European Archives of Oto-Rhino-Laryngology (2019) 276:761–765
https://doi.org/10.1007/s00405-018-05274-8

RHINOLOGY

Effects of montelukast on human nasal mucosa

Hsing-Won Wang[1,2] · Jih-Chin Lee[1] · Pei-Chuan Wu[3] · Yueng-Hsiang Chu[1] · Yuan-Yung Lin[1] · Li-Hsiang Cheng[1]

Received: 18 November 2018 / Accepted: 26 December 2018 / Published online: 2 January 2019

Abstract

Objective Montelukast is a selective and orally active leukotriene D_4 receptor antagonist often used in treating asthma and allergic rhinitis. Montelukast nasal spray was developed to avoid systemic adverse effects of the drug in vitro. However, the effects of montelukast on human nasal mucosa are not yet fully explored and potential nasal vascular side effects of the drug merit further exploration. First, the effects of montelukast on vasocontractile responses generated by smooth muscles in the vascular structures of human nasal mucosa were investigated directly in vitro.

Methods This study examined the effects of montelukast on human nasal mucosa in terms of mucosa resting tension, vasoconstriction caused by 10^{-6} M methoxamine as a sympathetic mimetic, and electrically induced vasoconstrictions.

Results The results indicated that addition of methoxamine to the incubation medium caused the nasal mucosa to vasocontract in a dose-dependent manner. Addition of montelukast at doses of 10^{-5} M or above elicited a significant vasodilation response to 10^{-6} M methoxamine-induced vasoconstriction. Montelukast could not inhibit electrical field stimulation-induced spike vasoconstriction. Moreover, increase in concentration of montelukast had minimal effect on basal tension of nasal mucosa.

Conclusions The study indicated significant vasodilation on human nasal mucosa under high concentrations of montelukast with a probable α-adrenoceptor antagonism. Hence, the nasal activity of α-adrenergic agonist nasal spray for nasal obstruction may be reduced in those using concomitant (oral or local spray) montelukast.

Keywords Montelukast · Sympathetic function · Human nasal mucosa

Introduction

Leukotrienes are inflammatory mediators, previously known as slow-reacting substances of anaphylaxis, produced by a number of cell types, including mast cells, eosinophils, basophils, macrophage, and monocyte. These mediators are generated by the metabolism of arachidonic acid via the 5-lipoxygenase pathway and exert their biologic effects by binding to and activating specific adaptors. These events lead to the contraction of human airway smooth muscle, cell chemotaxis, and increased vascular permeability [1]. Montelukast is a selective and orally active leukotriene D_4 receptor antagonist and it has shown efficacy in treating asthma and allergic rhinitis. [2–6]. Although montelukast is safe and well tolerated in adults and children, a number of systemic adverse effects were reported including the development of Churg–Strauss syndrome characterized by eosinophilic vasculitis associated with asthmatic disease, the development of reversible visual hallucinations in asthmatic children, parasomnias in the form of sleep-talking and sleepwalking, ecchymosis, eyelid angioedema, and generalized urticaria [7–11]. Montelukast nasal spray was developed to avoid systemic adverse effects of the drug in vitro [12]. However, the effects of montelukast on nasal mucosa are not yet fully known and potential nasal vascular side effects of the drug merit further exploration. Therefore, the primary goal of the present study was to test the direct effects of montelukast on vasocontractile responses of vascular smooth muscles in human nasal mucosa.

✉ Li-Hsiang Cheng
doc30478@gmail.com

1 Department of Otolaryngology-Head and Neck Surgery, Tri-Service General Hospital, National Defense Medical Center, 325, Cheng-Kung Road Section 2, Taipei 114, Taiwan, ROC

2 Graduate Institute of Clinical Medicine and Department of Otolaryngology, Taipei Medical University-Shuang Ho Hospital, New Taipei City, Taiwan, ROC

3 Department of Psychiatry, Far Eastern Memorial Hospital, New Taipei City, Taiwan, ROC

Materials and methods

Tissue preparation

After obtaining informed consent, mucosal specimens were obtained from 12 patients under general anesthesia during elective turbinectomies. We performed partial inferior turbinectomies with nasal angle scissors (cold instruments). Indications for surgery were severe nasal obstruction due to hypertrophic rhinitis or nasal allergy (eight patients with allergic rhinitis and four patients with hypertrophic rhinitis in the study). Strict criteria were applied to exclude those with a history of vasomotor rhinitis, nasal surgery, chronic rhinosinusitis, sinonasal pathology, as well as others who used vasoconstrictors for nasal obstruction prior to surgery.

