

Histamine and tryptase in nasal lavage fluid after allergen challenge: Effect of 1 week of pretreatment with intranasal azelastine or systemic cetirizine

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Background: Antihistamines (H₁-receptor antagonists) act by competitive antagonism of histamine at H₁-receptors. In addition, high concentrations of some antihistamines inhibit allergen-induced histamine release from mast cells in vitro.

Objective: The purpose of this study was to determine the effect of intranasal azelastine or systemic cetirizine (both potent antihistamines) on the allergen-induced release of mast-cell mediators from the human nasal mucosa in vivo.

Methods: Patients allergic to birch pollen (n = 11) and control subjects not allergic to birch pollen (n = 5) were included in a randomized, double-blind, placebo-controlled, 3-way crossover study outside the pollen season. Each subject was treated with azelastine nasal spray 0.14 mg per nostril twice daily, cetirizine tablets 10 mg every day, or placebo for 1 week using a double-dummy design. At the end of each treatment period, nasal allergen challenges were performed, and the number of sneezes were counted. In addition, nasal lavage fluid was collected, and the levels of mast-cell mediators (histamine and tryptase) were measured.

Results: The allergen challenge of patients allergic to pollen produced sneezing and a significant increase in the levels of histamine and tryptase. The challenge of subjects not allergic to pollen produced no such response. Azelastine and cetirizine significantly reduced allergen-induced sneezing and the associated increase in histamine and tryptase levels. No significant differences were found between the azelastine and cetirizine treatments.

Conclusion: Pretreatment with azelastine or cetirizine inhibits the allergen-induced release of mast-cell mediators from the human nasal mucosa. Our results are consistent with the hypothesis that both antihistamines reduce mediator release from nasal mucosa mast cells in vivo. However, further studies are necessary to test this hypothesis. (*J Allergy Clin Immunol* 1999;103:768-72.)

Key words: Allergic rhinitis, nasal allergen challenge, nasal mucosa, histamine, tryptase, cetirizine, azelastine

Abbreviations used

OPT: o-Phthaldialdehyde

SQU: Standardized quality units

Antihistamines (H₁-receptor antagonists) reduce the symptoms of allergic rhinitis by competitive antagonism of histamine at H₁-receptors. In addition, antihistamines may have other pharmacologic effects of potential therapeutic interest. For example, high concentrations of some antihistamines inhibit allergen-induced/IgE-dependent histamine release from human mast cells in vitro.¹⁻⁴ It is not clear whether antihistamines at currently prescribed doses also inhibit allergen-induced histamine release from human mast cells in vivo. However, several studies have demonstrated reduced levels of mast-cell mediators in nasal lavage fluid after nasal allergen challenge of patients allergic to pollen who were pretreated with either terfenadine or loratadine.⁵⁻⁸ These findings are consistent with the hypothesis that terfenadine and loratadine reduce mediator release from nasal mucosa mast cells in vivo.

Azelastine is a potent antihistamine that can be administered orally or as an intranasal spray. Clinical trials have demonstrated that azelastine is an effective treatment for both seasonal and perennial allergic rhinitis.⁹⁻¹¹ In addition to antagonizing the effects of histamine at H₁-receptors, high concentrations of azelastine also inhibit IgE-dependent histamine release from human lung mast cells and basophils in vitro.^{2,12} Cetirizine is another potent antihistamine that has proved to be an effective treatment for seasonal and perennial allergic rhinitis.^{13,14} Like azelastine, cetirizine has additional pharmacologic effects of potential therapeutic interest. In particular, considerable attention has been focused on the inhibitory effects of cetirizine on human eosinophils.¹⁵ However, cetirizine also inhibits IgE-dependent histamine release from human lung and tonsil mast cells in vitro.³

The aim of this study was to assess the effect of a 1-week pretreatment with intranasal azelastine or systemic cetirizine on allergen-induced release of mast-cell mediators from the human nasal mucosa in vivo. The release of mast-cell mediators was assessed by measuring histamine and tryptase levels in nasal lavage fluid collected before and after nasal challenge with allergen. Both hist-

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amine and tryptase are stored as preformed mediators in human mast cells.¹⁶ However, the level of tryptase in nasal lavage fluid seems to be a more reliable marker of nasal mucosa mast-cell activation than the level of histamine.¹⁷

METHODS

Study design

Patients allergic to birch pollen and control subjects not allergic to pollen were included in a randomized, double-blind, placebo-controlled, 3-way crossover study. All subjects were treated with cetirizine tablets 10 mg daily, azelastine nasal spray 0.14 mg per nostril twice daily, or placebo for 1 week; a double-dummy design was used. At the end of each treatment period, nasal allergen challenges were performed, and the number of sneezes were recorded. In addition, nasal lavage fluid was collected, and the levels of histamine and tryptase were measured.

