Allergology International (2001) **50**: 337–341

Short Communication

Effects of olopatadine hydrochloride on the release of thromboxane B_2 and histamine from nasal mucosa after antigen–antibody reaction in guinea pigs

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Abstract

Background: Various mediators, such as thromboxane (TX) A_2, peptide leukotrienes (P-LT) and histamine, are involved in allergic nasal obstruction. The aim of the present study was to investigate the mechanism whereby olopatadine hydrochloride, a novel anti-allergic drug, ameliorated the allergic nasal obstruction.

Methods: The levels of TXB_2, P-LT and histamine in nasal lavage fluid (NLF) were measured after intranasal antigen challenge in sensitized guinea pigs.

Results: Histamine and TXB_2 levels in the NLF increased eight- and threefold, respectively, 10 min after antigen challenge, whereas the P-LT level was under the detection limit. Oral administration of olopatadine at 0.1, 1 and 3 mg/kg significantly inhibited the increases in TXB_2 and histamine levels. At 3 mg/kg, olopatadine also ameliorated the nasal obstruction caused 10 min after antigen challenge, as determined by acoustic rhinometry.

Conclusions: These results suggest that the amelioration by olopatadine of the allergic nasal obstruction involves the inhibition of the release of TXA_2 and histamine.

Key words: allergic rhinitis, chemical mediators, guinea pigs, olopatadine.

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Received 4 December 2000. Accepted for publication 18 August 2001.

Introduction

Thromboxane (TX) A_2 and peptide leukotrienes (P-LT), as well as histamine, are involved in the pathogenesis of allergic nasal obstruction.[1–6] These mediators in nasal lavage fluid (NLF) are reported to be increased after antigen challenge in allergic human patients,[1,2] as well as in sensitized guinea pigs.[2–4] The intranasal application of U-46619, a stable TXA_2 mimetic, P-LT or histamine is known to cause nasal obstruction.[3] Moreover, ramatroban,[5] a TXA_2 receptor antagonist, and pranlukast,[6] a P-LT receptor antagonist, have been demonstrated to ameliorate nasal obstruction in allergic rhinitis patients.

Olopatadine hydrochloride ((*Z*)-11-(3-dimethylaminopropylidene)-6,11-dihydrobenz[*b*,*e*]oxepin-2-acetic acid monohydrochloride; CAS 140462-76-6; KW-4679) is a novel drug for the treatment of allergic diseases. Olopatadine has an antagonistic action against the histamine H_1 receptor.[7] A previous *in vitro* study demonstrated that olopatadine inhibited histamine release from rat peritoneal mast cells following the antigen–antibody reaction.[8] Moreover, olopatadine inhibits TXB_2 release from human neutrophils and P-LT release from guinea pig eosinophils.[9] Olopatadine has been shown to ameliorate the nasal symptoms of allergic rhinitis, including nasal obstruction, in humans,[10] as well as in guinea pigs.[11]

In the present study, we determined the concentrations of TXB_2, P-LT and histamine in NLF after intranasal antigen challenge in passively sensitized guinea pigs in order to elucidate whether olopatadine inhibits the release of these mediators from the nasal mucosa *in vivo*. We also examined the effects of ketotifen, an anti-allergic drug, on the release of the mediators because ketotifen, as well as olopatadine, inhibited allergic nasal obstruction in guinea pigs.[11] In addition, in the present

study we confirmed the inhibition of nasal obstruction by olopatadine.

METHODS

Materials

Olopatadine was synthesized at the Sakai Research Laboratories of Kyowa Hakko Kogyo (Osaka, Japan). Ketotifen fumarate (ketotifen) was purchased from Sigma Chemical (St Louis, MO, USA). Other reagents used were ovalbumin (OVA; Sigma Chemical), carbamic acid ethyl-ester (urethane; Tokyo Kasei, Tokyo, Japan), indomethacin (Sigma Chemical), EDTA and AA-861 (Wako Pure Chemical Industries, Osaka, Japan).

Sensitization

Male 5-week-old Hartley guinea pigs (Japan SLC, Shizuoka, Japan) were passively sensitized by intravenous injections of guinea pig anti-OVA antiserum in a volume of 1 mL/kg bodyweight. The antiserum was prepared by immunizing guinea pigs subcutaneously with 10 μg OVA and 1 mg alum. An additional injection was performed 3 weeks later and blood was collected 1 week after the injection. The antibody titer in the serum, determined by the 8 day homologous passive cutaneous anaphylaxis, was 64. The antibody in serum was inactivated by heating at 56°C for 2 h and, thus, the serum was confirmed to contain the IgE antibody.

