

Loratadine and desethoxycarbonyl-loratadine inhibit the immunological release of mediators from human FcεRI⁺ cells

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Summary

Background Loratadine, a novel histamine H₁-receptor antagonist, is effective in the treatment of patients with seasonal and perennial rhinitis and some allergic skin disorders. Histamine and other chemical mediators are synthesized and immunologically released by human peripheral blood basophils and tissue mast cells (FcεRI⁺ cells).

Objective To evaluate the effects of loratadine and its main metabolite, desethoxycarbonyl-loratadine (des-loratadine), on the immunological release of preformed (histamine and tryptase) and *de novo* synthesized mediators (leukotriene C₄: LTC₄ and prostaglandin D₂: PGD₂) from human FcεRI⁺ cells.

Methods Human FcεRI⁺ cells purified from peripheral blood and from skin (HSMC) and lung tissue (HLMC) were preincubated with loratadine and des-loratadine before immunological challenge with Der p 1 antigen or anti-FcεRI. The release of preformed mediators (histamine and tryptase) and *de novo* synthesized eicosanoids was evaluated in the supernatants of human FcεRI⁺ cells.

Results Preincubation (15 min, 37 °C) of purified (36–74%) basophils with loratadine (3×10^{-6} – 10^{-4} M) and des-loratadine before Der p 1 antigen or anti-FcεRI challenge concentration-dependently (5–40%) inhibited the release of histamine and LTC₄. Loratadine (3×10^{-6} – 10^{-4} M) and des-loratadine also inhibited (10–40%) histamine, LTC₄, and PGD₂ release from purified HLMC (16–68%) activated by anti-FcεRI. Loratadine (3×10^{-6} – 10^{-4} M) and des-loratadine caused concentration-dependent inhibition (10–40%) of histamine, tryptase, LTC₄, and PGD₂ release from purified HSMC (24–72%) immunologically challenged with anti-FcεRI.

Conclusion These results indicate that loratadine and its main metabolite have anti-inflammatory activity by inhibiting the release of preformed and *de novo* synthesized mediators from human FcεRI⁺ cells.

Keywords: basophils, histamine, leukotrienes, loratadine, mast cells, tryptase

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Introduction

Basophil granulocytes and tissue mast cells are the only cells synthesizing histamine and possessing high-affinity receptors for immunoglobulin-E (IgE) (FcεRI) [1,2].

Although the physiological role of human FcεRI⁺ cells has remained largely elusive, it has been suggested they play a role in the pathogenesis of several inflammatory disorders [1–3]. Mast cells and basophils play a role in allergic disorders affecting the upper and lower respiratory tract and the skin, through different immunological and inflammatory mechanisms. Human FcεRI⁺ cells may modulate the immune response through the release of

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various cytokines [4,5] or through interaction with the CD40 ligand on their surface [6]. In addition, human Fc ϵ RI⁺ cells play a considerable proinflammatory role through the release of preformed (histamine, tryptase, and chymase) [7,8] and the *de novo* synthesis of eicosanoids (prostaglandin D₂; PGD₂ and peptide-leukotriene C₄: LTC₄) [9] and of PAF [10]. Histamine is an important proinflammatory and vasoactive mediator in allergic rhinitis, urticaria and asthma [11,12]. Tryptase, a neutral protease almost exclusively synthesized by mast cells, may play a role through different mechanisms [13]. Lipid mediators synthesized *de novo* by human Fc ϵ RI⁺ cells presumably also play an important role in allergic disorders of the skin [14] and respiratory tract [15]. In 1949 Herxheimer showed that anti-histamines could be useful in the management of asthma [16]. However, the first generation of histamine-H₁ receptor antagonists were not considered of value because high doses resulted in side effects, which limited their use intravenously or by aerosol [17–19]. However, histamine-H₁ receptor antagonists are effective in the treatment of allergic rhinitis and allergic skin disorders [20,21]. Lichtenstein and Gillespie reported that several classes of H₁ receptor antagonists inhibited antigen-induced histamine release from human basophils [22]. This observation has since been confirmed and extended [23–27]. There is also evidence that high concentrations of histamine H₁ receptor antagonists *per se* can induce histamine release from human Fc ϵ RI⁺ cells [22,23]. Loratadine is a novel H₁-specific receptor antagonist effective in the treatment of allergic rhinitis in children [28] and adults [20,21,29], and chronic urticaria [30]. There is preliminary evidence that loratadine inhibits the release of slow reacting substance (SRS) from human lung fragments without affecting histamine release [31]. In addition, loratadine and its active metabolite inhibit the release of histamine from human basophils *in vitro* [32,33]. *In vivo*, loratadine modulates the release of histamine, tryptase, and PGD₂ after nasal challenge [34,35] and the late-phase reaction in conjunctiva [36]. These findings suggested that loratadine might influence the release of vasoactive mediators from human Fc ϵ RI⁺ cells. This study evaluated the effect of loratadine and its active metabolite (desethoxycarbonyl-loratadine: des-loratadine) *in vitro* on the immunological release of preformed and *de novo* synthesized mediators from human basophils and mast cells purified from human lung parenchyma and skin tissue.

