Azelastine nasal spray inhibiting sympathetic function on human nasal mucosa in patients with allergy rhinitis*

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Abstract

Background: Azelastine hydrochloride (azelastine) nasal spray is a histamine receptor-1 (H1) antagonist often used in treating allergic rhinitis to relieve its symptoms. However, the effects of azelastine to influence decongestion on human nasal mucosa in patients with allergic rhinitis are not yet fully explored and merit further exploration. The effects of azelastine on the vasocontractile responses generated by smooth muscles in the vascular structures of human nasal mucosa were investigated directly in vitro.

Methods: We examined the effectiveness of azelastine on isolated human nasal mucosa by testing: 1) the effect on mucosa resting tension; 2) the effect on mucosal contraction caused by 10^{-6} M methoxamine as a sympathetic mimetic; 3) the effect of the drugs on electrically induced mucosal contractions.

Results: The results indicated that addition of methoxamine to the incubation medium caused the nasal mucosa to contract in a dose-dependent manner. Addition of azelastine at doses of 10^{-6} M or above elicited a significant dilation response to 10^{-6} M methoxamine-induced mucosal contraction. Azelastine could inhibit electrical field stimulation-induced spike mucosal contraction. Moreover, increase in concentration of azelastine had minimal effect on basal tension of nasal mucosa.

Conclusions: The technique in our study is simple and reproducible. Azelastine could inhibit both EFS and methoxamine-induced nasal mucosal contractions in vitro. This study highlights that although azelastine nasal spray is often used in treating allergic rhinitis to improve symptoms, nasal obstruction may be not relieved immediately due to the anti-sympathetic effect of azelastine.

Key words: Azelastine, allergic rhinitis, nasal obstruction, human, nasal mucosa, in vitro study

Introduction

Allergic rhinitis (AR) is an inflammatory disease of nasal mucosa and one of most common medical conditions encountered by health professionals, including physicians, nurse practitioners, physician assistants and pharmacists. The prevalence of AR is difficult to accurately determine because many AR "sufferers" do not seek professional help but rather self-treat what they perceive to be annoying symptoms. The U.S. prevalence is estimated to be between 10-30% of adults and 40% of children, thus affecting 30 to 60 million individuals annually. It is characterized by symptoms of sneezing, itching, rhinorrhea, postnasal discharge, and congestion. Azelastine hydrochloride (azelastine) and MP-AzeFlu nasal spray comprising a novel formulation of fluticasone propionate, azelastine hydrochloride and excipients delivered in a single intranasal spray were used for treatment of allergic rhinitis. Azelastine nasal spray is a second-generation intranasal antihistamine and selectively antagonizes histamine receptor-1 (H1). Azelastine has mast-cell stabilizing and anti-inflammatory properties, reducing the concentration of leukotrienes, kinins, and platelet activating factor in vitro and in vivo, as well as inflammatory cell migration by downregulating intercellular adhesion molecule-1 expression. Azelastine nasal spray is an effective, rapid-acting, and well-tolerated drug to improve nasal symptoms due to its complex anti-inflammatory model of action [1-6]. However, the effects of azelastine in decongestion of human nasal mucosa in patient with allergic rhinitis are not

yet fully explored and merit further exploration. Therefore, the primary goal of the present study was to test direct effects of azelastine on the contractile responses on human nasal mucosa.

Materials and methods

Tissue preparation

After obtaining informed consent, mucosal specimens were obtained from 12 patients under general anesthesia during elective turbinectomies. Indications for surgery were severe nasal obstruction due to allergic rhinitis. Strict criteria were applied to exclude those with a history of vasomotor rhinitis and nasal surgery, as well as others who used vasoconstrictors for nasal obstruction prior to surgery.

