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The Therapeutic Effect of Capsaicin and/or Steroids on Inflammation in an Experimental Allergic Rhinitis Model

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ABSTRACT

Objective: Allergic rhinitis is a chronic upper respiratory disease characterized by inflammation of the nose due to the accumulation of inflammatory cells. We aimed to investigate the therapeutic efficacy of capsaicin and/or steroids in reducing the symptoms, proinflammatory cytokine levels, and inflammatory cell infiltrations in an animal model of allergic rhinitis.

Materials and Methods: Thirty-five male Wistar rats were divided into five groups. Following injection and initial intranasal challenge with ovalbumin, rats were treated with intraperitoneal capsaicin (50 mg/kg) and/or steroid (10 mg/kg) injection for seven days. After nasal symptom scorings, all rats were sacrificed under anesthesia, and blood samples and nasal septa were collected for hematologic, biochemical, and histopathologic examinations.

Results: The ovalbumin challenge increased nasal symptom scores, eosinophil and basophil counts, the serum IL-4, IL-5, IL-13 and IL-33 levels, and histopathologic damage. Capsaicin and/or steroid administration attenuated the allergic rhinitis symptoms. There was a therapeutic effect of capsaicin and the combined use of capsaicin and steroids on experimentally-induced allergic rhinitis as reflected by decreases in allergic inflammation and secretion of cytokines. There was no difference between the CAPS, CAPS-STR, and STR groups in terms of allergic rhinitis symptoms.

Conclusion: Parenteral administration of CAPS alone, and especially the combined use of CAPS and STR, effectively treats allergic rhinitis symptoms in a rat model of allergic rhinitis. Our results show that CAPS alone is not an effective alternative to STR but accelerates the recovery of allergic rhinitis.

Keywords: Allergic rhinitis, capsaicin, ovalbumin, rat, steroid

INTRODUCTION

Allergic rhinitis is a widespread chronic nasal inflammation characterized by sneezing, congestion, itching, and rhinorrhea (1). Due to its high frequency and numerous comorbidities and consequences, AR is an important factor regarding general health (2). This disorder is characterized by over-secretion of acetylcholine and pro-inflammatory cytokines from parasympathetic innervation to the nasal mucosa (1). Allergic rhinitis occurs when circulating inflammatory cells, including eosinophils and basophils, migrate to the inflammation area from the alveolar capillaries (3). Pharmacologic therapy for allergic rhinitis includes administration of immunotherapy, but this frequently causes some side effects or can lead to desensitization or reduced effectiveness when administered over a long term (3).

Currently, the primary treatment for AR is medication, whose therapeutic success has been substantially supported by mounting data. The available drugs or their combinations cannot completely treat allergic rhinitis symptoms. The fact that no definitive treatment has been found leads to studies for new molecules. Capsaicin (CAPS) is a rec-

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ommended treatment method for patients with chronic rhinosinusitis and nasal polyps (4,5). Capsaicin is also a recommended agent for allergic rhinitis (6).

The occurrence of allergic inflammation and innate and acquired immune responses are related to an alteration in the balance between the levels of different cytokines (7). Some cytokines, including the interleukins IL-4, IL-5, and IL-6, induce the release of Immunoglobulin E (IgE) and eosinophil cationic protein (ECP) to trigger eosinophil chemotaxis from TH2 cells (8). The airway inflammatory process has been evaluated using animal models, such as the nasal challenge model, to provide a therapeutic assessment of the administration of various therapeutic agents (9,10). One of these agents is CAPS, a compound extracted from hot peppers. CAPS administration promotes a selective degeneration/desensitization of peptidergic neurons in the nasal mucosa and has shown beneficial effects in neuronal stimulation (11,12).

In the present study, the anti-inflammatory and anti-allergic effects of CAPS were compared with those of steroids (STR) in a rat model of allergic rhinitis induced by ovalbumin (OVA) administration.

