

Azelastine nasal spray inhibiting parasympathetic function of tracheal smooth muscle*

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SUMMARY

Background: Azelastine hydrochloride is a histamine receptor-1 (H_1) antagonist with anti-inflammatory properties that is available in the United States as Astelin Nasal Spray for rhinitis patients who are suffering from sneezing and rhinorrhea. The effect of H_1 antagonists on nasal mucosa in vivo is well known; however, the effect of the drug on tracheal smooth muscle has rarely been explored. During administration via oral intake or inhalation of the H_1 antagonist for nasal symptoms, it might affect the trachea.

Methods: We examined the effectiveness of azelastine on isolated rat tracheal smooth muscle by testing: 1) the effect on tracheal smooth muscle resting tension; 2) the effect on contraction caused by 10^{-6} M methacholine as a parasympathetic mimetic; and 3) the effect on electrically induced tracheal smooth muscle contractions.

Results: The results indicated that addition of methacholine to the incubation medium caused the trachea to contract in a dose-dependent manner. Addition of azelastine at doses of 10^{-5} M or above elicited a significant relaxation response to 10^{-6} M methacholine-induced contraction. Azelastine could inhibit electrical field stimulation-induced spike contraction. It alone had a minimal effect on the basal tension of trachea as the concentration increased.

Conclusions: This study indicated that high concentrations of azelastine might actually inhibit parasympathetic function of the trachea. Azelastine might reduce asthma attacks in rhinitis patients because it could inhibit parasympathetic function and reduce methacholine-induced contraction of tracheal smooth muscle.

Key words: azelastine, histamine receptor-1 antagonist, trachea, smooth muscle, in vitro study

INTRODUCTION

The use of antihistamines in asthma has long been controversial. The early first-generation antihistamines were not valuable in the treatment of asthma because they were not effective at the doses recommended for allergic rhinitis, and higher doses caused intolerable side effects. These side effects limited their use intravenously or via aerosol⁽¹⁻⁴⁾. Azelastine hydrochloride (azelastine) is a histamine receptor-1 (H_1) antagonist with anti-inflammatory properties that is available in the United States as Astelin Nasal Spray[®] for rhinitis patients who are suffering from sneezing and rhinorrhea⁽⁵⁾. Most of the studies on azelastine were mainly clinical trials^(6,7). Histamine plays a prominent and diverse role in the pathophysiology of allergic diseases; therefore, therapeutic intervention is typically focused on blocking the effects of this biogenic amine. Azelastine is used as an antihistamine nasal spray and is frequently prescribed for various allergic diseases. During an asthmatic attack, the tracheal smooth muscle plays an important role in reducing pulmonary function as it becomes contracted.

Hence, the effect of azelastine nasal spray on tracheal smooth muscle merits further exploration.

To test the effects of drugs in tracheal constriction or relaxation, a simple in vitro technique was applied to rat tracheas. The technique used was developed from a previously described method^(8,9), in which 5-mm strips of rat trachea were suspended in a tissue bath containing 30 ml Krebs solution^(10,11). One end of the strip was attached to a steel plate and the other to an isometric transducer and a steel plate. A passive tension of 0.3 g was applied to the strips. The aim of this study was to determine the effects of azelastine on isolated tracheal smooth muscle in vitro.

MATERIALS AND METHODS

Chemicals

Chemicals used were of the highest purity available. Commercial azelastine nasal spray was obtained through the courtesy of Pharma Power Biotec Co., Taiwan. All other chem-

ical reagents were obtained from Sigma (St. Louis, MO, USA). We tested methacholine as a tracheal contraction drug.

Rat trachea experimental set-up

Eighteen rats were anesthetized by intraperitoneal administration of pentobarbital (45 mg/kg) and two pieces of trachea (~5 mm in length) were removed from each rat. This study was approved by an animal experiment review board (IACUC-07-133). The tracheal specimen was mounted using two steel plates and submersed in a 30 ml muscle bath at 37°C as previously reported^(3,4). Briefly, the bath was filled with 30 ml Krebs solution consisting of (mmol/l): NaCl, (118); KCl, (4.7); CaCl₂, (2.5); MgSO₄·7H₂O, (1.2); KH₂PO₄, (1.2); NaHCO₃, (25.0); and glucose, (10.0). The upper side of the tracheal strip was attached to a Grass FT-03 force displacement transducer (AstroMed, West Warwick, RI, USA) by 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 0.3 g was applied to the strips and subsequent changes in tension were recorded continuously using Chart V4.2 software (PowerLab, AD Instruments, Colorado Springs, CO, USA). Preliminary tests showed that the tracheal strip immersed in the bath solution used for subsequent experiments did not contract when basal tension was applied. Before drug assays were conducted, isolated tracheas were equilibrated in the bath solution for 15-30 min, during which they were continuously aerated with a mixture of 95% O₂ and 5% CO₂. Stepwise increases in the amount of drugs used were made to study contraction or relaxation responses of tracheal strips. All drugs were administered by adding a defined volume of stock solution to the tissue bath solution. In each experiment, one untreated strip served as a control.