The study was performed more than 2 months after the end of the pollen season, and all volunteers were free of nasal symptoms at the time of investigation. There was a 2-week interval (washout period) between each treatment period, and there was a 3-week interval between each nasal challenge with allergen. The protocol was approved by the Ethical Committee of Copenhagen.

Volunteers

Eleven patients who were sensitive to birch pollen (9 men, 2 women) volunteered to take part in the study. Their median age was 25 years (range, 22 to 31 years). They all had hay fever symptoms in April and May and a positive skin prick test response¹⁸ and specific IgE of class 2 or more to birch pollen (Pharmacia CAP System; Pharmacia Diagnostics AB, Uppsala, Sweden). Five subjects who were not allergic to pollen (4 men and 1 woman) of similar age participated as healthy control subjects. All volunteers were students or hospital staff members. None had asthma or perennial rhinitis, and rhinoscopy did not reveal any anatomic abnormalities. No medication was taken at the time of investigation (with the exception of the study medication), and none of the patients had received immunotherapy.

Study medication

Azelastine nasal sprays (0.14 mg/puff), matched placebo sprays, cetirizine tablets (10 mg/tablet), and matched placebo tablets were supplied by ASTA Medica AG (Frankfurt am Main, Germany). Tablets were taken once daily (morning), whereas nasal sprays were used twice daily (1 puff in each nostril, morning and evening). The doses of azelastine and cetirizine given in this study are the doses currently prescribed for the treatment of allergic rhinitis.

Allergen challenge

Nasal challenges were performed in the morning, 3 hours after the last administration of the study medication. One puff of the challenge solution was sprayed into each nostril from a metered-dose pump spray (delivering 0.14 mL/puff). A standardized birch pollen extract was used (AquaGen SQ; ALK, Hørsholm, Denmark), supplied as a freeze-dried stock solution. Ampules were stored at 5°C, and fresh solutions of allergen in 0.9% saline solution were made before the challenge testing. Doses of allergen are expressed in standardized quality units (SQU); the doses used for nasal challenge were 140 SQU, 1400 SQU, and 14,000 SQU per nostril. Both patients allergic to pollen and control subjects not allergic to pollen were challenged with 0.9% saline solution twice (control challenge) followed by allergen in increasing doses. The challenges were performed at 10-minute intervals. Saline and allergen solutions for nasal challenge were kept at room temperature for 1 hour before provocation.

Recording of nasal symptoms

The number of sneezes was counted throughout the study period. Nasal blockage and rhinorrhea could not be recorded in a reliable way because of the nasal lavage procedure.

Nasal lavage

We followed closely the lavage technique and protocol described by Naclerio et al.¹⁹ Five milliliters of prewarmed (30°C) physiological saline solution were instilled into each nostril while the volunteer was sitting with the neck extended approximately 30 degrees from the horizontal level. After 10 seconds, the fluid was expelled in a beaker made of polypropylene. The protocol included 4 prechallenge lavages to reduce the baseline level of histamine. Lavages were then performed 10 minutes after each challenge (saline or allergen solution).

To limit mucosal swelling that would interfere with subsequent lavages, the first challenge with saline solution was preceded by a spray of 0.1 mL of oxymetazoline hydrochloride (0.5 mg/mL) into each nostril. In a previous publication, Naclerio et al¹⁹ found no effect of this dose of oxymetazoline on histamine release after nasal allergen challenge.

Lavage fluid processing

The recovered lavage fluid was immediately put on ice. Mucus lumps were dissolved by pipetting, and the fluid was centrifuged for 10 minutes at 4°C and 1800g. The supernatant was separated from the cell pellet by pipetting and stored at -20°C. The supernatant was later assayed for histamine and tryptase.