MATERIALS and METHODS

Ethics

This research was reviewed and approved by the ethics committee of Atatürk University Animal Experiments Local Ethics Committee (Decision No: 36643897- 25 / 23). All procedures performed in studies involving human participants abided by the institutional and national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Animal housing procedure

Thirty-five male Wistar rats (12 weeks old) weighing 240 to 270 g were selected for this study. The animals were divided randomly into five groups (n=7 per group) as follows:

1- Control group,

2- Ovalbumin (OVA) group,

3- Ovalbumin plus steroids (STR) group,

4- Ovalbumin plus capsaicin (CAPS) group, and

5- Ovalbumin plus STR and CAPS (CAPS-STR) group,

All procedures were performed according to the ''Experimental Animal Care and Use'' protocol after obtaining ethics committee approval. Pairs of rats were placed in cages in a room with a 12-hour daylight/darkness cycle and an ambient temperature of 23 C±2 C with 55%±10% humidity throughout the study. The processes of the method are summarized in the Supplemental graphical abstract.

Allergic Rhinitis Induction

Experimental allergic rhinitis was induced in the rats by sensitization, according to Shimizu and coworkers' revised protocol (13). Ovalbumin (Sigma Chemical Co., St. Louis, MO) was freshly prepared at 0.4 mg/mL in saline and precipitated at a 1:1 ratio with Al[OH]3 (20 mg/ml). For sensitization, the rats were given a 1 mL intraperitoneal injection of ovalbumin-Al[OH]3 solution at 0.2- mg/kg daily for 14 days. After the sensitization period, the intranasal challenge was initiated by treating the rats with 0.1 mL saline containing 10 mg OVA, administered in the form of intranasal drops to each side of the nose with a micropipette, once a day for seven days (control rats were given saline drops). After the development of allergic rhinitis (day 21), the experimental rats were given intraperitoneal CAPS at a dose of 50 mg/kg and/or STR (prednisone) at a dose of 10 mg/kg for seven days. The timeline and allergic rhinitis protocol are shown in Figure 1.

Allergic Rhinitis Model Scoring

After the last nasal provocation, each animal was observed for nose scratching, sneezing, nasal discharge, and feeding behavior. Animal behavior was observed for 30 minutes and scored according to nasal symptoms, which were graded on a four-point scale (14). Each grade was assigned a numerical score (0–3) and the scores were graded as summarized in Table 1.

Blood and Tissue Sampling

On day 28, 30 minutes after the last treatment, all rats were anesthetized with xylazine (10 mg/kg) and ketamine (40 mg/kg), and cardiac blood samples were collected from the aortas into ethylenediaminetetraacetic acid (EDTA) blood collection and serum tubes and stored at 4°C. The animals were euthanized using a lethal injection of sodium pentobarbital (50 mg/kg) after ether anesthesia. The nasal mucosa of each rat was dissected out and immediately placed in 10% neutral formaldehyde solution for light microscopy analysis.

Biochemical and Hematologic Parameter Analysis

The blood in the serum tubes was separated by centrifugation, and the resulting sera were stored at −80°C for biochemistry analysis. For hematologic analysis, the numbers of eosinophils and basophils in the blood samples from the EDTA tubes were determined with a blood counter (Abacus Junior Vet5, Diatron, Austria).

Biochemical Measurement of Serum IL-4, IL-5, IL-13, and IL-33 Levels

Serum IL-4, IL-5, IL-13, and IL-33 concentrations were measured using rat-specific sandwich enzyme-linked immunosorbent assay kits for IL-4 (EBioscience, USA, Cat no: BMS/628), IL-5 (Sunred, Cat. No: 201-11-0135), IL-13 (Sunred, Cat. No: 201-11-0113), and IL-33 (Sunred, Cat. No: 201-11-3102). Analyses were performed according to the manufacturers' instructions.

Table I: Variables of allergic rhinitis scoring method.