Electrical field stimulation (EFS) (5 Hz, 5-ms pulse duration, at 50 V, trains of stimulation for 5 s) was applied to the trachea strip with two wire electrodes placed parallel to the trachea strip and connected to a direct-current stimulator (Grass S44, Quincy, MA, USA). An interval of 2 min was imposed between each stimulation period to allow recovery from the response. Stimulation was applied continuously to the trachea at 37°C.

Azelastine assessments

The following assessments for azelastine were performed: 1) Effect on tracheal smooth muscle resting tension: this test was to examine the effect of the drug on the simulating condition of resting trachea condition. 2) Effect on contraction caused by 10⁻⁶ M methacholine (a parasympathetic mimetic): this procedure was concerned with the examination of postsynaptic events such as muscle-receptor blockade, enhancement, and second messengers. 3) Effect of azelastine on electrically induced contractions: electrical stimulation of this tissue causes parasympathetic nerve remnants in the trachea to release the transmitter acetylcholine. If there is interference with transmitter release, electrical stimulation does not cause con-

traction. Thus, presynaptic events were seen more easily with this procedure.

Statistical analysis

The 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 t-test. Differences were assumed to be significant at $p < 0.05$.

RESULTS

The degree of contraction or relaxation of tracheal strips was estimated from the tension applied to the transducer. Tracheal contraction induced by a small dose of methacholine 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 1). It resulted in relaxation of the trachea when introduced after the addition of a constricting agent such as 10⁻⁶ M methacholine (Figure 2). Low doses of azelastine resulted in a mild effect on contraction while higher doses relaxed significantly the trachea smooth muscle (Figures 2 and 3). At 10⁻⁸ M azelastine, the tension was 99% ± 1.1% of the control values (Figure 3). At 10⁻⁵ M and 10⁻⁴ M azelastine, the tensions were 85% ± 12.8% and 31% ± 18.8%, respectively (Figure 3). The difference in tension among the specimens treated with 10⁻⁸ M azelastine and 10⁻⁵ M or 10⁻⁴ M azelastine was statistically significant ($p < 0.05$).

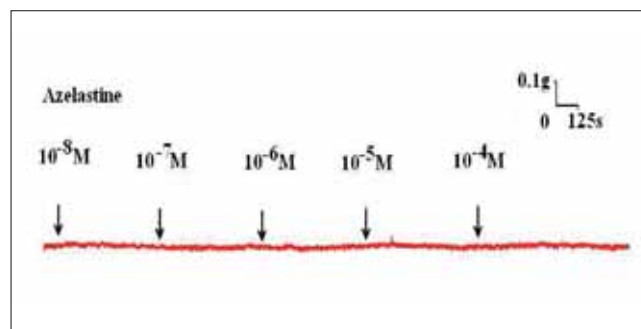


Fig. 1. Tension changes in rat trachea after application of various azelastine concentrations. Azelastine alone had a minimal effect on the basal tension of trachea as the concentration increased. Original basal tension was 0.3 g.

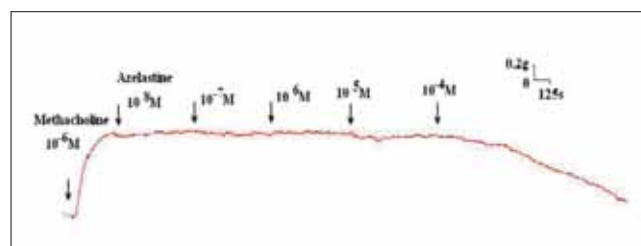


Fig. 2. Original recording of the effects of azelastine on 10⁻⁶ M methacholine-induced contraction of rat trachea.

