

Anti-inflammatory effects of intranasal cyclosporine for allergic rhinitis in a mouse model

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Background: Although topical corticosteroids are considered a safe and effective drug for allergic rhinitis (AR), some AR patients do not show sufficient symptomatic improvement by use of topical corticosteroids. Topical cyclosporine is a safe and effective drug for patients with allergic conjunctivitis, particularly for those with steroid-resistant allergic conjunctivitis. We investigated the anti-inflammatory effects of intranasal cyclosporine for AR using a mouse model.

Methods: After establishment of allergic inflammation in 5-week-old BALB/c mice, cyclosporine was administered intranasally 3 times per week for 2 weeks. To confirm its anti-inflammatory effects, triamcinolone acetonide (TAC) was utilized as a control drug. Histopathologic changes were evaluated using Sirius red and Giemsa staining for eosinophilic and mast cell infiltration, respectively. The levels of cytokines in sinonasal tissues, including tumor necrosis factor (TNF), interleukin (IL)-4, IL-5, and IL-13, were assessed based on a cytometric bead array.

Results: The degree of eosinophilic infiltration was significantly decreased by instillation of cyclosporine, the

potency being similar to TAC. However, the number of mast cells was not decreased by cyclosporine or TAC. The levels of TNF, IL-4, IL-5, and IL-13 were significantly decreased after treatment with cyclosporine.

Conclusion: The anti-inflammatory effects of topical cyclosporine for AR were equivalent to those of topical corticosteroids. Topical cyclosporine may be useful for the treatment of AR, although human studies are required.
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Key Words:

cyclosporine; allergic rhinitis; anti-inflammatory agents; mice; cytokines; corticosteroid

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Allergic rhinitis (AR) is the most common noninfectious rhinitis caused by an immunoglobulin E (IgE)-mediated response. It not only affects social life, sleep, school, and work, but also increases socioeconomic costs.¹ Oral H₁-antihistamines and intranasal corticosteroids are currently the most effective and commonly used medications for the treatment of AR. In particular, intranasal corticosteroids can alleviate nasal stuffiness and are the drugs of choice for persistent, moderate-to-severe AR.¹ However, some patients with AR do not show sufficient subjective and objective improvement while using intranasal

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corticosteroids.² Accordingly, the development of alternative drugs for AR is important in the clinical setting.

Cyclosporine is a potent inhibitor of T-cell activation. Its systemic administration causes significant adverse effects such as nephrotoxicity, hepatotoxicity, neurotoxicity, and leukopenia.³ However, topical cyclosporine can be a safe and efficacious drug for several ophthalmologic and dermatologic diseases, including dry eye, allergic conjunctivitis, psoriasis, atopic dermatitis, and connective tissue diseases.^{4–6} Stinging or burning sensation is the most common adverse event by cyclosporine eye drops, which is well tolerated by most patients. On the other hand, no serious adverse events related with cyclosporine eye drops have been reported in long-term use.^{7,8} Therapeutic drugs for allergic conjunctivitis are similar to those for AR based on patients' responses to oral H₁-antihistamines and topical corticosteroids. Because prolonged use of steroid eye drops can cause increased ocular pressure and subsequent glaucoma, topical cyclosporine is the drug of choice, particularly in patients with steroid-resistant, moderate-to-severe allergic conjunctivitis.^{5,9,10}

We hypothesized that topical cyclosporine can be an alternative drug for patients with steroid-resistant, moderate-to-severe AR. In the present study, the anti-inflammatory effect of intranasal cyclosporine on AR was investigated using a mouse model.

Materials and methods

Animals and experimental protocols

Forty-eight 4-week-old BALB/c mice (Koatech Laboratory Animal, Inc., Pyeongtaek, Korea) were used in this study. They were housed in a specific-pathogen-free biohazard containment facility maintained at 22°C to 24°C and 50% to 60% humidity. The mice were categorized into 5 groups, and allergic inflammation of the sinonasal mucosa in the experimental groups was induced according to previously reported protocols shown in Figure 1.¹¹ In the control group (group A; n = 8), phosphate-buffered saline (PBS) was administered both systemically and intranasally. Mice in the experimental groups (groups B, C, D, and E; n = 10 each) were systemically sensitized with 25 µg of ovalbumin (OVA, Grade V; Sigma, St. Louis, MO) dissolved in 200 µL of PBS and 2 mg of aluminum hydroxide gel as an adjuvant by intraperitoneal injection on days 0, 7, and 14. One week after the last intraperitoneal injection, 3% OVA diluted in 20 µL of PBS was administered into each nostril of the mice daily for a week. Following the establishment of AR using the above-described technique, therapeutic drugs were continually administered while maintaining allergic inflammation by intranasal instillation of 3% OVA (3 times/week) for 2 consecutive weeks. The experimental groups were designated as follows: intranasal instillation of vehicle, which was a mixture of glycerol and distilled water (group B); 0.5 mg/kg of cyclosporine dissolved in vehicle (Restasis®; Allergan Inc., Irvine, CA) (group C); 0.5 mg/kg

