Differential modulation of mediator release from human basophils and mast cells by mizolastine

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**Summary**

**Background** Basophils and mast cells play a major role in the pathogenesis of allergic disorders by releasing several proinflammatory mediators. Some histamine H₁ receptor antagonists exert anti-inflammatory activities by modulating mediator release from basophils and mast cells.

**Objective** To study the *in vitro* effects of mizolastine, an H₁ receptor antagonist, on the release of eicosanoids, histamine and IL-4 from human basophils and lung mast cells.

**Methods and results** Mizolastine (10⁻⁷–10⁻⁵ M) concentration-dependently inhibited the release of cysteinyl leukotriene C₄ from anti-IgE-stimulated basophils (IC₅₀: 3.85 ± 0.28 μM) and mast cells (IC₅₀: 3.92 ± 0.41 μM). The same concentrations of mizolastine did not affect anti-IgE-induced prostaglandin D₂ release from lung mast cells. In contrast, mizolastine enhanced up to 80% IgE-mediated histamine release (EC₅₀: 4.63 ± 0.14 μM) from basophils, but not from mast cells and it significantly potentiated IL-4 release from basophils induced by anti-IgE. Mizolastine did not affect histamine release from basophils induced by formyl peptide, whereas it inhibited cysteinyl leukotriene C₄ release (IC₅₀: 1.86 ± 0.24 μM). Blockade of cytosolic phospholipase A₂ and arachidonic acid mobilization by pyrrolidine-1 did not alter the effect of mizolastine on histamine release from basophils, thereby excluding accumulation of arachidonic acid metabolic intermediates as the cause of this effect. Mizolastine did not influence anti-IgE-induced activation of extracellular signal-regulated kinase-1 and -2 (ERK-1 and -2) in human basophils.

**Conclusions** Mizolastine efficiently inhibits LTC₄ synthesis in human basophils and mast cells presumably by interfering with 5-lipoxygenase. In contrast, it enhances histamine and IL-4 release only from anti-IgE-stimulated basophils. Therefore, mizolastine differentially regulates the production of mediators from basophils and mast cells in a cell- and stimulus-specific fashion.

**Keywords** basophils, cysteinyl leukotriene C₄, histamine, IL-4, mast cells, mizolastine

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**Introduction**

Basophils, mast cells and their pre-formed and *de novo* synthesized mediators play a pivotal role in the pathogenesis of allergic disorders. These molecules are potent vasoactive and bronchoconstrictor agents and they modulate local immune responses and inflammatory cell infiltration [1–4]. Histamine is a central mediator in the pathogenesis of allergic and inflammatory disorders. Its biological effects are mediated by the activation of specific receptors denominated H₁, H₂, H₃ and H₄ [5, 6]. The effect of histamine in urticaria, allergic rhinitis and bronchial asthma is mediated mainly by H₁, which explains the clinical efficacy of histamine H₁ receptor antagonists in the treatment of most of these disorders [6, 7].

Some antihistamines seem to possess anti-inflammatory activities that are unrelated to the H₁ receptor blockade [8, 9]. First-generation antihistamines, like other related drugs, inhibit at low concentrations and enhance at high concentrations histamine release from the human lung [10]. Certain second-generation antihistamines inhibit cytokine and histamine release from human basophils [11–14] and cysteinyl leukotriene C₄ (LTC₄) production from human basophils and eosinophils [11, 12] and suppress skin reactivity to platelet-activating factor (PAF) [15], whereas others inhibit ICAM-1 expression on epithelial cells [16] and reduce eosinophil infiltration during the late-phase response [17]. These data indicate that antihistamines possess various anti-inflammatory activities and that this property is not general to this class of compounds.

