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Characterization of the Ocular Antiallergic and Antihistaminic Effects of Olopatadine (AL-4943A), a Novel Drug for Treating Ocular Allergic Diseases

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ABSTRACT

Olopatadine (AL-4943A; KW-4679) [(*Z*)-11-[3-(dimethylamino)propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepine-2-acetic acid hydrochloride] is an antiallergic/antihistaminic drug under development for topical ocular use. The effects of the compound on release of proinflammatory mediators (histamine, tryptase and prostaglandin D_2) from monodispersed human conjunctival mast cells were assessed. Histamine receptor subtype binding affinities and functional potencies were determined with ligand binding and phosphoinositide turnover assays, respectively. Olopatadine inhibited the release of histamine, tryptase and prostaglandin D_2, in a concentration-dependent manner (IC_{50} = 559 μM). Evaluation of the interaction of olopatadine with histamine receptors revealed a relatively high affinity for the H_1 receptor (K_i = 31.6 nM, pK_i = 7.5 ± 0.1, n = 7) but lower affinities for H_2 receptors (K_i = 100 μM, pK_i = 4.0 ± 0.19, n = 7) and H_3 receptors (K_i = 79.4 μM, pK_i = 4.1 ± 0.16, n = 7). The H_1 selectivity of olopatadine was superior to that of other ocularly used antihistamines studied, such as ketotifen, levocabastine, antazoline and pheniramine. The profiling of olopatadine in 42 nonhistamine receptor binding assays revealed that olopatadine interacts with only two nonhistamine receptor/uptake sites to any significant degree ($pIC_{50} \leq 5–6$). Olopatadine inhibited histamine-induced phosphoinositide turnover in human conjunctival epithelial cells (IC_{50} = 10 nM, pIC_{50} = 8.0, n = 4) and in other human ocular cells (IC_{50} = 15.8–31.6 nM, pIC_{50} = 7.5–7.8) and exhibited apparent noncompetitive antagonist properties in these cells, with an estimated dissociation constant (K_b) of 19.9 nM (pK_b = 7.7, n = 6). This combination of mast cell mediator release inhibition and selective H_1 receptor antagonism suggests that olopatadine may be particularly useful in the treatment of ocular allergic diseases. Indeed, olopatadine has recently shown clinical efficacy in an allergic conjunctivitis model in human subjects.

Allergic conjunctivitis is characterized clinically by the presence of hyperemia, itching, edema and tearing (Berdy *et al.*, 1991; Abelson and Schaefer, 1993; Li *et al.*, 1993). Degranulation of the resident mast cell population in human conjunctival tissue, release of preformed and newly synthesized proinflammatory mediators and subsequent activation of appropriate receptors produce the symptoms noted above. Because histamine is the predominant preformed mast cell mediator initiating the symptoms, topical histamine antagonists, alone or in combination with *alpha* adrenergic agonist vasoconstrictors, or mast cell stabilizers (Abelson and Schaefer, 1993; Berdy *et al.*, 1991) have been the drugs of choice to alleviate and control ocular allergic diseases.

It has been demonstrated that mast cells from different species and from different tissues within the same animal differ in morphological, cytochemical and functional properties (Katz *et al.*, 1985; Irani *et al.*, 1990). Mast cell populations also differ in their responses to pharmacological agents (Befus *et al.*, 1987; Irani and Schwartz, 1989; Irani *et al.*, 1990). These observations strongly suggest that experimental antiallergic drugs for treating conjunctivitis should be evaluated by using HCMC as target cells. Recently, methods have been developed to allow drug evaluation in these target cells (Miller *et al.*, 1996).

Histamine has a well-established role in evoking a cascade of proinflammatory events upon mast cell degranulation during an allergic response (Beavan, 1982). Therefore, the use of antihistaminic drugs to attenuate an immediate hypersensitivity response is well known. However, many of the currently available histamine antagonists for treating ocular conjunctival hypersensitivity have a combination of problems, such as low affinity/selectivity for H_1 receptors, slow onset of action and short duration of action (Berdy *et al.*, 1991), that limit their clinical use in humans. These deficiencies have prompted further research to discover better H_1 antagonists. Although this strategy has recently culminated

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ABBREVIATIONS: HCE, human conjunctival epithelial; HCF, human corneal fibroblasts; HCMC, human conjunctival mast cell(s); 5HT, 5-hydroxytryptamine; HTM3, human trabecular meshwork; IP, inositol phosphate; PGD, prostaglandin D_2; PI, phosphoinositide(s).

in the discovery and clinical development of emedastine (Sharif *et al.*, 1994; Yanni *et al.*, 1994), antihistamines with additional therapeutic properties are still sought.