Variable	0	1	2	3
No. of nasal itching motion (scratches)	None	2	4-6	>6
No. of sneezes (time/minute)	None	2	4-6	>6
Amount of nasal flow (time/minute)	None	In one nostril	In both nostril	Out-flowing

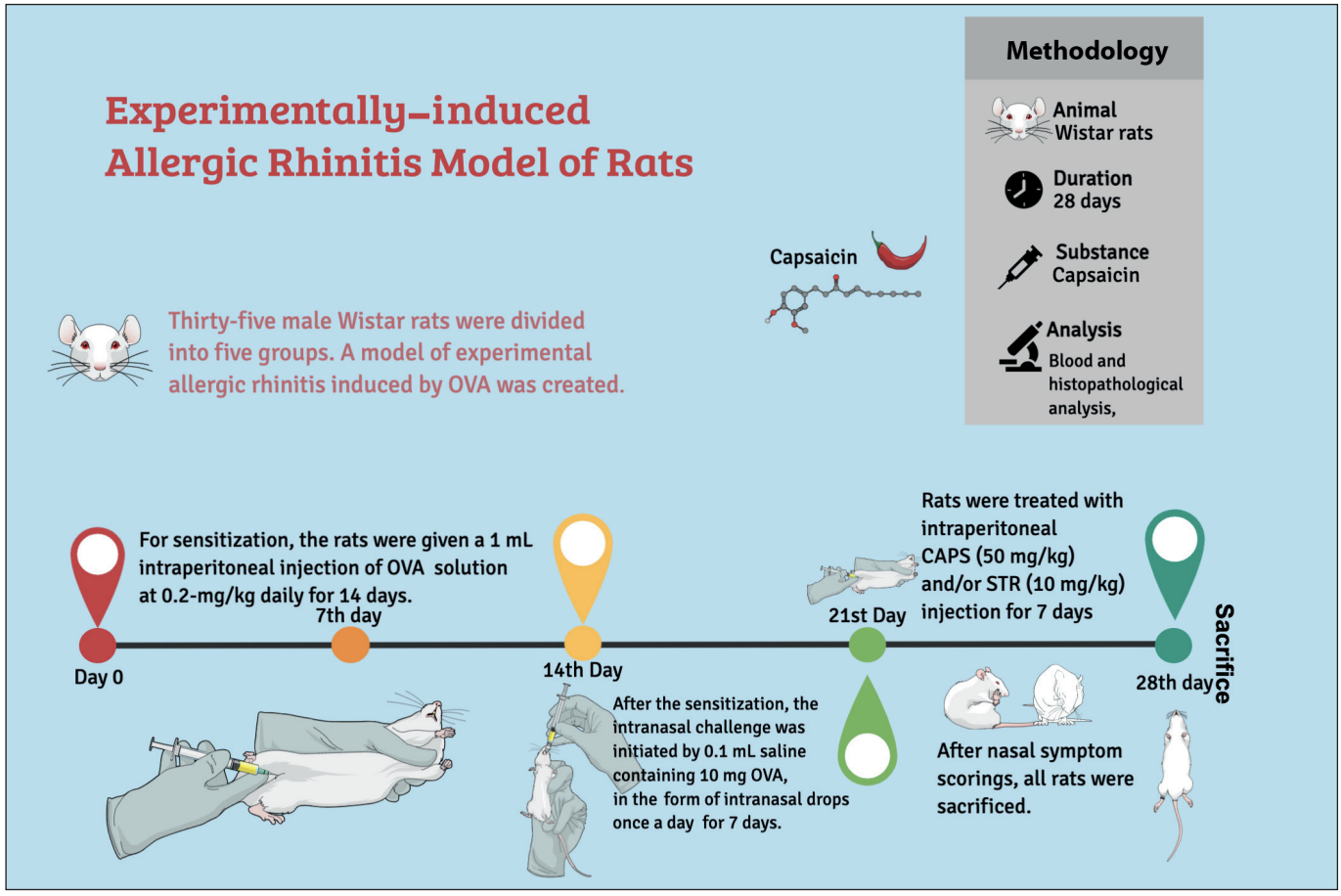

Figure 1. Timeline for intraperitoneal (ip) sensitization and intranasal challenge (Drops) with OVA for the rat model of allergic rhinitis. **Supplemental Graphical Abstract;** the methodology of the allergic rhinitis model experimentally created in rats.

