responds by increasing sIgA formation against an allergic reaction. Azelastine also increases sIgA and raises the antiallergic effect.

On the other hand, studies on the immunomodulatory role of SIgA have increased in recent years. It is known that allergic diseases are seen more frequently in patients with IgA deficiency [17]. High salivary SIgA levels were associated with a lesser development of allergic symptoms in sensitized children [18]. As a result of decreased or altered activity of SIgA, the immune response could be inappropriate or allergic reactions could be against aeroallergens [19].

Azelastine blocks histamine release from rat mast cells and from human basophils, and inhibits the response to histamine via antagonism of airway H1 receptors [3]. In vitro and in vivo studies have demonstrated that azelastine may affect early and late reactions in allergic rhinitis through its effect on histamine, leukotrienes B4 and C4, kinins, superoxide/oxygen free radicals, cytokines (TNF- α , GM-CSF, IL-1 β , IL-4, IL-6, IL-8, ECP and nitric oxide) and ICAM-1 expression and/or release [20–24]. According to the American Journal of Otorhinolaryngology Allergic Rhinitis Treatment Guideline 2015, intranasal antihistamines can be used as a first or second line of therapy and they are especially useful in patients with episodic nasal symptoms or as a pretreatment prior to nasal allergen exposure [25]. In our study, significant increase was observed in sIgA levels of the group of normal rats to which azelastine was applied. There are no clear data in the literature to explain to us how that increase occurred. It was revealed in conducted studies that azelastine provides a reduction in leukotriene concentration [26, 27]. There are studies, which were carried out related to the effect of SIgA on interleukins, eosinophils and basophils [28, 29]. In these studies, there is limited information related to the relationship between sIgA and histamine and no additional studies were conducted after this time. However, we consider with the available data that the relationship between azelastine and sIgA might be effective through these mechanisms.

Although there is no clear relationship between the development of an allergic patient and mucosal IgA production, a high level of sIgA may prevent allergen absorption and the interaction between IgE antibodies and allergen in sensitized individuals, thus preventing the development of allergic symptoms [18]. We consider that sIgA levels increasing based on azelastine use might have affected both by preventing mucosal absorption and by preventing interaction of Ig E antibodies with an antigen. Though having different effective pathways, azelastine shows its antiallergic effect by increasing sIgA.

Dilek et al. reported that children with AR have decreased total nasal fluid SIgA levels independent of treatment [30]. Hsin et al. also showed similar results of adults with AR compared to healthy volunteers [31]. Cortesina et al. investigated SIgA titration in nasal secretions of AR patients as determined by the immuno-isoelectric focusing method. And they showed that SIgA ratios were significantly decreased compared to the control group [32]. Two different studies on AR patients have shown that the ratio of allergen-specific SIgA in nasal secretions does not decrease with intranasal budesonide use [33, 34]. In an experimental study, the SIgA levels in nasal secretions significantly increased following intranasal mometasone furoate application [7].

Although azelastine nasal spray has been used for a long time in the treatment of allergic rhinitis, we did not find any study about the effects on SIgA of azelastine nasal spray in the English literature. In our study, rats were divided into three groups based on the intranasal route of administration of azelastine nasal spray (Group 1), saline (Group 2) and non-drug control (Group 3). On days 0, 15 and 30 of the study, nasal secretion samples were obtained by nasal lavage of all rats. SIgA levels were measured by ELISA method and compared. In the group with applied azelastine nasal spray, SIgA levels were significantly higher on days 15 and 30 compared to the other two groups. In addition, SIgA levels on the 30th day were also significantly higher than the 15th day measurements.

Conclusion

In this study it was found that azelastine nasal spray caused a significant increase in SIgA levels in nasal secretions. Thus, for the first time in the literature, the relationship between intranasal antihistamines and SIgA in intranasal secretions has been established. Since this study is an experimental animal study, randomized and prospective clinical studies are needed.

Compliance with ethical standards

Funding source and financial disclosures None.

Research involving human participants and/or animals All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed consent For this type of study, formal consent is not required.

Conflict of interest All authors declare that they have no conflict of interest.

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