Olopatadine (AL-4943A, KW-4679) [(*Z*)-11-[13-(dimethylamino)propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepine-2-acetic acid hydrochloride] is a new antiallergic/antihistaminic drug that prevents immunologically stimulated HCMC histamine release *in vitro* and potently blocks histamine-induced conjunctival microvascular permeability *in vivo* (Yanni *et al.*, in press). Olopatadine exhibited a relatively fast onset of action and a long duration of action (up to 24 hr after topical administration) in the guinea pig model of conjunctivitis (Yanni *et al.*, in press). Olopatadine has also shown good efficacy and potency in animal models of allergic conditions such as bronchial asthma, allergic rhinitis and chronic urticaria (Ishii *et al.*, 1991; Ohshima *et al.*, 1992). Inhibition of passive anaphylaxis was maintained for 9 hr after p.o. administration of olopatadine, indicating an acceptable duration of action of this drug (Ishii *et al.*, 1991; Ohshima *et al.*, 1992).

The aims of the present studies were 4-fold, as follows: 1) to correlate the effects of olopatadine on the release of histamine with release of other mast cell-derived mediators (tryptase and PGD_2) from HCMC; 2) to compare histamine receptor subtype affinity and selectivity of olopatadine with those of other relevant, ocularly used antihistamines and pertinent reference compounds; 3) to determine the pharmacological specificity of olopatadine and ketotifen, an antiallergic/antihistaminic compound, by studying the relative affinities of the compounds at a range of other receptor systems unrelated to histamine; and 4) to determine the *in vitro* potency, efficacy and possible modes of action of olopatadine in antagonizing histamine-induced PI hydrolysis in cultured human ocular cells and to correlate these parameters with receptor binding affinities.

Materials and Methods

HCMC mediator release. Methods detailing preparation of monodispersed HCMC and mediator release studies with these cells have been described (Miller *et al.*, 1996; Yanni *et al.*, in press). Briefly, HCMC were isolated from postmortem donor tissues obtained, within 8 hr after death, at various eye banks and transported in Dexol corneal preservation medium. The conjunctival tissue was placed in supplemented RPMI 1640 culture medium and equilibrated overnight at 37°C in an incubator. After this time, the tissue was transferred to Tyrode's buffer containing 0.1% gelatin and 200 U of collagenase and 200 U of hyaluronidase per gram of tissue, for 30 min at 37°C. The incubation mixture was filtered over Nitex filter cloth and washed with an equal volume of the latter buffer. This digestion procedure was performed twice, followed by a more stringent digestion with 2000 U each of collagenase and hyaluronidase per gram of tissue, for 30 min at 37°C. The combined filtrates from each of the digestion procedures were centrifuged at 825 × *g* for 7 min, and the pelleted cells were resuspended in calcium/magnesium-free Tyrode's buffer and centrifuged (825 × *g* for 30 min) over a Percoll (1.058 g/liter) cushion. The mast cells that were pelleted in this fashion were resuspended in Tyrode's buffer and washed by another centrifugation step. Toluidine blue O staining and trypan blue exclusion by the cells permitted the counting and viability assessment of the isolated mast cells.

To measure the release of endogenous mediators from these cells, suspensions of 5000 mast cells were treated with the test compound(s) for 15 min and then exposed to 10 μg/ml anti-human IgE in ice-cold gelatin-containing Tyrode's buffer and centrifugation at 500 × *g* for 7 min. Supernatants from these experiments were collected and stored at −20°C until analyzed for histamine, tryptase and PGD_2 by radioimmunoassays. The sensitivities of these kits are 1 nM, 2 ng/ml and 3.125 pg/ml, respectively. Total preformed mediator (histamine and tryptase) concentrations were determined by assaying supernatants collected from mast cells that had been completely disrupted by 30-min exposure to 0.1% Triton X. The concentrations of histamine and tryptase were determined to be 4.6 ± 1.4 pg/cell and 23.6 ± 13.9 pg/cell, respectively.

