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RHINOLOGY

Assessment of the effectiveness of cyclosporine nasal spray in an animal model of allergic rhinitis

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Abstract The aim of this study is to show if cyclosporine has an antiallergic role in a rat model of ovalbumin-induced allergic rhinitis. The 54 rats were divided into six equal groups. The first group was a negative control group without induced allergic rhinitis; the second group a positive control with induced allergic rhinitis not receiving treatment. The remaining four groups, after induction of allergic rhinitis, received intranasal cyclosporine treatment in doses of 0.05, 0.1, or 0.2% or nasal steroid treatment. In the biochemical examination, on the surface of the tissue tumor necrosis factor (TNF) interferon (IFN), interleukin (IL)-5, IL-13, as well as IL-2, IL-4, IL-17A, and IgE were studied. Histologically, ciliary loss, increase of goblet cells, vascular congestion, and the degree of eosinophil infiltration were rated. In all treatment groups, on average, a significant reduction in all histological and biochemical values was found compared to the positive control group. Comparing each of the three cyclosporine-using groups with the group of nasal corticosteroid did not show any significant difference in the average scores. Cyclosporine nasal drops are effective to be used in an animal model of experimental allergic rhinitis without systemic effects.

Keywords Allergic rhinitis · Rat · Nasal spray · Immunosuppressive · Cyclosporine · Corticosteroid

Introduction

Rhinitis is an inflammatory disease of the nasal mucosa that can occur with allergic or non-allergic factors. Allergic rhinitis is a specific reaction of the nasal mucosa to an allergen mediated by IgE. In the pathogenesis, a disruption of the interaction between inflammatory cells and cytokines as a result of an imbalance between allergen-specific T helper 1 cells (Th1) and T helper 2 cells (Th2) plays a fundamental role. Commonly used treatments are topical and oral antihistamines, topical and oral corticosteroids, topical and oral decongestants, mast cell stabilizers, mucolytics, anticholinergic agents, and physiological saline sprays.

Cyclosporine is an immunosuppressive molecule inhibiting interleukin synthesis by inhibiting calcineurin in Th cells. To this day, it is used systemically against rejection of transplanted organs. In many eye diseases, it is also used locally [1]. There are studies showing it to be safe for use in conditions that include allergic reactions in their pathogenesis, such as allergic conjunctivitis, atopic dermatitis, and asthma [2]. There are a few studies about its use in allergic rhinitis, which is constituted by similar mechanisms. The aim of this study is to demonstrate if cyclosporine plays an antiallergic role in a rat model of ovalbumin-induced allergic rhinitis.

This study was presented at the 38. Turkish National Congress Otorhinolaryngology Head and Neck Surgery was chosen the best rhinology study (Abstract Number SB-006).

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Materials and methods

This study was carried out using 54 healthy female Sprague Dawley rats weighing on average of 250–300 g, which were obtained from the Experimental Application and Research Center of the medical faculty of our university. The study was started after obtaining approval from our university's experimental animal ethics committee (Ethics Committee Approval No. 112/2015 dated 3/31/2015). Expenses of the study were covered by the Scientific Research Support Unit (6.2015/12).

The rats were kept in cages with a maximum of eight animals each under a 12-h-light–12-h-dark cycle at an ambient temperature of 24 ± 2 °C and fed standard laboratory diet. The animals were allowed to drink water and eat food ad libitum during the experiment.

The animals were divided into six groups of nine rats each:

1. Negative control group (physiological saline (PS)-sensitization + intranasal PS provocation).
   In this group, rats were sensitized with intraperitoneal PS and intranasal PS was given.

2. Positive control group (ovalbumin [OVA] sensitization and provocation + intranasal PS).
   In this group of rats, sensitization and provocation were made with OVA; PS was given as intranasal therapy.

3. Steroid group (OVA sensitization and provocation + intranasal corticosteroid).
   In this group, rats were sensitized and provoked with OVA, and corticosteroid was given as intranasal therapy.

4. Experimental group 1 (OVA sensitization and provocation + intranasal cyclosporine 0.05%).
   Rats in this group were sensitized and provoked with OVA, and 0.05% cyclosporine was given as intranasal treatment.

5. Experimental group 2 (OVA sensitization and provocation + intranasal cyclosporine 0.1%).
   Rats in this group were sensitized and provoked with OVA, and 0.1% cyclosporine was given as intranasal treatment.