Measurement of mediators

Eight days after passive sensitization, animals were anesthetized with urethane (1.2 g/kg, i.p.) and a cannula was inserted from the trachea into the nasal cavity. The antigen was challenged by applying 20 μL OVA solution (1% w/v in saline) into each nasal cavity through each nostril. In the sham group, saline, instead of the OVA solution, was applied intranasally. Olopatadine, ketotifen or distilled water was administered orally 1 h before the intranasal antigen challenge. Distilled water was administered in the control and sham groups. Ten minutes after antigen challenge, the nasal cavity was perfused with saline at a rate of 0.3 mL/min and the NLF was collected into a tube, containing indomethacin (10 μmol/L), the 5-lipoxygenase inhibitor AA-861 (1 μmol/L) and EDTA (7.7 mmol/L), for 10 min. These three reagents were added to block the release of TXA_2, P-LT and histamine, respectively, from inflammatory cells into the NLF in the tube. The supernatants obtained after centrifugation were used for analyses.

The concentration of TXB_2 has been determined to study the dynamics of TXA_2 because TXA_2 is degraded to the stable product TXB_2. The concentrations of TXB_2 and P-LT were measured by enzyme immunoassay (Cayman, Ann Arbor, MI, USA) and radioimmunoassay (Amersham, Little Chalfont, UK), respectively, after pretreatment of samples as follows. Samples were mixed with ethanol and centrifuged and the supernatants were applied to Sep-pak C18 columns (Waters, Milford, MA, USA) and eluted into ethyl acetate containing methanol for the measurement of TXB_2 or into methanol for measurement of P-LT. The concentration of histamine was measured by radioimmunoassay (Eiken Kagaku, Tokyo, Japan) without pretreatment of the samples. The detection limits for TXB_2, P-LT and histamine were 0.2 pg/mL, 156 pg/mL and 0.06 ng/mL, respectively.

Evaluation of nasal obstruction

Eight days after passive sensitization, nasal obstruction was evaluated by observing the decrease in nasal cavity volume, which was measured by acoustic rhinometry (GJ Elektronik, Skanderborg, Denmark), in guinea pigs anesthetized with urethane (1.2 g/kg, i.p.). The details of the acoustic reflection technique have been reported elsewhere.[12] The volume of the right and left nasal cavities between the nostril and 2 cm into the nasal cavity was determined by averaging three measurements. The nasal obstruction in each animal was evaluated by the decrease in the total volume of the right and left nasal cavities. The antigen was challenged by applying 20 μL OVA solution (1% w/v in saline) into each nasal cavity through each nostril. In the sham group, saline, instead of the OVA solution, was applied intranasally. The nasal cavity volume was measured before and 10 min after intranasal challenge and nasal obstruction after the challenge is expressed as a percentage change from the nasal cavity volume before challenge. Olopatadine (3 mg/kg) or distilled water was administered orally 1 h before the intranasal antigen challenge. Distilled water was administered to the control and sham groups.

Statistical analysis

Data are shown as the mean±SEM. The Wilcoxon rank sum test or Student's *t*-test were used for analysis of differences between two groups and the least significant

difference (LSD) test was used for analysis among multiple groups. $P < 0.05$ was considered significant.

Results

The concentration of TXB_2 in the NLF was higher in the control group than that in the sham group (185.3 ± 40.5 *vs* 58.1 ± 14.9 pg/mL, respectively; $n = 8$). The TXB_2 concentrations in the olopatadine (0.01–3 mg/kg) groups and ketotifen (0.01–3 mg/kg) groups were lower than in the control group. Significant inhibitions were observed following olopatadine at 1 and 3 mg/kg and ketotifen at 0.1 mg/kg (75.6 ± 21.0, 84.3 ± 25.0 and 57.0 ± 10.7 pg/mL, respectively; $n = 8$; Fig. 1).

The concentration of histamine was higher in the control group than in the sham group (2.36 ± 0.48 *vs* 0.28 ± 0.15 ng/mL, respectively; $n = 8$). The concentrations of histamine in the olopatadine (0.01–3 mg/kg) groups and ketotifen (0.1–3 mg/kg) groups were lower than in the control group. Olopatadine at 0.1 and 3 mg/kg and ketotifen at 1 mg/kg significantly inhibited the increase in histamine levels (1.04 ± 0.19, 1.12 ± 0.25 and 1.09 ± 0.31 ng/mL, respectively; $n = 8$; Fig. 2).

The concentration of P-LT in both the control and the sham groups was lower than the detection limit (468 pg/perfusate).