Histamine receptor subtype binding assays. Histamine receptor subtype binding assays were conducted as described below. Briefly, frozen-thawed guinea pig forebrains (for H_1 and H_2 receptors) and rat forebrains (for H_3 receptors) were homogenized in 20 ml of ice-cold phosphate-buffered saline (pH 7.4), with a Brinkman Polytron tissue disrupter (setting 5–7 for 10 sec), and were centrifuged at 40,000 × *g* for 15 min at 4°C. The supernatants were discarded and the tissue pellets were rehomogenized in fresh phosphate-buffered saline and centrifuged as described above. The final pellets were homogenized in 50 mM sodium potassium phosphate buffer (pH 7.5) and frozen at −40°C until used in the binding assays. The H_1 (Hill *et al.*, 1977; Chang *et al.*, 1979), H_2 (Gajtkowski *et al.*, 1983) and H_3 (Korte *et al.*, 1990) histamine receptor subtype binding assays were performed as previously described, with minor modifications (Sharif *et al.*, 1994). Briefly, 50 μl of [^{3}H]pyrilamine (1–2 nM), [^{3}H]tiotidine (4–5 nM) or *N*-[^{3}H]methylhistamine (1–2 nM) were incubated with 10 to 20 mg/ml frozen-thawed brain homogenates in the presence or absence of unlabeled test compounds, in a total volume of 500 μl. The nonspecific binding was determined with 5 mM histamine. The assays were conducted at 23°C for 40 min and then terminated by rapid filtration over Whatman GF/B glass fiber filters (prewetted with 0.3% polyethyleneimine) with 2 × 6 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The radioactivity bound to filters was determined with a Wallac Big-Spot β-counter, with four samples being counted simultaneously.

To determine the relative affinity of olopatadine for nonhistamine receptors, it was tested at 1 nM, 100 nM and 10 μM in a battery of 38 receptor binding assays at NovaScreen (Oceanix Biosciences Corp., Hanover, MD) and in four additional prostanoid receptor assays performed in-house with standard receptor binding techniques. For comparison, the broad profiling at NovaScreen was also determined in parallel for ketotifen, another ocularly utilized antiallergic/antihistaminic compound.

PI turnover studies. The determination of histamine receptor-mediated PI hydrolysis was performed as described previously (Sharif and Whiting, 1990), with minor modifications (Sharif *et al.*, 1994). Briefly, primary HCE cells, immortalized HTM3 cells and HCF, which are known to express H_1 receptors (Sharif *et al.*, 1994, in press, a,b), were used for these functional studies. The cells were cultured in 24-well plates and incubated with 1 μCi/0.5 ml myo-[^{3}H]inositol in Dulbecco's modified Eagle medium (supplemented with 4 mM L-glutamine, 50 μg/ml gentamicin and 10% fetal bovine serum) for 24 hr at 37°C, to label the cell membrane PI. After this time the medium was aspirated and the cells were exposed to histamine (10 nM to 1 mM) in Dulbecco's modified Eagle medium (containing 10 mM LiCl) for 60 min at 23°C, to stimulate the production and subsequent accumulation of [^{3}H]IPs (Berridge *et al.*, 1982). To determine the potencies of the antagonists, the drugs were added to the cells 30 min before the addition of histamine and were kept in contact with the cells for a total of 90 min. The medium was aspirated at the end of this incubation, and the assay was stopped by the addition of 1 ml of ice-cold 0.1 M formic acid. After 15 min, 0.9 ml of the cell lysates were transferred to Bio-Rad Econo-columns containing 1 ml of AG1X8 ion-exchange resin in formate form, which had been previously wetted with 10 ml of deionized water. The columns were washed with 10 ml of deionized water to remove the free myo-[^{3}H]inositol, and the water-soluble [^{3}H]IPs were then eluted

M formic acid. A water-accepting scintillation fluid (15 ml) was then added to the eluates and the [^{3}H]IPs were quantified by scintillation counting with a Beckman LC 6000 β-scintillation counter.