Mizolastine is highly selective for the H₁ receptor and it has been proven effective in the treatment of urticaria and rhinitis [18–20]. In addition to its antihistamine effects, mizolastine exerts anti-inflammatory effects that may contribute to its clinical efficacy [21, 22]. For example, mizolastine inhibited the passive cutaneous anaphylactic reaction in guinea-pigs, rats and mice [21, 23]. It also protected rats from lethal shock induced by compound 48/80 and prevented bronchospasm...
induced by PAF and LTD₄ [23]. In isolated mast cells obtained by peritoneal and bronchoalveolar lavage of sensitized rats and guinea-pigs, respectively, mizolastine prevented antigen-induced histamine release [23, 24]. Recently, mizolastine has been reported to inhibit LT₄ and LTC₄ release from nasal polyp cells [25].

Experiments in cell-free systems provided evidence that mizolastine inhibits 5-lipoxygenase (5-LO), but not cyclooxygenase activity [21, 26]. Therefore, mizolastine inhibits, at least in vitro, the production of both cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄) and LTB₄. Together, these findings suggest that mizolastine might interfere with the production of two major classes of mediators produced by basophils and mast cells (histamine and cysteinyl leukotrienes).

Human basophils and mast cells differ in many functional aspects, including the response to stimuli [27], the profile of mediators they produce [1, 27] and the pharmacological modulation of mediator release [1, 27, 28]. For example, basophils respond to several stimuli that do not affect mast cells. In addition, whereas both basophils and mast cells synthesize LTC₄ only mast cells produce prostaglandin D₂ (PGD₂) [29]. Finally, pharmacological agents that inhibit mediator release from human basophils fail to do so in mast cells [28]. Given the complex heterogeneity of human basophils and mast cells, there is clearly a need for pharmacological studies of both cell types.

To understand whether pharmacological concentrations of mizolastine influence the release of pre-formed or de novo synthesized mediators from human cells involved in allergic inflammation, we studied the effects of this drug on the release of histamine, arachidonic acid metabolites and IL-4 from human basophils and lung mast cells.

**Materials and methods**

**Reagents**

Human serum albumin (HSA), Percoll, DMSO, L-glutamine, antibiotic-antimycotic solution (10000 U/mL penicillin, 10 mg/mL streptomycin and 25 μg/mL amphotericin B) and elastase were from Sigma (St Louis, MO, USA). Iscove’s modified Dulbecco medium (IMDM) and non-essential amino acids were from Gibco (Grand Island, NY, USA). DNase, pronase, collagenase and f-Met-Leu-Phe (fMLP) were from Calbiochem Behring Co. (La Jolla, CA, USA). Chymopapain, RPMI-1640 and FCS were from ICN Biomedicals (Costa Mesa, CA, USA). LTC₄ and PGD₂ standards were from Biomol (Plymouth Meeting, PA, USA). ³H-LTC₄ and ³H-PGD₂ were from New England Nuclear (Boston, MA, USA). The rabbit anti-LTC₄ Ab was supplied from Dr J. Rakoch (Merck Frosst Canada, Montreal, Canada). The rabbit anti-PGD₂ Ab was supplied by Dr L. M. Lichtenstein (The Johns Hopkins University, Baltimore, MD, USA). Rabbit anti-human Fcε Ab was supplied from Drs T. Ishizaka and K. Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA). Rabbit anti-phospho-ERK 1/2 Ab and rabbit anti-ERK 1/2 Ab were purchased from New England Biolabs (Beverly, MA, USA). HRP-conjugated donkey anti-rabbit IgG Ab was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Pyrrolidine-1 was synthesized as described [30]. Mizolastine was donated by Synthelabo Recherche (Paris, France); stock solutions of 10⁻² M were prepared in DMSO. All other reagents were from Carlo Erba (Milan, Italy).

**Buffers**

P buffer was composed of 25 mM piperazine-N,N'-bis-2-ethanesulphonic acid (Pipes, Sigma), 110 mM NaCl and 5 mM KCl, pH 7.4. PCG buffer is P buffer containing 1 mM CaCl₂ and 1 g/L D-glucose, pH 7.4. PGMD contains 1 mM MgCl₂, 6 H₂O, 10 mg/L DNase and 1 g/L gelatin in addition to P buffer, pH 7.4. PBS contains 150 mM NaCl, 10 mM Na₂HPO₄, 3 mM KCl and 1 mM KH₂PO₄, pH 7.4.

