Effects of Olopatadine Hydrochloride on the Increase of Histamine and Peptide-Leukotrienes Concentrations in Nasal Lavage Fluid Following the Antigen-Antibody Reaction in Actively Sensitized Guinea Pigs

Kiyomi Miyake, Kaori Horikoshi, Yoshimi Ikeda, Akio Ishii and Akira Karasawa*

Drug Development Research Laboratories, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.,
1188 Shimotogari, Nagatzumi-cho, Santo-gun, Sizuoka 411-8731, Japan

Received December 25, 2000 Accepted February 6, 2001

ABSTRACT—To investigate the mechanism for the amelioration by olopatadine hydrochloride (olopatadine) of allergic rhinitis, we determined its effects on the increase of chemical mediator concentrations in nasal lavage fluid following the intranasal antigen challenge in guinea pigs actively sensitized with DNP-Ascaris. The concentrations of histamine and peptide-leukotrienes increased 10 min after the challenge. Olopatadine at 10 mg/kg (p.o.) significantly prevented the increase of histamine and tended to inhibit the increase of peptide-leukotrienes. The inhibition by olopatadine of the nasal symptoms seems to involve the inhibitory effect on the releases of histamine and, possibly, p-LTs into the nasal cavity.

Keywords: Chemical mediator, Nasal lavage fluid, Olopatadine

Allergic rhinitis is a typical allergic disease that presents nasal symptoms such as sneezing, itching and nasal blockage. Several chemical mediators, including histamine and arachidonic acid metabolites, are considered to play important roles in the pathogenesis of allergic rhinitis (1 – 3).

Olopatadine hydrochloride (olopatadine, KW-4679), (Z)-11-[(3-dimethylamino)propylidene]-6,11-dihydrodibenz[b,e]oxepin-2-acetic acid monohydrochloride, is an orally-active antiallergic drug with selective and potent histamine H1-receptor antagonistic activity (4). The previous in vitro studies demonstrated that olopatadine inhibited the mediator release from several inflammatory cells (5, 6). Kaise et al. reported that olopatadine inhibited the sneeze and nasal rubbing responses and the nasal blockage caused by antigen challenge in sensitized guinea pigs (7, 8). Moreover, olopatadine has been shown to be useful for the treatment of allergic rhinitis in human patients (9). In the present study, to analyze the mechanism for the inhibition by olopatadine of the nasal symptoms, we investigated the changes of histamine, peptide-leukotrienes (p-LTs) and thromboxane (TX) B2 concentrations in nasal lavage fluid (NALF) following the antigen-antibody reaction and the effect of olopatadine on the releases of these mediators in actively sensitized guinea pigs.

Olopatadine (Sakai Research Laboratories of Kyowa Hakko Kogyo, Osaka) was dissolved in distilled water for oral administration. Ascaris suum allergenic extract (Funakoshi, Tokyo) was reacted with 2,4-dinitrobenzene-sulfonic acid sodium salt (Tokyo Kasei, Tokyo) to couple with dinitrophenyl (DNP-Ascaris) by the method of Eisen et al. (10).

Animal experiments were performed in accordance with the protocol approved by the Animal Ethical Committee of Kyowa Hakko Kogyo Co., Ltd. (Shizuoka). Active sensitization was performed as previously described by Kaise et al. (8). Briefly, male Hartley guinea pigs (Japan SLC, Shizuoka), weighing 251 – 300 g (4 weeks of age), were intraperitoneally injected with DNP-Ascaris (3.12 μg protein) and aluminium hydroxide (1 mg) 4 times at 2-week intervals, followed by inhalation of DNP-Ascaris (3.12 μg protein) and aluminium hydroxide (1 mg) 4 times at 2-week intervals, followed by inhalation of DNP-Ascaris (15.6 μg protein/ml saline, for 3 min), repeated every day for 5 days, beginning 10 days after the fourth intraperitoneal injection. The animals were used at 10 – 18 days after the final intranasal sensitization.

The animal was anesthetized with urethane (1.2 g/kg, i.p.) and a cannula was inserted from the trachea into the nasal cavity for nasal lavage. The challenge was performed by instilling either 20 μl of the antigen solution (DNP-Ascaris, 1.8 mg protein/ml saline) into bilateral nostrils.

*Corresponding author. FAX: +81-559-86-7430
E-mail: akira.karasawa@kyowa.co.jp
In the sham group, the vehicle (saline) was given instead of the antigen. Nasal cavities were perfused with 3 ml of saline for 10 min before (pre) and at various time points after the challenge, and NALF was collected. Olopatadine at 10 mg/kg, a dose exhibiting the inhibitory effect on experimental allergic rhinitis (8), was orally administrated 1 h before the antigen challenge. In the control and the sham group, the vehicle (distilled water) was given instead of the drug.

The supernatant of NALF obtained after centrifugation was used for the analyses. The concentration of histamine in the sample was measured with the enzyme immunoassay (EIA) kit (Immunotech, Marseille, France). The TXB\(_2\) and p-LTs in the sample were extracted as follows: To remove proteins, the sample was incubated with ethanol at \(-20^\circ C\) overnight, and thereafter was centrifuged. The supernatant was mixed with 8 ml of 0.1 mmol/l phosphate buffer (pH 4.0) and applied to the Sep-Pak C18 cartridge column (Waters Assoc., Milford, MA, USA), which had been washed with ethanol and ultrapure water. After washing the column with ultrapure water and of hexane, TXB\(_2\) and p-LTs were eluted with methanol and were evaporated to dryness by vacuum centrifugation. The dry eluate was then dissolved in the buffer of the EIA kit and used in the assay of TXB\(_2\) or p-LTs. The concentration of TXB\(_2\) or p-LTs was determined with each EIA kit (Cayman Chemical Co., Ann Arbor, MI, USA).