Materials and methods

Reagents and buffers

HClO₄ (Baker Chemical Co., Deventer, The Netherlands), BSA, piperazine-N,N'-bis (2-ethanesulfonic acid), hyaluronidase, collagenase type II, chymopapain, elastase type I,

synthetic LTC₄ and PGD₂ (Sigma Chemical Co., St Louis, USA), Hanks' balanced salt solution (HBSS), fetal calf serum (FCS) (GIBCO, Grand Island, New York, USA), deoxyribonuclease I and pronase (Calbiochem, La Jolla, California, USA), RPMI 1640 with 25 mM Hepes buffer, Eagle's minimum essential medium (Flow Laboratories, Irvine, UK), Dextran 70, Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) (³H)-LTC₄ (39.3 Ci/mmol) and (³H)-PGD₂ (210 Ci/mmol) (New England Nuclear, Boston, Massachusetts, USA), gelatin (Serva Feinbiochemica, Heidelberg, Germany), Nytex cloth (Tetko, Elmsford, New York, USA) were obtained commercially. Loratadine and des-loratadine were kindly donated by Schering-Plough (Milan, Italy). Rabbit anti-human-Fc ϵ antibody was a generous gift from Drs Teruko and Kimishige Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, California, USA). Monoclonal antibody anti-Fc ϵ RI (29C6) was kindly donated by Dr J. Hakimi (Hoffmann-LaRoche, Nutley, New Jersey, USA) [37]. Rabbit anti-sulfidopeptide leukotriene C₄ was a generous gift from Dr E. J. Kusner (Zeneca Pharmaceuticals, Wilmington, Detroit, USA) [38]. Rabbit anti-PGD₂ antisera was provided by Dr L. M. Lichtenstein (Johns Hopkins University, Baltimore, Maryland, USA). Tryptase radioimmunoassay kits (Pharmacia Tryptase RIACT 50, Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) were kindly donated by Kabi Pharmacia Diagnostics (Milan, Italy). The Pipes buffer used in these experiments was made up of 25 mM Pipes, 110 mM NaCl, 5 mM KCl, pH 7.4, referred to as P [8]. PC contained 1 mM CaCl₂ [9], in addition to P; pH was titrated to 7.4 with sodium bicarbonate. PGMD contained 0.25 g/L MgCl₂ × 6H₂O, 10 mg/L DNase, and 1 g/L gelatin in addition to P, pH 7.4.

Peripheral blood leucocytes containing basophils and basophil purification

Venous blood from normal and *Dermatophagoides*-allergic volunteers aged 24–44 years (mean age 36.5 ± 4.1 years) was drawn into a final concentration of 0.008 M ethylenediaminetetra-acetic acid (EDTA) and 1.1% dextran T 70. Erythrocytes were allowed to settle (90 min at 22 °C) and the leucocyte-rich upper layer was drawn off, pelleted (200 × g, 8 min, 4 °C), and washed twice in P as previously described [39]. This preparation, containing about 0.5% basophils, is hereafter referred to as 'basophils'. Contaminating cells were lymphocytes, neutrophils, eosinophils, monocytes and platelets. Basophils were purified as previously described [40]. In brief, 'buffy coat' cell packs were reconstituted in phosphate-buffered saline (PBS) containing 0.5 g/L human serum albumin (HSA) and 3.42 g/L Na citrate, and loaded onto a countercurrent elutriator (model J2–21, Beckman Instruments Inc., Fullerton, California, USA). Several fractions were collected and fractions

containing basophils ($>20 \times 10^6$ basophils) of improved purity ($>15\%$) were further enriched by discontinuous Percoll gradients. Yields by this technique ranged from 3 to 8×10^6 basophils and purities from 75 to 90%, as assessed by basophil staining with Alcian blue [40].