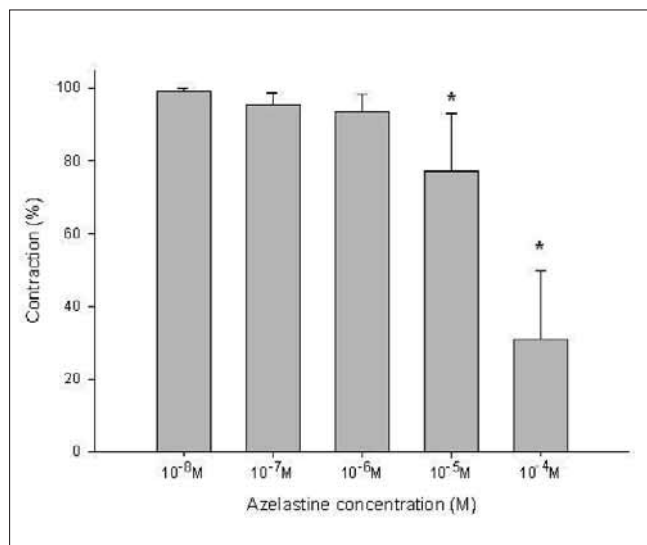


Fig. 3. Effects of azelastine on 10^{-6} M methacholine-induced contraction (contraction area calculated at 100% with no addition of azelastine) of rat trachea. The difference in tension between 10^{-8} M azelastine and 10^{-5} M azelastine or 10^{-4} M azelastine was statistically significant ($p < 0.05$). Results were mean \pm SD ($n = 7$).

Azelastine also inhibited the spike contraction induced by EFS (Figures 4 and 5). The peak tension of the tracheal strip evoked by EFS upon the addition of 10^{-8} M azelastine was $99\% \pm 1.3\%$, whereas at 10^{-5} M and 10^{-4} M azelastine the peaks were $87\% \pm 8.4\%$ and 0% , respectively (Figure 5). The peak tension of the tracheal strip evoked by EFS at 10^{-4} M azelastine addition was significantly less than that at 10^{-8} M azelastine ($p < 0.001$).

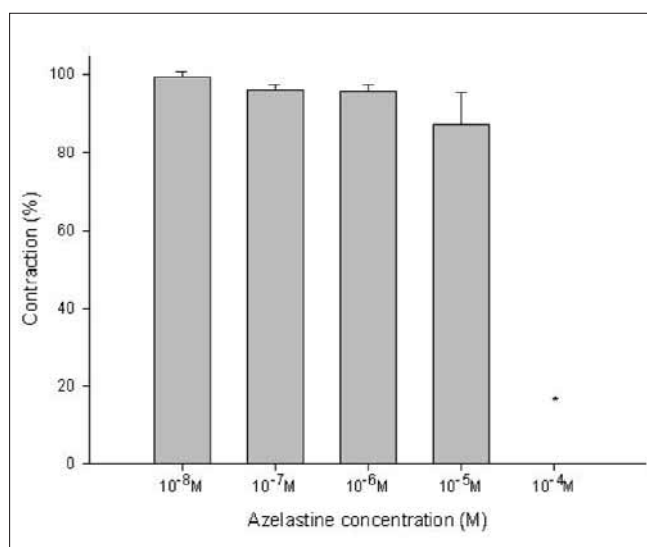


Fig. 5. Effects of azelastine on electrically induced tracheal smooth muscle contractions (contraction area calculated at 100% with no addition of azelastine). The peak tension of the tracheal strip evoked by EFS during the addition of 10^{-4} M azelastine was significantly less than that at the addition of 10^{-8} M azelastine ($p < 0.001$). Results were mean \pm SD ($n = 6$).

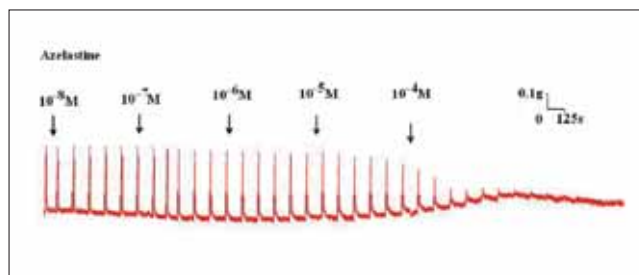


Fig. 4. Original recording of effects of azelastine on electrically induced tracheal smooth muscle contractions was noted. Higher doses of azelastine also decreased the spike contraction induced by EFS.

DISCUSSION

This study was simple and effective. An intact tracheal ring was an important component of our technique^(3,4). Such an intact tracheal ring is much more representative of a physiological setting than smooth muscle strips. The results of our experiments should be interpreted within the context of the test materials used. Although it was difficult to determine which tissue component of the trachea was responsible for drug-induced contraction, the nature of specific tissues and their responses to specific drugs provided some indication. First, the tracheal strips used in our study were crude preparations that contained cartilage and tracheal smooth muscle. The smooth muscle of the trachea appeared to be the main tissue component responsible for contraction, as the other components (epithelium, glands, connective tissue, nerves, and cartilage) did not contract to a significant extent. Because this method involved cross contraction, changes in tension were caused by radial contraction of the tracheal ring. Although responses to drugs and electrical stimulation have been verified for similar preparations^(9,12,13), the contractile response observed in this study was probably an aggregate of the responses of various types of muscle tissue. Second, the isolated tracheal preparations used in our experiments were excised from rats without damaging the endothelium or smooth muscle. Therefore, it is reasonable to assume that tracheal responses to test agents in our study are comparable to those observed after application of a spray to the trachea during an asthmatic attack. It was not easy to obtain human tissue for similar studies. The effect of this drug on isolated human tracheal smooth muscle still needs further investigation. Because this was an in vitro study, there are reservations as to its comparability with an in vivo situation in humans. In the in vivo situation, the response might be much more complicated than that in the in vitro situation.

