

In vivo and *ex vivo* inhibitory effects of loratadine on histamine release in patients with allergic rhinitis

Miadonna A, Cottini M, Milazzo N, Tosi D, Danzig M, Tedeschi A. *In vivo* and *ex vivo* inhibitory effects of loratadine on histamine release in patients with allergic rhinitis.

Allergy 1998; 53: 1183–1188. © Munksgaard 1998.

Background The aim of this study was to evaluate the *in vivo* and *ex vivo* effects of the H₁-antagonist loratadine on histamine release.

Methods The study was designed as a double-blind, crossover trial. Ten patients with allergic rhinitis due to *Dermatophagoides pteronyssinus* were treated with loratadine (10 mg daily p.o.) and with placebo for 1 week, with a 2-week interval between the two treatments. Nasal lavages with saline solution were done before and after challenge with the relevant allergen at the end of treatments with loratadine and placebo. Venous blood was taken after treatments, and basophil histamine release induced by anti-IgE (10 µg/ml), N-formyl-methionyl-leucyl-phenylalanine (fMLP, 1 µM), and Ca²⁺ ionophore A23187 (1 µM) was evaluated by an automated fluorometric method.

Results Treatment with loratadine attenuated early antigen-induced nasal obstruction, rhinorrhea, and itching. Nasal symptoms were accompanied by a significant histamine release in the nasal lavages collected 5 min after stimulation when the patients received placebo (median 4 ng/ml, range 1–28; $P < 0.05$). After treatment with loratadine, histamine release in the 5-min postchallenge lavages was almost abrogated (median 0.5 ng/ml, range 0–3; $P < 0.01$ vs placebo). Median anti-IgE-induced histamine release from basophils was 41.9% (range 27.8–79.2) after placebo and 30.0% (range 1.7–73.3, $P < 0.05$) after loratadine. Active treatment exerted an inhibitory effect also on basophil histamine release induced by fMLP and Ca²⁺ ionophore A23187.

Conclusions Treatment for 1 week with loratadine reduces allergen-induced nasal symptoms and inhibits *in vivo* and *ex vivo* histamine release in patients with allergic rhinitis.

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Key words: allergic rhinitis; basophil; histamine release; loratadine.

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Accepted for publication 30 July 1998

Loratadine is a nonsedating H₁-antagonist (1, 2) which has been successfully employed in the treatment of allergic disorders; in particular, allergic rhinitis and urticaria (3–5). Recent studies have shown that loratadine is not a simple H₁-antagonist, and that it has a more complex antiallergic activity. Studies in guinea pigs or rats sensitized to ovalbumin have demonstrated that loratadine can block antigen-induced bronchoconstriction in a dose-dependent fashion (6). *In vitro* studies have shown that loratadine can inhibit histamine and leukotriene C₄ (LTC₄) release from rat and human lung mast cells (7, 8). We and others

have found that loratadine can reduce *in vitro* histamine and LTC₄ release from human basophils stimulated by IgE-dependent (anti-IgE) and IgE-independent (fMLP, Ca²⁺ ionophore A23187) agents (9–11). The antiallergic activity of loratadine has also been reported in clinical studies in man using skin prick tests and nasal challenge methods (12–14).

This study aimed to determine whether loratadine maintains its inhibitory effect on histamine release from mast cells and basophils when administered orally to patients with allergic rhinitis. We investigated the effect of 1-week treatment

with 10 mg loratadine p.o. on *in vivo* allergen-induced histamine release in nasal lavage fluids and *ex vivo* basophil histamine release in patients with allergic rhinitis.

Material and methods

Subjects

Ten patients (nine men and one woman, aged 19–45 years) with allergic rhinitis due to *Dermatophagoides pteronyssinus* were included in the study. Allergic rhinitis was diagnosed on the basis of clinical history, skin prick tests, and positive nasal challenge. All patients had a positive nasal stimulation test to *D. pteronyssinus*. They were not using any systemic or local medication at the time of the study; informed consent was obtained from all subjects.

Study design

The study was designed as a randomized, double-blind, two-period, crossover trial comparing loratadine with placebo. Five patients were randomized to the treatment sequence loratadine→placebo, and five patients were randomized to the treatment sequence placebo→loratadine. Each treatment period was 1 week, separated by a 2-week washout period. All the patients received loratadine (10 mg daily p.o.) and placebo.

In vivo study

Nasal lavages with saline solution were done before and after challenge with the relevant allergen after each treatment. Nasal lavage fluids were collected according to a technique previously described (15, 16). Briefly, 5 ml of sterile saline solution (0.9% w/v NaCl) was instilled into each nostril, while patients extended their necks and abstained from breathing and swallowing. After 10 s, the subjects expelled the mixture of mucus and saline, which was collected into conical polypropylene tubes (Falcon, Becton Dickinson Labware, Oxnard, CA, USA) and maintained on ice until the end of the experiment. The antigen (2000 biologic units lyophilized, purified allergen extract, provided by Lofarma Allergeni, Milan, Italy, and diluted in saline solution) was insufflated in both nostrils by a nasal pump spray delivering a volume of 100 μ l for each puff. Nasal lavages were performed 5, 10, 20, 30, and 60 min after antigen provocation. Soon after collection, nasal lavage fluids were centrifuged at 4°C for 15 min at 300 g in order to separate the cells and the liquid phase. The supernatants were aspirated, divided in aliquots, diluted 1/2 with

perchloric acid (Carlo Erba-Pharmacia, Milan, Italy), and stored at –20°C until assayed. Histamine concentration was measured by an automated fluorometric method with a sensitivity of 1 ng/ml (17).