**Purification of peripheral blood basophils**

Basophils were purified from peripheral blood cells of healthy donors. Buffy coat cell packs from healthy donors, provided by the Immunohematology Service (University of Naples Federico II), were loaded onto a countercurrent elutriator (model J2-21; Beckman, Fullerton, CA, USA). Several fractions were collected, and fractions containing basophils in large number (>20 x 10⁶ basophils) and of good purity (>15%) were enriched by discontinuous Percoll gradients [31]. Basophils were further purified to near homogeneity by negative immunomagnetic selection (MidiMACS, Milteny Biotec, Bergisch Gladbach, Germany) [31]. Yields ranged from 3 to 10 x 10⁶ basophils, with purity usually >98%, as assessed by staining with Alcian blue.

**Isolation and purification of human lung mast cells**

Human lung tissue obtained during surgical resection was processed as previously described [32]. Macroscopically normal lung tissue was finely chopped with scissors. The fragments were dispersed into their cellular elements by sequential incubation with pronase (2 mg/g of tissue), chymopapain (0.5 mg/g), collagenase (1 mg/g) and elastase (10 U/g). The cells were resuspended in PGMD and further purified by countercurrent elutriation centrifugation. After this procedure, the fractions containing the highest percentages of mast cells (15–20%) were collected and further purified (>80%) by flotation over Percoll density gradients as described elsewhere [32]. The mast cell number was determined by Alcian blue staining.

**Histamine release**

Basophils (~6 x 10⁶/tube) or mast cells (~3 x 10⁶/tube) resuspended in PCG were pre-incubated with increasing concentrations of mizolastine (10⁻⁸–10⁻⁴ M) and stimulated with anti-IgE (0.3 and 1 μg/mL in basophils and mast cells, respectively) or fMLP (10⁻⁶ M). Incubation was continued at 37°C for 30 min. The reaction was stopped by centrifugation (1000 g, 22°C, 5 min), and the cell-free supernatants were stored at −20°C for subsequent assay of histamine content [33]. Histamine release was expressed as the percentage of total cellular histamine content determined in cell aliquots lysed with 8% HClO₄. Replicates differed in histamine content by <10%.
**LTC_{4} and PGD_{2} assays**

LTC_{4} release was measured by radioimmunoassay, as previously described [34]. The linearity of the assay ranged between 150 and 10 000 pg/mL. The results were expressed as ng of LTC_{4}/10^6 cells.

PGD_{2} release was measured by radioimmunoassay as previously described [34]. The linearity of the assay ranged between 300 and 10 000 pg/mL. The results were expressed as ng of PGD_{2}/10^6 cells.

**IL-4 assay**

Basophils (2.5 X 10^5 cells/well) were incubated in polystyrene plates (Falcon, Becton Dickinson, Franklin Lakes, NJ, USA) in 150 µL of IMDM containing 1% non-essential amino acids, 5% FCS and 10 µg/mL gentamicin, and were pre-incubated (20 min, 37°C) with mizolastine (5 X 10^{-6} or 10^{-5} M). The cells were then stimulated with anti-IgE (0.3 µg/mL) and incubation was continued for 4 h at 37°C. At the end of the incubation, supernatants were collected and centrifuged (1000 g, 22°C, 5 min). IL-4 release in the supernatants was measured in duplicate determinations using commercially available ELISA kits for IL-4 (R&D System, Minneapolis, MN, USA). The linearity range of the assay was between 31.2 and 1000 pg/mL. The results were expressed as pg of IL-4/10^6 basophils and were considered as not detectable when they were below 20 pg/10^6 basophils.