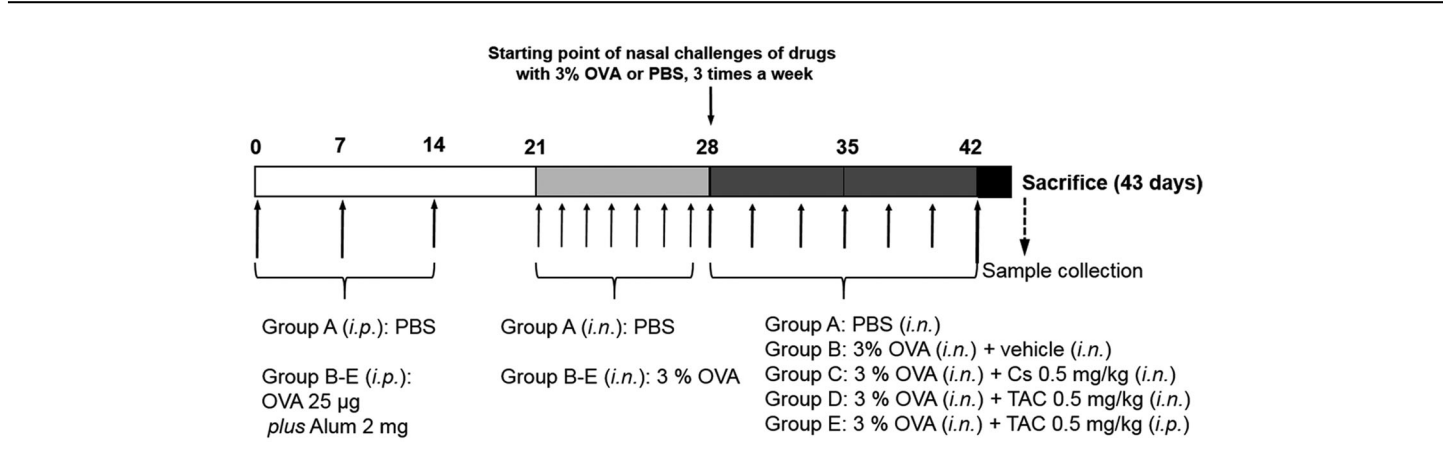
triamcinolone acetonide (TAC; group D); and intraperitoneal injection of 0.5 mg/kg TAC (group E). At day 42, final nasal instillation of the drugs and 3% OVA diluted in 20 µL of PBS was performed; 24 hours later, the mice were euthanized and decapitated. Three control mice and 5 from each experimental group were prepared for histologic examination; 5 mice from each group were used for quantitative measurement of cytokines using a cytometric bead array (CBA). This study was approved by the Committee on the Use and Care of Animals of Gyeongsang National University.

Histological analyses

The heads of the mice were immediately fixed in 2% paraformaldehyde and decalcified in 5% nitric acid for 4 to 5 days at 4°C. The tissues were dehydrated and processed according to standard paraffin wax-embedding procedures. The tissue was cut in coronal sections at 4-µm thickness. The superior and inferior maxillary turbinates were identified, and 2 coronal consecutive sections were chosen for evaluation. Sirius red staining was adopted to examine eosinophilic infiltration, and Giemsa staining was used to identify characteristic cytoplasmic granules of the mast cells. Under high-power fields (original magnification ×400), the number of eosinophils was counted at the transition zone of the olfactory and respiratory epithelia by an independent researcher blinded to the experimental groups. Because mast cell infiltration was not apparent at the transition zone of the olfactory and respiratory epithelia, the superior turbinates covered with olfactory epithelia were chosen for mast cell counting.

