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Short Communication

Inhibitory effect of olopatadine hydrochloride (KW-4679), a novel antiallergic drug, on peptide leukotriene release from human eosinophils

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ABSTRACT

Olopatadine hydrochloride (olopatadine; KW-4679), (Z)-11-[(3-dimethylamino)propylidene]-6,11-dihydrodibenz[b,e]oxepin-2-acetic acid monohydrochloride, is an antiallergic drug with selective and potent histamine H_1 receptor antagonist activity. In the present study, we investigated the effect of olopatadine on the release of peptide leukotrienes (P-LT), potent inflammatory mediators, from human eosinophils. Human eosinophils were purified from venous blood of healthy donors by negative selection using the anti-CD16 antibody. When human eosinophils were stimulated with the calcium ionophore A23187 (1 μmol/L), the amount of P-LT release was approximately 1200 pg/10^5 cells, while thromboxane (TX) B_2 release was under the detection limit (< 20 pg/10^5 cells). Olopatadine inhibited the A23187-induced P-LT release from human eosinophils with an IC_{50} of 4.5 μmol/L. Ketotifen also inhibited this reaction with an IC_{50} of 39.4 μmol/L. The inhibitory effect of olopatadine on the P-LT release may contribute to the antiallergic efficacy of this drug.

Key words: antiallergic drug, human eosinophils, leukotriene, olopatadine.

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INTRODUCTION

Eosinophil accumulation at sites of inflammation is a characteristic feature of various allergic diseases.[1,2] Activation of eosinophils results in the release of various inflammatory mediators, including peptide leukotrienes (P-LT), such as leukotriene (LT) C_4, LTD_4 and LTE_4, which induce the enhancement of vascular permeability and mucus secretion.[3,4] Thus, eosinophil-derived mediators are considered to play important roles in the pathogenesis of allergic diseases.

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 H_1 receptor antagonist activity.[5,6] Moreover, olopatadine inhibits the release of thromboxane (TX) B_2 and LTB_4 from human neutrophils and that of P-LT from guinea pig eosinophils.[7] However, it has not been determined whether olopatadine inhibits the activation of human eosinophils. As far as we are aware, few studies have examined the effects of antiallergic drugs on the P-LT release from human eosinophils.[8,9] Thus, in the present study, we investigated the effect of olopatadine on the release of P-LT from human eosinophils compared with the effects of ketotifen fumarate (ketotifen), an established antiallergic drug.

METHODS

Materials

Olopatadine, which was synthesized in our laboratories, and ketotifen (Sigma Chemical Co., St Louis, MO, USA) were dissolved in distilled water so as to make a

10 mmol/L solution. The calcium ionophore A23187 (Sigma Chemical Co.) was dissolved in dimethyl sulfoxide (Wako Pure Chemical, Osaka, Japan) to make a 10 mmol/L solution. These solutions were diluted with Hanks' buffer (Nissui Pharmaceutical Co., Tokyo, Japan) containing 0.1% bovine serum albumin (BSA; Sigma Chemical Co.). Heparinized peripheral venous blood was obtained from healthy volunteers. Informed consent was obtained from all donors.

Purification of human blood eosinophils

Eosinophils were purified by the combination of density gradient centrifugation and negative selection with the anti-CD16 antibody. Briefly, after erythrocytes were sedimented with dextran, the granulocyte fraction was separated by centrifugation on a Ficoll–Paque cushion (Amersham Pharmacia Biotech, Uppsala, Sweden) followed by hypotonic lysis of contaminating erythrocytes. Isolated granulocytes were mixed with anti-CD16-bound immunomagnetic beads (Miltenyi Biotec, Bergish-Gladbach, Germany) and were incubated for 30 min. Cells were then separated using a magnetic cell separation system (MACS; Miltenyi Biotec). The eluate was collected and the cell number and eosinophil purity were determined. Eosinophils were suspended in Hanks' buffer containing 0.1% BSA at a concentration of 5×10^4 cells/mL. The purity of eosinophils was 95.6 ± 1.4% as determined by Wright–Giemsa staining and the viability was 99.4 ± 0.1% as measured by trypan blue dye exclusion ($n = 10$). Each single experiment was performed using cells from a separate donor.

P-LT release from eosinophils

To 400 μL of cell suspension, 50 μL of the test drug solution or Hanks' buffer containing 0.1% BSA (control) was added and the cell suspension was preincubated for 10 min at 37°C. Thereafter, cells were stimulated by adding 50 μL A23187 solution (final concentration 1 μmol/L) for 20 min at 37°C. The reaction was terminated in an ice-bath and the amount of P-LT or TXB_2 in the supernatant was measured with enzyme immunoassay (EIA) kits (Cayman Chemical, Ann Arbor, MI, USA).