6. Experimental group 3 (OVA sensitization and provocation + intranasal cyclosporine 0.2%).
   Rats in this group were sensitized and provoked with OVA, and 0.2% cyclosporine was given as intranasal treatment.

Sensitization with ovalbumin

The AR model was prepared according to a previously published protocol [3]. Each group of rats was sensitized with intraperitoneal (IP) injections of 1 ml physiological saline (PS) containing 0.3 mg ovalbumin (OVA) (Grade V, Sigma–Aldrich Chemical Co. St. Louis, MO) and 30 mg Al(OH)3 every other day over 14 days.

Provocation test with ovalbumin

Provocation of allergic rhinitis in the rats sensitized with IP OVA was made with daily application of drops prepared with 20 µl 10% OVA solution dissolved in 1 ml PS into both nostrils.

Drug treatment

In the first group, allergic rhinitis was not induced and intranasal ps was applied (negative control group). In the second group, after 2 week sensitization with IP OVA and use of intranasal OVA during the last 2 weeks, allergic rhinitis without treatment was achieved (positive control). In the third group, after 2-week sensitization with IP OVA, in the last 2-week intranasal OVA and 1 h later, a treatment with intranasal 0.5 mg/kg triamcinolone acetonide (Nasocort, Aventis Pharma Ltd. Holmes Chapel, England) was given. The other groups, after 2-week sensitization with IP OVA, were given intranasal OVA and 1 h later intranasal cyclosporine (Restasis, Allergan Inc. Irvane, CA) in concentrations of 0.05, 0.1, and 0.2%, respectively, for the last 2 weeks. The intranasal drugs were applied after sedation with 2% sevoflurane. Attention was given to avoid cessation of respiration in that the animals. Twenty-four hours after the last drug application, the animals were anesthetized with intramuscular ketamine hydrochloride (ketamine® flacon, Parke-Davis) and sacrificed. An intracardiac blood sample was taken. Subsequently, the animals' nasal cavity, nasal septum, paranasal sinus, and conchae were removed en bloc. For this purpose, an incision was performed beginning at the level right in front the incisors and stretching to the end of the hard palate. For histological examination and biochemical analysis, tissue samples were taken from the mucosa of the nasal septum.

Evaluation of symptoms

The immediate and late reactions of all animals after nasal challenge were evaluated on day 1, 7, 14, 21, and 28 after the

administration OVA. Observations were performed by the same person for 10 min, following an adaptation period of 10 min. AR was evaluated regarding the severity of typical clinical symptoms that included nasal irritation, sneezing, and nasal secretion. Nasal symptom scores were graded on a four-point scale, and each grade was assigned a numerical score (0–3): (1) nasal itching motion: 0—none; 1—rubbing nose 2/min; 2—rubbing nose 4–6/min; and 3—rubbing nose > 6/min; (2) sneeze: 0—none; 1–1–3/10 min; 2–4–9/10 min; and 3— > 10/10 min; and (3) nasal mucus: 0—none; 1—in a nostril; 2—outside a nostril; and 3—overflowing. On day 14, which was the first day of sensitization, nasal scratching, sneezing, and nasal flow were scored. The AR model was considered successful if the total score exceeded 5 [3].

Histological examination

The septal mucosa samples extracted from the sacrificed animals were fixated for 72 h in 10% neutral buffered formalin (pH 7.2). After fixation, the tissues were dehydrated in an ascending alcohol series of 70, 80, 90, 96, and 100% ethyl alcohol. The samples were then left in xylene for 3 h to soften and were molded in paraffin at 60 °C. After molding, ca. 5 µm-thick sections were sliced by microtome. The sections were routinely stained with hematoxylin eosin. Stained samples were examined with an optical microscope (Nikon Eclipse i5, Tokyo, Japan).

In microscopic examination, ciliary loss, increase in goblet cells, vascular congestion, and degree of eosinophil infiltration were rated [4]. The scoring was done semiquantitatively, according to Ercan et al., giving each parameter "0" points in the absence of any modification, "1" for slight change, "2" for noticeable change, and "3" points for severe change [5]. Averages of the results of the count were evaluated statistically.

Biochemical assessment

Hemogram and biochemical parameters (AST, ALT, BUN, creatinine, total bilirubin) of the intracardiac blood sampled immediately before sacrification at the end of the experiment were assessed.

