

Desloratadine partially inhibits the augmented bacterial responses in the sinuses of allergic and infected mice

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Summary

Background Allergic rhinitis (AR) is considered a major predisposing factor for the development of acute bacterial rhinosinusitis. How AR augments a bacterial infection is unknown.

Objective Our purpose in this study was to test whether an H₁ receptor antagonist, desloratadine, could reduce the augmented effect of an ongoing allergic reaction on acute bacterial rhinosinusitis.

Methods Three groups of infected and ovalbumin (OVA)-sensitized mice were studied: (1) infected and allergic mice treated with desloratadine, (2) infected and allergic mice treated with placebo, and (3) infected mice. A fourth group of uninfected, non-sensitized mice served as a control for the cellular changes. BALB/c mice were sensitized by two intraperitoneal injections of OVA given 8 days apart. One day after the second injection, the mice were nasally exposed daily to 6% OVA (the groups treated with desloratadine or placebo) or phosphate-buffered saline (PBS) (the infection-only group) for 5 days. After the second OVA exposure, the mice were intranasally inoculated with *Streptococcus pneumoniae*. Desloratadine or placebo was given daily throughout the OVA exposure period. Nasal allergic symptoms were observed by counting of nasal rubbing and sneezing for 10 min after OVA or PBS nasal challenge. On day 5 post-infection, nasal lavage culture was done, and the inflammatory cells in the sinuses were evaluated by flow cytometry.

Results Mice that were made allergic, infected, and treated with placebo showed more organisms and phagocytes than did only infect mice. They also manifested allergic nasal symptoms and eosinophil influx into the sinuses. Desloratadine treatment during allergen exposure reduced allergic symptoms and reduced sinonasal infection ($P < 0.05$). There tended to be less myeloid cell and neutrophil influx ($P = 0.09$ both), but not eosinophil influx ($P = 0.85$) compared with that in the placebo-treated group.

Conclusion Desloratadine treatment during nasal challenge inhibited allergic symptoms and reduced sinonasal infection, suggesting that histamine via an H₁ receptor plays a role in the augmented infection in mice with an ongoing allergic reaction.

Keywords allergic rhinitis, desloratadine, H₁ antihistamine, infection, *S. pneumoniae*, sinusitis

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Introduction

Acute bacterial rhinosinusitis is one of the 10 most common diagnoses made in the ambulatory practice of medicine and is the fifth most common diagnosis for which antibiotics are prescribed [1]. Allergic rhinitis (AR) is also a common health problem, affecting 10–30% of adults and up to 40% of children [2]. Additionally, AR is often considered a major predisposing factor for the development of acute bacterial rhinosinusitis.

Epidemiologic studies have shown a clinical association between AR and acute bacterial rhinosinusitis. Savolainen reported the incidence of allergy to be 25% in acute maxillary rhinosinusitis, which was significantly greater than a 16%

incidence in a control group [3]. Chen et al. [4] found the prevalence of rhinosinusitis to be significantly higher in children with than in children without AR. Holzmann et al. [5] found that the prevalence of orbital complications of acute bacterial rhinosinusitis was higher in children with AR than in children with non-AR. However, the mechanism underlying the way in which AR predisposes to acute bacterial rhinosinusitis remains unclear.

In an attempt to uncover the mechanisms underlying the association between AR and acute bacterial rhinosinusitis, we developed a mouse model of infection during an ongoing allergic reaction [6–8]. We previously showed in this model that when mice with an ongoing allergic reaction become infected, they develop an augmented infectious and inflammatory response.

H₁ receptor antagonists are widely used in the treatment of AR. Many studies have shown the efficacy of H₁ receptor antagonists in the relief of the sneezing, itching, and nasal discharge of AR [9–14]. No study has shown the benefit of an

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H₁ receptor antagonist in reducing infectious complications of AR.