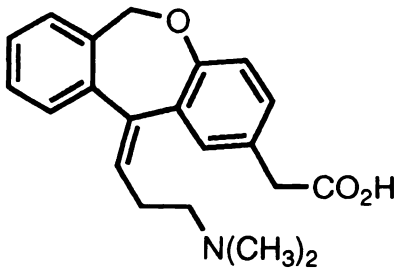
Data analyses. Competition binding and functional data were analyzed by using a nonlinear, iterative, sigmoidal curve-fitting computer program (Michel and Whiting, 1984; Sharif *et al.*, 1991). The drug inhibition constants (drug dissociation constants) derived from the receptor binding assays (K_i) and from the functional assays (K_b) represent the molar drug concentrations required to produce 50% inhibition of the binding/response, whereas pK_i and pK_b represent the negative logarithms of these parameters. All data from three or more experiments are shown as mean ± S.E.M.

Materials. [^{3}H]Pyrilamine (24 Ci/mmol), [^{3}H]tiotidine (87 Ci/mmol), *N*-[^{3}H]methylhistamine (84 Ci/mmol) and myo-[^{3}H]inositol (15–17 Ci/mmol) were all purchased from DuPont-NEN (Boston, MA). Percoll was purchased from Sigma Chemical Co. (St. Louis, MO). Frozen guinea pig and rat brains were purchased from Pel-Freez (Rogers, AR). Histaminergic agonists and antagonists were purchased from Research Biochemicals International (Natick, MA). Olopatadine (AL-4943A, KW-4679) was synthesized at Kyowa Hakko Kogyo Co. (Shizuoka, Japan). Anti-human IgE was purchased from Cortex Biochemicals (San Leandro, CA). Radioimmunoassay systems for proinflammatory mediator quantitation were purchased from the following companies; histamine, Immunotech Inc. (Westbrook, ME); tryptase, Kabi-Pharmacia (Fairfield, NJ); PGD_2, Amersham Corp. (Arlington Heights, IL).

Results

HCMC mediator release. Olopatadine (fig. 1), when added to monodispersed preparations of HCMC 15 min before challenge with anti-IgE, inhibited histamine release in a concentration-dependent manner (fig. 2). Calculated IC_{50} values from these three independent experiments were 314 μM, 504 μM and 859 μM. No mast cell cytotoxicity, as evidenced by enhanced mast cell mediator release, was observed with concentrations of olopatadine 10-fold higher than the maximally effective concentrations tested.

In separate experiments, the effects of olopatadine upon the mast cell release of tryptase and PGD_2 were correlated with the effect upon histamine release. Olopatadine at concentrations ranging from 100 μM to 2 mM inhibited HCMC mediator release. Concentrations of tryptase released into culture supernatants were positively correlated (r = 0.98) (fig. 3a) with those of histamine in the same supernatants. Similarly, the concentrations of PGD_2 were also correlated (r = 0.905) (fig. 3b) with those of histamine in supernatants obtained from HCMC cultures treated with olopatadine before the immunological challenge.



Olopatadine

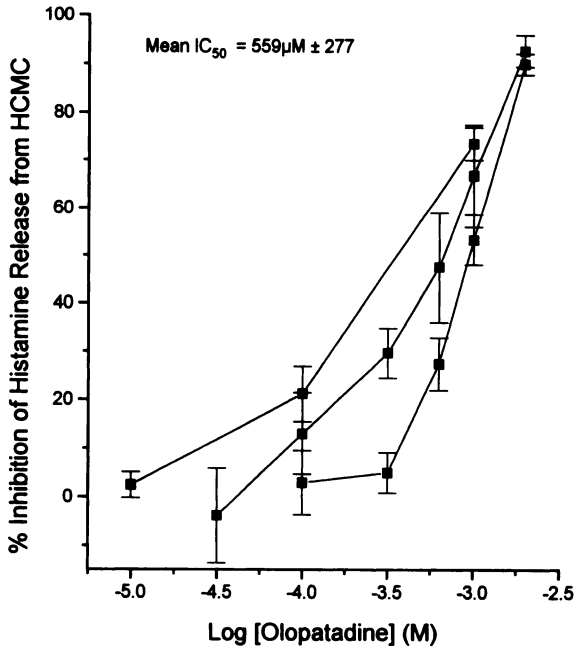


Fig. 2. Inhibition of histamine release from HCMC by olopatadine. Calculated IC_{50} values for the three separate experiments were 314 μM, 504 μM and 859 μM. Data shown are mean ± S.D.