Experimental protocol

In vitro preparation of human nasal mucosal strip was used (Figure 1) [7]. Following immediate removal (because our operation room and lab room were close in the same building, less than 5 minutes elapsed between the harvesting and testing of samples and tissue was conserved in 0.9% saline solution with low temperature during this period), a nasal mucosal strip measuring 20 × 8 mm was mounted using two steel plates and submersed in a water-jacketed 30-ml glass chamber at 37°C. The bath was filled with 30 ml Krebs solution consisting of (mmol/l) NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; MgSO4·7H_2O, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; and glucose, 10.0. The upper side of the mucosal strip was attached to a Grass FT-03 force displacement transducer (AstroMed, West Warwick, RI, USA) using a steel plate and a 3-0 silk ligature. The other side of the strip was fixed to a steel plate attached to the bath. A passive tension of 0.5 g was applied to the strips and subsequent changes in tension were recorded continuously using Chart V4.2 software (Power Lab, AD Instruments, Colorado Springs, CO, USA). Preliminary tests showed that a nasal mucosal strip immersed in the bath solution used for subsequent experiments did not contract when basal tension was applied. The response of this preparation to drugs and electrical stimulation has been described previously [8-10]. The study tested methoxamine, an α-adrenergic agonist, as a nasal mucosal vasocontraction drug. There was no wear off when stimulating with methoxamine and direct application of 10^{-4} M azelastine inhibited the effects. Before drug assays were conducted, nasal mucosal strips were equilibrated in the bath solution for 15–30 mins, during which continuous aeration with a mixture of 95% O_2 and 5% CO_2 was applied. Stepwise increases in the amount of drugs used were employed to study the contraction or dilation responses of the nasal mucosal strips. All drugs were administered by adding a defined volume of stock solution to the tissue bath solution. Electrical field stimulation (EFS) (5 Hz, 5 ms pulse duration, at a voltage of 50 V, trains of stimulation for 5 seconds) was applied to the nasal mucosal strip through two wire electrodes placed parallel to the nasal mucosal strip and connected to

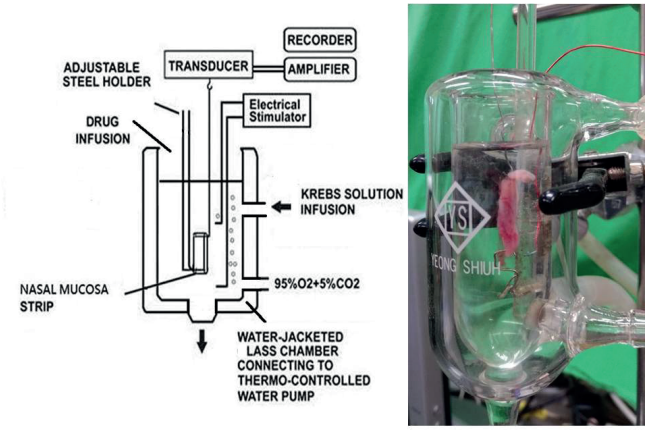

Figure 1. Schematic diagram and actual photo of tension measurements in an isolated human nasal strip.

a direct-current stimulator (Grass S44, Quincy, MA, USA). There was an interval of 2 minutes between each stimulation period to allow recovery from the response. Stimulation was applied continuously to the nasal mucosal strip at 37°C. The chemicals used were of the highest purity available and were obtained from Sigma-Aldrich (St Louis, MO, USA). This study was approved by the institutional review board of the Tri-Service General Hospital.

Azelastine assessments

The following assessments for azelastine were performed: 1) the effect on nasal mucosal resting tension: this test examined the effect of azelastine on the stimulated condition of the resting nasal mucosa. 2) the effect on nasal mucosal contraction caused by 10^{-6} M methoxamine: this procedure examined postsynaptic events such as muscle receptor blockade, enhancement, and second messengers; and 3) the effect of the azelastine on electrically induced on nasal mucosal contraction: electrical stimulation of the tissue causes sympathetic nerve remnant in the nasal mucosa to release norepinephrine. If there is interference with transmitter release, then electrical stimulation does not cause contraction. Stepwise increases in the amount of test agent were used to study the contraction or dilation responses of nasal mucosal strips.

In each experiment, one untreated strip served as a control and at least three technical replicate measurements were made.

Statistical analysis

Concentrations of drugs were expressed as concentrations present in the 30 ml bath solution. Data were presented as mean values and standard deviations (SD). Differences between mean values were compared using Student's t-test. Differences were assumed to be significant at $p < 0.05$.

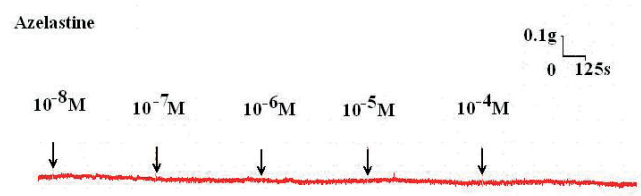

Figure 2. Changes in tension of human nasal mucosa after application of azelastine at various concentrations. Increased concentration of azelastine alone had a minimal effect on basal tension of human nasal mucosa. Original basal tension was 0.5 g.