Isolation and purification of human lung and skin mast cells

Macroscopically normal lung tissue obtained from patients undergoing thoracotomy and lung resection, mostly for lung cancer, was dissected free from pleura, bronchi and blood vessels, minced into 5–10 mm fragments and dispersed into single-cell suspension as described [40]. Yields with this technique ranged between 3×10^6 and 20×10^6 lung mast cells, and purities between 1% and 8%. Skin tissue from patients undergoing mastectomies for breast cancer was immediately placed in Eagle's minimum essential medium (MEM) (4 °C) and used within 3 h. The skin, separated from the subcutaneous fat by blunt dissection, was cut into 1–2 mm fragments with scissors. The fragments, washed twice in Ca^{2+} - and Mg^{2+} -free HBSS (22 °C), were incubated 3 h at 37 °C under constant stirring in Ca^{2+} - and Mg^{2+} -free HBSS containing 2500 U collagenase, 5 mg hyaluronidase, and 1000 U deoxyribonuclease I per g of wet tissue [39]. Isolated cells were separated from the partially digested tissue fragments by filtration through Nytex cloth (150- μ pore size) and stored (Tetko, Elmstard, New York). The remaining tissue was digested a second time for 2 h at 37 °C. Cells were separated from the tissue fragments by filtration through 150- μ pore Nytex cloth, washed twice, combined with the cells from the first digestion, and washed again in Ca^{2+} - and Mg^{2+} -free HBSS. Yields with this technique ranged between 1×10^6 and 20×10^6 skin mast cells, and purities between 1 and 6%. Mast cells were partially purified by a multidensity Percoll gradient [39]. Mast cells were stained with Alcian blue and counted in a Spiers-Levy eosinophil counter [40].

Histamine release assay

Basophils ($\approx 6 \times 10^4$ basophils/tube), purified or not purified, or mast cells ($\approx 3 \times 10^4$ cells/tube), were resuspended in PC, and 0.4 mL of the cell suspension was placed in 12 $\times$ 75 mm polyethylene tubes (Sarstadt Inc., Princeton, New Jersey, USA) and warmed to 37 °C (basophils and HLMC) or to 30 °C (HSMC); 0.2 mL of each prewarmed stimulus for release was added, and incubation was continued at 37 °C for 60 min (basophils) or for 20 min (mast cells). Experiments with HSMC were performed at 30 °C, close to the temperature of the human skin, rather than 37 °C because it has been shown that these cells reach optimal mediator release at 30 °C [41]. The reaction was stopped by centrifugation (1000 $\times g$, 22 °C, 2 min), and the cell-free

supernatants were stored at -70 °C for subsequent assay of histamine and LTC_4 content. Histamine was assayed with an automated fluorometric technique [42]. To calculate histamine release as a percentage of total cellular histamine, the 'spontaneous' release from basophils or mast cells (0–10% of the total cellular histamine) was subtracted from both numerator and denominator. The percentage histamine release was calculated according to the following equation:

$$\frac{A-B}{T-B} \times 100$$

where A is the sample, B the spontaneous histamine release and T is the total histamine content [39]. All values are based on means of duplicate or triplicate determinations. Replicates differed from each other by less than 10% in histamine content. Radioimmunoassay of PDG_2 , LTC_4 and tryptase PGD_2 and LTC_4 were analysed on 100 μL fractions of the supernatant fluids. The PGD_2 and LTC_4 assays were carried out by a previously described RIA [9,39]. Tryptase was assayed by a solid-phase immunoassay (Pharmacia Tryptase RIACT 50) [7].

Statistical analysis

Results are expressed as the mean $\pm$ SEM. Statistical significance was analysed by two-way non-parametric analysis of variance (Friedman χ^2 test); the level of significance was $P < 0.05$ [43].