The cholinergic contracting agent tested in this preparation is commonly used for research purposes. It is noteworthy that azelastine-induced relaxation of tissue was dependent on prior partial contraction of smooth muscle after application of methacholine. Thus, it should be possible to assess the effects of common drugs and potential therapeutic agents supposedly

responsible for relieving asthma attacks. It is now well known that histamine exerts its effects by activating histamine receptors, of which four types are now identified. All of these receptors belong to the G protein-coupled receptor family. Histamine receptors were initially subdivided by Ash and Schild in 1966^(14,15). They introduced the term H₁ receptor to describe the class of histamine receptors that was sensitive to inhibition by promethazine and mepyramine, which are now regarded as H₁ antagonists. The classic H₁-mediated responses include contraction of many visceral smooth muscles including guinea pig trachea, uterus, and the longitudinal smooth muscle of the ileum. Azelastine, an H₁ antagonist⁽¹⁶⁾, could reduce methacholine-induced contraction. Commercial azelastine nasal spray contains 0.1% azelastine HCl, which is approximately 2×10^{-3} M azelastine. When applying a spray, one gets immediately a 1/10 dilution resulting in a concentration of 2×10^{-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 smooth muscles. Therefore, commercial azelastine nasal spray could decrease the contraction of tracheal smooth muscle during an asthmatic attack. The actual concentration of azelastine in tracheal smooth muscle when used in a spray requires further investigation. The mechanism by which this H₁ antagonist affected the trachea smooth muscle is unknown and further studies are needed to elucidate the answer. We did similar experiments for cetirizine, which is also a water-soluble H₁ antagonist and obtained the same effects on isolated tracheal smooth muscle⁽¹⁷⁾.

Electrical-field stimulation is a common experimental tool; it activates the nerve terminals within the tissue to be tested and induces the release of endogenous neurotransmitters thereby triggering smooth muscle contraction. EFS-induced spike contraction of canine nasal mucosa, which is believed to result from the contraction of vascular smooth muscles, disappeared after ipsilateral cervical sympathetic ganglionectomy⁽¹⁸⁾. Thus, EFS-induced spike contraction of isolated canine nasal mucosa has been proven to be mediated by sympathetic innervation⁽¹⁸⁾. In this study, EFS-induced spike contraction of the tracheal smooth muscle was believed to be due to stimulation of parasympathetic innervation. Therefore, EFS-induced contraction of the trachea was decreased as the azelastine concentration was increased. These findings suggested that an H₁ antagonist could antagonize the parasympathetic innervation responsible for trachea smooth muscle contraction. In addition, basal tension elicited a minimal effect at various concentrations of azelastine. H₁ antagonists can have a direct cholinergic effect⁽¹⁶⁾. Azelastine showed no such effects. Oxymetazoline, another nasal spray used as a nasal decongestant, could reduce methacholine-induced contraction as well, but it also had minimal effect on basal tension. It is known as a direct-acting α -adrenergic agonist⁽¹¹⁾. Clearly, what was observed in this study is very interesting, but further study is needed to clarify these phenomena.

Azelastine is a chemically novel compound that can be administered orally in a twice-a-day regimen⁽¹⁹⁾. It has been demonstrated to have bronchodilator properties in humans. Clinical trials in the United States have shown treatment with azelastine being significantly better than placebo in the treatment of bronchial asthma⁽²⁰⁾. Its precise mechanism of action for the treatment of chronic asthma, however, remains to be established. It has been demonstrated to have antimediator properties as well as some significant bronchodilating action in both short- and long-term studies. The results of this study showed that high concentrations of azelastine might actually inhibit parasympathetic function of the trachea. It could reduce methacholine-induced contraction as well. In addition to nasal symptoms, azelastine treats effectively an asthmatic attack. In the in vivo situation, the response might be much more complicated than that in the in vitro situation.

CONCLUSION

This study indicated that high concentrations of azelastine might actually inhibit parasympathetic function of the trachea. Azelastine might reduce asthmatic attacks when administered to rhinitis patient because it could inhibit parasympathetic function and reduce methacholine-induced contraction of tracheal smooth muscle.

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