Histamine in lavage fluid

The concentration of histamine in nasal lavage fluid was determined by using the fluorometric glass fiber method. The assay has been described in detail elsewhere.²⁰ Briefly, 25-μL aliquots of nasal lavage fluid were pipetted into a microtiter plate (provided by the Reference Laboratory, Copenhagen, Denmark) coated with a glass-fiber matrix, which selectively binds histamine. After a 1-hour incubation at 37°C, the plate was rinsed with deionized water. Then, interfering substances from the nasal lavage fluid were further dissolved by adding a solution of triton and renafix²¹ into the wells for 30 minutes at 37°C, followed by another rinse. Subsequently, histamine bound to glass fibers was resolved by adding a mixture of 75 μL o-phthalaldehyde (OPT) reagent (10 mg of OPT dissolved in 2 mL methanol, alkalinized by 18 mL of 0.05 mol/L NaOH). After 10 minutes, the histamine-OPT reaction was stopped by adding 75 μL 0.6% HClO₄. Histamine fluorescence was measured with a fluorometer (Gilson 122; Gilson Co, Washington, Ohio) and autosampler (Gilson Co). The concentration of histamine is expressed as nanograms of histamine dihydrochloride per milliliter, and the lower limit of detection was 1 ng/mL.

Anderson et al²² have previously demonstrated that the fluorometric glass fiber method is valid for the determination of histamine in nasal lavage fluid. Azelastine (final concentration, 0.1 mg/mL) and cetirizine (final concentration, 0.1 mg/mL) did not interfere with the measurement of histamine in the lavage fluid samples.

Tryptase in lavage fluid

The concentration of tryptase in nasal lavage fluid was assayed by using the Pharmacia UniCap system (Pharmacia Diagnostics AB). A monoclonal antitryptase antibody (clone B12²³) is used for the detection of tryptase in this system. The samples were assayed according to the instructions of the manufacturers. The concentration of tryptase is expressed as nanograms per milliliters, and the lower limit of detection was 1 ng/mL.

Recovery experiments and tests of samples in various dilutions

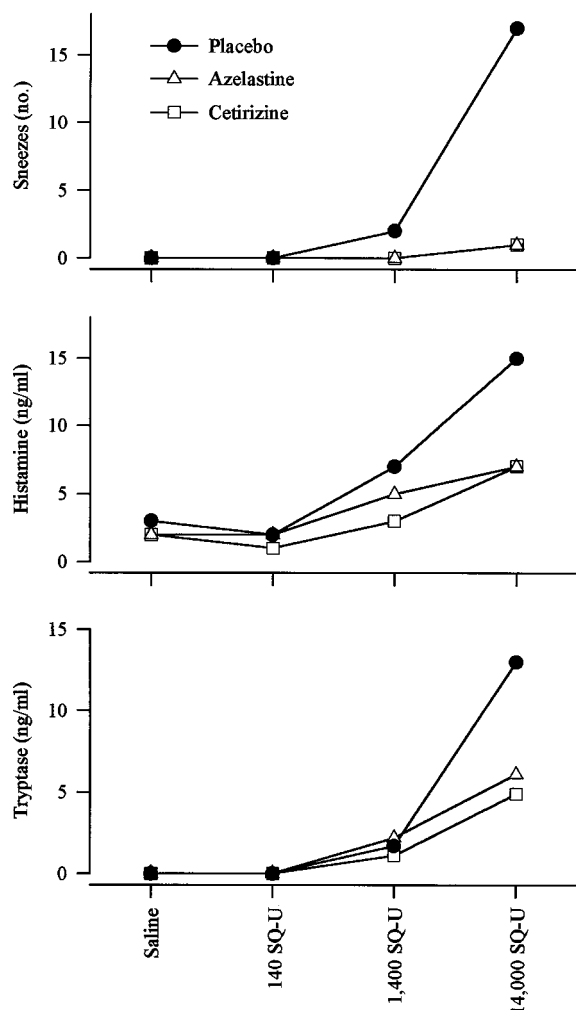


FIG 1. Allergen challenge of patients allergic to pollen after 1 week of pretreatment with placebo, azelastine, or cetirizine. The nasal challenge protocol is indicated on the abscissa. The median number of sneezes and median levels of histamine and tryptase are shown.

confirmed the absence of interference in nasal lavage fluid. Azelastine (final concentration, 0.1 mg/mL) and cetirizine (final concentration, 0.1 mg/mL) did not interfere with the measurement of tryptase in the lavage fluid samples.