Desloratadine is a potent, once daily, orally active non-sedating H₁ antihistamine. Several studies have shown its efficacy in treatment of AR and additional non-H₁ receptor effects [15–20]. We hypothesized that histamine, in part, mediated the augmented infectious and inflammatory response in mice with an ongoing allergic reaction and that treatment with an H₁ receptor antagonist, desloratadine, would reduce the augmented bacterial and inflammatory response seen in mice with an ongoing allergic reaction.

Materials and methods

Mice

BALB/c mice of either sex, aged 6–8 weeks, were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and kept in microisolation cages in the Carlson Biocontainment Suite at The University of Chicago. These animals were on an ovalbumin (OVA)-free diet. The Animal Safety Committee of The University of Chicago approved the protocol. All manipulations of the animals before killing them were conducted in a class II biosafety hood following strict biosafety control measures.

Study design

The number of mice in each group was determined by a power calculation. Based on prior data, we calculated the number of mice per group by using 90% power to detect a difference among groups of animals killed on day 5 for the primary outcome variable, the number of bacteria recovered, using a two-tailed significance level of 0.05. We needed at least eight mice per group. We assumed that two to three mice would be lost from each group for technical reasons.

Because of the large number of mice, the study was conducted in three parts, and the data were pooled.

Mice were divided into four groups:

- (1) *Group A*: 11 mice were given OVA/alum intraperitoneal (i.p.) injection, nasally challenged with 6% OVA solution in PBS, intranasally (i.n.) inoculated with *Streptococcus pneumoniae* (*S. pneumoniae*), and gavaged with 100 µL of 2.4 mg/mL desloratadine suspension in 0.4% methylcellulose.
- (2) *Group B*: 10 mice were given OVA/alum i.p. injection, nasally challenged with 6% OVA solution in PBS, i.n. inoculated with *S. pneumoniae*, and gavaged with 0.4% methylcellulose.
- (3) *Group C*: 10 mice were given OVA/alum i.p. injection, nasally challenged with PBS, i.n. inoculated with *S. pneumoniae*, and gavaged with 0.4% methylcellulose.
- (4) *Group D*: Three mice had no intervention before killed and served as controls for flow cytometry.

Mice were sensitized, challenged, inoculated, and gavaged according to the protocol shown in Fig. 1.

Allergen sensitization

Groups A–C were sensitized with 20 µg OVA as follows: a 100 µg/mL OVA solution in alum was prepared by dilution of OVA (Sigma Chemical Co., St Louis, MO, USA) with PBS and Imject[®] Alum (Pierce, Rockford, IL, USA). Two hundred microlitres of this solution were administered by i.p. injection.

Allergen exposure

Nasal allergic reactions in sensitized mice were created by daily i.n. challenge with 50 µL of 6% OVA solution in PBS. Group C mice were challenged with 50 µL of PBS.