Results

Effect of loratadine and des-loratadine on antigen- and anti-Fc ϵ RI-induced mediator release from basophils

Aggregation of the receptor-bound IgE on basophils triggers a series of signal transduction events which culminate in the release of histamine and the *de novo* synthesis of LTC_4 [24]. Aggregation of the IgE-Fc ϵ RI network can be induced by multivalent antigens [8], by bivalent anti-IgE antibody [40], and by a monoclonal antibody against the α chain of Fc ϵ RI [7,11]. There are biochemical differences between the activating properties of multivalent antigens and bivalent ligands [44]. We evaluated the *in vitro* effects of pharmacological concentrations of loratadine (3×10^{-6} – 10^{-4} M) on the release of histamine and the *de novo* synthesis of LTC_4 from human basophils challenged *in vitro* with Der p 1, a major allergen of *Dermatophagoides pteronyssinus* [8]. Preincubation (15 min, 37 °C) of enriched preparations (36–74%) of peripheral blood basophils with loratadine concentration-dependently inhibited the release of histamine and LTC_4 from antigen-activated cells (Fig. 1). Maximal inhibition of histamine release of $59.9 \pm 3.9\%$ was obtained with 10^{-4} M loratadine, with an IC_{50} of $5.5 \pm 1.5 \times 10^{-5}$ M, comparable to the inhibition of Der p

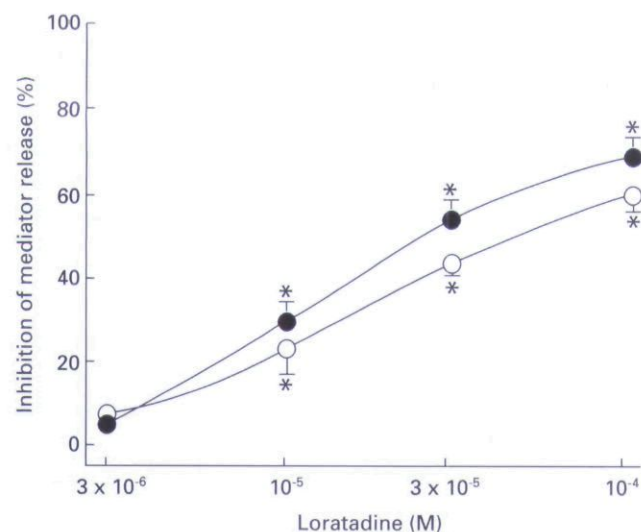


Fig. 1. Effect of increasing concentrations of loratadine on Der p 1-induced release of histamine (○) and LTC₄ (●) from basophils. Cells were preincubated (15 min, 37 °C) with the concentrations of loratadine indicated, then challenged with Der p 1 (0.1 µg/mL) (60 min, 37 °C). Each bar represents the mean ± SEM of four preparations in which Der p 1 induced the release of $34.1 \pm 3.2\%$ of total histamine and 47.7 ± 5.5 ng of LTC₄/10⁶ basophils. **P* < 0.01 compared with mediator release induced by Der p 1 alone.

1-induced release of LTC₄ ($IC_{50} = 3.1 \pm 0.5 \times 10^{-5}$ M). Loratadine alone (3×10^{-6} – 10^{-4} M) did not modify the spontaneous release of histamine from basophils. Concentrations of loratadine higher than 3×10^{-4} M caused histamine release (data not shown). Autoantibodies against the IgE-FcεRI network occur naturally in some patients with allergic disorders [45–47] and *in vitro* [11,47] as well as *in vivo* [11] they induce the release of mediators from human FcεRI⁺ cells. We investigated the effect of increasing concentrations of loratadine on anti-FcεRI-induced