The percentage change in nasal cavity volume 10 min after intranasal antigen challenge (–14.8 ± 2.8%; $n = 15$) was significantly greater than that after the intranasal instillation of saline (–2.0 ± 1.6%; $n = 15$). At 3 mg/kg, olopatadine tended to inhibit the decrease in nasal cavity volume to –5.3 ± 3.8% ($n = 15$; Table 1).

Discussion

In the present study, we used a guinea pig model of nasal allergy induced by passive sensitization, but not by active sensitization,[11] because the concentration of TXB_2 released into NLF varies considerably in actively sensitized guinea pigs.[2] The present study demonstrated that olopatadine and ketotifen inhibited the increase in TXB_2 and histamine levels in NLF after antigen challenge in sensitized guinea pigs, indicating that both drugs inhibit the releases of TXA_2 and histamine from the nasal mucosa. This is the first demonstration that anti-allergic drugs inhibit TXA_2 release from the nasal mucosa *in vivo*.

The present study failed to detect an increase in P-LT levels, although P-LT levels in the NLF have been reported

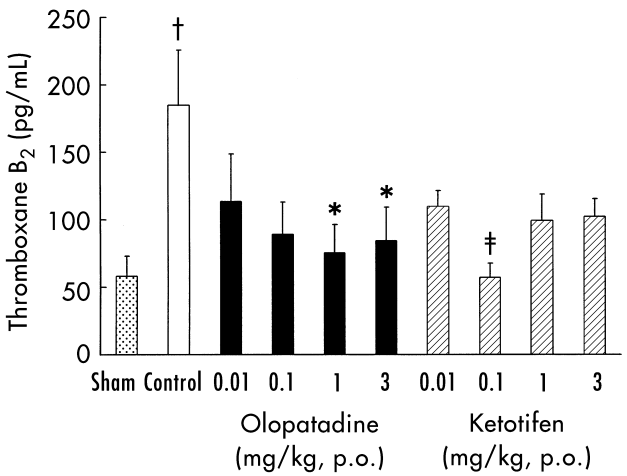

Fig. 1 Effects of olopatadine and ketotifen on the increase in thromboxane B_2 concentrations in nasal perfusates after antigen challenge in sensitized guinea pigs. Drugs were administered orally 1 h before antigen challenge. In the sham group, saline, instead of the antigen solution, was applied into the nasal cavity. Results are the mean±SEM of 10 animals in the 0.01 mg/kg groups and eight animals in the other groups. †$P < 0.01$ compared with the sham group; *$P < 0.05$, ‡$P < 0.001$ compared with the control group.

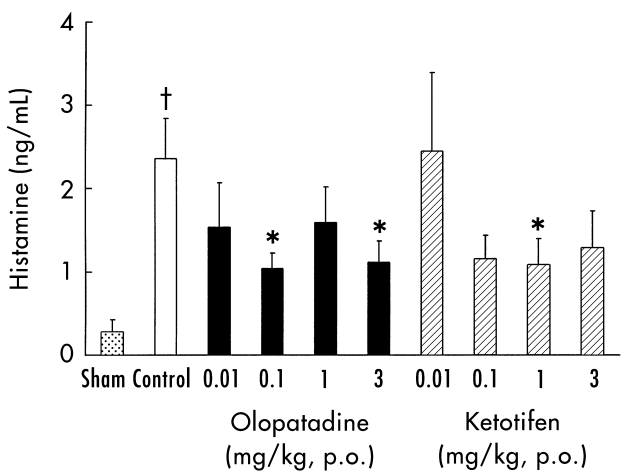

Fig. 2 Effects of olopatadine and ketotifen on the increase in histamine concentrations in nasal perfusates after antigen challenge in sensitized guinea pigs. Drugs were administered orally 1 h before antigen challenge. In the sham group, saline, instead of the antigen solution, was applied into the nasal cavity. Results are the mean±SEM of 10 animals in the 0.01 mg/kg groups and eight animals in the other groups. †$P < 0.01$ compared with the sham group; *$P < 0.05$ compared with the control group.

Table 1 Effect of olopatadine on the decrease in the nasal cavity volume after antigen challenge in sensitized guinea pigs

Group	% Volume change	*P**	% Inhibition
Sham	–2.0 ± 1.6	0.0005	
Control	–14.8 ± 2.8		
Olopatadine (3 mg/kg, p.o.)	–5.3 ± 3.8	0.0526	74.2

Data are the mean±SEM of 15 animals.