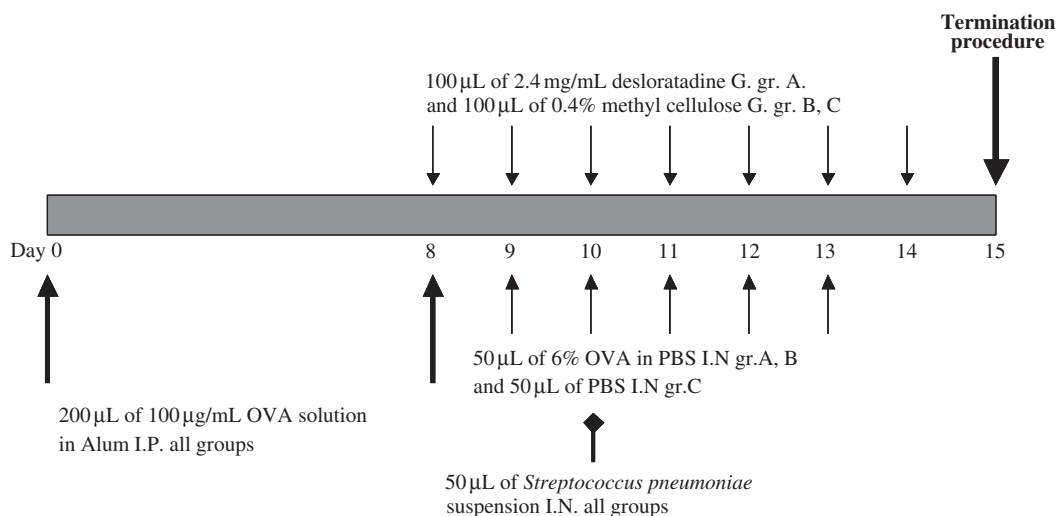


Fig. 1. Schematic of the protocol. Mice in Groups A–C were ovalbumin (OVA)-sensitized by intraperitoneal (i.p.) injection on days 0 and 8 and then infected by intranasal (i.n.) inoculation with *Streptococcus pneumoniae* on day 10. Mice in Group A were gavaged with desloratadine, and 0.4% methylcellulose in Groups B and C from days 8 to 14. Mice in Groups A and B were subsequently challenged i.n. with OVA and Group C with phosphate buffered saline (PBS) from days 9 to 13. Mice in Group D had no any intervention before killing. Small downward arrows represent gavages. Largest downward arrow represents termination time point. Small upward arrows represent intranasal (i.n.) challenges with OVA or PBS. ↑ represents *Streptococcus pneumoniae* i.n. inoculation. Large upward arrows represent intraperitoneal injection.

Inoculation

Groups A–C were i.n. inoculated with *S. pneumoniae* (ATCC49619) for induction of acute bacterial rhinosinusitis. The *S. pneumoniae* was grown on blood agar plates, and colonies were suspended in sterile saline solution immediately at a McFarland no. 3 concentration. This is equivalent to approximately 1.2×10^9 colony-forming units (CFU)/mm. A 25 μ L of suspension *S. pneumoniae* was placed in each nostril. Because mice are obligatory nasal breathers, the fluid was drawn into the nasal passage during inhalation.

Medication

A suspension of 100 μ L of 2.4 mg/mL desloratadine in 0.4% methylcellulose was fed via gavage tube in Group A. Groups B and C were gavaged with 100 μ L of 0.4% methylcellulose.

Nasal symptoms

Nasal symptoms were evaluated for each mouse in each group by counting of the number of nasal rubbings and sneezes that occurred in the 10-min interval after nasal challenge with 6% OVA or PBS.

Nasal lavage culture

Five days after infection, mice were sedated with a respiratory-failure dose of pentobarbital sodium (Nembutal, 120 mg/kg). While sedation ensued, nasal lavage with 200 μ L of PBS was performed. The lavage fluid was serially diluted in 10-fold increments by PBS from 1 : 1 to 1 : 10^4 . Ten microlitres of each dilution were dropped and streaked on Columbia sheep blood agar plates. Plates were incubated for 24 h. Bacterial colonies were counted and reported in CFU/mL.

Harvesting of sinus mucosa

After nasal lavage, mice were decapitated. Skin, mandible, and tongue were removed, and then the skull was split in the midline to expose the sinuses. Sinus tissue was removed and placed in 2 mL of PBS and then mixed with 2 mL of 2 mg/mL collagenase P (Roche Diagnostics, Indianapolis, IN, USA) to give a final concentration of 1 mg/mL. The tissue was incubated at 37 °C for 1 h in a shaker for increasing the recovery of cells from sinus tissue. The material was passed through a Nyltex filter, and cells were recovered in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal calf serum (FCS). Next, this cell medium suspension was spun for 5 min at $15000 \times g$. We discarded the supernatant and resuspended the pellet in 2 mL of DMEM with 5% FCS. We counted cells by using 0.4% Trypan blue at 1 : 10 dilution. The volume of solution that contained 5×10^5 cells was calculated, and this amount was placed in each fluorescence-activated cell sorting (FACS) tube. Tubes were filled with FACS buffer, spun for 5 min at $15000 \times g$, and decanted. Twenty microlitres of anti-Fc γ R2/3 antibody (2.4 G2) were added, and the tubes incubated for 15 min at room temperature in order to stop non-specific staining by fluorochrome-conjugated antibodies that is mediated by Fc receptors. Ten microlitres of antibody was added and the tubes were incubated for 60 min at 4 °C.