**Phosphorylation of extracellular signal-regulated kinases (ERKS)**

Purified basophils (5 X 10^5 cells/0.5 mL) were suspended in PCG buffer, pre-incubated (20 min, 37°C) with mizolastine (10^{-6} and 10^{-5} M) and then stimulated (5 min, 37°C) with anti-IgE (0.3 µg/mL). At the end of the incubation, the reaction was stopped by adding ice-cold P buffer containing 5 mM EDTA and the samples were microfuged for 15 s. Cell pellets were immediately lysed in Electrophoresis Sample Buffer (ESB, Novex, Invitrogen, Milan, Italy) [35]. Proteins were separated on 10% Bis–Tris Gels (NuPAGE®, Novex) and transferred onto a nitrocellulose membrane (Biorad, Hercules, CA, USA). After immersion overnight in TBST (50 mM Tris pH 7.5, 150 mM NaCl and 0.05% Tween 20) containing 4% BSA, the membranes were washed three times (10 min each) with TBST and then blotted (2 h, 22°C) with anti-phospho-ERK 1/2 Ab. The membranes were then incubated (1 h, 22°C) with HRP-conjugated anti-rabbit IgG Ab and, after washing, membrane-bound anti-rabbit IgG Ab was visualized with the ECL Western blotting detection reagent (Amersham Pharmacia Biotec) and HyperECL luminescence detection film (Amersham Pharmacia Biotec). Although comparisons were made on the basis of an equal number of cells, membranes were stripped with stripping buffer (7 M guanidine hydrochloride in distilled water) and then rebotted with anti-ERK 1/2 Ab to verify equal protein content of each sample.

**Statistical analysis**

The data are expressed as the mean ± SE of the indicated number of experiments. P-values were determined with a t-test for unpaired samples with Bonferroni’s correction. The thresholds for statistical significance were set at P<0.05 and at P<0.01.

**Results**

**Effects of mizolastine on mediator release from human basophils**

We first evaluated the effects of mizolastine on the release of pre-formed (histamine) and de novo synthesized (LTC_{4} and IL-4) mediators from basophils isolated from peripheral blood of healthy donors. As shown in Fig. 1 (upper panel), mizolastine (10^{-5}–10^{-4} M) enhanced histamine release from human basophils induced by anti-IgE (0.3 µg/mL). Mizolastine alone, at concentrations up to 10^{-4} M, did not induce histamine release. The IC_{50} of mizolastine for potentiation of histamine release was 3.14 ± 0.57% vs. 2.91 ± 0.53% in unstimulated cells. Mizolastine-induced enhancement of histamine release was concentration-dependent and reached a maximum of ~80% at 10^{-5} M. The IC_{50} of mizolastine for potentiation of histamine release was 4.63 ± 0.14 µM. Mizolastine also concentration-dependently inhibited LTC_{4} release from anti-IgE-stimulated basophils (Fig. 1, lower panel). At 10^{-5} M, mizolastine completely suppressed LTC_{4} synthesis with an IC_{50} of 3.85 ± 0.28 µM. Interestingly, the enhancement of histamine release mirrored the mizolastine-induced inhibition of LTC_{4}.

We next evaluated the effect of mizolastine on anti-IgE-induced release of IL-4, a cytokine that is crucial for Th2 polarization of immune responses and is produced in large quantities by human basophils [4, 36]. Table 1 reports the results of four experiments showing that mizolastine (5 X 10^{-6} and 10^{-5} M) enhanced IL-4 release from basophils stimulated with anti-IgE. Basophils pre-incubated with 10^{-5} M of mizolastine (M) were challenged (30 min, 37°C) with mizolastine alone, at concentrations up to 10^{-5} M, did not induce histamine release (3.14 ± 0.57% vs. 2.91 ± 0.53% in unstimulated cells). Mizolastine-induced enhancement of histamine release was concentration-dependent and reached a maximum of ~80% at 10^{-5} M. The IC_{50} of mizolastine for potentiation of histamine release was 4.63 ± 0.14 µM. Mizolastine also concentration-dependently inhibited LTC_{4} release from anti-IgE-stimulated basophils (Fig. 1, lower panel). At 10^{-5} M, mizolastine completely suppressed LTC_{4} synthesis with an IC_{50} of 3.85 ± 0.28 µM. Interestingly, the enhancement of histamine release mirrored the mizolastine-induced inhibition of LTC_{4}.