Histamine receptor subtype binding. Olopatadine and histaminergic agonists and antagonists competed for specific [^{3}H]pyrilamine, [^{3}H]tiotidine and *N*-[^{3}H]methylhistamine binding to H_1, H_2 and H_3 histamine receptors, respectively, in a concentration-dependent manner (figs. 4–6). Well-known key H_1-selective ligands, such as triprolidine, pyrilamine, tripelennamine and promethazine, had high H_1 receptor affinities. Likewise, the H_2-selective compound aminopentidine had a high H_2 affinity. In addition, known H_3-selective compounds like clobenpropit, thioperamide, imetit and *N*-methylhistamine exhibited high H_3 receptor affinities (figs. 4–6; table 1).

Olopatadine exhibited a relatively high affinity for H_1 receptors (K_i = 31.6 nM, pK_i = 7.5 ± 0.10, n = 7) and a significantly lower affinity at H_2 receptors (K_i = 100 μM, pK_i = 4.0 ± 0.19, n = 7) and H_3 receptors (K_i = 79.4 μM, pK_i = 4.1 ± 0.16, n = 7) (figs. 4–6; tables 1 and 2). The H_2:H_1 and H_3:H_1 receptor affinity ratios for olopatadine, indicating H_1 selectivity over H_2 and H_3 receptors, were 3164 and 2500, respectively, demonstrating that this is an H_1-selective compound (table 2). This H_1-selective profile of olopatadine was shown to be superior to that of some other ocularly used antihistamines evaluated, such as ketotifen (H_2:H_1 affinity ratio = 1032), levocabastine (H_2:H_1 affinity ratio = 500), antazoline (H_2:H_1 affinity ratio = 1000) and pheniramine (H_2:H_1 affinity ratio = 406) (table 2), but olopatadine was less H_1-selective than chlorpheniramine (H_2:H_1 affinity ratio = 6270) and pyrilamine (H_2:H_1 affinity ratio = 12,589) (table 2).

To define the specificity of olopatadine, it was tested (at 1 nM to 10 μM) in 38 nonhistamine receptor binding assays, pertaining to neurotransmitters, regulatory sites, peptides, prostaglandins, uptake sites, second messengers and immunological factors, at NovaScreen (Oceanix Biosciences Corp.) and in another four prostanoid receptor assays in our labo-