activation of human basophils. In a series of three experiments with purified (49–78%) basophils loratadine (3×10^{-6} – 10^{-4} M) concentration-dependently (5–60%) inhibited the release of histamine and LTC₄ (Table 1). The concentrations inhibiting mediator release by 50% were $8.5 \pm 2.5 \times 10^{-5}$ M (histamine) and $6.9 \pm 1.6 \times 10^{-5}$ M (LTC₄). Loratadine is metabolized primarily to the water-soluble compound, des-loratadine [48], reported to be more active than the parent compound in binding to the histamine H₁-receptor [49]. We therefore investigated the potential inhibitory effect of des-loratadine on mediator release from human basophils (46–64% pure) challenged *in vitro* with Der p 1 and anti-FcεRI. The results presented in Table 2 show that the release of histamine and the *de novo* synthesis of LTC₄ induced by cross-linking of the IgE-FcεRI network by antigen Der p 1 were inhibited in a dose-dependent fashion. Inhibition was seen at concentrations greater than 3×10^{-6} M, reaching a maximum at 10^{-4} M. Concentrations of des-loratadine higher than 10^{-4} M caused histamine release from basophils (data not shown). Similar results were obtained in three experiments in which cells were challenged with anti-FcεRI (data not shown). Effect of loratadine and des-loratadine on the release of preformed and *de novo* synthesized mediators from HLMC Aggregation of FcεRI⁺ on HLMC induced the release of preformed (histamine and tryptase) and the *de novo* synthesis of PGD₂ and LTC₄ [40]. We investigated whether preincubation (15 min, 37 °C) with loratadine and its metabolite (3×10^{-6} – 10^{-4} M) inhibited the release of preformed and *de novo* synthesized mediators from enriched preparations (18–68%) HLMC. Figure 2 shows that loratadine concentration-dependently inhibited the release of histamine and of eicosanoids induced by anti-IgE. Inhibition of histamine ($54.6 \pm 9.4\%$) and LTC₄ ($69.5 \pm 3.3\%$) was maximal at 10^{-4} M loratadine, with IC_{50} of $5.5 \pm 1.7 \times 10^{-5}$ M and $2.2 \pm 0.4 \times 10^{-5}$ M. In these experiments des-loratadine

Table 1. Effect of loratadine on the release of histamine and LTC₄ from human basophils induced by anti-FcεRI*

Drug (M)	Exp. No. 1		Exp. No. 2		Exp. No. 3	
	% H.R.	LTC ₄ (ng/10 ⁶ cells)	% H.R.	LTC ₄ (ng/10 ⁶ cells)	% H.R.	LTC ₄ (ng/10 ⁶ cells)
No drug	46	62.0	42	62.3	60	76.3
3×10^{-6}	45	58.6	43	50.1	58	70.9
10^{-5}	34	46.2	34	44.2	55	55.6
3×10^{-5}	26	34.4	28	29.5	45	42.1
10^{-4}	14	15.7	22	25.5	35	37.8

* Cells were preincubated (15 min, 37 °C) with the concentrations of loratadine indicated, then challenged with anti-FcεRI (10^{-1} µg/mL). H.R. Histamine release.

Table 2. Effect of des-loratadine on the release of histamine and LTC₄ from human basophils induced by Der p 1*

Drug (M)	Exp. No. 1		Exp. No. 2		Exp. No. 3	
	% H.R.	LTC ₄ (ng/10 ⁶ cells)	% H.R.	LTC ₄ (ng/10 ⁶ cells)	% H.R.	LTC ₄ (ng/10 ⁶ cells)
No drug	33	49.7	26	43.5	33	52.3
3 × 10 ⁻⁶	32	48.2	23	40.6	ND	ND
10 ⁻⁵	26	41.1	ND	ND	26	49.5
3 × 10 ⁻⁵	19	37.2	22	35.1	23	44.1
10 ⁻⁴	16	26.4	14	23.4	18	36.3

* Cells were preincubated (15 min, 37 °C) with the concentrations of des-loratadine indicated, then challenged with Der p 1 (10⁻¹ µg/mL). H.R. Histamine release. ND=Not done.