**Fig. 1.** Effect of mizolastine on histamine (upper panel) and LTC_{4} release (lower panel) induced by anti-IgE from human basophils. Basophils pre-incubated (20 min, 37°C) with mizolastine were challenged (30 min, 37°C) with anti-IgE (0.3 µg/mL). The data are expressed as percent enhancement (histamine) or inhibition (LTC_{4}) of the release induced by anti-IgE. Spontaneous release was 2.91 ± 0.53% for histamine and 2.36 ± 0.61 ng/10^6 cells for LTC_{4}. Release induced by anti-IgE was 34.5 ± 7.8% for histamine and 39.5 ± 7.9 ng/10^6 cells for LTC_{4}. Values are the mean ± SE of six experiments.
mizolastine produced up to twofold larger quantities of IL-4 as compared to cells stimulated with anti-IgE alone. Mizolastine alone did not induce IL-4 release from basophils. Under the same incubation conditions, i.e., in IMDM containing FCS, mizolastine efficiently inhibited LTC4 release with an IC50 of 3.55 ± 0.43 μM.

Table 1. Effect of mizolastine on IL-4 release induced by anti-IgE from human basophils

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<tr>
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<td>139.6</td>
<td>38.3</td>
<td>50.7</td>
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Basophils were pre-incubated (20 min, 37°C) with mizolastine and were then challenged (4 h, 37°C) with anti-IgE (0.3 μg/mL). At the end of the incubation, supernatants were collected and centrifuged (1000 g, 22°C, 5 min) for subsequent determination of IL-4 release. The values are the mean of duplicate determinations of four experiments with different basophil preparations. ND: not detectable.

Fig. 2. Reversibility of the effects of mizolastine on histamine (upper panel) and LTC4 (lower panel) release from human basophils. Basophils were pre-incubated (20 min, 37°C) with mizolastine. An aliquot of cells was washed (3 × ) with P buffer at 4°C. Unwashed and washed cells were then challenged (30 min, 37°C) with anti-IgE (0.3 μg/mL). Values are the mean ± SE of four experiments. *P < 0.05 vs. anti-IgE alone. § P < 0.01 vs. anti-IgE alone.

Fig. 3. Effect of mizolastine on histamine (white columns, left axis) and LTC4 (black columns, right axis) release induced by fMLP from human basophils. Basophils pre-incubated (20 min, 37°C) with mizolastine were challenged (30 min, 37°C) with fMLP (1 μM). Values are the mean ± SE of four experiments. *P < 0.05 vs. fMLP alone. § P < 0.01 vs. fMLP alone.

Kinetics experiments revealed that a pre-incubation time of 20 min was optimal for both the enhancement of histamine and IL-4 secretion and the inhibition of LTC4 release (data not shown).

We next determined whether the effects of mizolastine on mediator release from human basophils were reversible. When we washed the cells pre-incubated with mizolastine (10⁻⁷–10⁻⁵ M) before the addition of anti-IgE, both the enhancement of histamine release (Fig. 2, upper panel) and the inhibition of LTC4 production (lower panel) were completely reverted. Data are shown in the concentration range of 10⁻⁶–10⁻⁵ M. No effect of mizolastine was detected at concentrations lower than 10⁻⁸ M and no additional effect was observed at concentrations higher than 10⁻⁵ M.

To understand whether the effect of mizolastine on mediator release from basophils was stimulus-specific, i.e., restricted to IgE-mediated activation, we stimulated the cells pre-incubated with mizolastine with fMLP. fMLP induces histamine release from human basophils by activating a G-protein-coupled, seven transmembrane cell receptor (FPR) unrelated to the IgE receptor [31]. Mizolastine did not affect fMLP-induced histamine release, whereas it concentration-dependently inhibited the fMLP-induced de novo synthesis of LTC4 with an IC50 of 1.86 ± 0.24 μM (Fig. 3). Data are shown in the concentration range of 10⁻⁶–10⁻⁵ M. No effect of mizolastine was detected at concentrations lower than 10⁻⁸ M and no additional effect, i.e., no effect on histamine release, was observed at concentrations up to 10⁻⁴ M.

