

Effect of intranasal azelastine on substance P release in perennial nonallergic rhinitis patients

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ABSTRACT

Background: Rhinitis symptoms can be produced or augmented by neural mechanisms. Azelastine, a pharmacologic agent with potent H₁-receptor blocking activity can inhibit the release of various mediators implicated in the pathogenesis of nasal hyperresponsiveness. The therapeutic benefits of topical intranasal azelastine on symptoms of perennial nonallergic rhinitis (NAR) are in part because of an impact on neural mechanisms. Assessment of changes in the concentration of substance P (SP) in nasal lavage fluid before and after saline hypertonic challenge may be a means of assessing the effect of intranasal azelastine on neuropeptide release and severity of rhinitis symptoms.

Methods: Twenty-three patients with perennial NAR (negative skin-prick tests with inhalant allergens and concentration of total IgE in the normal range) were studied. Thirteen of 23 patients were treated with intranasal azelastine 0.15% spray at a dosage of 2 sprays (137 micrograms/spray) twice daily for 10 days. The control group consisted of 10 untreated patients with rhinitis. Nasal provocation using 4.5% saline solution was after 15 minutes by lavage before and after 10 days of treatment with intranasal azelastine. The concentration of SP in nasal lavage fluid was determined by enzyme immunoassay methods.

Results: Nasal lavage fluid baseline concentrations of SP were similar in both groups. After azelastine treatment, significantly greater concentrations of SP were seen in nasal lavage fluid 15 minutes after hypertonic saline challenge in the untreated patients (56.8 ± 13.8 pg/mL) in comparison with azelastine-treated patients (44.5 ± 16.5 pg/mL; $p < 0.05$). Total vasomotor rhinitis symptoms scores were substantially reduced in the azelastine-treated subjects compared with the control group.

Conclusion: Azelastine intranasal spray reduces SP release into nasal lavage fluid of NAR patients immediately after hypertonic nasal saline challenge. Reduction of neuropeptide release may be an important aspect of the clinical efficacy of topical azelastine in perennial NAR patients.

(Am J Rhinol Allergy 27, 514–516, 2013; doi: 10.2500/ajra.2013.27.3955)

Rhinitis is an increasing global problem. The World Health Organization has recognized the significance of rhinitis through the Allergic Rhinitis and Its Impact on Asthma initiative launched in 2000 with subsequent periodic updates. Up to 60 million people in the United States suffer from rhinitis including both allergic rhinitis (AR) and nonallergic rhinitis (NAR). Common forms of NAR are vasomotor or idiopathic rhinitis classified by the Nonallergic Rhinitis Consensus Panel Proceedings as nonallergic rhinopathy.¹ It is estimated that it affects ~20–30% of patients with rhinitis presenting as NAR and, overall, 44–87% have mixed allergic rhinitis and NAR.² The economic burden of rhinitis makes it the fifth most common chronic disease in the United States. The overall economic impact of perennial NAR is not fully known.³

Rhinitis symptoms can be produced or augmented by neural mechanisms. In susceptible individuals, exposure to airborne environmental triggers results in enhanced migration of inflammatory cells into the nasal mucosa and subsequent release of inflammatory mediators.

Although histamine is a less important mediator in NAR, other mediators such as leukotrienes (leukotriene B₄ and leukotriene C₄), prostaglandins (prostaglandin D₂ and prostaglandin F₂), neuropeptides (substance P [SP], neurokinin A, and calcitonin gene-related peptide [CGRP]), kinins (bradykinin), cytokines, and chemokines likely have a role. The neuropeptides and kinins can cause vasodilation, increased vascular permeability, and inflammatory cell migration. Animal studies have indicated that neuropeptides have great biological potential impact on inflammatory mechanisms in the nasal

mucosa. These mediators applied alone may provoke clinical symptoms connected with the immediate nasal mucosal response including sneezing, rhinorrhea, and pruritus.^{4,5} Recently, a study⁶ using an animal model showed that SP released from the nasal mucosa because of allergen challenge activated kinin receptors NK1 and induced allergic nasal symptoms. Intranasal allergen challenge performed on dust-mite-allergic patients induces SP and bradykinin release not only during the early phase of the allergic response but also during the late-phase response.⁷ Histopathology studies have confirmed the presence of regulatory peptide-associated with immunoreactivity in the human nasal mucosa. Peptide immunoreactive nerve fibers and vasoactive intestinal peptide, CGRP, SP, or neurokinin A have been detected in human nasal mucosa.⁸