Statistical analysis

All results are expressed as the mean ± SEM. After the variance was evaluated by the Bartlett test, statistical differences were examined by the Steel multiple comparison test following the Kruskal–Wallis test. $P < 0.05$ was considered statistically significant. The 50% inhibitory concentration (IC_{50}) of a test drug was calculated from the concentration–inhibition curve using probit plot analysis.

RESULTS

In preliminary experiments, the appropriate concentration of the calcium ionophore A23187 was determined. In human eosinophils, stimulation with A23187 at concentrations of 0.5, 1 and 2 μmol/L induced P-LT release by 127, 601 and 1005 pg/10^5 cells, respectively, while 0.2 μmol/L A23187 failed to induce detectable P-LT release ($n = 2$). Moreover, we confirmed that this reaction reached a plateau level at around 10 min stimulation (data not shown). Therefore, in the following experiments, human eosinophils were stimulated with 1 μmol/L A23187 for 20 min, as was the case with the previous study examining the effect of olopatadine on the P-LT release from guinea pig eosinophils.[7]

The amount of P-LT released from human eosinophils following A23187 (1 μmol/L) stimulation for 20 min was 1168 ± 311 pg/10^5 cells, whereas spontaneous P-LT release was below the detection limit of the EIA (< 19.5 pg/10^5 cells). In contrast, the release of TXB_2, the degradation product of TXA_2, from eosinophils was not observed with A23187 stimulation.

Olopatadine at concentrations of 3, 10, 30 and 100 μmol/L inhibited the A23187-induced P-LT release from human eosinophils by 39.6 ± 4.9, 69.8 ± 4.7, 82.5 ± 3.9 and 92.6 ± 1.8%, respectively (Fig. 1). The IC_{50} value for olopatadine was 4.5 μmol/L. Ketotifen at 3, 10, 30 and 100 μmol/L inhibited this reaction by 13.9 ± 7.3, 14.2 ± 7.2, 34.8 ± 9.7 and 82.5 ± 5.2%, respectively (Fig. 2). The IC_{50} value for ketotifen was 39.4 μmol/L.

To examine whether or not the inhibitory effects of olopatadine and ketotifen on P-LT release were derived from their cytotoxicity, the release of lactate dehydrogenase (LDH) was determined in the supernatant of eosinophil suspensions treated with the drug by using the LDH-cytotoxic test kit (Wako Pure Chemical). As a result, olopatadine and ketotifen did not show any cytotoxicity at concentrations used in this study.

DISCUSSION

In the present study, we observed that human eosinophils, when stimulated with A23187, released P-LT but not

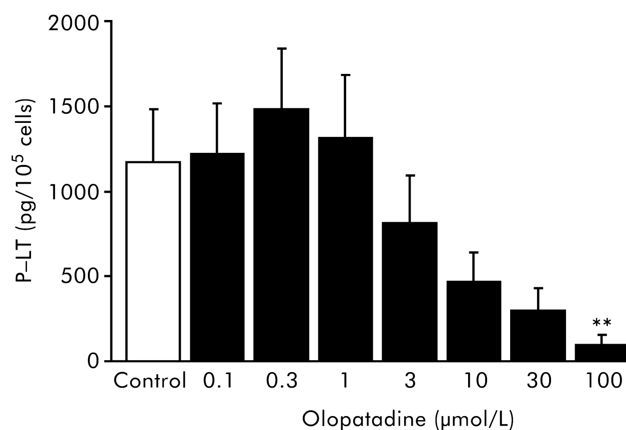

Fig. 1 Effect of olopatadine on peptide leukotriene (P-LT) release from human eosinophils induced by A23187 (1 μmol/L). Eosinophils were preincubated with Hanks' buffer containing 0.1% bovine serum albumin (control; □) or olopatadine (■) for 10 min at 37°C. The cell suspension was then incubated with A23187 (1 μmol/L) for 20 min at 37°C. Each column indicates the mean±SEM ($n = 10$). $^{**}P < 0.01$ compared with the control response.

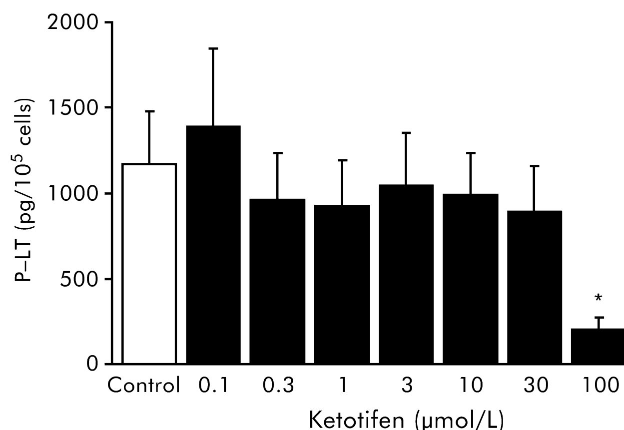

Fig. 2 Effect of ketotifen on peptide leukotriene (P-LT) release from human eosinophils induced by A23187 (1 μmol/L). Eosinophils were preincubated with Hanks' buffer containing 0.1% bovine serum albumin (control; □) or ketotifen (■) for 10 min at 37°C. The cell suspension was then incubated with A23187 (1 μmol/L) for 20 min at 37°C. Each column indicates the mean ± SEM ($n = 10$). $^{*}P < 0.05$ compared with the control response.