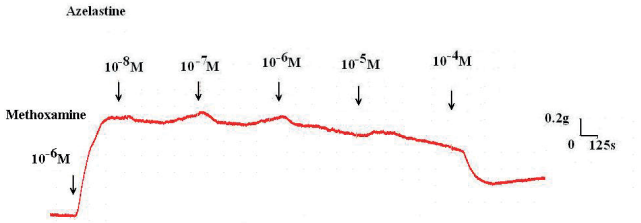

Figure 3. Original recording of effects of azelastine on 10^{-6} M methoxamine-induced contraction of human nasal mucosa.

Results

The degree of contraction or dilation of nasal mucosal strips was estimated from the tension applied to the transducer. Mucosal contraction induced by a small dose of methoxamine was easily detected, and the tissue remained in a contracted state until the drug was rinsed from the tissue.

Addition of the H1 antagonist, azelastine, to the basal tension elicited a negligible effect (Figure 2). It resulted in dilation of the mucosa when introduced after the addition of a vasoconstricting agent such as 10^{-6} M methoxamine (Figure 3). Low doses of azelastine resulted in a mild effect on dilation while higher doses caused significant dilation of human nasal mucosa (Figures 3, 4). At 10^{-8} M azelastine, the tension was 97.33% ± 1.36% of control values (Figure 4). At 10^{-6} M and 10^{-5} M azelastine, the tensions were 78.33% ± 4.72% and 67.83% ± 3.43% respectively (Figure 4). The difference in tension between 10^{-8} M and 10^{-6} M or 10^{-5} M azelastine was statically significant ($p < 0.05$). Azelastine also inhibited electrical field stimulation-induced spike contraction (Figures 5, 6). The peak tension of the nasal mucosal strip evoked by EFS upon the addition of 10^{-8} M azelastine was 100.0 % ± 0 %, whereas at 10^{-5} M and 10^{-4} M azelastine the peaks were 87.67% ± 1.97% and 6.17% ± 6.94%, respectively (Figure 6). The difference in tension between 10^{-8} M azelastine and 10^{-5} M or 10^{-4} M azelastine was statically significant.

All the experiments were performed with a control substance. Azelastine can be dissolved by water. The solvent used in this study is Krebs solution. During the test process, the control substance was added. It will not affect the results (Figure 7).

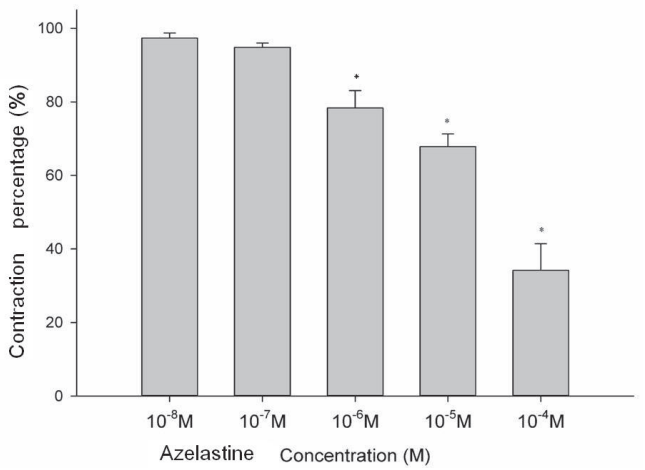

Figure 4. Effects of azelastine on 10^{-6} M methoxamine-induced contraction (contraction area calculated at 100% with no addition of azelastine) of human nasal mucosa. The difference in tension between 10^{-8} M and 10^{-6} M or 10^{-5} M azelastine was statistically significant ($p < 0.05$). Results were mean ± SD ($n = 6$).

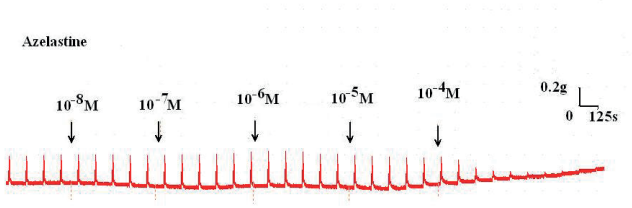

Figure 5. Original recording of the effects of azelastine on electrically induced nasal mucosal contractions. Higher doses of azelastine could decrease EFS-induced spike contraction.