After incubation, tubes were filled with FACS buffer, spun, and decanted. We then added 300 μ L of FACS buffer.

Flow-cytometric analysis

Cells were stained and then analysed on a BD LSR II flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) with CellQuest software. For staining, all conjugated antibodies were purchased from BD Biosciences except anti-CCR3 fluorescein isothiocyanate (FITC) from R&D Systems Inc., Minneapolis, MN, USA: anti-CD3 FITC for CD3⁺ T lymphocytes, anti-CD4 phycoerythrin (PE) for CD4⁺ T lymphocytes, anti-CD8 FITC for CD8⁺ T lymphocytes, anti-CD11b FITC for CD11b⁺ Myeloid cells (mainly macrophages and neutrophils) [21, 22] and double stained anti-GR1 PE & CCR3 FITC for GR1^{hi} CCR3[−] neutrophils, and GR1^{lo-int} CCR3⁺ Eosinophils [21, 23–26]. Spleen cells were used as a positive control.

Statistical analysis

Parametric statistics were used for analysis of the data. The number of bacterial colonies grown in nasal lavage culture and positively stained cells in flow cytometry were normalized by conversion on to logarithm base 10 before analysis. Statistical analyses were performed by use of ANOVA followed by an unpaired *t*-test, if ANOVA showed a significant difference between groups. In all tests, two-tailed alternatives were used. A *P*-value of <0.05 was considered to indicate statistical significance.

Results

Clinical symptoms

Both nasal rubbing and sneezing were observed for 10 min after each i.n. challenge with OVA or PBS. The number of nasal rubbings and sneezes increased in the allergic and infected group (Group B) after each daily nasal challenge. The infected mice (Group C) had few, if any symptoms (Fig. 2). The infected, allergic mice treated with desloratadine (Group A) showed significantly decreased symptoms, compared with the infected and allergic mice and no significant difference from the infected-only mice at 3 and 4 days after nasal challenge.

Nasal lavage culture

Mice in Groups A–C were inoculated with *S. pneumoniae*, and nasal lavage culture was performed 5 days after inoculation. The number of bacterial colonies grown in infected and allergic mice was statistically significantly higher than in infected mice; i.e., allergic inflammation led to more bacterial growth. The infected, allergic mice treated with desloratadine showed statistically significantly lower bacterial growth compared with untreated infected and allergic mice, although the bacterial counts were higher than in the infected mice (Fig. 3).

Flow-cytometric analysis

Five days after infection, the sinus mucosa was harvested and processed, and the number of positively stained cells was quantified (Fig. 4).