TXB_2. Sun *et al.*[10] have reported that human eosinophils mainly release P-LT, which was in contrast with the guinea pig eosinophils releasing both TXB_2 and LTB_4, following the A23187 stimulation. This difference between human eosinophils and guinea pig eosinophils has been suggested to be due to the difference between the two species of the capacity to synthesize arachidonic acid metabolites. In the present study, we found that human eosinophils produced approximately 50-fold more P-LT (1168 ± 311 pg/10^5 cells) than that shown in guinea pig eosinophils (23 ± 3 pg/10^5 cells).[7] Therefore, humans eosinophils are considered to be more appropriate than guinea pig eosinophils in order to determine the efficacy of human antiallergic drugs on P-LT release from eosinophils.

We have previously reported that olopatadine inhibited A23187-induced P-LT release from guinea pig eosinophils with an IC_{50} of 66.9 μmol/L.[7] In the present study, we evaluated the effect of olopatadine on the A23187-induced P-LT release from human eosinophils. As a result, olopatadine inhibited P-LT release with an IC_{50} of 4.5 μmol/L. Our results indicate that the inhibitory effect of olopatadine on the activation of human eosinophils is more prominent than that of guinea pig eosinophils. In contrast, ketotifen inhibited P-LT release from human eosinophils with an IC_{50} of 39.4 μmol/L while, in a previous study using guinea pig eosinophils, the inhibition was 60.7% at 100 μmol/L.[7] These results demonstrate that olopatadine, at relatively low concentrations, inhibits P-LT release from human eosinophils, suggesting that this action may be involved in the antiallergic efficacy of olopatadine in humans.

We have previously reported that olopatadine suppressed the release of arachidonic acid, the precursor of LT and TX, from membrane phospholipids in human neutrophils.[7] Thus, the suppressive effect of olopatadine on arachidonic acid release may be involved in the inhibitory effect of this drug on P-LT release in human eosinophils. In the study using human neutrophils,[7] olopatadine similarly inhibited the releases of TXB_2, a cyclo-oxygenase-derived product, and LTB_4, a 5-lipoxygenase-derived product, with IC_{50} values of 5.9 and 6.0 μmol/L, respectively. In contrast, the present study showed that olopatadine inhibited P-LT release from human eosinophils with an IC_{50} value of 4.5 μmol/L. These observations demonstrate that all the inhibitory effects on mediator release are observed at similar concentrations, suggesting that the mechanism for the inhibition may be the same between neutrophils and eosinophils in humans. Further studies, however, are needed to clarify the precise mechanism for the inhibitory effect of olopatadine on P-LT release from human eosinophils.

It has been reported that the antioxidant inhibits the production of LTB_4 in human neutrophils.[11] Ketotifen, at 100 μg/mL (235 μmol/L), is reported to exhibit a

prominent radical-scavenging effect in human neutrophils.[12] From these reports, it is considered that the radical-scavenging effect of ketotifen is involved in its inhibitory effect on P-LT release. In contrast, olopatadine, at concentrations up to 100 μmol/L, has no effect on reactive oxygen species in guinea pig eosinophils (K Miyake *et al.*, unpubl. obs., 1994). Thus, the mechanism for the inhibition of P-LT release may differ between ketotifen and olopatadine, the action of which is supposedly mediated by the suppression of arachidonic acid release.

Kaise *et al*. have reported that oral administration of olopatadine inhibited nasal mucosal swelling, an index of nasal blockage, caused by antigen challenge in actively sensitized guinea pigs.[13] Moreover, olopatadine has been shown to be useful for the treatment of allergic rhinitis in a double-blind clinical trial.[14] Recent evidence suggests that P-LT play an important role in the pathogenesis of allergic rhinitis, especially that of nasal blockage,[15] while the classic histamine H_1 receptor antagonists, such as chlorpheniramine and clemastine, have little effect on nasal blockage in allergic rhinitis.[16,17] The present study, as well as previous studies[7] using guinea pig eosinophils, demonstrates that olopatadine inhibits the release of P-LT from eosinophils. These observations suggest that the inhibitory action on P-LT release is involved in the therapeutic efficacy of olopatadine on nasal blockage.

In summary, we have demonstrated that olopatadine prevents P-LT release from human eosinophils. The inhibitory effect on P-LT release may contribute to the antiallergic efficacy of olopatadine.

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