Inflammatory parameters

The sinonasal mucosa samples were homogenized mechanically and again suspended in 500 µl PBS. These homogenates were filtrated using 70 µm filters (SPL Life Sciences, Pocheon, Republic of Korea). These filtrates were centrifuged for 5 min at 12,000× g at a temperature of 4 °C. After centrifugation, supernatants were collected and stored at − 80 °C until the samples were worked up. To measure rat cytokines in the supernatant, Flex Sets and Enhanced Sensitivity Flex Sets (BD Biosciences, San Jose, CA) were used. Tumor necrosis factor (TNF), interferon (IFN), interleukin (IL)-5, IL-13, and IL-2, IL-4, and IL-17A were studied. In accordance with the producing company's instructions, a cytometric bead array was applied. Fifty µl of each sample were mixed with 50 µl bead and incubated under room air in darkness for 2 h. Then, 50 µl phycoerythrin-conjugated antibodies were added to sandwich the analyte. As a standard, recombinant proteins (TNF, IL-2, IFN, IL-4, IL-5, IL-13, and IL-17A) were dissolved in buffer and diluted serially. After rinsing the unbound antibodies, each sample was worked up with a flow cytometer (BD LSR II instrument; BD Biosciences). For these measurements, the FACSDiva software package (BD Biosciences) was used.

Nasal serum irrigation samples were assessed with IgG *ELISA*.

Statistical analyses

Statistical data were analyzed using the SPSS ver. 16.0 package (SPSS Inc. Chicago, IL, USA). Descriptive statistics in this study are given as means and standard deviation. Normal distribution has been assessed by Kolmogorov–Smirnov test. For non-normally distributed parameters, nonparametric tests have been used.

To establish significance in the comparison of quantitative data between groups, Kruskal–Wallis test was performed. To compare groups pairwise, Mann–Whitney *U* test was used. A value of $p < 0.05$ for the test results was considered statistically significant.

Results

During the course of the study, six rats died of respiratory depression under sevoflurane anesthesia and were removed from the study (two from the nasal steroid group, two from the positive control group, one from the cyclosporine 0.1, and one from the cyclosporine 0.2 group, respectively).

Experimental allergic rhinitis model

In the negative control group, no allergic symptoms were seen. In all other groups, after the first topical intranasal OVA application, typical symptoms of allergic rhinitis such as frequent sneezing, nasal scratching, and watery rhinorrhea were observed in each animal, which on average continued for 1.5 h. Since the total score in all animals was > 5 at the first subjective evaluation of AR carried out on day 14, the AR model was considered successful. With repeated topical intranasal OVA application, duration and intensity of the symptoms increased. Symptoms were

reduced and disappeared on day 2 in animals treated with cyclosporine or steroid. Furthermore, subsequent intranasal challenges with OVA did not trigger AR symptoms. At the end of the study, all symptom scores decreased significiently in treated groups 3, 4, 5, and 6. Statistically significant differences were not observed in groups 1 and 2.

Histological examination

In the negative control group, no significant change in the histological parameters was observed. Mean of histological parameters was found to be 0.33.

In the positive control group (allergic rhinitis group), an increase in all histological parameters was observed. These mean values were found to differ significantly from those of the control group.

In the group that was given nasal steroids, scores for ciliary loss, eosinophil infiltration, goblet cell increase, and vascular congestion were significantly lower than the positive control group. In comparison with the negative control group, all scores except that for vascular congestion were significantly higher.

In the group treated with cyclosporine 0.05, mean scores were significantly lower than in the positive control group. Compared to the negative control group, no significant difference was noted in ciliary loss and vascular congestion, while eosinophil infiltration and goblet cell increase differed significantly.

In the group treated with cyclosporine 0.1, mean scores were significantly lower than in the positive control group. Compared to the negative control group, none of the scores showed a significant difference.

In the group treated with cyclosporine 0.2, mean scores were significantly lower than in the positive control group. In comparison with the negative control group, no significant difference was found in any of the scores.

Comparing among the study groups, no statistically significant difference in the mean histological parameters was found between the nasal steroid group and the cyclosporine 0.05 group, cyclosporine 0.1 group, and cyclosporine 0.2 group (Fig. 1).

Biochemical assessment

Comparing among the study groups, no statistically significant difference between ALT, AST, BUN, creatinine, bilirubin, and hemoglobin values was found at the end of the study ($p > 0.05$). We can say cyclosporine was found to be safe without causing any systemic side effects like nephrotoxicity or hepatotoxicity.