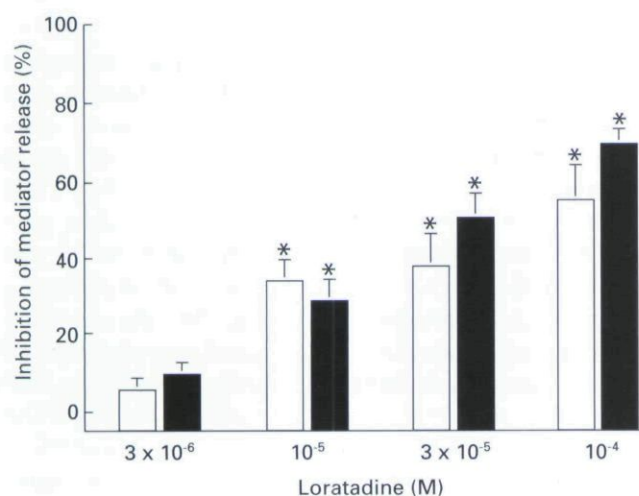


Fig. 2. Effect of increasing concentrations of loratadine on anti-IgE-induced release of histamine (□) and LTC₄ (■) from HLMC. Cells were preincubated (15 min, 37 °C) with the concentrations of loratadine indicated, then challenged with anti-IgE (2 µg/mL) (20 min, 37 °C). Each bar represents the mean ± SEM of three preparations in which anti-IgE induced the release of 38.9 ± 5.0% of total histamine and 56.5 ± 7.6 ng of LTC₄/10⁶ cells. **P* < 0.01 compared with mediator release induced by anti-IgE alone.

(3 × 10⁻⁶–10⁻⁴ M) also concentration-dependently inhibited the *de novo* synthesis of eicosanoids (PGD₂ and LTC₄) from enriched preparations (16–46%) of HLMC induced by anti-FcεRI (Table 3). Effect of loratadine and des-loratadine on the release of preformed and *de novo* synthesized mediators from HSMC. Mast cells isolated from human skin tissue contain high concentrations of tryptase (≈ 30 µg/10⁶ cells) which can be released after aggregation of receptor-bound IgE [50]. Autoantibodies to FcεRI are present in the sera of some patients with chronic idiopathic urticaria [11] and play a pathogenic role through the release of mediators from

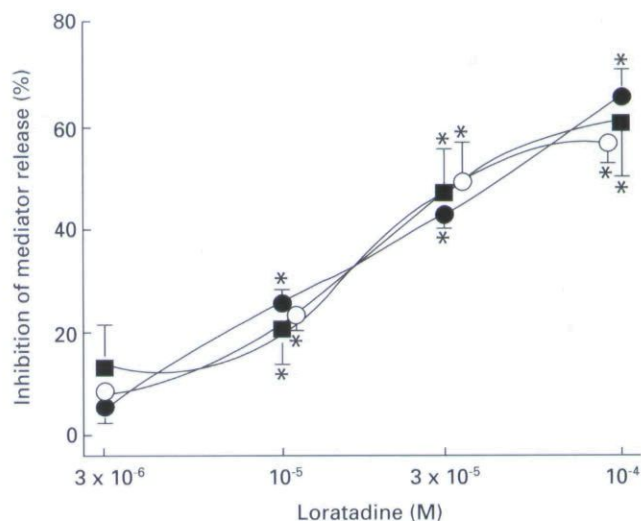


Fig. 3. Effect of increasing concentrations of loratadine on anti-FcεRI-induced release of histamine (○), tryptase (■) and PGD₂ (●) from HSMC. Cells were preincubated (15 min, 37 °C) with the concentrations of loratadine indicated, then challenged with anti-FcεRI (1 µg/mL) (20 min, 30 °C). Each bar represents the mean ± SEM of three experiments in which anti-FcεRI induced the release of 29.0 ± 6.0% of total histamine, 10.6 ± 1.1 µg of tryptase/10⁷ mast cells and 38.2 ± 1.2 ng of PGD₂/10⁶ mast cells. **P* < 0.01 compared with mediator release induced by anti-FcεRI alone.

FcεRI⁺ cells [11]. We evaluated the effect of preincubation (15 min, 30 °C) of HSMC with increasing concentrations of loratadine and des-loratadine on anti-FcεRI-induced release of histamine, tryptase and the *de novo* synthesis of eicosanoids. In three experiments loratadine (3 × 10⁻⁶–10⁻⁴ M) significantly inhibited the release of preformed (histamine and tryptase) mediators and of PGD₂ from enriched-preparations (24–72%) of HSMC activated by anti-FcεRI (Fig. 3). Maximal inhibition of histamine (62.3 ± 4.2%),

Table 3. Effect of des-loratadine on the release of histamine, PGD₂, and LTC₄ from HLMC induced by anti-FcεRI*