Depolarization of immunopeptide reactive nerves leads to release of neuropeptides such as SP and CGRP. SP is a vasodilator, causing vascular leakage and it stimulates mucus secretion from submucosal glands.^{9,10} SP can also induce release of histamine from mast cells.^{11,12} All of these mechanisms taken together suggest a role of this mediator in provoking characteristic rhinitis symptoms.

Azelastine, a drug with potent H₁-receptor blocking activity could inhibit the release of various mediators implicated in the pathogenesis of hyperresponsiveness and allergic reactions. Azelastine nasal spray is the only prescription topical antihistamine that has been approved in the United States for the treatment of both allergic and nonallergic vasomotor rhinitis. This study assesses the therapeutic benefit of topical intranasal azelastine on NAR symptoms and explores its impact on neural mechanisms. The aim of this study was to determinate the concentrations of SP in nasal lavage fluid after saline hypertonic challenge before and after use of azelastine nasal spray to examine the impact of this agent on neuropeptide release. This study also assessed the effect of azelastine on total vasomotor rhinitis symptoms (TVRSs).

METHODS

Subjects

Patients with NAR with no evidence of mechanical obstruction or infectious disease were included in the study. At the screening visit

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(14 days before the baseline visit), medical history was assessed and a physical examination (including nasal examination) was performed along with laboratory assessments and skin-prick tests. All patients had a nasal cytology examination test that was negative for eosinophils, a normal sinus x ray, and no clinically significant nasal anatomic deformities.

Twenty-three patients (13 female and 10 male patients) having a mean age of 29.2 ± 4.3 years were investigated. All patients experienced chronic rhinitis symptoms. Thirteen patients (7 female and 6 male patients) aged 28.8 ± 5.6 years were treated with intranasal azelastine spray (Astelin; Meda, Somerset, NJ) 2 sprays ($137 \mu\text{g}/\text{spray}$) twice daily for 10 days. The control group consisted of 10 untreated perennial NAR patients (6 female and 4 male patients) with a mean age of 30.1 ± 4.8 years. Skin-prick tests with biologically standardized extracts of important perennial and seasonal inhalant allergens (Allergopharma, Rheinbek, Germany) were performed in all patients. To be eligible for the study, all skin-prick tests had to be negative. Concentrations of serum total IgE (UniCAP; Phadia, Uppsala, Sweden) were required to be in the normal range ($<85.0 \text{ kU/L}$) to enter the study.

All intranasal topical allergy medications were withheld for 2 weeks before the study. Before the start of the study, patients were required to have stopped using oral decongestants for 24 hours, oral antihistamines for 96 hours, systemic corticosteroids for 1 month (intramuscular for 3 months), and nasal corticosteroids or nasal cromolyn for 2 weeks. Use of these drugs was not allowed during the study.

Nasal provocation was performed according to the method of Naclerio *et al.*¹³ Four lavages each nostril using 5 mL of 0.9% NaCl at room temperature were performed to reduce baseline mediator levels to a stable level. Subsequently, 4.5% saline solution was administered into the same nostril by a hand-held nebulizer bottle, which provided 0.1 mL/puff. Each challenge was followed by lavage with 0.9% NaCl at room temperature performed at 15 minutes after the nasal challenge. Nasal lavage fluid was collected in tubes positioned on the same side of the anterior nasal septum as the challenge was performed.

The nasal lavage fluid was immediately put on ice and phosphoramidon was added and centrifuged at $3600 \times g$ for 10 minutes at 4°C to remove cellular fragments and mucus and then stored at -70°C until analysis.

The cell pellet was stained with May–Grunwald–Giemsa and inspected by light microscopy.

SP and Protein Measurement

The concentration of SP in nasal lavage fluid was determined by RIA (Incstar, Stillwater, MN).