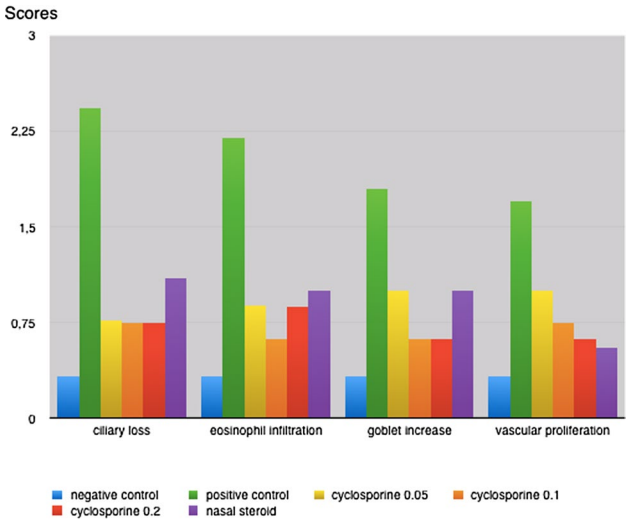

Fig. 1 Mean histological scores. 0—absence of any modification, 1—slight change, 2—noticeable change, and 3—severe change. In order of groups; negative control, positive control, cyclosporine 0.05, cyclosporine 0.1, cyclosporine 0.2, and nasal steroid

Inflammatory parameters

In the positive control group (allergic rhinitis group), a significant increase was observed in all biochemical parameters compared to the control group.

In the nasal steroid, cyclosporine 0.05, cyclosporine 0.1 group, and cyclosporine 0.2 group means of biochemical parameters were significantly lower compared to the positive control group (Table 1).

Compared to the negative control group, there was no significant difference except for IgE in the nasal steroid group, except for IFN and IL-4 in the cyclosporine 0.05 group, except for TNF and IgE in the cyclosporine 0.1 group, and except for IL-2 and IgE in the cyclosporine 0.2 group.

Comparing among the study groups, mean values for IFN, IL-13, IL-4, and IgE were not different between the nasal steroid group and the cyclosporine 0.05 group. The remaining parameters (IL-2, IL-5, TNF, and IL-17) were found to be significantly lower in the steroid group.

Between the nasal steroid and the cyclosporine 0.1 group, no statistically significant difference in the mean biochemical parameters was found except for IL-13, and the cyclosporine 0.2 group except for IL-2.

Comparing the cyclosporine 0.05 and 0.1 groups, while there was no significant difference in the mean biochemical parameters IFN, IL-4, and IgE, the other values were significantly lower in the 0.1 group. Equally, between the cyclosporine 0.05 and cyclosporine 0.2 groups, no statistically significant difference was found in the IL-2, IL-4, and IgE values. The other values were found to be significantly lower in the 0.2 group.

Table 1 Mean biochemical values (pg/ml)

	IL-2 (pg/ml)	TNF (pg/ml)	IL-17 (pg/ml)	IL-4 (pg/ml)	IL-5 (pg/ml)	IL-13 (pg/ml)	IFN (pg/ml)	IgE (pg/ml)
Negative control	1217[a]	2392[a]	442[a]	80[a]	64[a]	161[a]	137[a]	58[a]
Positive control	4173	10,806	5353	362	867	997	680	195
Cyclosporine 0.05	2385[a]	1337[a]	1337[a]	130[a]	195[a]	296[a]	193[a]	112[a]
Cyclosporine 0.1	1323[a]	1049[a]	590[a]	72[a]	85[a]	115[a]	137[a]	129[a]
Cyclosporine 0.2	1829[a]	2931[a]	535[a]	85[a]	68[a]	166[a]	89[a]	121[a]
Nasal steroid	1157[a]	2293[a]	522[a]	83[a]	64[a]	190[a]	170[a]	134[a]

[a]Comparison between positive control group and the other group, $p < 0.05$

Between cyclosporine 0.1 and cyclosporine 0.2 groups, no statistically significant difference was found for the mean biochemical parameters except for IFN and IL-5 (Fig. 2a, b).

Discussion

Allergic rhinitis is a disease with no mortality but increasing morbidity, characterized by membrane inflammation developing as a result of contact with allergens [6] found in 10–30% of the general population and in 35% of the pediatric age group. In therapy, the first and most important step is protection from the specific allergen that is responsible for the condition. In pharmacological therapy, antihistamines, leukotriene inhibitors, systemic and topical steroids, cromolyn sodium, and decongestants are in use. When adequate drug and environment control are insufficient, immunotherapy can be applied [7].