Statistics

Statistical analyses were performed with SigmaStat for Windows, Version 1.0 (Jandel Scientific, Erkrath, Germany). Nonparametric techniques were used. Results are presented as medians and quartiles. For the number of sneezes and for each mediator, the Friedman test was used to determine whether there was an overall difference between the treatments. To isolate the treatment or treatments that differed from each other, multiple comparisons were made with Dunn's method. Two-tailed *P* values of less than .05 were considered significant.

RESULTS

All volunteers completed the study. Side effects were few and mild. The 2 most frequent side effects were nasal

irritation (reported by 2 subjects who were using the azelastine spray and 1 subject who was using the placebo spray) and the bitter taste of the spray (reported by 2 subjects who were using the azelastine spray).

Nasal challenge with 0.9% saline solution (control challenge) produced no sneezing. In contrast, the allergen challenge of the patients allergic to pollen caused marked and dose-dependent sneezing, with a median number of 17 sneezes at the highest dose of allergen (Fig 1). This response was almost completely inhibited by pretreatment with azelastine or cetirizine, with no significant differences between the 2 active treatments (Table I). Allergen challenge of control subjects not allergic to pollen produced no sneezes (data not shown).

The allergen challenge of patients allergic to pollen also produced a marked and dose-dependent increase in the levels of both tryptase and histamine. After the nasal challenge with 0.9% saline solution (control challenge), tryptase levels were below the detection limit and histamine levels were low (3 ng/mL). The nasal challenge with allergen significantly increased the levels of these mediators; and after the highest dose of allergen, the median levels of tryptase and histamine were 13 ng/mL and 15 ng/mL, respectively (Fig 1). Interestingly, pretreatment with azelastine or cetirizine significantly inhibited this allergen-induced increase in the levels of tryptase and histamine (Table I). Again, there were no significant differences between the azelastine and cetirizine treatments (Table I). The allergen challenge of subjects not allergic to pollen produced no increase in tryptase or histamine levels (data not shown).

DISCUSSION

It is well established that high concentrations of some antihistamines can inhibit allergen-induced/IgE-dependent histamine release from human mast cells *in vitro*.¹⁻⁴ The mechanism is not clear, but it is probably unrelated to the H₁-receptor-blocking activity of these drugs (reviewed by Rimmer and Church²⁴). Azelastine concentrations of 5 μ mol/L or more inhibit IgE-mediated histamine release from human lung mast cells *in vitro*.² For comparison, the concentration of the azelastine solution used in this study was 2390 μ mol/L (1 mg/mL in the nasal spray).

Cetirizine concentrations of 25 μ mol/L or more also inhibit histamine release from human lung and tonsil mast cells *in vitro* (but apparently have no effect on human skin mast cells).³ This concentration is higher than the steady state plasma concentration (which is approximately 0.4 μ mol/L) obtained after treatment with cetirizine tablets 10 mg daily.²⁵ However, in the *in vitro* situation, mast cells are treated with collagenase/hyaluronidase and incubated with antihistamines for relatively short time periods (5 to 30 minutes) before stimulation with optimal concentrations of anti-IgE.³ It is obvious that such experiments cannot be directly compared with the *in vivo* situation. Thus the high cetirizine concentration necessary to achieve an effect in the *in vitro* experiments does not necessarily