Effects of mizolastine on mediator release from human lung mast cells

Mediator release from human basophils and mast cells is, at least in part, differentially regulated [27]. We therefore examined the effects of mizolastine on the release of histamine and eicosanoids from mast cells purified from human lung tissue. In these experiments, purified mast cells were pre-incubated (20 min, 37°C) with increasing concentrations of mizolastine (10⁻⁷–10⁻⁵ M) and then stimulated with an optimal concentration of anti-IgE (1 μg/mL). Figure 4 shows that mizolastine inhibited anti-IgE-induced LTC4 release
from human lung mast cells in a concentration-dependent manner. At $10^{-5}$ M, mizolastine completely blocked LTC4 release from lung mast cells. The IC$_{50}$ for LTC4 inhibition in mast cells (3.92 ± 0.41 μM) was similar to that found in basophils challenged with anti-IgE. These data indicate that mizolastine inhibits IgE-mediated release of LTC4 from human basophils and mast cells to a similar extent.

Immunologically challenged human mast cells metabolize arachidonic acid also via the cyclooxygenase pathway, the main metabolite being PGD$_2$ [34]. Interestingly, pre-incubation (20 min, 37°C) with increasing concentrations of mizolastine ($10^{-7}$–$10^{-5}$ M) did not affect PGD$_2$ release from human lung mast cells stimulated with anti-IgE (unstimulated: 2.34 ± 0.46 ng of PGD$_2$/10$^6$ mast cells; anti-IgE: 57.2 ± 7.8; mizolastine $10^{-5}$ M + anti-IgE: 55.1 ± 8.6), even at concentrations that completely suppressed LTC4 production.

Figure 5 shows that mizolastine did not affect IgE-mediated histamine release from lung mast cells at concentrations up to $10^{-5}$ M, even with a pre-incubation time of 60 min.

**Influence of cytosolic PLA$_2$ inhibition on the modulation of mediator release by mizolastine**

Our findings that mizolastine inhibits LTC4 production in basophils and mast cells without affecting the cyclooxygenase pathway, and that mizolastine selectively enhances IgE-mediated histamine release from basophils are compatible with the hypothesis that mizolastine blocks 5-LO activity in basophils and mast cells. Enzyme 5-LO has two functions: it oxidizes arachidonic acid to endoperoxides (5-HPETE and 5-HETE), and then converts endoperoxides to LTA$_4$. Previous studies showed that 5-HPETE and 5-HETE potentiate histamine release from human basophils and rat mast cells selectively activated by IgE-mediated stimuli (anti-IgE or antigen) [37–39]. Therefore, if mizolastine selectively blocked the second function of 5-LO, 5-HPETE and 5-HETE would accumulate and this would enhance histamine release.

To test this hypothesis, we used pyrrolidine-1, a novel, selective inhibitor of cytosolic PLA$_2$ [30]. Cytosolic PLA$_2$ is the main enzyme involved in arachidonic acid mobilization in immunologically stimulated human basophils [40]. In preliminary experiments, pre-incubation with pyrrolidine-1 ($10^{-5}$ M) inhibited arachidonic acid mobilization in anti-IgE-stimulated basophils by 98.2 ± 2.4%. This concentration of pyrrolidine-1 was not cytotoxic since more than 99% of the cells remained viable at the end of the experiment, as assessed by trypan blue exclusion. Under these conditions, basophils are not expected to produce 5-HPETE or 5-HETE. Figure 6 shows that pyrrolidine-1 ($10^{-5}$ M) completely suppressed LTC4 production from anti-IgE-stimulated basophils. As previously shown, mizolastine ($10^{-5}$ M) also inhibited LTC4 release from anti-IgE-activated basophils. The combination of pyrrolidine-1 and mizolastine did not alter the inhibitory effect of each compound. In contrast, pyrrolidine-1 per se did not affect anti-IgE-induced histamine release from basophils (Fig. 7). In addition, pre-incubation with pyrrolidine-1 did not alter the enhancement of histamine release induced by mizolastine (Fig. 7). These data are compatible with the hypothesis that the accumulation of arachidonic acid metabolite intermediates of the 5-LO pathway is unrelated to the potentiating effect exerted by mizolastine on IgE-mediated histamine release from basophils.