As is the case of a number of other diseases today, target-specific drugs with minimal side effects that are immunologically effective at the molecular level are needed for treating allergic rhinitis. It is imperative to develop new, effective, and safe therapeutic approaches to a condition as frequently seen as allergic rhinitis. In animal models of allergic rhinitis, intranasal anti-IL-1β/anti-TNF-α IgY administration has been shown to reduce inflammation as well as eosinophils in the peripheral blood and in nasal and bronchoalveolar lavage [8]. A study by Shimizu et al. is shown that suplatast tosilate, a Th2 cytokine inhibitor, reduces eosinophil infiltration in the nasal epithelium and mucus hypersecretion in allergic rhinitis by decreasing interleukins IL-4 and IL-13 [9]. Another study demonstrated that the T cell inhibitors cyclosporine and tacrolimus inhibited the histamine secretion in cultures derived from basophils of persons suffering from allergic rhinitis [10]. Another study showed that cyclosporine inhibited histamine secretion in an ovalbumin-induced allergic rhinitis guinea pig model [11]. While this and other studies have recently been dealing with the use of cyclosporine in allergic rhinitis, we could not find

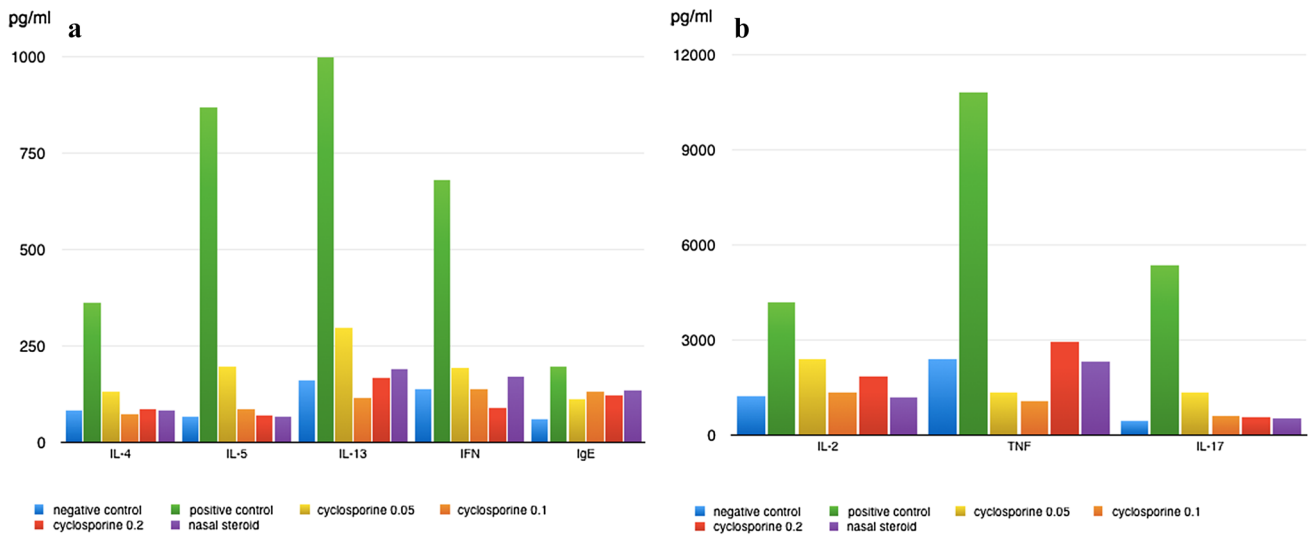

Fig. 2 **a, b** Mean biochemical values (pg/ml). In order of groups, negative control, positive control, cyclosporine 0.05, cyclosporine 0.1, cyclosporine 0.2, and nasal steroid

any study in the English literature dealing with its effect on the histology of the nasal mucosa and on cytokines. Our study distinguishes itself as a comprehensive animal model for the use of cyclosporine in allergic rhinitis.