Experimental protocol

In vitro preparation of human nasal blood vessels was used (Fig. 1) [13, 14]. Following immediate removal, 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; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO4, 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 vasocontract when basal tension was applied. The response of this preparation to drugs and electrical stimulation has been described previously [13–17]. The study tested methoxamine, an α-adrenergic agonist, as a nasal mucosal vasoconstriction drug. Before drug assays were conducted, nasal mucosal strips were equilibrated in the bath solution for 15–30 min, 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 vasoconstriction or vasodilation 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 s) was applied to the nasal mucosal strip through two wire electrodes placed parallel to the nasal mucosal strip and connected to a direct-current stimulator (Grass S44, Quincy, MA, USA). There was an interval of 2 min 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).

Montelukast assessments

The following assessments for montelukast were performed: (1) the effect on nasal mucosal resting tension: this test examined the effect of montelukast on the stimulated condition of the resting nasal mucosa; (2) the effect on nasal

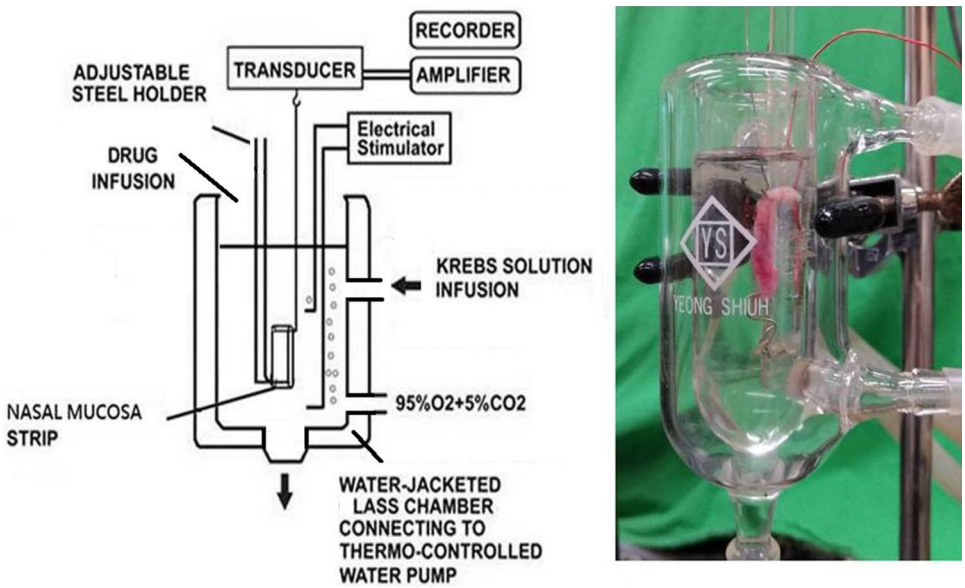

Fig. 1 Schematic diagram and actual photo of tension measurements in an isolated human nasal strip

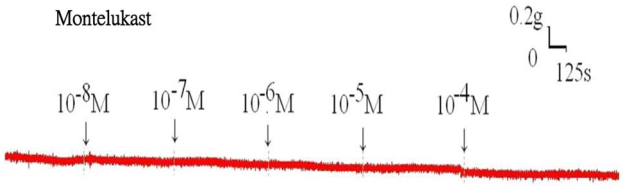

Fig. 2 Changes in tension of human nasal mucosa after application of montelukast at various concentrations. Increased concentration of montelukast alone had a minimal effect on basal tension of human nasal mucosa. Original basal tension was 0.5 g

mucosal vasoconstriction 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 montelukast on electrically induced nasal mucosal vasoconstriction: 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 vasoconstriction. Stepwise increases in the amount of test agent were used to study the vasoconstriction or vasodilation 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 $p < 0.05$.