Quantitative measurement of cytokines

The nasal and sinus mucosae of mice were dissected and harvested using sharp dissecting devices and then resuspended in 500 µL of PBS. The homogenates were filtered using a 70-µm cell strainer (SPL Lifesciences, Pocheon, Korea); the filtrates were then centrifuged at 12,000 × g for 5 min at 4°C. Next, the supernatants were collected and cryopreserved at –70°C until analysis. To measure cytokine concentrations, Enhanced Sensitivity Flex Sets (BD Biosciences, San Jose, CA) were purchased for interleukin (IL)-4, and Flex Sets (BD Biosciences) were purchased for tumor necrosis factor (TNF), IL-5, and IL-13. Cytometric bead array was performed according to the manufacturer's instructions. Briefly, 50 µL of each sample or recombinant standards were added to 50 µL of mixed capture beads and incubated for 2 hours at room temperature in the dark, and 50 µL of mixed phycoerythrin (PE)-conjugated detection antibodies were added to form sandwich complexes. For the standard, recombinant proteins (TNF, IL-4, IL-5, and IL-13 from the assay kits) were dissolved in buffer medium and serially diluted. Each sample was measured using a flow cytometer (BD LSR II instrument; BD Biosciences) in conjunction with the FACS-Diva software package (BD Biosciences) after washing to



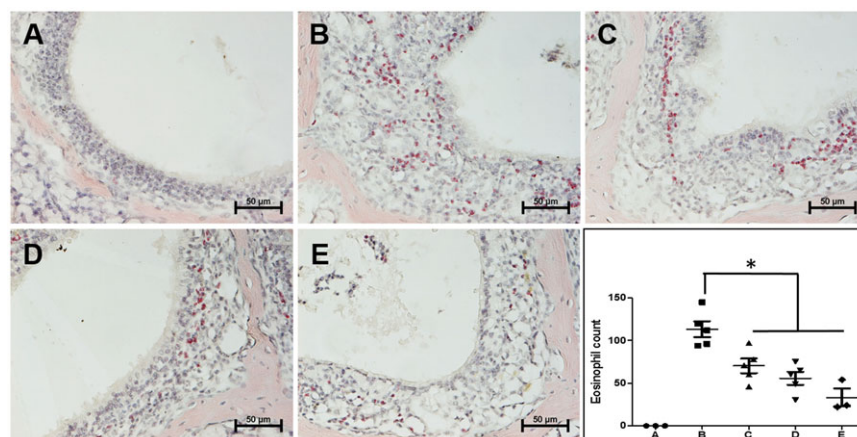


FIGURE 2. Representative photographs of mucosal lesions and comparison of eosinophil counts among groups. Eosinophilic infiltration was remarkably lower in groups C, D, and E than in group B (* $p < 0.05$, Sirius red staining, $\times 400$).

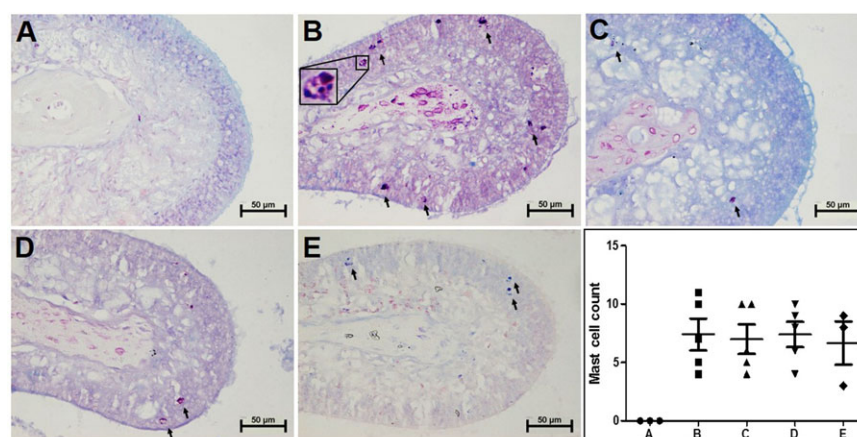


FIGURE 3. Representative photographs of mucosal lesions and comparison of mast cell counts among groups. The degree of mast cell infiltration was not significantly different among the experimental groups. Black arrows indicate mast cells containing cytoplasmic granules (Giemsa staining, $\times 400$).

corticosteroids are less effective than antihistamines in blocking early-phase symptoms.¹² It was reported that topical corticosteroids have no influence on mast cell density and the histamine concentration in nasal tissues from patients with hay fever.²³ Similarly, in the other study, topical cyclosporine did not improve early-phase symptoms such as itching and tearing in patients with allergic conjunctivitis.⁹ Cyclosporine also did not affect the vascular permeability or the number of mast cells in a murine model of allergic conjunctivitis, although it inhibited eosinophilic infiltrations and T-cell-related late-phase reactions.²⁴ Likewise, neither cyclosporine nor corticosteroids exhibit mast cell-reducing effects in the present study.