Cyclosporine is a neutral, lipophilic peptide obtained from certain aerobic fungi, esp. *Hypocladium inflatum*. Initially, it was used as a weak antifungal agent [12]. Nowadays, it is applied systemically as an immunomodulator to prevent solid organ rejection. Cyclosporine is known as a calcineurin inhibitor. Calcineurin is required for IL-2 transcription in CD4 + helper T (Th1) cells. In this way, cyclosporine reduces lymphocyte proliferation and antigen sensitization [1]. While its main target are T helper cells, it shows effects on suppressor T cells, too, as IL-2 is a necessary mediator for all functions of T cells. In systemic application, cyclosporine has serious undesired side effects such as nephrotoxicity, hepatotoxicity, neurotoxicity, and leukopenia [1]. In contrast, it is commonly and safely used topically in a number of ophthalmological and dermatological conditions such as dry eye syndrome, allergic conjunctivitis, psoriasis, and atopic dermatitis, without serious side effects [13–15]. Ophthalmological use has been licensed by the Food and Drug Administration (FDA) since 2003 [16]. In a study with 33 steroid-resistant asthmatic patients, Alexander et al. found that a 12-week low-dose use of cyclosporine was effective against the symptoms [17]. Coren et al. treated five steroid-resistant pediatric asthma patients with cyclosporine over 18 months and found it to be effective [18]. In a study with 20 patients suffering from idiopathic urticaria, patients not responding to antihistaminic therapy showed remission after treatment with cyclosporine [19]. Considering the etiopathogenesis of all these patients studied with cyclosporine, atopy and type 1 allergic reactions are conspicuous. Thus, we felt the need for more studies investigating allergic rhinitis. As in the previous literature in an animal model of ovalbumin-sensitized experimental allergic rhinitis, we compared the effects of three different doses of nasal cyclosporine application with a negative control group, a positive control group with allergic rhinitis, and a group using nasal corticosteroid as the currently accepted gold standard [4, 9].

Between the groups, there was no significant difference in hemogram, AST, ALT, BUN, creatinine, and total bilirubin levels, demonstrating that there was no systemic impact. Cyclosporine was found to be safe in intranasal local application without causing any systemic side effects such as nephrotoxicity or hepatotoxicity. No remarkable side effects such as hair loss, decreased activity, and demise were noted during the study.

Comparison between the negative control group, which was not receiving any medication, and the positive control group that had only been sensitized with ovalbumin, but had not received allergic rhinitis therapy found a significant change in the positive control group regarding histological as well as biochemical parameters, demonstrating that we had succeeded in creating an allergic rhinitis model.

In the previous studies with cyclosporine and allergic rhinitis, we found that cyclosporine was applied in a single concentration [10, 11]. In our study, like ophthalmological use, we applied three different cyclosporine concentrations (0.05, 0.1, and 0.2%). Ophthalmological studies have reported that systemic transition is minimal and undesirable side effects are not found [20].

Immunohistochemical studies performed with nasal tissues in the allergic rhinitis rat model have shown an intense accumulation of eosinophil and basophil/mast cells in the lamina propria and the epithelium. In addition, in the nasal secretions from allergic rhinitis patients, a high amount of eosinophils has been found [21]. In our study, we also rated ciliary loss, goblet cell increase, vascular congestion, and eosinophil infiltration on histological sections and compared scores between groups. Comparing the mean histological scores of each of the three cyclosporine groups with the group treated with corticosteroid, considered the gold standard of therapy, no significant difference was found. Histologically, cyclosporine was found to be as effective as nasal steroids.

Cytokines are playing an important role in allergic rhinitis. The main cytokine of Th1 cells, interferon, activates gamma macrophages, stimulating phagocyte-complement binding. As the cytokines produced by Th1 cells are proinflammatory, they can lead to tissue damage in the course of fighting pathogens. Th1 cells reacting to autoantigens can cause tissue damage-creating autoimmune diseases such as juvenile diabetes and multiple sclerosis [22].

While cytokines produced by Th1 cells are providing cellular immunity, cytokines produced by Th2 cells such as IL-4 and IL-5 lead to the production of IgE and the differentiation of eosinophils. Thus, cytokines produced by Th2 cells suppress potentially damaging effects of the Th1 immune reaction [23]. Active Th2 lymphocytes produce IL-4, IL-13, and IL-5, which induce the production of IgE from B cells, activation and accumulation of eosinophils, and the production of mucus. In view of immunologic pathophysiology, in our study, we have examined IFN, IL-13, IL-2, IL-5, IL-4, TNF, and IL-17 in the rats' nasal mucosa tissue with flow cytometry and IgE in the nasal irrigation with the ELISA method.