Discussion

The results of the present experiments should be interpreted within the context of the test materials used. The most obvious point to consider is which tissue component of nasal mucosa is responsible for drug-induced nasal mucosa contraction. Although it is difficult to establish through direct experimentation, the answer can be inferred by observing the nature of specific tissues and their response to particular drugs. First of all, the mucosal strips used in our study were crude preparations containing arteries, arterioles, capillaries, venous sinusoids, venules and veins. The smooth muscle of nasal blood vessels appeared to be the only tissue component able to contract. The other components (epithelium, nasal glands, connective tissue, and nerves) appeared unable to contract [11]. In view of that, the contractile responses should be regarded as coming from the vascular smooth muscles. Indeed, the capacity of such a preparation to respond to drugs and electrical stimulation has been verified previously [8-10]. However, the contractile responses are likely to represent the sum total of the various tissues. Secondly, the human nasal mucosa used in these experiments was obtained from patients with a clinical diagnosis of allergic rhinitis.

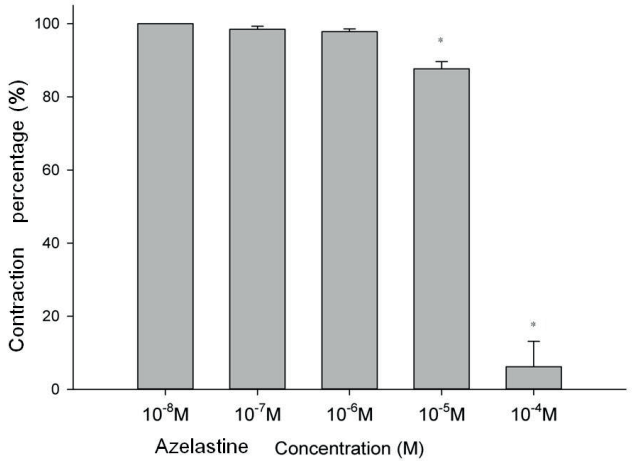

Figure 6. Effects of azelastine on electrically induced nasal mucosal contractions (contraction area was calculated at 100% with no addition of azelastine). The difference in tension between 10^{-8} M and 10^{-5} M or 10^{-4} M azelastine was statistically significant. Results were mean ± SD (n = 6).

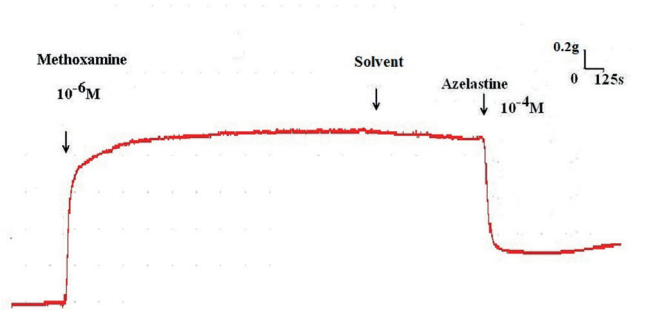

Figure 7. The solvent used in this study is Krebs solution. A single dose of 10^{-4} M azelastine inhibited 10^{-6} M methoxamine-induced contraction of mucosa strip. The same volume of solvent had neglected effects on 10^{-6} M methoxamine-induced contraction of mucosa strip.

Although the mucosal strips were taken from patients suffering from disorder varying in degrees, our experimental results had only negligible overall variabilities.

It is now well known that histamine exerts its effects by activating histamine receptors, of which four types are now identified. H1 and H2 receptors are widely expressed, in contrast to H3 and H4 receptors. All types of histamine receptor are heptahelical transmembrane molecules that transducer extracellular signals by way of G proteins to intracellular second messenger systems [12]. Azelastine is a phthalazinone derivative with H1 receptor binding approximately tenfold greater than chlorpheniramine on a milligram – per-milligram basis [5]. Commercial azelastine nasal spray contains 0.1% azelastine HCl, which is approximately 2 x 10^{-3} M azelastine. When applying a spray, one gets immediately a 1/10 dilution resulting in a concentration of 2 x 10^{-4} M azelastine at the nasal mucosal side. It remains to be shown that a concentration of 10^{-5} M can be reached at the vascular smooth muscles.