The subjects were asked to rate the severity of rhinorrhea, nasal obstruction, itching, and sneezing 5, 10, 20, 30, and 60 min after challenge. Scores for each symptom ranged from 0 to 4 (none=0, mild=1, moderate=2, intense=3, very intense=4). Sneezes were classified according to the number (no sneezes=0, 1–5 sneezes=1, 6–10 sneezes=2, 11–20 sneezes=3, more than 20 sneezes=4).

Ex vivo study

Peripheral venous blood was obtained from each subject after treatments. Leukocyte suspensions were prepared by dextran sedimentation of peripheral venous blood, anticoagulated with 0.01 M Na₂-ethylenediaminetetraacetate (EDTA) and mixed with 6% dextran in saline solution and 30 mM dextrose. The cells were allowed to settle for 60–90 min at room temperature, according to Lichtenstein & Osler (18). The leukocyte-rich plasma was centrifuged and the cell pellet washed twice in Tyrode's buffer, pH 7.4, containing (mM): NaCl 135; dextrose 5.5; KCl 2.7; NaH₂PO₄ 0.36; and NaHCO₃ (12). After centrifugation at 400 g for 15 min at 4°C, the cells were suspended in Tyrode's buffer with 1.8 mM CaCl₂ and 0.5 mM MgCl₂. Basophil concentration in leukocyte suspensions was evaluated by Alcian blue staining (19) and subsequent counting in a Fuchs-Rosenthal chamber (Walter Schrenk, Hofheim am Taunus, Germany). A final concentration of 8×10^4 basophils/ml was obtained by adjusting the volume of Tyrode's buffer. The basophil purity range was 0.4–2.3%. The cell viability of all leukocyte suspensions, assessed by trypan blue (Merck, Darmstadt, Germany) exclusion, was greater than 95%.

Leukocyte stimulation was performed in 12 $\times$ 75 mm polyethylene tubes (LP Italiana, Milan, Italy). The cells, suspended in 0.5 ml Tyrode's buffer with Ca²⁺ and Mg²⁺, were added to prewarmed (37°C) tubes containing 0.5 ml of the same buffer with anti-IgE (final protein concentration 10 μ g/ml; Sigma Chemical, St Louis, MO, USA), fMLP (1 μ M; Sigma Chemical), or Ca²⁺ ionophore A23187 (1 μ M; Sigma Chemical). In a series of preliminary experiments, the concentrations of the stimulating agents were proved to be optimal for histamine release. After mixing and incubating for 30 min at 37°C, the reaction was stopped by centrifugation at 1200 g for 15 min at 4°C. The supernatants were aspirated, mixed with an equal volume of 6% v/v