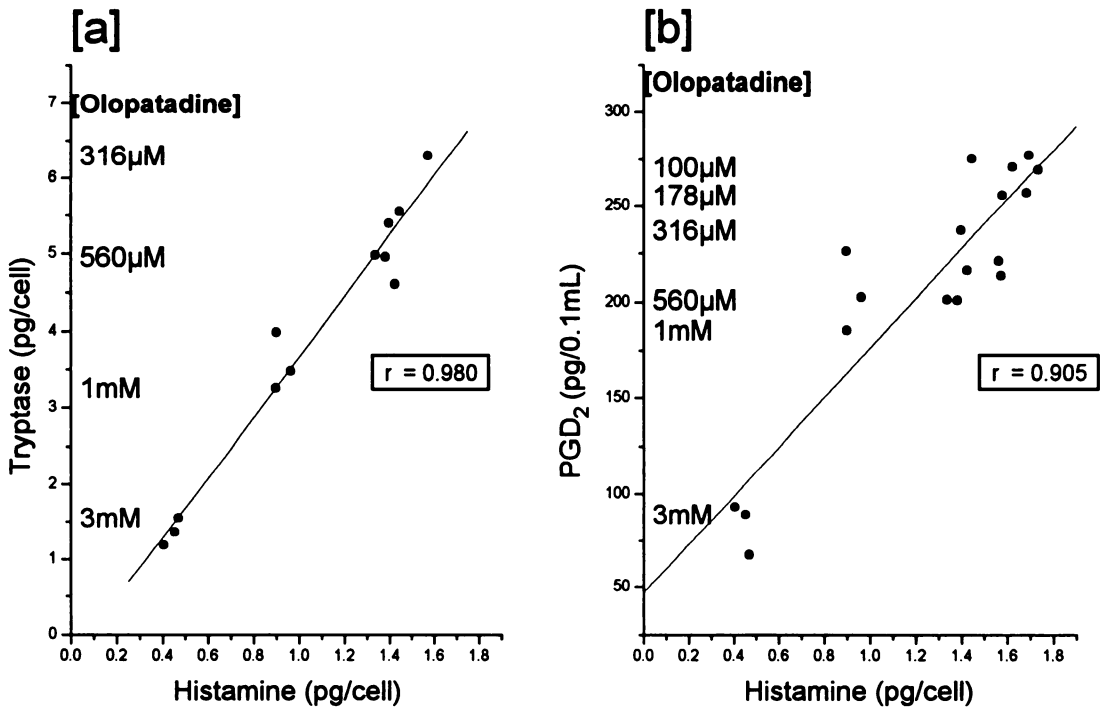


Fig. 3. a, correlation between concentrations of histamine and tryptase released from HCMC upon immunological challenge after treatment with various concentrations of olopatadine. b, correlation between concentrations of histamine and PGD_2 released from monodispersed HCMC preparations upon immunological challenge after treatment with olopatadine.

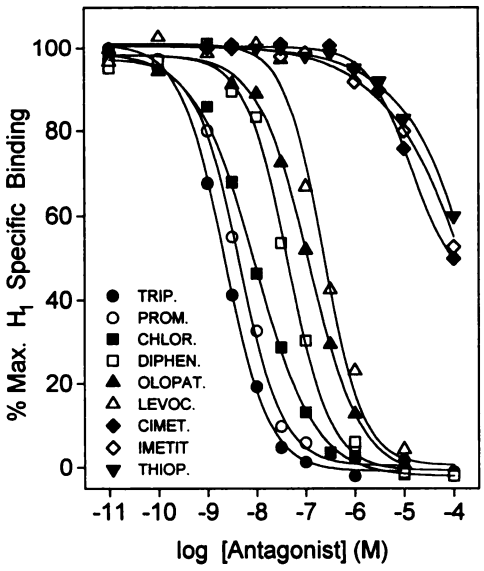


Fig. 4. Inhibition of specific [^{3}H]pyrilamine binding to H_1 receptors by olopatadine and a variety of histamine antagonists. Data are the average results from three to eight experiments. The error bars are not shown for clarity. ●, triprolidine; ○, promethazine; ■, chlorpheniramine; □, diphenhydramine; ▲, olopatadine; △, levocabastine; ♦, cimetidine; ◇, imetit; ▼, thioperamide.

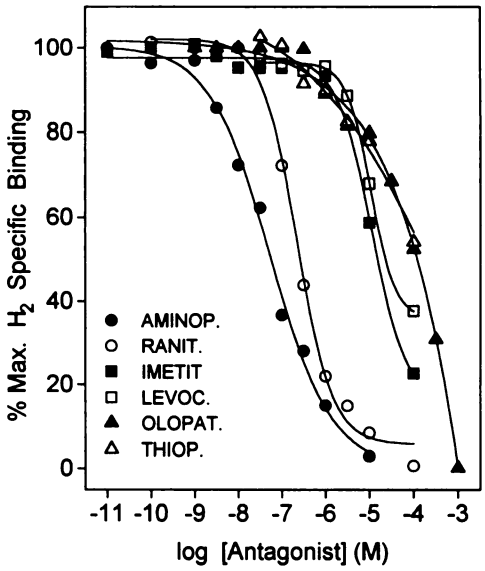


Fig. 5. Inhibition of specific [^{3}H]tiotidine binding to H_2 receptors by olopatadine and a variety of histamine antagonists. Data are the average results from three to eight experiments. The error bars are not shown for clarity. ●, aminopotentidine; ○, ranitidine; ■, imetit; □, levocabastine; ▲, olopatadine; △, thioperamide.