Results

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

Addition of the leukotriene D_4 receptor antagonist, montelukast, to the basal tension elicited a negligible effect (Fig. 2). It resulted in vasodilation of the mucosa when introduced after the addition of a vasoconstricting agent such as 10^{-6} M methoxamine (Fig. 3). Low doses of montelukast resulted in a mild effect on vasodilation while higher doses caused significant vasodilation of the vascular smooth muscle of human nasal mucosa (Figs. 3, 4). At 10^{-8} M montelukast, the tension was 99.33% ± 0.81% of control values (Fig. 4). At 10^{-5} M and 10^{-4} M montelukast,

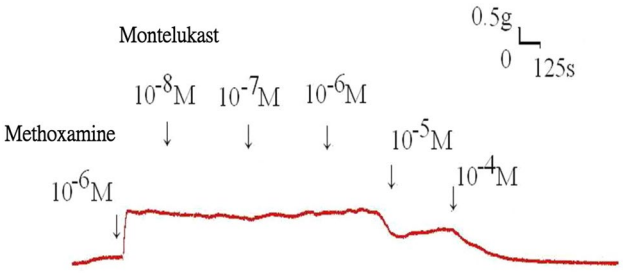

Fig. 3 Original recording of effects of montelukast on 10^{-6} M methoxamine-induced vasoconstriction of human nasal mucosa

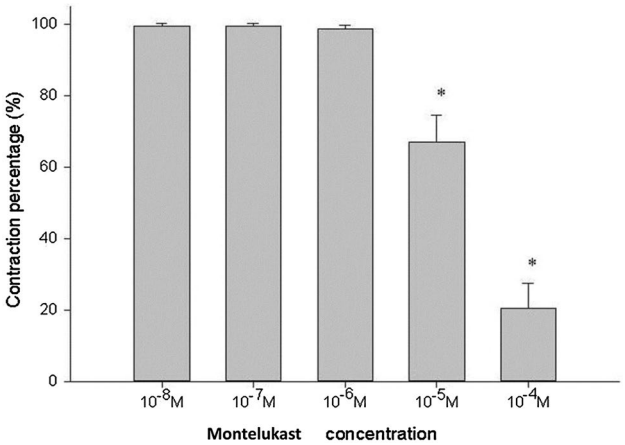

Fig. 4 Effects of montelukast on 10^{-6} M methoxamine-induced vasoconstriction (vasoconstriction area calculated at 100% with no addition of montelukast) of human nasal mucosa. The difference in tension between 10^{-8} M and 10^{-5} M or 10^{-4} M montelukast was statistically significant ($p < 0.05$).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

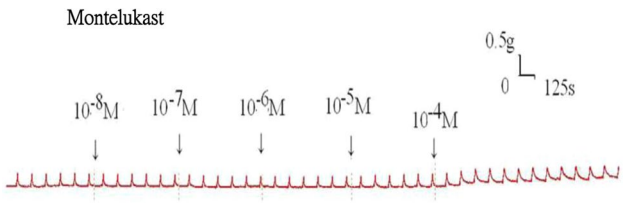

Fig. 5 Original recording of the effects of montelukast on electrically induced nasal mucosal smooth muscle vasoconstrictions. Higher doses of montelukast could not decrease EFS-induced spike vasoconstriction

the tensions were 66.83% ± 7.57% and 20.50% ± 6.89%, respectively (Fig. 4). The difference in tension between 10^{-8} M and 10^{-5} M or 10^{-4} M montelukast was statistically significant ($p < 0.05$). However, higher dose of montelukast did not inhibit electrical field stimulation-induced spike vasoconstriction (Figs. 5, 6). The peak tension of the nasal mucosal strip evoked by EFS upon addition of 10^{-8} M montelukast was 100.0% ± 0%, whereas at 10^{-5} M and

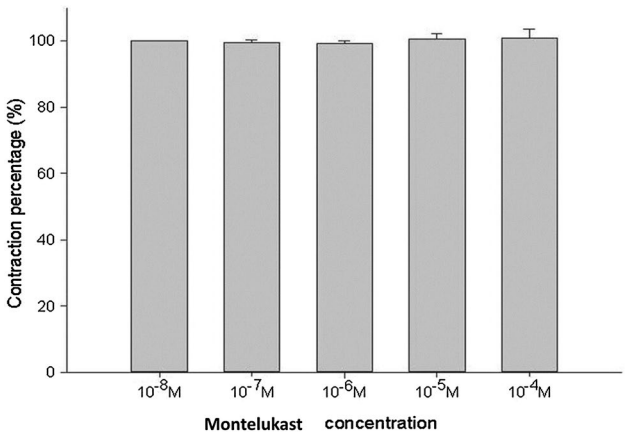

Fig. 6 Effects of montelukast on electrically induced nasal mucosal smooth muscle vasoconstrictions (vasoconstriction area was calculated at 100% with no addition of montelukast). The difference in tension between 10^{-8} M and 10^{-5} M or 10^{-4} M montelukast was not statistically significant. Results were mean ± SD ($n = 6$)

10^{-4} M montelukast, the peaks were 100.50% ± 1.76% and 100.83% ± 2.63%, respectively (Fig. 6). The difference in tension between 10^{-8} M and 10^{-5} M or 10^{-4} M montelukast was not statistically significant.