Azelastine is more than just anti-histamine and has a well-known anti-inflammatory mode of action. Azelastine's anti-inflammatory activity is widespread. Azelastine inhibits TNF-α release, granulocyte macrophage colony-stimulating factor generation and reduces the number of a range of inflammatory cytokines, including IL-1β, IL-4, IL-6 and IL-8. These cytokines perpetuate the inflammatory response. In vitro, azelastine decreases free-radical production by human eosinophils and neutrophils, and calcium influx induced by platelet-activating factor. It reduces inflammatory cell migrations in patients with rhinitis, most likely as a consequence of the downregulation of ICAM-1 expression, and inhibits kinin (e.g., bradykinin and substance P), platelet-activating factor and leukotriene release in vitro and in vivo. Leukotrienes are associated with dilation of vessels, increased vascular permeability and edema, which results in nasal congestion, mucus production and recruitment of inflammatory cell in vitro and in vivo. Bradykinin and substance P are associated with the AR symptoms such as nasal itching and sneezing [3-5]. Furthermore, both AR and nonallergic rhinitis (NAR) are amongst the most common chronic diseases with a significant impact on quality of life [13]. Previous studies with repeated intranasal applications of capsaicin demonstrated reduction in nasal symptoms, nasal hyperreactivity and transient receptor potential vanilloid 1 (TRPV1) overexpression in patients with NAR [14-18]. Singh and colleagues published the effects of azelastine on TRPV1channels, demonstrating a direct activity effect of azelastine on TRPV1 and desensitization of TRPV1 through the modulation Ca^{2+} signaling on sensory neurons and in nasal epithelial cells after repeated applications azelastine. Azelastine, similar to capsacine, exhibits direct activity on TRPV1 ion channels that may represent a novel mechanistic pathway explaining its clinical efficacy in NAR [19, 20].

This study observed that increased concentrations of azelastine had minimal effect on the basal tension of nasal mucosa, demonstrating that azelastine can cause neither direct vasoconstriction nor vasodilation in nasal blood vessels. Electrical field stimulation is a common experimental tool for activating the nerve terminals within the tissue to be tested and inducing the release of endogenous neurotransmitters, thereby triggering the smooth muscle to contract. EFS could induce a spike contraction of canine nasal mucosa, which was believed to result from the contraction of vascular smooth muscles, disappearing after ipsilateral cervical sympathetic ganglionectomy [8]. Thus, EFS-induced spike contraction of isolated canine nasal mucosa was proved to be mediated by sympathetic innervations [8]. Moreover, a concentration of 10^{-5} M or above azelastine could block electrically induced nasal mucosal contractions. Therefore, EFS-induced contraction of the nasal mucosa was decreased as the azelastine concentration was increased. These findings suggested that azelastine could antagonize the sympathetic innervations responsible for vascular smooth muscle contraction. Regarding effects of azelastine on contraction caused by 10^{-6} M

of methoxamine, the procedure examined postsynaptic events such as muscle receptor blackade, enhancement, and secondary messengers. Azelastine at a concentration of 10^{-6} M or above reduced the contraction induced by 10^{-6} M of the α-adreoceptor agonist methoxamine; hence it is possible that these contractions actually antagonize α-adreoceptor functions. However, how does azelastine antagonize the α-adreoceptor agonist and affect the nasal mucosal smooth muscle? Further studies are needed to elucidate this question. Briefly, this study observed significant inhibitory effect of azelastine on human nasal vascular smooth muscle during field stimulation and only minimal effect of azelastine on basal tension of turbinate mucosa. Finally, azelastine antagonizes methoxamine and it is known as a direct-acting α-adrenergic agonist [21]. These pointed out azelastine also had an anti-sympathetic effect on nasal vascular smooth muscle in patients with allergic rhinitis to cause vasodilation. The study indicated that although in clinical studies demonstrated that azelastine nasal spray is an effective, rapid-acting, and well-tolerated drug to improve symptoms of allergy rhinitis [4,5], nasal obstruction may be not relieved immediately due to the anti-sympathetic effect of azelastine.

Conclusion

The technique in our study is simple and reproducible. Azelastine could inhibit both EFS and methoxamine-induced nasal mucosa contractions in vitro. This study highlights that although azelastine nasal spray is often used in treating allergic rhinitis to improve symptoms, nasal obstruction may be not relieved immediately due to the anti-sympathetic effect of azelastine.

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Authorship contribution

HWW, LHC, and PCW: data collection, analysis, and writing the manuscript. JCL, YYL, and YHC: data collection and analysis. All Authors have reviewed the final paper.

Conflict of interest

No conflicts of interest.

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