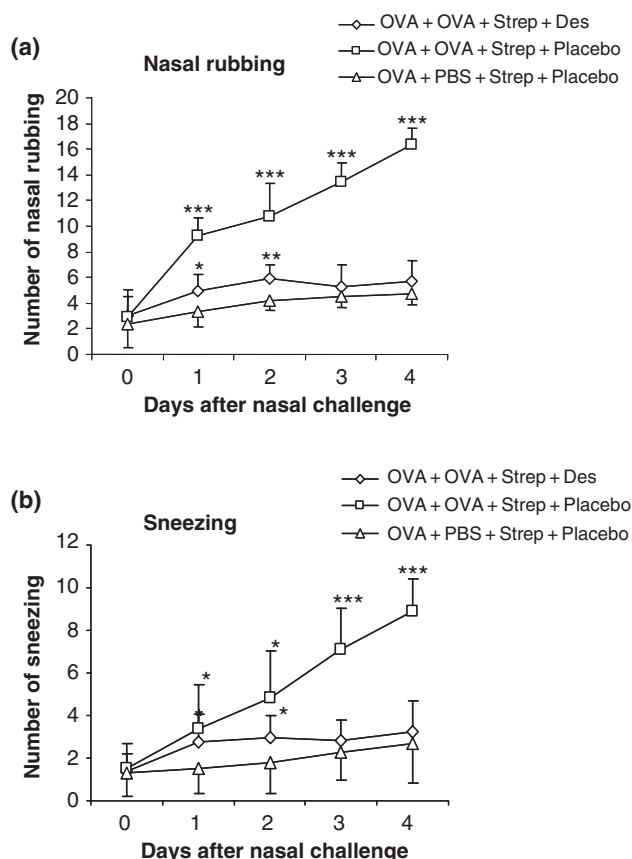


Fig. 2. Symptoms after nasal challenge. The number of nasal rubbings (a) and sneezings (b) was counted for 10 min after each nasal challenge. Each point represents the mean for each group. Bars represent standard deviation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with Group C.

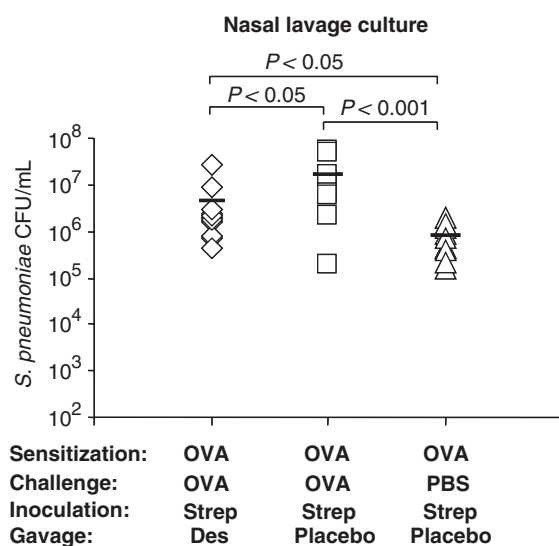


Fig. 3. Nasal lavage culture. Each symbol represents the number of bacterial colonies grown in colony-forming units (CFU)/mL for each mouse.

The infected mice showed significantly more inflammatory cells than did control, except for $CD8^+$ cells. The infected and allergic mice showed significantly higher numbers of $CD11b^+$, $GR1^{hi+}$ $CCR3^-$ and $GR1^{lo-int+}$ $CCR3^+$ cells compared with

the infected-only mice. The number of $CD11b^+$ and $GR1^{hi+}$ $CCR3^-$ cells in infected, allergic mice treated with desloratadine showed a tendency to be lower than untreated infected and allergic mice, although there was no statistically significant difference ($P = 0.09$ both).

There was no significant difference in $GR1^{lo-int+}$ $CCR3^+$ cells between infected, allergic mice treated with desloratadine and untreated infected and allergic mice. There were also no significant differences in $CD3^+$ and $CD4^+$ cells between infected, allergic mice treated with desloratadine, infected and allergic mice, and infected mice, although the cell counts were higher than in the control group.

Discussion

AR is an inflammatory disease of the nasal mucosa mediated by IgE [27]. The acute response to nasal challenge with antigen in sensitized individuals is accompanied by sneezing, itching, rhinorrhoea, and nasal congestion [28]. The acute response is followed by cellular infiltration of lymphocytes, eosinophils, and neutrophils [29]. In this study, we used nasal challenge with OVA to create allergic reactions in the noses of mice. These challenges resulted in significantly increased allergic nasal symptoms and influx of cells into the nose and sinus mucosa.

Acute bacterial rhinosinusitis was induced in the mice by inoculation with *S. pneumoniae*, the most common bacteria obtained from maxillary sinuses during acute infection in man [30]. We cultured the organism from the infected mice and showed an inflammatory response marked by a cellular influx.