**Effect of mizolastine on anti-IgE-induced phosphorylation of ERK-1 and -2**

Binding of anti-IgE to FcεRI-linked IgE has been shown to activate intracellular signalling through the ERK 1/2 pathway [35]. The activation of this pathway has been shown to be crucial for LTC4 synthesis in human basophils [35]. To investigate the effect of mizolastine on this intracellular pathway, we pre-incubated (20 min, 37°C) cells with mizolastine ($10^{-6}$ and $10^{-5}$ M) and then stimulated (5 min, 37°C) them with anti-IgE (0.3 μg/mL). The activation of basophils with anti-IgE induced the phosphorylation of both ERK-1 and -2 (Fig. 8). At concentrations that efficiently inhibited LTC4 synthesis, mizolastine had no effect on phosphorylation of ERKs (Fig. 8).
Discussion

Second-generation H1 antihistamines remain a cornerstone in the treatment of allergic rhinitis and urticaria [41, 42]. Some display anti-inflammatory properties unrelated to H1 receptor antagonism and mostly based on interference with the production and/or release of mediators such as histamine, cysteinyl leukotrienes, PAF and cytokines [9, 11–14]. However, the clinical relevance of these effects in vivo is presently unclear [43]. The present study demonstrates that mizolastine, a second-generation H1 blocker, inhibits LTC4 production in anti-IgE-stimulated human basophils and lung mast cells without affecting PGD2 production (in mast cells). In contrast, mizolastine potentiates histamine (up to 80%) and IL-4 (up to 130%) release only from basophils selectively activated by anti-IgE. These effects of mizolastine are very rapid and are completely reversed if the drug is removed before cell activation. The observation that mizolastine suppresses LTC4 but not PGD2 production in mast cells suggests that the drug inhibits 5-LO activity in human inflammatory cells, which is in agreement with the studies of rodent cells [21] and of mixed populations of human nasal polyp cells [25].

A novel finding of our study is the enhancement of histamine and IL-4 release caused by mizolastine from human basophils challenged with IgE-mediated stimuli. This effect occurs at exactly the same concentrations that inhibit LTC4 production. In addition, the enhancing effect on histamine release caused by mizolastine appears to be cell- and stimulus-specific, being absent from mast cells and...
restricted to IgE-activated basophils. Our data depict a clearly divergent effect of mizolastine on the release of histamine and IL-4 vs. that of LTC₄. This is in agreement with observations showing that the intracellular signals involved in LTC₄ production in human basophils are distinct from those regulating histamine and IL-4 release [4, 35]. In this regard, it is worth pointing out that mizolastine does not influence the activation of ERK 1/2, a major signalling pathway for LTC₄ synthesis in human basophils [35]. Moreover, it has been reported that the magnitude of IL-4 production after anti-IgE-triggered activation of basophils correlates with the level of histamine release [44]. Taken together, these results reinforce the concept that the release of histamine and IL-4 from basophils is differentially regulated compared with LTC₄ release.