Drug (M)	Exp. No. 1		Exp. No. 2			Exp. No. 3		
	% H.R.	LTC ₄ (ng/10 ⁶ cells)	% H.R.	PGD ₂ (ng/10 ⁶ cells)	LTC ₄ (ng/10 ⁶ cells)	% H.R.	PGD ₂ (ng/10 ⁶ cells)	LTC ₄ (ng/10 ⁶ cells)
No drug	27	41.2	45	63.8	82.5	24	52.6	48.9
3 × 10 ⁻⁶	25	40.2	45	52.1	76.2	21	45.1	46.1
10 ⁻⁵	16	36.1	40	48.2	64.2	21	28.4	37.2
3 × 10 ⁻⁵	17	27.3	38	25.6	51.6	11	23.5	26.4
10 ⁻⁴	12	18.5	23	18.7	32.4	6	15.7	14.7

* Cells were preincubated (15 min, 37 °C) with the concentrations of des-loratadine indicated, then challenged with anti-FcεRI (1 µg/mL). H.R. Histamine release.

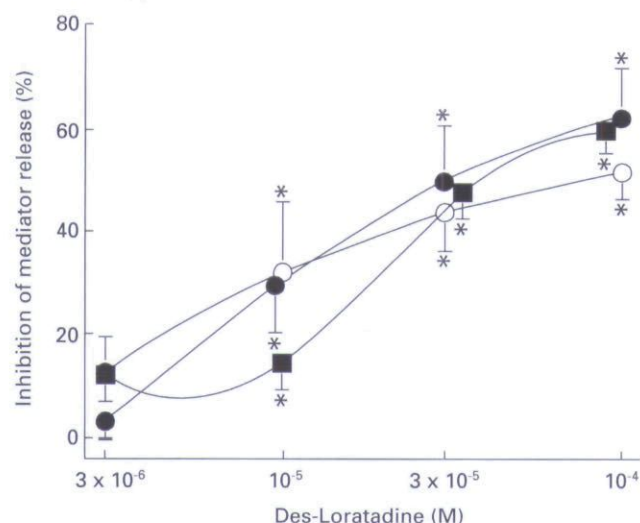


Fig. 4. Effect of increasing concentrations of des-loratadine on anti-FcεRI-induced release of histamine (○), tryptase (■) and PGD₂ (●) from HSMC. Cells were preincubated (15 min, 30 °C) with the concentrations of des-loratadine indicated, then challenged with anti-FcεRI (1 µg/mL) (20 min, 30 °C). Each bar represents the mean ± SEM of four experiments in which anti-FcεRI induced the release of 27.2 ± 1.0% of histamine, 9.8 ± 0.8 µg of tryptase/10⁷ cells and 34.8 ± 10.6 ng of PGD₂/10⁶ mast cells. *P* < 0.01 compared with mediator release induced by anti-FcεRI alone.

tryptase (68.2 ± 6.7%), and PGD₂ (56.1 ± 6.6%) was obtained at 10⁻⁴ M loratadine, with IC₅₀ of 5.1 ± 2.4 × 10⁻⁵ M, 5.4 ± 2.7 × 10⁻⁵ M, and 5.5 ± 0.8 × 10⁻⁵ M, respectively. In another series of three experiments, des-loratadine (3 × 10⁻⁶–10⁻⁴ M) caused concentration-dependent inhibition of anti-FcεRI-induced histamine, tryptase, and PGD₂ release from HSMC (29–47% pure), with maximal inhibition of 52.0 ± 5.5%, 59.7 ± 4.6%, and 62.3 ± 9.9% at 10⁻⁴ M (Fig. 4).