AR is a basically T-helper 2 (Th2)-skewed inflammation mediated by inflammatory cytokines such as IL-4 and IL-13.²⁵ In previous studies, these inflammatory cytokines were suppressed by corticosteroids. For example, topical corticosteroids inhibited the release of IL-4 and IL-13 in nasal tissues obtained from grass-pollen-sensitive patients with AR.¹⁵ Similar findings regarding cyclosporine have also been reported. In a single study using an allergic conjunctivitis mouse model, cyclosporine inhibited

inflammatory cell infiltration in conjunctival tissues and suppressed the production of Th2 cytokines including IL-4 and IL-5.²⁴ In another study on children with severe atopic dermatitis, inflammatory cytokines including IL-2, IL-4, and IL-13 were significantly decreased with symptom improvement after topical cyclosporine treatment.²⁶ Likewise, all Th2-related cytokines were reduced by topical cyclosporine in the present study (Fig. 4).

Collectively, the anti-inflammatory effects, particularly for eosinophils, of intranasal cyclosporine were equivalent to those of topical corticosteroids, although a mast cell-inhibiting effect was not confirmed. Anti-inflammatory effects by topical cyclosporine were also previously proven in a rat model of asthma that has the same pathomechanism with AR.²⁷ However, in a double-blind, placebo-controlled, randomized clinical trial on steroid-dependent asthmatics, cyclosporine, even in systemic use, exhibited only a slight beneficial effect on asthma severity and limited steroid-sparing effect.²⁸ Given such discrepancy, further human study is imperative to confirm the therapeutic effects of intranasal cyclosporine in AR patients.

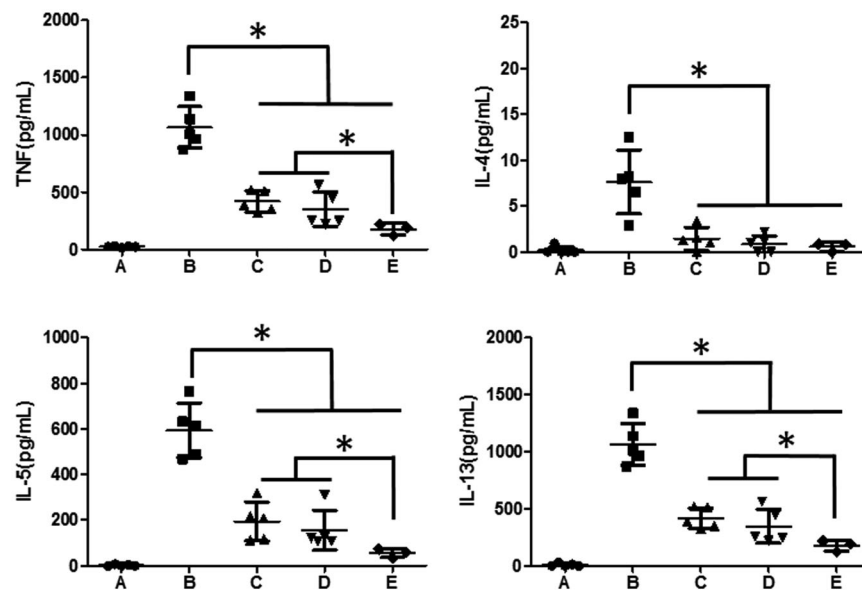


FIGURE 4. Cytometric multiplex bead array results of cytokines. Groups C and D showed inhibitory effects on production of TNF, IL-4, IL-5, and IL-13 similar to those in group B. Furthermore, the decreases in the levels of TNF, IL-5, and IL-13 were more pronounced in group E than in group C or group D. * $p < 0.05$. IL = interleukin; TNF = tumor necrosis factor.

In terms of safety, systemic cyclosporine was previously reported to cause serious side effects such as hypertension and renal impairment although it had notable benefits as a corticosteroid-sparing agent.²⁹ However, topical cyclosporine was safe and well tolerated without systemic side effects such as nephrotoxicity or hepatotoxicity, even after long-term use, although minor side effects such as stinging and a burning sensation have been reported.^{30,31} Given that topical cyclosporine has been established as a safe and efficacious drug for allergic conjunctivitis, it appears that it can play a useful role in the treatment of AR, especially for patients with moderate-to-severe symptoms and steroid resistance.^{5,9}

The main limitation of this study is lack of symptom measurement. The inhibitory effect of cyclosporine on nasal

symptoms was not measured. As corticosteroids effectively alleviate nasal obstruction rather than sneezing and rhinorrhea, cyclosporine may have also suppressed nasal obstruction. Because we were not able to measure the degree of nasal obstruction in mice, however, we focused on objective measurement of allergic inflammation instead of symptom measurement.

Conclusion

The anti-inflammatory effects of topical cyclosporine for AR were similar to those of topical TAC in a mouse model. Topical cyclosporine may be a useful steroid-sparing drug, particularly for patients with steroid-intolerant, moderate to severe AR. ☯

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