Patients were given daily symptom diaries and instructed to record their individual symptoms once daily (evening) for the duration of the study by using the scoring scale. Four nasal symptoms (rhinorrhea, nasal stuffiness/congestion, sneezing, and postnasal drip) were scored using a 4-point rating scale (0, none; 1, mild; 2, moderate; and 3, severe), which reflected symptom severity during the previous 12-hour period. TVRS score (TVRSS) was calculated as the sums of these, so that it could range from 0 to 12.

All subjects provided written informed consent before participating in the study. The study was conducted under the auspices and supervision of the Silesian Medical University Institutional Review Board.

Statistics

Statistical analysis was performed using Statistica for Windows (Statsoft, Inc, Tulsa, OK). Wilcoxon's signed-rank test was used to compare mediator release at each time point. Correlations were performed using the Spearman rank method.

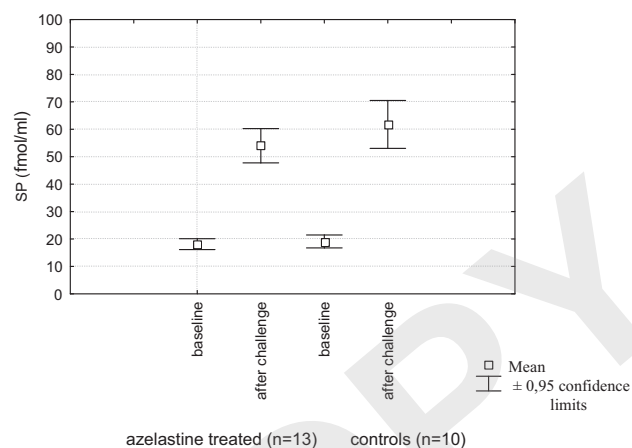


Figure 1. Concentration of substance P (SP) in nasal lavage fluid of non-allergic rhinitis (NAR) patients treated with azelastine (before and after saline challenge).

RESULTS

The hypertonic saline solution challenge induced an increase in nasal SP concentrations. The volume of nasal lavage fluid recovered from active treated rhinitis patients ($62.6 \pm 9.2\%$) was comparable with the untreated controls ($67.6 \pm 8.4\%$) before and after 10 days of treatment ($66.8 \pm 8.4\%$ versus $64.2 \pm 9.6\%$, respectively). Compared with the levels found after diluent challenge, there was a statistically significant increase in SP in the first 15 minutes after hypertonic provocation.

Significantly greater concentrations of SP were seen in nasal lavage fluid from both groups initially at baseline immediately (15 minutes) after hypertonic saline provocation, with SP levels of $64.17 \pm 14.8 \text{ fmol/mL}$ in treated patients and $62.34 \pm 16.8 \text{ fmol/mL}$ in the control group (Fig. 1).

Lower concentrations of SP in nasal washings after hypertonic provocation were observed in patients treated with azelastine at $54.25 \pm 14.6 \text{ fmol/mL}$ versus $61.76 \pm 17.6 \text{ fmol/mL}$ in the control group.

A weak positive correlation was seen between SP level after saline challenge on the 10th day of treatment and TVRSS ($r = 0.40$; $p < 0.05$).

Patients treated with azelastine had reduced TVRSS from 7.46 ± 1.64 at the start of the study to 5.91 ± 1.20 after 10 days of therapy compared with control subjects who ranged from 7.4 ± 1.56 before treatment to 7.1 ± 1.44 after 10 days (Fig. 2).

Treatment with azelastine nasal spray resulted in statistically significant improvements versus untreated controls in rhinorrhea ($p < 0.01$), sneezing ($p < 0.02$), nasal congestion ($p < 0.038$), and postnasal drip ($p < 0.042$). The end point analysis of sneezes also was statistically significant ($p < 0.03$), in favor of azelastine.

There were almost no eosinophils in the recovered nasal lavages from subjects in both groups.

Good tolerability of azelastine was seen; 3 patients reported bitter taste.

DISCUSSION

Neural mechanisms probably make an important contribution to rhinitis symptoms but few studies have been performed to evaluate the possible involvement of neurogenic mechanisms in human nasal inflammation.