Discussion

The isolated nasal mucosa preparations used in our experiments were excised from humans without adding any constricting agent (oxymetazoline or epinephrine) or local anesthetic agent (Xylocaine, Fujisawa Pharmaceutical Co., Ltd., Chuo-Ku Osaka, Japan), which would present increased difficulty for surgeons performing turbinectomy. Therefore, the specimens were obtained from patients under general anesthesia only. The result 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 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 muscles of nasal blood vessels appeared to be the only tissue component with the ability to vasocontract. The other components (epithelium, nasal glands, connective tissue, and nerves) seemed unable to contract. 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 [13–17]. However, the contractile responses are likely to represent the sum total of the various vessels. Second, the human nasal mucosa used in these experiments was obtained from patients with clinical diagnoses of hypertrophic rhinitis or nasal allergy. Although the mucosal strips were taken from patients suffering from disorders varying in degree and nature, our experimental results showed only negligible overall variabilities. Moreover, obtaining healthy human turbinate to perform the study was impractical.

This study observed that increased concentrations of montelukast had minimal effect on the basal tension of nasal mucosa, demonstrating that montelukast 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 induced a spike contraction of canine nasal mucosa, which is believed to result from the contraction of vascular smooth muscles, disappearing after ipsilateral cervical sympathetic ganglionectomy [16]. Thus, EFS-induced spike vasoconstriction of isolated canine nasal mucosa was proved to be mediated by sympathetic innervations. Moreover, high concentration of 10^{-4} M montelukast could not block electrically induced nasal mucosal vasoconstrictions. Regarding effects of montelukast on vasoconstriction caused by 10^{-6} M of methoxamine, the procedure examined postsynaptic events such as muscle receptor blockade, enhancement, and secondary messengers. Montelukast at a high concentration of 10^{-5} M or above reduced the vasoconstriction induced by 10^{-6} M of the α-adrenoceptor agonist methoxamine; hence, it is possible that these vasoconstrictions actually antagonize α-adrenoceptor functions. However, how does montelukast antagonize the α-adrenoceptor agonist and affect the nasal mucosal smooth muscle? Further studies are needed to elucidate this question. The recommended once-daily oral dose of montelukast is 10 mg for adults, 4 mg for children aged 1–5 years, and 5 mg for children aged 6–14 years. The plasma concentration of montelukast was in the range of 1.31–1.76 μg/ml ($2.15–2.89 \times 10^{-6}$ M) at 0.5–12 h with C_{max} value of 1.59 ± 0.16 μg/ml at 3.71 ± 0.64 ($2.61 \pm 0.26 \times 10^{-6}$ M) hours in indigenous healthy males [18]. However, some accidents of unintentional administration of 65 mg, 80 mg, and 135 mg montelukast were reported in asthmatic children of 3–5 years old and the plasma concentration of montelukast exceeds 10^{-5} M [19, 20]. Clinical trials of montelukast for acute asthma exacerbations are being planned in patients aged 5–17 years using doses of 50 mg and a high plasma concentration of 10^{-5} M will be approached [21].

In general, the route of localized nasal drug delivery can reduce the amount of drugs entering human body and also avoids systemic adverse effects of drug. Although montelukast nasal spray was developed, there are limited reports in medical literature. To our knowledge, there have been no reports on the direct effect of montelukast on human nasal

mucosa. This study observed no significant inhibitory effect of montelukast on human nasal vascular smooth muscle during field stimulation and only minimal effect of montelukast on basal tension of turbinate mucosa in vitro. Finally, montelukast at high concentrations antagonizes methoxamine and it is known as a direct-acting α-adrenergic agonist [22]. Hence, it can be deduced that the nasal effectiveness of α-adrenergic agonist nasal spray for nasal obstruction may be reduced in those using concomitant (oral or local spray) montelukast.

Conclusion

The study indicated significant vasodilation on human nasal mucosa under high concentrations of montelukast with a probable α-adrenoceptor antagonism. Hence, the nasal activity of α-adrenergic agonist nasal spray for nasal obstruction may be reduced in those using concomitant (oral or local spray) montelukast.