When mice were infected during an ongoing allergic reaction, they showed an augmented infectious and inflammatory response, mimicking the epidemiologic data in man that show worsening of sinusitis in allergic individuals. Acute bacterial rhinosinusitis was confirmed by positive bacterial recovery and a phagocytic cell influx into the sinus mucosa that was significantly greater than in mice that were only infected.

In this study, we evaluated whether treatment of the allergic reaction with an H_1 receptor antagonist (desloratadine) could reduce the augmented bacterial and inflammatory responses to acute bacterial rhinosinusitis. The infected, allergic mice treated with desloratadine had significantly decreased allergic nasal symptoms, compared with untreated infected and allergic mice. These results showed the efficacy of desloratadine in the treatment of an acute nasal allergic reaction, which is mediated in large part by histamine. Interestingly, the number of bacteria recovered in infected, allergic mice treated with desloratadine was significantly lower than that in untreated infected and allergic mice.

Macrophages and neutrophils, effector cells that engulf pathogenic microbes and contain intracellular vacuoles of toxic molecules, such as nitric oxide, superoxide, and degradative enzyme, which destroy the microbes [31], showed a tendency to be reduced in infected, allergic mice treated with desloratadine compared with untreated infected and allergic mice, but the number was still higher than in infected mice. Decreased macrophage and neutrophil influx into the sinus mucosa could reflect a lesser bacterial infection.