**P* values are for comparisons with the control group.

Olopatadine or its vehicle was administered orally 1 h before antigen challenge. In the sham group, saline, instead of the antigen solution, was applied intranasally.

to increase 10 min after antigen challenge in humans.[1] In contrast, Fujita *et al*.[4] recently demonstrated that P-LT levels in the NLF after antigen challenge in sensitized guinea pigs were 374.5 ± 88.2 pg/lavage, which is much lower than that in humans[1] and slightly lower than the detection limit in the present study. Further studies, using a more sensitive analytical method to detect P-LT, are required to elucidate the effects of olopatadine on P-LT release.

In the present study, we demonstrated that the levels of TXB_2 and histamine increased in NLF collected 10 min after antigen challenge and that olopatadine inhibited these increases. The increases in TXB_2 and histamine levels 10 min after antigen challenge are consistent with previous results in guinea pigs[2,3] and humans.[1,2] Because the source of both TXA_2 and histamine, released immediately after intranasal antigen challenge, is assumed to be mast cells,[2,3] it seems that olopatadine inhibited the release of TXA_2 and histamine from nasal mast cells.

In *in vitro* studies, olopatadine inhibited histamine release from rat peritoneal mast cells at a concentration of 100 μmol/L.[8] The peak plasma drug concentration was reported to be 3.1 μmol/L after the oral administration of 3 mg/kg olopatadine,[13] a dose that inhibited histamine release in the present study. The difference in the concentrations inhibiting the histamine release between the *in vitro* and *in vivo* studies may be ascribed to the following two reasons. First, there may be an involvement of antihistaminergic action in the *in vivo*, but not *in vitro*, inhibition by olopatadine of histamine release. The intranasal application of histamine releases neuropeptides from sensory nerves in the nasal mucosa,[14] while substance P, one of the neuropeptides released, stimulates nasal mast cells to release histamine.[15] These results suggest that histamine, once released from nasal mast cells, can stimulate sensory nerves to potentiate the release of histamine in a positive-feedback manner. It is thus possible that the antagonistic action of olopatadine against histamine H_1 receptors contributes to the inhibition of histamine release in the nasal mucosa, although the antagonism against H_1 receptors may inhibit only the secondary histamine release mediated by the neuropeptides and not the primary release immediately after the antigen–antibody reaction. Second, the direct inhibitory effect of olopatadine on neruopeptide release[16] may have contributed to the *in vivo* inhibition of histamine release. As mentioned above, the inhibition of neruopeptide release is assumed to inhibit the neuropeptide-mediated histamine release. These effects of olopatadine, in addition to its direct action on mast cells,[8] may have played a role in the inhibition of histamine release *in vivo*.

In the present study, olopatadine tended to ameliorate nasal obstruction at a dose of 3 mg/kg, the dose that inhibited the release of both TXA_2 and histamine. The study by Nabe *et al*.[17] demonstrated that the histamine H_1 receptor antagonist mepyramine did not affect nasal obstruction caused 10 min after intranasal antigen challenge in passively sensitized guinea pigs, suggesting that the blockade of histamine H_1 receptors alone is not sufficient to inhibit nasal obstruction. Indeed, although the histamine H_1 receptor antagonist is the first choice of recommended therapy in mild cases of allergic rhinitis,[18] it has only limited therapeutic effects on nasal obstruction.[19] It is thus possible that olopatadine inhibited nasal obstruction by its inhibition of TXA_2 and histamine release and not solely by its antagonistic action against histamine H_1 receptors. Considering the suppressive action of ramatroban against nasal obstruction,[5] it is assumed that the amelioration by olopatadine of nasal obstruction involves its inhibitory effect on TXA_2 release. However, histamine H_2 and H_3 receptors, as well as H_1 receptors, are reported to have significant roles in the pathogenesis of nasal obstruction.[20–22] These results suggest that the inhibition of histamine release has some advantage over the antagonism against H_1 receptor in

the treatment of nasal obstruction, because the former is supposed to result in inhibited responses mediated by H_2 and H_3 receptors in addition to H_1 receptors. Thus, olopatadine, which inhibits both TXA_2 and histamine release from the nasal mucosa, is assumed to be an effective drug in the treatment of nasal obstruction in allergic rhinitis patients. In fact, olopatadine has been shown to prominently ameliorate nasal obstruction in humans.[10]

In conclusion, we have elucidated that olopatadine inhibits the release of TXA_2 and histamine from guinea pig nasal mucosa after antigen challenge. The inhibited release of TXA_2 and histamine is suggested to be involved in the amelioration by olopatadine of allergic nasal obstruction.

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