Acknowledgements No source of financial support or funding.

Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

Ethical approval The study was approved by the institutional review board of the Tri-Service General Hospital. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study, and each patient had given a written informed consent about the use of the samples for medical research.

References

1. Cingi C, Muluk NB, Ipci K, Sahin E (2015) Antileukotrienes in upper airway inflammatory diseases. Curr Allergy Asthma Rep 15:64
2. Jones TR, Labelle M, Belley M, Champion E, Charette L et al (1995) Pharmacology of montelukast sodium (Singulair), a potent and selective leukotriene D4 receptor antagonist. Can J Phsiol Pharmacol 73:191–201
3. Joachim RA, Quarcoo D, Arck PC, Herz U, Renz H et al (2003) Stress enhances airway reactivity and airway inflammation in an animal model of allergic bronchial asthma. Psychosom Med 65:811–815
4. Perry TT, Corren J, Philip G, Kim EH, Conover-Walker MK et al (2004) Protective effect of montelukast on lower and upper respiratory tract responses to short-term cat allergen exposure. Ann Allergy Asthma Immunol 93:431–438
5. Papadopoulous NG, Philip G, Giezek H, Watkins M, Smugar SS et al (2009) The efficacy of montelukast during the allergy season in pediatric patients with persistent asthma and seasonal aeroallergen sensitivity. J Asthma 46:413–420
6. Philip G, Nayak AS, Berger WE (2004) The effect of montelukast on rhinitis symptoms in patients with asthma and seasonal allergic rhinitis. Curr Med Res Opin 20:1549–1558
7. Tuggey JM, Hosker HS (2000) Churg-Strauss syndrome associated with montelukast therapy. Thorax 55:805–806
8. Aypak C, Turedi O, Solmaz N, Yikilkan H, Gorpelioglu (2013) A rare adverse effect of montelukast treatment: ecchymosis. Respir Care 58:e104–e106
9. Kocyigit A, Gulcan Oksuz B, Yarar F, Uzun F, Igde M et al (2013) Hallucination development with montelukast in a child with asthma: case presentation. Iran J Allergy Asthma Immunol 12:397–399
10. Alkhuja S, Gazizov N, Alexander ME (2013) Sleeptalking! Sleepwalking! Side effects of montelukast. Case Rep Pulmonol 2013:813786
11. Minciullo PL, Saija A, Bonanno D, Feriazzo E, Gangemi S (2004) Montelukast-induced generalized urticaria. Ann Pharmacother 38:999–1001
12. Jullaphant T, Nakpeng T, Srichana T (2018) Montelukast nasal spray: formulation development and in vitro evaluation. Pharm Dev Technol 27:1–10
13. Jackson RT (1979) A new in vitro method of drug assay of nasal blood vessels. Arch Otorhinolarygol 225:33–38
14. Jackson RT (1980) An in vitro technique for testing nasal vasodilating agent. Otolarygol Head Neck Surg 88:434–448
15. Wang HW, Jackson RT (1988) Effects of lidocaine and procaine on canine nasal blood vessels. Ann Oto Rhinol laryngol 97:409–413
16. Wang HW, Jackson RT (1988) Do cholinergic neurons directly innervate nasal blood vessels? Rhinology 26:139–146
17. Wang HW, Wang SW, Wang JY, Su. WY (1996) Effects of amphetamine on human nasal mucosa. Eur Arch Otorhinolaryngol 253:498–500
18. Fayyaz A, Khan JA, Ashraf MM et al (2017) Pharmacokinetic behavior of montelukast in indigenous healthy male volunteers. Park J Pharm Sci 30(6 suppl):2435–2439
19. Cobb DB, Abbott CL, Waston WA, Fernandez MC (2002) High-dose montelukast exposures in a 3-year-old and 5-year-old. Vet Hum Toxicol 44:91–92
20. Geller M, Melo LR, Coutinho SV (2000) Successful outcome of montelukast overdosage in an asthmatic child. Ann Allergy Asthma Immunol Mar 84:370
21. Donald H, Arnold N, Bowan TF, Seger DL (2018) Adverse events are rare after single-dose montelukast exposures in children. Clin Toxicol 56:25–29
22. Kao CH, Chu YH, Wang HW (2008) Effects of oxymetazoline on isolated rat's tracheal smooth muscle. Eur Arch Otorhinolaryngol 265:695–698