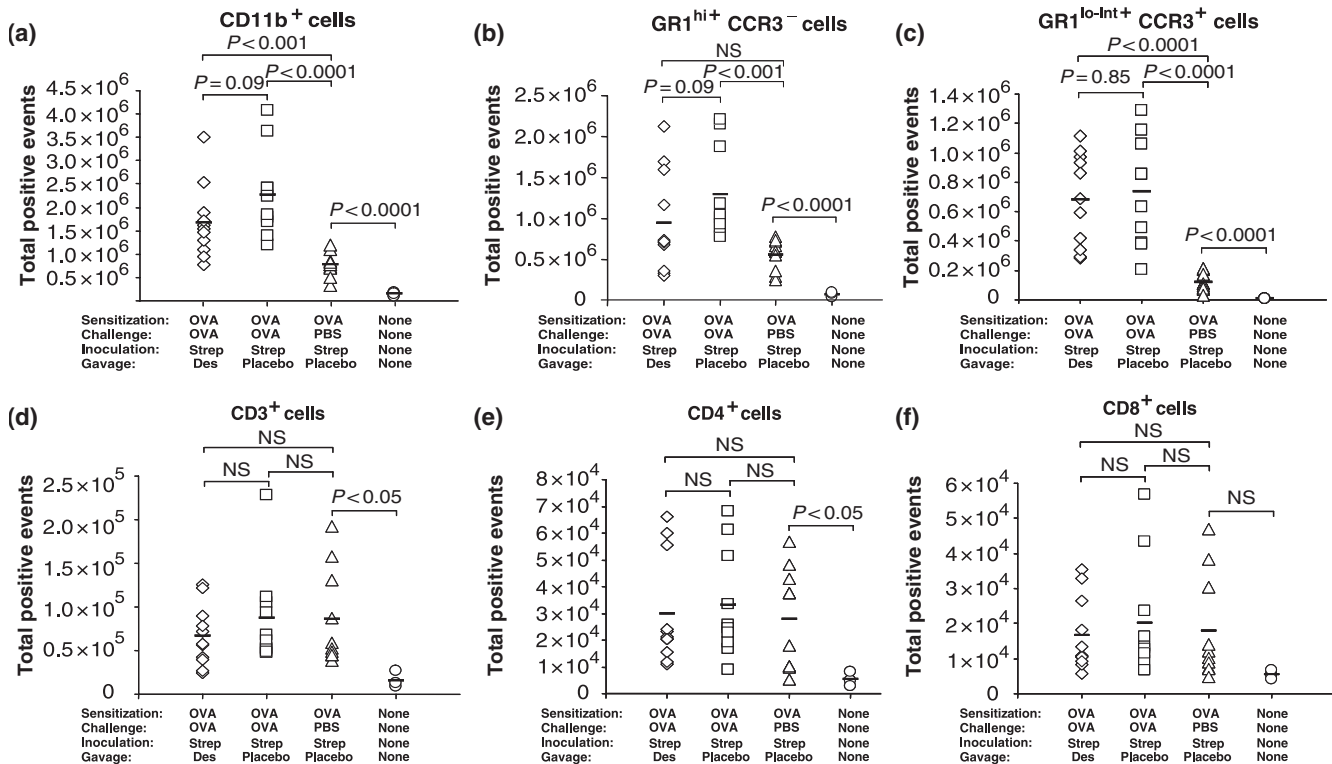


Fig. 4. Flow-cytometric analysis. The number of CD11b⁺ (a), GR1^{hi} CCR3⁻ (b), GR1^{lo-int} CCR3⁺ (c), CD3⁺ (d), CD4⁺ (e), and CD8⁺ (f)-stained cells is displayed. Each symbol represents the number of positively stained cells for each mouse.

The number of T lymphocytes and CD3- and CD4-positive cells in all active groups were higher than in the control group, but did not differ among the infected groups. Possible explanations are differences among groups related to the T-helper (Th) cell profile rather than absolute number; total T cells in sinus mucosa were too few and our sample sizes were not large enough to demonstrate a statistically significant reduction; or the T lymphocyte state of activation was different, but not detectable by our techniques.

Histamine has been demonstrated to affect the Th1/Th2 balance. Histamine affects immune regulation directly via histamine receptors on monocytes, dendritic cells, and T lymphocytes [32]. Several studies showed enhancement of Th2 and reduction of the Th1 profile by histamine. In addition, histamine inhibited neutrophil activation, superoxide formation, and degranulation [33]. These effects of histamine could interfere with the normal bacterial immune response. Histamine receptor antagonists might be helpful, possibly through interaction with histamine receptor in the immune system.

Histamine also elicits vasodilatation, mucous hypersecretion, mucosal oedema, and increased vascular permeability, as part of the immediate allergic reaction [34]. The allergic mucosal inflammation and oedema could impair mucociliary clearance that interferes with drainage from the sinuses, increasing mucous accumulation, serum transudation, decreasing oxygenation, and promoting bacterial proliferation within the sinuses, which could be reduced by a histamine receptor antagonist.

In our study, desloratadine did not affect eosinophil influx into the sinuses. This finding agreed with several studies that showed that antihistamines, *in vivo*, did not inhibit migration

of inflammatory cells [35, 36]. Most data on the anti-inflammatory property of desloratadine came from *in vitro* studies with higher doses of the medication than used in this study [15, 37]. However, it is possible that the reduction in infected, allergic mice treated with desloratadine might be related to anti-inflammatory rather than anti-histaminic properties of desloratadine. It is also possible that desloratadine had anti-infective actions.

Our findings suggest that desloratadine can ameliorate the bacterial and possible inflammatory responses in infected sinuses of patients with AR, through the histamine H₁ receptor. Clinical trials would be necessary for testing this hypothesis.

Conclusion

Our data demonstrated that mice sensitized and then exposed to OVA develop an allergic reaction in the nose and sinuses, as shown by significantly increased allergic nasal symptoms and eosinophil influx into the sinuses. Mice can be infected with *S. pneumoniae*, as shown by positive bacterial recovery and inflammatory cell influx into the sinuses. An ongoing local allergic reaction augments bacterial infection, as indicated by significantly higher bacterial culture recovery and inflammatory cell influx into the sinus mucosa than in non-allergic mice. Desloratadine treatment during allergen exposure can inhibit allergic symptoms and reduce sinonasal infection, as indicated by a significantly lower bacterial recovery.

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