revealed that olopatadine had some detectable affinity (≥50% inhibition of binding at 10 μM) for 5HT uptake sites ($pIC_{50} \geq 5$) and $5HT_2$ receptors ($pIC_{50} \geq 6$), but these reflected lower affinities than its H_1 receptor affinity (pK_i = 7.3). Olopatadine possessed a very low affinity for the other 38 nonhistamine receptor systems examined (table 3). In contrast, ketotifen tested in the same 42 assays exhibited for *alpha*-2 adrenergic, D_2 dopamine, $5HT_2$, histamine H_2 and M_1 and M_2 muscarinic receptors (table 4; NovaScreen task order 0251), in addition to its high affinity for H_1 histamine receptors (tables 1 and 4).

Functional studies. To determine the *in vitro* potency of olopatadine, it was tested for its ability to antagonize histamine-induced PI turnover in a variety of human ocular cell types possessing H_1 histamine receptors. Olopatadine con-

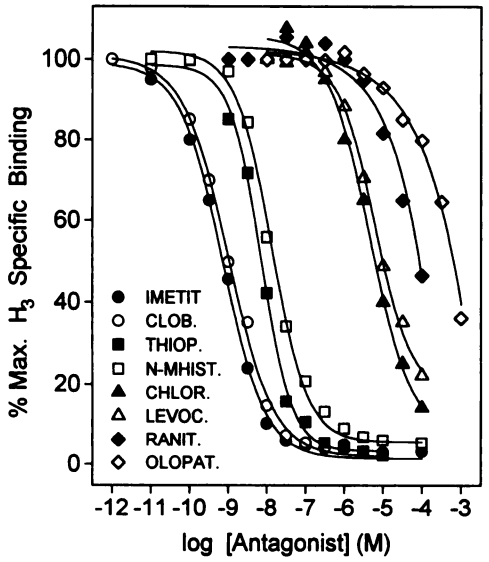


Fig. 6. Inhibition of specific *N*-[^{3}H]methylhistamine binding to H_3 receptors by olopatadine and a variety of histamine antagonists. Data are the average results from three to eight experiments. The error bars are not shown for clarity. ●, imetit; ○, clobenpropit; ■, thioperamide; □, *N*-methylhistamine; ▲, chlorpheniramine; △, levocabastine; ◆, ranitidine; ◇, olopatadine.

duced PI turnover in primary cultures of HCE cells (IC_{50} = 10 nM, pIC_{50} = 8.0 ± 0.06, n = 4), in HCF (IC_{50} = 15.8 nM, pIC_{50} = 7.8 ± 0.04, n = 4) and in immortalized HTM3 cells (IC_{50} = 31.6 nM, pIC_{50} = 7.5 ± 0.1, n = 9), which respond to histamine as primary HTM3 cells do (Pang *et al.*, 1994) (figs. 7 and 8; table 5). The potencies of some ocularly used antihistamines for blocking histamine-induced PI turnover in HCE cells, HCF and HTM3 cells are also shown in table 5 for comparison.

In additional mechanistic studies, olopatadine (1–300 nM) preincubated with HTM3 cells produced a rightward shift of the histamine concentration-response curves, with a progressive decrease in the histamine-induced maximal response (fig. 9). Thus, olopatadine exhibited apparent noncompetitive antagonist properties in the HTM3 cells under the present experimental conditions. The estimated dissociation constant (pK_b) (Furchgott, 1972; Brown *et al.*, 1984) of olopatadine from these studies was calculated to be 7.7 ± 0.2 (n = 6) (K_b = 19.9 nM). Similar studies conducted in the HTM3 cells

TABLE 2

H_1 selectivity of olopatadine and various histaminergic ligands relative to their H_2 and H_3 receptor selectivities

Data represent the affinity ratios at the different histamine receptor subtypes based on the molar values derived from data in Table 1. The respective H_2 selectivities of ranitidine and cimetidine, relative to H_1 receptors, were 250 and 16. The respective H_3 selectivities of *N*-methylhistamine, thioperamide and histamine, relative to H_1 receptors, were 63,000, 202,532 and 50,000.