TABLE I. Allergen challenge of allergic patients

	Placebo			Azelastine			Cetirizine			Friedman's ANOVA	Dunn's test (<i>P</i> < .05)		
	Median	25th	75th	Median	25th	75th	Median	25th	75th	(<i>P</i> value)	P vs A	P vs C	A vs C
Sneezes (no)													
Saline solution	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	>.05	—	—	—
140 SQU	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	>.05	—	—	—
1400 SQU	2	0.3	9.8	0	0.0	1.8	0	0.0	1.0	.003	Yes	Yes	No
14,000 SQU	17	10.8	22.0	1	0.0	2.8	1	1.0	3.0	.0001	Yes	Yes	No
Histamine (ng/mL)													
Saline solution	3	1.3	4.0	2	1.3	3.0	2	1.0	3.0	>.05	—	—	—
140 SQU	2	1.0	3.0	2	1.0	2.0	1	0.3	3.0	>.05	—	—	—
1400 SQU	7	3.3	11.0	5	2.3	5.8	3	1.3	5.5	.002	Yes	Yes	No
14,000 SQU	15	8.3	19.8	7	4.0	13.8	7	5.3	12.5	.002	Yes	Yes	No
Tryptase (ng/mL)													
Saline solution	<1	<1	<1	<1	<1	<1	<1	<1	<1	>.05	—	—	—
140 SQU	<1	<1	<1	<1	<1	<1	<1	<1	<1	>.05	—	—	—
1400 SQU	1.7	<1	6.4	2.2	<1	2.9	1.1	<1	3.2	>.05	—	—	—
14,000 SQU	13.0	4.9	34.8	6.1	2.6	8.6	4.9	1.9	21.8	.03	Yes	Yes	No

25th, Lower quartile; 75th, upper quartile; P, placebo treatment; A, azelastine treatment; C, cetirizine treatment.

exclude that cetirizine, at currently prescribed doses, can inhibit allergen-induced mediator release from human mast cells in vivo.

In this study the allergen challenge of patients allergic to pollen produced sneezing and a dose-dependent increase in histamine and tryptase levels, indicating that nasal mucosa mast cells were activated.¹⁷ Pretreatment with azelastine or cetirizine almost completely suppressed allergen-induced sneezing. In addition, both antihistamines significantly suppressed the allergen-induced increase in histamine and tryptase levels. These findings are consistent with the hypothesis that both antihistamines (in addition to antagonizing the effects of histamine at H₁ receptors) also reduce mediator release from nasal mucosa mast cells in vivo. This could be a direct effect on the mast cells (similar to the effect observed in vitro) or the result of the inhibition of another component of the acute allergic response causing a synergistic release of mediators from the nasal mucosa mast cells (eg, histamine-stimulated neural release of neuropeptides that caused additional release of mediators from the mast cells). However, as the discussion below will indicate, there are other possible explanations of our findings.

Until now, only few nasal lavage studies have examined the effect of azelastine and cetirizine on allergen-induced histamine release from the nasal mucosa. In 1 study, Shin et al²⁶ examined the effect of a single oral 2-mg dose of azelastine. In this study azelastine produced a small and insignificant reduction in histamine levels. In another study Naclerio et al²⁷ examined the effect of pretreatment with 20 mg cetirizine daily for 2 days. In this study there was also a small and insignificant reduction in histamine levels. Tryptase levels were not determined in either study. The 2 studies differ from the present study with respect to the duration of the treatment periods (single dose/2 days vs 1 week) and the administra-

tion of azelastine (oral route vs intranasal administration). It is possible that these factors contribute to the different outcome of these studies and the present study.

The present study demonstrates that 1 week of pretreatment with azelastine or cetirizine inhibits the allergen-induced release of mast-cell mediators from the human nasal mucosa. This effect was demonstrated with 2 different mast-cell markers, and the results are clear cut. However, it is less clear how to interpret this finding. In theory, reduced levels of mast-cell mediators in nasal lavage fluid may result from a reduced release of mediators from mast cells and/or a reduced transfer of released mediators from the nasal mucosa to the lavage fluid. Thus it is well established that antihistamines reduce histamine-induced plasma exudation and secretion from nasal glands,²⁸ and it is possible that this inhibition of fluid movement also reduces the transfer of released mast-cell mediators from the nasal mucosa to the lavage fluid during nasal challenge with allergen.

In summary, pretreatment with intranasal azelastine 0.14 mg per nostril twice daily or cetirizine tablets 10 mg daily inhibit the allergen-induced release of mast-cell mediators from the human nasal mucosa. The results are consistent with the hypothesis that both antihistamines reduce mediator release from nasal mucosa mast cells in vivo. However, further studies are necessary to test this hypothesis.

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