Histopathologic Analysis

After fixation, the removed nasal mucosa specimens were decalcified in 6% nitric acid for five days. These tissues were then dehydrated, embedded in paraffin, and sectioned and stained with Crossman- modified Mallory triple staining for histologic evaluation. The nasal mucosa epithelium was evaluated, and photographs were taken by high-power light microscopy (Nikon Eclipse i50, Tokyo, Japan).

Statistical analysis

Because the data represent a normal distribution and the coefficient variables were more than 20%, the differences between the five groups were tested with a one-way analysis of variation, followed by a post-hoc Tukey test using SPSS 17.0 (SPSS Inc., Chicago, USA). $p<0.05$ was considered statistically significant.

RESULTS

Allergic Rhinitis

The nose scratch, discharge, and sneeze scores for allergic rhinitis are presented in Figure 2. The lowest allergic rhinitis score counts for nose scratching, nasal discharge, and sneezing were found in the control group and the highest counts in the OVA group ($p<0.05$). No differences were noted among the CAPS, CAPS-STR, and STR groups for nose scratch, discharge, and sneeze counts ($p>0.05$).

Hematologic and Biochemical Parameters

The hematologic and biochemical parameters are presented in Figure 3, respectively. The eosinophil and basophil count, as well as cytokine levels (IL-4, IL5- IL-13, and IL-33), were higher for the OVA group than for the other groups ($p<0.05$), but the differences among the control, CAPS, and CAPS-STR groups were not significant ($p>0.05$).

Histopathologic Results

The nasal cavity mucosa had a healthy histologic structure in the control group, including regular cilia and goblet cells in the pseudostratified ciliated cylindrical respiratory epithelium cells. However, the OVA group had typical signs of inflammatory histopathology, including edema, congestion, increased connective tissue, inflammatory cell infiltration, and vascular dilatation in the nasal wall mucosa. The CAPS and STR groups, and especially the CAPS-STR groups showed normal pseudostratified ciliated cylindrical respiratory epithelium cells with only mild congestion evident in the nasal mucosa (Figure 4).

DISCUSSION

In this study, we evaluated the therapeutic potential of CAPS and combined administration of CAPS and STR for the treatment of OVA-induced allergic rhinitis after stimulation, including the release of cytokines and the formation of an inflammatory pathology, in a rat model of OVA-induced allergic rhinitis (7).

For the therapy of AR, various pharmacological alternatives are available, including first-line medications, oral and/or intranasal H1-antihistamines, intranasal STR, and the combination of STR and H1-antihistamines. (5,15) Although CAPS relieves the symptoms of AR, nasal itch-