All results are expressed as means ± standard error (S.E.M.). The statistical differences were examined by the sign-Wilcoxon test for the comparison with the pre value or by the Wilcoxon rank sum test for the comparison with each group. \(P\) values less than 0.05 were considered statistically significant.

Figure 1 shows the time courses of changes of histamine, p-LTs and TXB\(_2\) concentrations in NALF following the antigen-antibody reaction. In the control group, the intranasal administration of antigen increased histamine and p-LTs concentrations in the NALF, peaking 10 min after the antigen challenge. In the sham group and the non-sensitized animals challenged with DNP-Ascaris, these concentrations did not change after the challenge. The TXB\(_2\) concentrations were similar in all groups until 360 min after the antigen challenge, although at some time points, the concentrations were lower than the pre values.

Olopatadine at 10 mg/kg, when given orally 1 h before the antigen challenge, significantly inhibited the increase of histamine concentration in NALF at 10 min after the challenge by 53.6%. Moreover, in the olopatadine-treated group, the p-LTs concentration in the NALF at 10 min after the challenge was lower by 48.4% than that in the control group, although it was not statistically significant. At 30 min and 60 min after the challenge, however, there was no difference in the concentrations of histamine and p-LTs in NALF between the control and the olopatadine-treated group (Fig. 2). In the previous in vitro studies, olopatadine inhibited the histamine release from rat mast cells (5) and the p-LTs release from human eosinophils (6). The present results elucidated that olopatadine exhibits the inhibitory effect on the mediator release in vivo as well.

In this study, the concentrations of histamine and p-LTs in NALF increased 10 min after intranasal administration of the antigen similarly to the previous observation in humans (1). Although the TXB\(_2\) concentration in NALF is reported to increase after the antigen challenge in guinea pigs and humans (2, 3), the present study failed to induce the increase of TXB\(_2\) concentration in the NALF following the antigen-antibody reaction. Yamasaki et al. demonstrated that the TXB\(_2\) concentration in NALF increased after the antigen challenge and seratrodast, a TX-receptor antagonist, at 30 mg/kg significantly inhibited the swelling of the
nasal mucosa in the guinea pigs actively sensitized with ovalbumin (3). The discrepancy between the present study and the previous reports (2, 3) (i.e., the effects on TXB2 in NALF) may be due to the difference in the antigen for sensitizing the animal. Thus, the involvement of TXB2 seems to be minimal, if any, in the present experimental condition employing DNP-Ascaris as the antigen. In fact, in the DNP-Ascaris-sensitized animal, seratrodast only partially inhibited the nasal blockage even at a high dose of 80 mg/kg (11).

We confirmed that olopatadine significantly inhibited the sneeze and nasal rubbing responses in the actively sensitized guinea pig (T. Kaise et al., unpublished data) as well as in the passively sensitized animal (7). In addition, we have reported that chlorpheniramine, a classic histamine H1-antagonist, prominently inhibited the sneeze and nasal rubbing responses in the actively sensitized animal, while pranlukast, a LT-receptor antagonist, and seratrodast failed to inhibit these responses (11). These results indicate that histamine is mainly involved in the development of the sneeze and nasal rubbing responses in this model. In the present study, the increase of histamine concentration in NALF was observed at 10 min after the antigen challenge and olopatadine inhibited this increase. It is thus considered that the inhibitory effect of olopatadine on the histamine release, in addition to its H1 antagonistic action, contributes to the inhibition of sneeze and nasal rubbing responses.

In patients with allergic rhinitis, the classical histamine H1-receptor antagonists have little effect on the nasal blockage (12). Kaise et al. previously reported that olopatadine inhibited the nasal blockage in the actively sensitized guinea pig that yields a biphasic nasal response similar to the response observed in patients with allergic rhinitis (8). In contrast, chlorpheniramine did not affect the nasal blockage in this model (11). It was reported that histamine, via activation of H2 and H3 receptors rather than H1 receptors, is involved in the nasal blockage (13, 14). Thus, the inhibition of histamine release may have some advantage over the H1 receptor blockade alone for the treatment of nasal blockage.

Accordingly, the inhibitory effect of olopatadine on histamine releases seems to play a role in the inhibition by this drug of nasal blockage. In fact, olopatadine has been shown to be useful for the treatment of allergic rhinitis, especially that of nasal blockage, in a double-blind clinical trial (9). The present study demonstrated that the p-LTs concentration in NALF increased following the antigen-antibody reaction. Additionally, pranlukast significantly inhibited the nasal blockage in this model (11). These results indicate that the development of nasal blockage involves p-LTs. Thus, the tendency of olopatadine to inhibit the p-LTs release may have contributed to the inhibition of nasal blockade.

In summary, we demonstrated that olopatadine significantly prevented the increase of histamine concentration and tended to inhibit that of p-LTs in NALF following the antigen-antibody reaction in actively sensitized guinea pigs. The inhibition by olopatadine of the nasal symptoms seems to involve the inhibitory effect on the release of histamine and, possibly, p-LTs into the nasal cavity.

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