A high IgE level is an important sign in the investigation of the presence of allergic diseases. One study reported testing for the presence of IgE antibodies as a specific method for the diagnosis of allergic diseases [24]. Chen et al. suggested that high serum IgE levels in children with perennial allergic rhinitis have a diagnostic value, and Winther et al. showed that serum IgE levels in patients with seasonal allergic rhinitis are correlated with the severity of rhinitis [25, 26]. In addition in our study,

the lowest values were found in the negative control group and the highest values in the positive control group. In all other treatment groups, a significant decrease was seen. We can say that the treatments are effective towards IgE, but an increase in the cyclosporine dose does not show an additional benefit.

IL-4 and IL-13 are playing an important role in the Th2 response [27]. They induce the production of IgE, are necessary for the lymphocytes' task of delivering antigens, and induce allergic inflammation [17]. Our study found the lowest values for these two cytokines in the negative control group and the highest values in the positive control group. All other treatment groups showed a significant reduction. Indeed, in the cyclosporine 0.1, cyclosporine 0.2, and nasal steroid groups, the value went down to the level of the negative control group.

IL-5 is a hematopoietic factor produced in lymphocytes, mast cells, and eosinophils. It is required for the activation of eosinophils [28]. Treatment of eosinophil-infiltrated tissues with anti-IL-5 mAb causes apoptosis of eosinophils and reduces eosinophilia [29]. This is why in the pathogenesis and treatment of allergic rhinitis, IL-5 levels are very important. In our study, in all treatment groups, the values decreased. With increasing cyclosporine dose, the values decreased significantly.

IL-2 is the basic growth factor for Th cells. Cyclosporine shows its principal effect in suppressing IL-2 production. An ophthalmological study showed that cyclosporine reduced the IL-2 levels in lacrimal fluid [30]. In our study, an increase in the cyclosporine dose led to a significant decrease of the values.

TNF is another potent proinflammatory cytokine secreted by eosinophils. An ophthalmologic study showed that cyclosporine reduced TNF levels in lacrimal fluid [31]. In our study, in all treatment groups, the values showed a decrease to the levels observed in the negative control group.

A role of IL-17 in the pathogenesis of allergic rhinitis is being considered but has not yet been entirely clarified. IL-17 is a proinflammatory cytokine. In all treatment groups, a reduction of the values was observed. This reduction was seen to be dose-dependent.

IFN is a cytokine that increases when damage to epithelial tissue occurs. Previous studies have shown a correlation with the corneal damage score [31]. In all treatment groups, a reduction of the values was observed. This reduction was seen to be dose-dependent.

In allergic rhinitis therapy, nasal corticosteroids can inhibit Th2 cytokines, while their effect on Th1 is minimal. Cyclosporine is effective in both pathways. A study carried out in an animal model for chronic sinusitis with nasal polyposis has shown that cyclosporine has a similar inhibitor effect as topical steroids not only on IFN, but also on TNF, IL-2, IL-4, IL-5, IL-13, and IL-17A [32]. It appears that cyclosporine can play a useful role in the treatment of AR, especially for patients with steroid resistance [14].

In a recent study, topical cyclosporine was found to be useful for the treatment of AR like our study [33]. This study is a mouse model unlike our study and there are not a symptom measurement. In addition, there are some differences at biochemical and histological methods. Therefore, these studies can be considered as different works supporting each other.

Conclusion

Cyclosporine nasal spray can be safely used in an animal model of experimental allergic rhinitis without short-term systemic effects. In the experimental allergic rhinitis animal model, cyclosporine is histologically and biochemically as effective as nasal corticosteroids, which are considered as the gold standard in therapy. In the histological parameters, an increase of the cyclosporine dose does not change the effect, but in the biochemical parameters, an increase in the cyclosporine dose raises the effect. Clinical studies for the use in humans are required.

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Compliance with ethical standards

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Conflict of interest Author Erol Senturk declares that he has no conflict of interest. Author Yavuz Selim Yildirim declares that he has no conflict of interest. Author Remzi Dogan declares that he has no conflict of interest. Author Orhan Ozturan declares that he has no conflict of interest. Author Eray Metin Guler declares that he has no conflict of interest. Author Mehmet Serif Aydin declares that he has no conflict of interest. Author Abdurrahim Kocyigit declares that he has no conflict of interest. Author Mukaddes Esrefoglu declares that she has no conflict of interest. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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