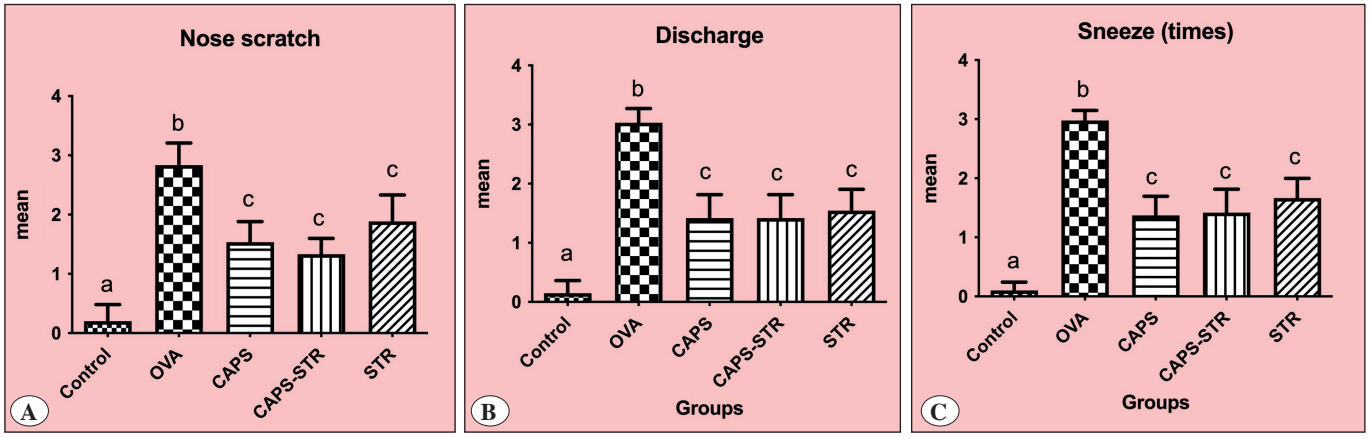

Figure 2. The scores of allergic rhinitis model results for all groups, **A)** Nose Scratch, **B)** Discharge, **C)** Sneeze a; no statistical difference, b; p<0,05, c: p>0,05.

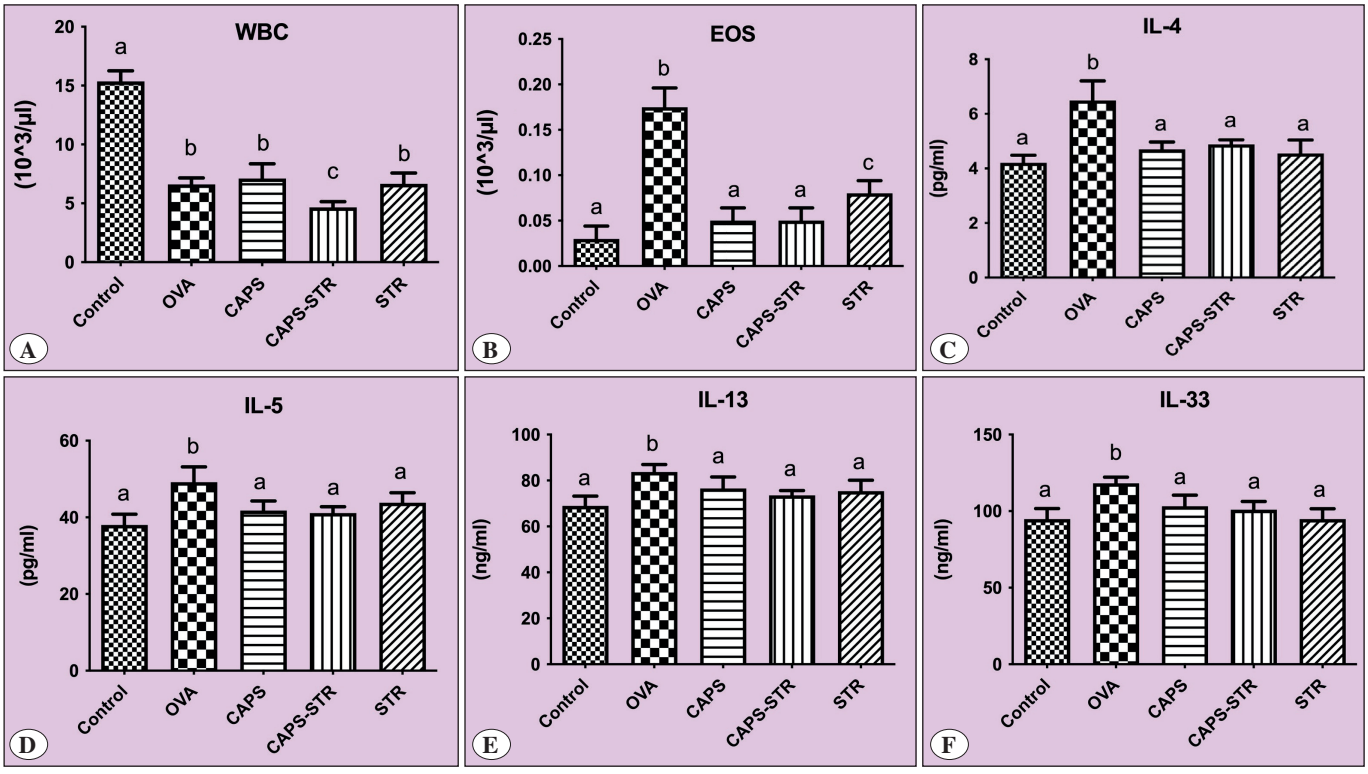

Figure 3. The graphics of hematologic eosinophil counts and basophil counts **(A and B)**. The biochemical interleukin IL-4, IL-5, IL-13, and IL-33 levels **(C, D, E, and F)** for all groups. a; no statistical difference, b; p<0,05, c: p>0,05.