Discussion

The results of this study indicate that pharmacological concentrations of loratadine and its active metabolite exert antiinflammatory activity by inhibiting the release of pre-formed and *de novo* synthesized mediators from human basophils and mast cells. Clinical studies have shown that loratadine is effective in the treatment of patients with allergic seasonal and perennial rhinitis [20,21,28,29] and chronic urticaria [30], two conditions in which histamine release *in vivo* is important [8,11]. Therefore, the inhibitory effect of loratadine and its active metabolite on mediator release from human basophils and mast cells might contribute to its clinical efficacy in these allergic disorders. Our results also show that pharmacological concentrations of loratadine inhibit the IgE-mediated release of *de novo* synthesized mediators (LTC₄ and PGD₂) from human basophils and mast cells purified from human lung and skin tissue. These cells and their immunologically released eicosanoids play a role in the early and late phases of allergic disorders [12]. Therefore, these findings provide additional evidence of the anti-inflammatory activity of loratadine. It has been recently reported that loratadine reduces allergen-induced mucosal output of tryptase in patients with allergic rhinitis [35]. This suggests the drug might attenuate mast cell release *in vivo*. We found that loratadine and its metabolite *in vitro* inhibited the release of tryptase from human mast cells. Substantial amounts of tryptase, 10–35 pg/cell, have been found in human mast cells, depending on the subtype [7,13]. Tryptase is released together with other mast cell mediators [7] and might contribute to allergic inflammation through different biochemical mechanisms [13]. This study demonstrates for the first time that loratadine can inhibit the release of tryptase from human mast cells immunologically activated *in vitro*. Taken together, these *in vitro* and *in vivo* findings identify an additional mechanism by which loratadine can

exert anti-inflammatory activity. The biological and clinical relevance of our findings derives also from the different types of immunological stimuli employed. Basophils were activated by Der p 1, a major antigen of *Dermatophagoides pteronyssinus* [8] and anti-IgE. HLMC and HSMC were activated by anti-FcεRI. Naturally occurring anti-FcεRI [11] and anti-IgE autoantibodies [45–47] are found in patients with various allergic disorders and they may play a pathogenic role through the release of proinflammatory mediators [11,47]. Therefore, the inhibitory effect of loratadine and its active metabolite on the release of mediators from FcεRI⁺ cells caused by antigen and antibodies against the IgE-FcεRI network seems clinically important. Our findings confirm and extend previous results showing the inhibitory effects of loratadine on the release of mediators from human FcεRI⁺ cells. Klein-Tebbe *et al.* showed that loratadine and its active metabolite inhibited the immunological release of histamine from human basophils [32]. A similar inhibitory effect was observed on basophils challenged *in vitro* with IgE- and non-IgE-mediated stimuli [33]. Loratadine also inhibits peptide leukotriene release from human bronchial strips [51] and lung parenchyma [31]. Our results were obtained with enriched preparations of human basophils and mast cells, bearing out the concept that loratadine may have direct anti-inflammatory action on these cells. Several H₁-receptor antagonists can modulate the release of mediators from human FcεRI⁺ cells [22–27]. However, the inhibitory effect of loratadine and its metabolite appears to differ. First, loratadine is a potent inhibitor of mediator release from FcεRI⁺ cells whereas other antihistamines have marginal inhibitory effects [23,27]. Second, the drug and its metabolite have *in vitro* inhibitory activity at pharmacological concentrations [48]. Third, human mast cell heterogeneity has been demonstrated with respect to H₁-antihistamine modulation of IgE-dependent mediator release [27], whereas loratadine appears equally active on basophils and mast cells from different anatomical sites. Moreover, loratadine appears to exert an *in vitro* anti-inflammatory effect comparable to that of desloratadine which is its main metabolite *in vivo*. Obviously this might have clinical implications. There are several possible explanations for the anti-inflammatory effects of loratadine and its metabolite on human basophils and mast cells. Different theories have been advanced to explain the inhibition of mediator release by H₁-receptor antagonists. Antihistamines are lipophilic cationic drugs and can permeate the cell membrane, thus stabilizing FcεRI⁺ cells, preventing the perturbation caused by immunological stimuli. However, Okayama *et al.* recently found that a lack of correlation between the lipophilicity of several anti-histamines and their inhibitory effects. Alternatively, anti-histamines might interfere with a rise in the intracellular concentrations of Ca²⁺ induced by IgE-mediated stimuli [27]. Finally, there

is the possibility that certain histamine H₁-receptor antagonists inhibit calmodulin which appears to be an integral part of the mediator release process [24]. Additional studies are needed to characterize the mechanisms of loratadine's anti-inflammatory activities. Whatever the mechanism of action, though, the drug's inhibitory effects on the release of preformed and *de novo* synthesized mediators from primary effector cells of allergic inflammation, demonstrated here, might help explain its efficacy in the treatment of allergic disorders.

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