The inhibitory effect on LTC₄ production and the enhancement of histamine and IL-4 release by mizolastine occurs at concentrations that are close to those reached in vivo during pharmacological treatment [45]. Therefore, it is conceivable that these two effects contribute to the overall pharmacological action of mizolastine. However, the pharmacological relevance in vivo of our findings is difficult to appreciate due to the complexity of the effects exerted by mizolastine on the immunological release of mediators from human basophils and mast cells. It is tempting to speculate that the inhibitory effect of mizolastine on the release of cysteinyl leukotrienes might prevail pharmacologically over the enhancing effect on histamine release for two reasons. First, LTC₄ production is inhibited in basophils, mast cells and presumably other cells synthesizing this mediator (e.g., eosinophils), whereas histamine release is enhanced only in basophils. Second, the enhancing effect on IgE-mediated histamine release should be antagonized by the H₁-blocking effect of mizolastine. However, it is possible that the enhanced release of histamine may induce in vivo a stronger stimulation of other receptors, such as H₂, that are expressed on monocytes [46], macrophages [47] and dendritic cells [48]. These observations raise the possibility that in vivo administration of mizolastine to allergic patients might have multiple and complex effects not only on basophils and mast cells but also on other immune cells that express histamine H₂, H₃ and H₄ receptors [5, 46–48].

The finding that mizolastine inhibits histamine release from rat and guinea-pig mast cells [23, 24], but not from human lung mast cells, is further evidence that human mast cells react in a unique manner to various stimuli and inhibitors. In addition, our results reinforce the concept that rodent mast cells are immunologically, biochemically and pharmacologically different from human mast cells [1]. Therefore, pharmacological results obtained with rodent mast cells cannot be automatically translated to human systems.

The enhancing effect of mizolastine on histamine release is another facet of the functional heterogeneity of human basophils and mast cells. Our data demonstrate that mizolastine potentiates the histamine release pathway activated by anti-IgE in basophils but not in mast cells. Other compounds, such as non-steroidal anti-inflammatory agents, deuterium oxide and cytochalasin B, which are not activating stimuli per se, amplify histamine secretion from basophils but not from mast cells by regulating different steps in the secretory process [49, 50]. Interestingly, none of these compounds inhibit LTC₄ synthesis in human basophils and mast cells. Thus, mizolastine is an additional biochemical tool with which to investigate differences in the mechanisms of histamine release between human basophils and mast cells.

Metabolites of arachidonic acid generated through 5-LO, particularly 5-HPETE and 5-HETE, modulate IgE-mediated histamine release from human basophils [38, 39]. Interestingly, 5-HPETE enhances histamine release from human basophils but not mast cells. This prompted the notion that mizolastine blocks only the LTA₄ synthase activity of 5-LO thereby leading to 5-HPETE accumulation. To test this hypothesis, we used a novel, specific inhibitor of cytosolic PLA₂, pyrrolidine-1 [51]. This molecule completely blocked arachidonic acid mobilization and suppressed LTC₄ formation from anti-IgE-stimulated basophils. However, pyrrolidine-1 did not revert the enhancing effect of mizolastine on histamine release, which shows that this effect was not caused by 5-LO metabolic intermediates. Further studies are required to clarify the biochemical mechanisms of the selective enhancing effect of mizolastine on histamine release and to define the biochemical basis of its cell and stimulus specificity.

This study underscores the complexity of the anti-inflammatory activities of certain antihistamines. The effects of antihistamines cannot be predicted and new antihistamines should be investigated in appropriate models. Mizolastine appears rather unique among other antihistamines since its anti-inflammatory activity depends strictly on the cell examined (basophil vs. mast cell), on the mediator produced (eicosanoids vs. histamine and IL-4) and the type of stimulation (IgE-mediated vs. FMLP-mediated). These results have both biochemical and clinical implications. They support the concept of a differential regulation of histamine
release and eicosanoid synthesis in human basophils and mast cells and suggest that mizolastine may be a pharmacological tool with which to explore the regulatory steps in immunologically activated basophils. From a clinical viewpoint, the mizolastine-induced inhibition of leukotriene synthesis in cells relevant to the pathogenesis of allergic disorders may help to clarify further the mechanism by which this drug exerts its beneficial effect in urticaria and allergic rhinitis [18, 19]. The enhancement of IgE-mediated histamine and IL-4 release from basophils may have an impact on the overall pharmacological activity of mizolastine and should be taken into account when evaluating its long-term therapeutic effects in allergic diseases.

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