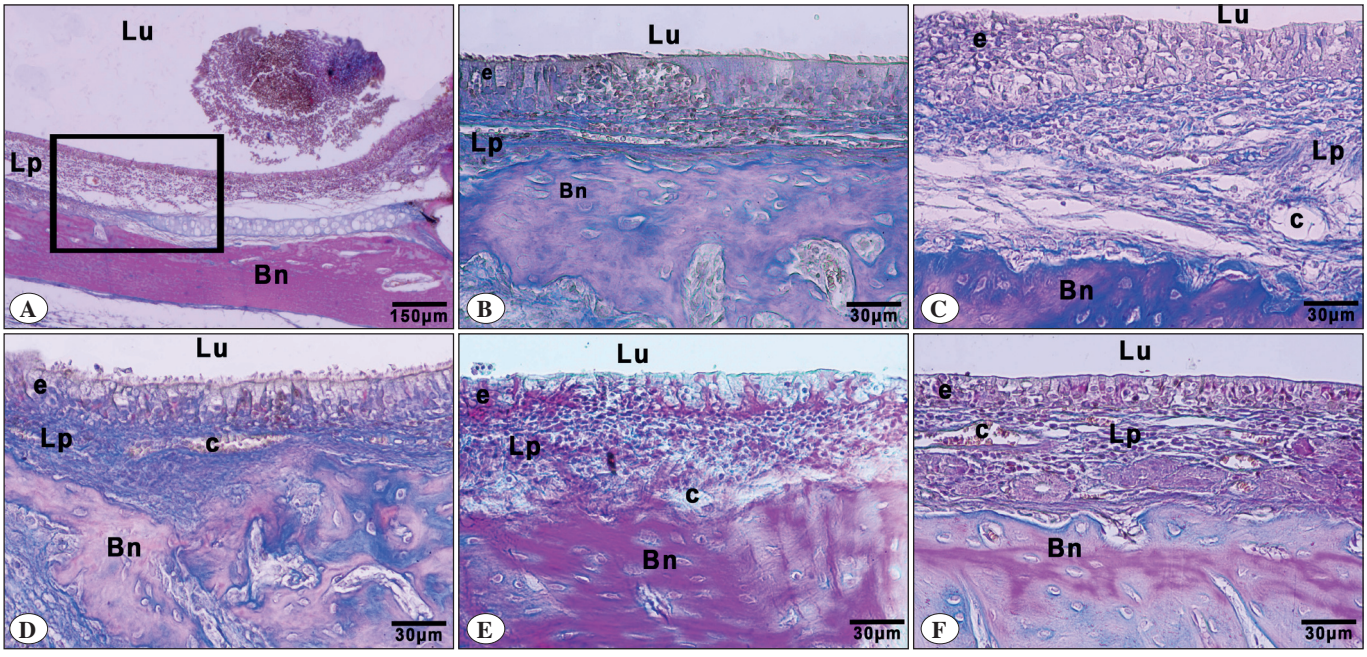

Figure 4. Illustration of histology of the nasal mucosa sections for all groups, **A)** lower magnification of histologic view belonging to the nasal mucosa of the rats, **B)** higher magnification of nasal mucosa of the control groups, **C)** nasal mucosa of the OVA group, **D)** nasal mucosa of the CAPS group, **E)** nasal mucosa of the CAPS-STR group, **F)** nasal mucosa of the STR group, square; magnification area for the nasal mucosa, **Lu:** lumen of the nasal cavity, **Bn:** alveolar bone, **c:** capillary, **Lp:** lamina propria, **e:** epithelium of the nasal mucosa, Crossman-modified Mallory triple staining.

ing, sneezing, and nasal congestion, it has not been shown whether it affects cytokine levels. Two studies have reported that CAPS released neuropeptides and induced neurogenic inflammation in rats (12,16). Another suggested that CAPS, at a concentration that produces intense pain, does not induce mucosal exudation of plasma in humans (12). Our study results indicate that the parenteral application of CAPS alone or the CAPS-STR combination can reduce clinical symptoms of allergic rhinitis in a rat model. Other studies using CAPS and STR or a single administration of STR have reported reduced allergic rhinitis scores in rats, like our results (7,17).