SP is considered as one of the neuromodulators involved in nasal mucosal inflammatory regulation. It is undecapeptide, belonging to the tachykinin family, and was the first neuropeptide identified.

Azelastine 0.15% nasal spray after 10 days of treatment showed a significant improvement in TVRSS.

In this study it was shown that SP can be recovered in significantly augmented concentrations from nasal lavage fluid after hypertonic saline challenge.

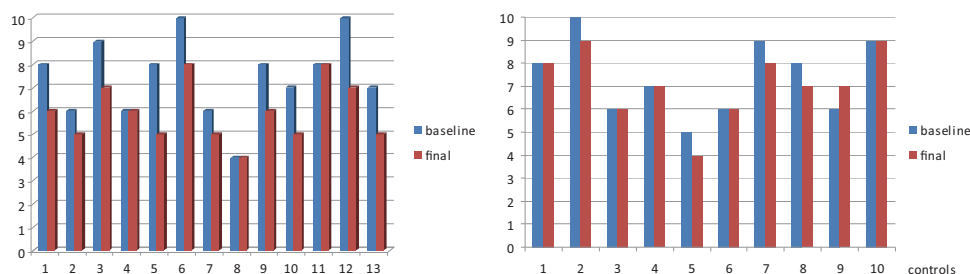


Figure 2. Total vasomotor rhinitis symptom scores (TVRSS) in patients treated with azelastine (left) and in the untreated control group (right).

The present study describes the nasal lavage SP release in NAR patients 15 minutes after a nonspecific nasal challenge. There was an increase in levels of SP after hypertonic nasal provocation in both groups, although these were considerably less after treatment with azelastine. An increase in SP in nasal secretions immediately after hypertonic saline challenge has also been shown in another study.¹⁴ The neuropeptide SP is associated with several rhinitis symptoms such as nasal itching and sneezing, but may also contribute to the onset of these and other nonallergic vasomotor rhinitis symptoms. Because neuropeptides such as SP have been shown to have a major role in airway hyperresponsiveness, their down-regulation is likely an important value. Whether SP is a principle mediator responsible for symptoms observed in patients with AR and NAR remains to be determined. This phenomenon has been shown in a study using an animal model.¹⁵ There is substantial evidence suggesting that SP liberated from sensory nerve endings is involved in generation of chronic AR and NAR symptoms. Some mechanisms and cell involvement are common in AR and idiopathic rhinitis.¹⁶

The participation of SP in inducing release of histamine from human nasal mucosa connects the neurogenic and immunologic mechanisms of pathophysiological changes in the nasal mucosa of mixed rhinitis patients.^{17,18} SP can modulate chemotaxis of eosinophils and neutrophils and also proliferation and activation of B and T lymphocytes, mast cells, and macrophages.¹⁹ SP, which may be released by a variety of stimuli including allergens, capsaicin, cold air, and ozone, may also augment the release of cytokines.

Topical azelastine may be more effective than oral antihistamines in reduction of SP release because the topical application route may create high local concentrations allowing for more effective pharmacologic activity. These anti-inflammatory actions may be part of the reason why azelastine is effective in relieving many symptoms of perennial vasomotor NAR. Also, use of another topical antihistamines olopatadine resulted in protection from an experimental hyperosmolar nasal challenge significantly reducing symptoms compared with controls although effects on neuropeptides have not been noted.²⁰

In general, azelastine nasal spray was well tolerated with occasionally reported bitter taste, seemingly associated in part with incorrect techniques of application.

Neuropeptides released by nonspecific stimuli administration can provoke nasal symptoms, suggesting a relationship between neuropeptides, rhinitis symptoms, and inflammatory reactions in the nasal mucosa. Additional studies are required to determine the relative importance of changes in SP release in the nasal mucosa with respect to the disease course of perennial NAR.

CONCLUSIONS

Azelastine nasal spray reduces SP release into nasal lavage of perennial NAR patients immediately after hypertonic saline challenge. Reduction of nonspecific nasal hyperresponsivity-induced neuropeptide release may be an important aspect of the clinical efficacy of topical azelastine in perennial NAR patients.

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