Compounds	H_1 Selectivity Relative to H_2	H_1 Selectivity Relative to H_3
H_1 ligands		
Pyrilamine	12,589	12,589
Chlorpheniramine	6,270	2,460
Triprolidine	4,750	6.6
Olopatadine	3,164	2,500
Ketotifen	1,032	1,953
Antazoline	1,000	800
Tripelennamine	731	6,454
Levocabastine	500	64
Promethazine	251	25,280
Pheniramine	406	197
Diphenhydramine	126	2,000
H_2 ligands		
Cimetidine	0.1	0.8
Ranitidine	0	0.4
H_3 ligands		
Imetit	0.2	0
N-Methylhistamine	0.8	0
Thioperamide	0.3	0
Histamine	0.1	0

TABLE 1

Affinity estimates for olopatadine (AL-4943A) and other histaminergic ligands for histamine receptor subtypes

Data are mean ± S.E.M. from three to eight independent experiments. The values shown in parentheses represent the mean Hill coefficients of the inhibition plots.

Compounds	Receptor Affinities (pK_i) and Hill Coefficients		
	H_1 Receptors	H_2 Receptors	H_3 Receptors
H_1 ligands			
Triprolidine	9.1 ± 0.06 (1.1)	5.5 ± 0.12 (0.6)	5.3 ± 0.10 (0.8)
Pyrilamine	9.1 ± 0.06 (0.9)	5.0 ± 0.05 (0.8)	5.0 ± 0.05 (0.9)
Ketotifen	8.9 ± 0.05 (1.0)	5.9 ± 0.05 (0.7)	5.6 ± 0.04 (1.0)
Chlorpheniramine	8.9 ± 0.10 (0.8)	5.1 ± 0.04 (0.8)	5.5 ± 0.01 (1.1)
Promethazine	8.9 ± 0.10 (1.0)	6.5 ± 0.31 (0.5)	4.5 ± 0.41 (0.7)
Tripelennamine	8.5 ± 0.10 (1.0)	5.6 ± 0.03 (0.6)	4.8 ± 0.25 (0.7)
Diphenhydramine	7.9 ± 0.20 (1.1)	5.8 ± 0.06 (0.6)	4.6 ± 0.30 (0.7)
Pheniramine	7.5 ± 0.03 (0.9)	4.9 ± 0.03 (0.6)	5.2 ± 0.22 (0.9)
Antazoline	7.4 ± 0.05 (1.0)	4.4 ± 0.05 (0.8)	4.5 ± 0.12 (1.2)
Olopatadine	7.5 ± 0.10 (1.1)	4.0 ± 0.19 (0.7)	4.1 ± 0.16 (0.9)
Levocabastine	7.3 ± 0.08 (1.0)	4.6 ± 0.08 (0.6)	5.5 ± 0.19 (0.8)
H_2 ligands			
Aminopotentidine	4.6 ± 0.20 (0.6)	7.4 ± 0.03 (0.6)	7.1 ± 0.20 (0.7)
Cimetidine	4.3 ± 0.30 (0.6)	5.8 ± 0.13 (0.6)	4.7 ± 0.03 (0.7)
Ranitidine	4.6 ± 0.20 (1.0)	6.8 ± 0.03 (0.8)	4.8 ± 0.09 (0.9)
H_3 ligands			
Clobenpropit	5.6 ± 0.03 (0.6)	5.7 ± 0.21 (0.7)	9.7 ± 0.10 (0.7)
Imetit	4.3 ± 0.10 (0.4)	5.1 ± 0.26 (0.7)	9.7 ± 0.03 (0.9)
Thioperamide	4.2 ± 0.30 (0.8)	4.3 ± 0.07 (0.9)	9.1 ± 0.09 (0.9)
N-Methylhistamine	4.2 ± 0.34 (1.0)	4.3 ± 0.27 (0.4)	9.0 ± 0.17 (0.9)
Histamine	3.7 ± 0.08 (0.4)	4.8 ± 0.09 (0.7)	8.4 ± 0.18 (0.9)

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Sync your system to PACER to automate legal marketing.