Eosinophil and basophil infiltrations are the predominant feature of the upper respiratory allergic reactions in the mucosa. Hematologic analysis showed significantly increased eosinophil and basophil counts in the OVA-induced allergic rhinitis group. If we compare the effect of blood parameters on eosinophil counts, another report stated that the number of eosinophils and basophils in sputum increased in patients with allergic rhinitis (18). Another report has demonstrated a similar increase in eosinophil counts in humans during the pollen season (19). Administration of CAPS and CAPS-STR significantly decreased the eosinophil and basophil counts in the OVA-induced rats. Previous studies have reported an inhibitory effect of steroids on eosinophil and basophil counts in an allergic rhinitis rat model (17). CAPS administration did not affect the inflammatory cell number or the eosinophil counts in rat lungs (20). One study reported that CAPS's subacute effect was to decrease the total white blood cell count in rats, including eosinophil count, but the basophil count was unchanged (21).

Cytokines are produced by white blood cells and regulate the activation of the immune system in inflammation (22). IL-4 is responsible for IgA production and the expression of VCAM-1, and it stimulates an increase in lymphocyte, monocyte, and especially eosinophil binding to endothelial cells (22,23). IL-5 promotes the infiltration of eosinophils in allergic rhinitis (24). IL-13 is necessary for IgA production and serves as the primary mediator for the striking histologic changes observed in the airway (23). IL-33 is also an important contributor to allergic inflammation (25). Many previous studies on allergic rhinitis have also reported increased IL-4 and IL-5 (which are Th-2 type cytokine receptors involved in allergic rhinitis and that respond to topical steroids), as well as IL-13 and IL-33 (26,27). In our study, the levels of IL-4, IL-5, IL-13, and IL-33 unchanged in the rats treated with CAPS, CAPS-STR, or STR when compared with the control group.

There was edema, congestion, and increases in connective tissue, inflammatory cell infiltration, and vascular dilatation in the nasal wall mucosa of the OVA-induced rats. These pathologies were consistent with previous responses observed in other studies that utilized animal models of allergic rhinitis (7,9). The inflammatory pathologies were decreased by treatment with CAPS, STR, and CAPS-STR, possibly linked to CAPS's anti-inflammatory effect (28). Moreover, due to its desensitization effect, inflammatory responses were alleviated by augmentation of vasodilation and permeability (16). Both CAPS alone and a combined use of CAPS and STR may block allergic mediators in the OVA-induced allergic rhinitis pathology in this rat model.

In conclusion, parenteral administration of CAPS alone, and particularly the combined use of CAPS and STR, was an effective treatment for allergic rhinitis symptoms in a rat model of allergic rhinitis. Data show that CAPS alone is not an effective alternative to STR, but enhances recovery from allergic rhinitis. It has been observed that when STR is used as an addition to the combination, it affects the treatment positively, but does not provide a statistically significant improvement. For future studies, CAPS in allergic rhinitis can be tested with different doses and administration forms, as monotherapy or combination treatment.

Conflict of Interest

The author declares no conflict of interest.

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Authorship Contributions

Concept: **Fatih Oner, Adem Kara,** Design: **Fatih Oner, Gunay Kozan, Adem Kara,** Data collection or processing: **Fatih Oner, Gunay Kozan, Adem Kara,** Analysis or Interpretation: **Fatih Oner, Adem Kara,** Literature search: **Fatih Oner, Adem Kara,** Writing: **Fatih Oner, Adem Kara,** Approval: **Fatih Oner, Gunay Kozan, Adem Kara.**

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