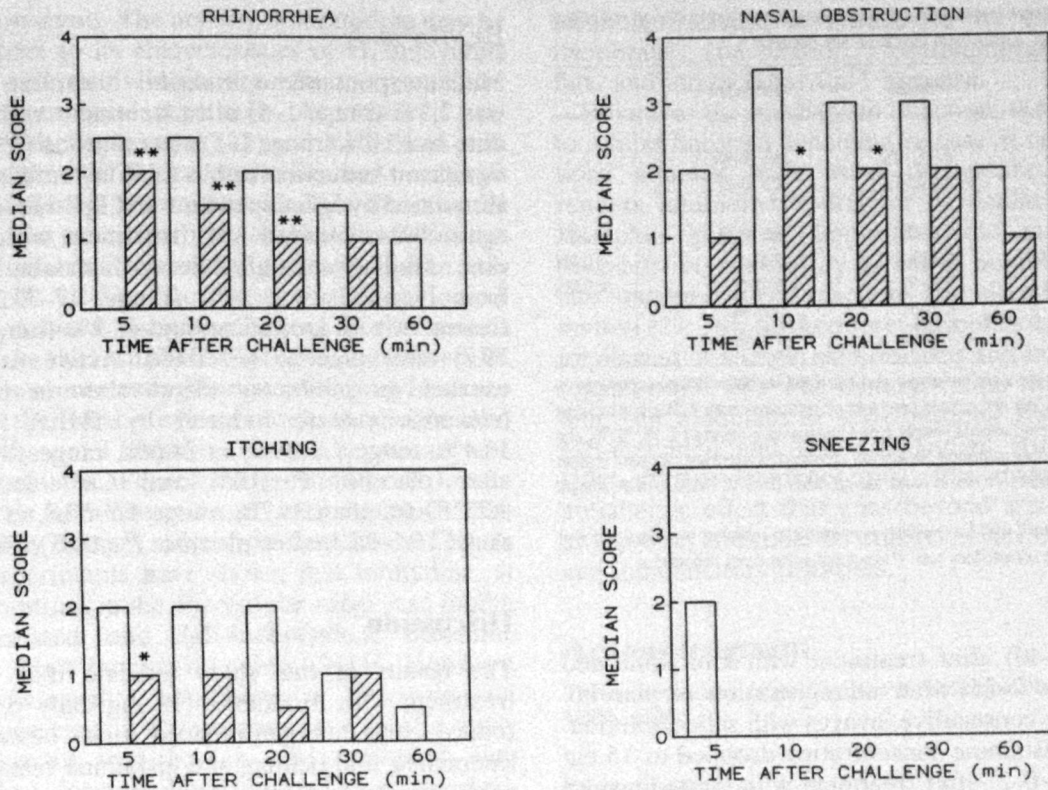


Fig. 1. Time courses of symptom scores for rhinorrhea, nasal obstruction, itching, and sneezing after nasal challenge with allergen extract (2000 biologic units *Dermatophagoides pteronyssinus*) in 10 patients with allergic rhinitis. Nasal challenge with the relevant allergen was done after administration of placebo for 1 week (empty bars) and after 1-week treatment with 10 mg loratadine p.o. (hatched bars). Data are expressed as median values. **Significantly different from corresponding values after administration of placebo at $P < 0.01$; *significantly different from corresponding values after administration of placebo at $P < 0.05$.

perchloric acid, and centrifuged at 2000 g for 15 min at 4°C. Histamine concentration was then measured by an automated fluorometric method. Spontaneous histamine release was evaluated by measuring the histamine concentration in the supernatant of unstimulated cells; the total histamine content was obtained by adding 0.5 ml 6% v/v perchloric acid to 0.5 ml cell suspension. Net histamine release was expressed as percentage of total histamine content, after subtraction of spontaneous histamine release. Every experiment was carried out in duplicate.

Statistical analysis

The results were expressed as median and range. The Wilcoxon signed rank sum test for paired data was used to compare basophil histamine release after treatment with loratadine and placebo. The Friedman nonparametric repeated measures test and Dunn's multiple comparisons test were used to analyze the effects of loratadine and placebo on nasal symptoms and histamine levels in nasal lavage fluids. P values lower than 0.05 were considered significant.

Results

In vivo study

Nasal symptoms. Nasal allergen challenge provoked nasal symptoms such as obstruction, rhinorrhea, itching, and sneezing in all subjects. Treatment with loratadine for 1 week caused a reduction in rhinorrhea, nasal obstruction, and itching (Fig. 1). A significant reduction in the scores for rhinorrhea was observed at 5, 10, and 20 min after challenge; the scores for nasal obstruction were reduced at 10 and 20 min, and the scores for itching were reduced at 5 min. The scores for sneezing were not reduced to a significant extent at any time after challenge. No adverse effects were reported by the patients either after treatment with loratadine or after placebo administration.

Histamine release in nasal lavages. As demonstrated previously (16), histamine was found in prechallenge nasal lavages, and its presence was not associated with clinical symptoms. Median histamine value in the first nasal lavages was 6.5 ng/ml

Table 1. Histamine levels in nasal lavage fluids after antigen challenge and effects of treatment for 1 week with loratadine and placebo

Nasal lavage	Placebo	Loratadine	P
Prechallenge			
I	6.5 (2–25)	6.5 (2–40)	
V	0.5 (0–1)	0.5 (0–2)	
Postchallenge			
5 min	4 (1–28)	0.5 (0–3)	<0.01
10 min	0.5 (0–5)	0 (0–1)	<0.001
20 min	0 (0–2)	0 (0–0)	<0.01
30 min	0 (0–1)	0 (0–0)	
60 min	0 (0–0)	0 (0–0)	

Five nasal lavages were performed at 4-min intervals before antigen challenge in order to obtain low and uniform prechallenge histamine levels (I, first prechallenge lavage; V, fifth prechallenge lavage). Nasal lavages were repeated 5, 10, 20, 30, and 60 min after antigen challenge. Results are expressed as ng/ml and represent median (range) of histamine levels in nasal lavage fluids from 10 subjects with allergic rhinitis.

Statistical analysis was by Friedman nonparametric repeated measures test and Dunn's multiple comparisons test. *P* is not significant if not indicated.

(range 2–40) after treatment with loratadine and 6.5 (range 2–25) after administration of placebo. After five consecutive lavages with saline solution, median histamine concentration dropped to 0.5 ng/ml (range 0–2) after treatment with loratadine and to 0.5 ng/ml (range 0–1) after administration of placebo. When the patients received placebo, antigen stimulation was followed by a significant, but short-lasting (5 min), increase in histamine concentration in nasal lavages ($P < 0.05$ vs the fifth prechallenge nasal lavages values); no significant histamine release was observed in the nasal lavages collected 10–60 min after challenge. Treatment with loratadine markedly reduced ($P < 0.01$ vs placebo) histamine release in the 5-min postchallenge lavages (Table 1).

Ex vivo study

Median spontaneous basophil histamine release was 2.2% (range 1–5) after treatment with loratadine and 3.0% (range 1–5) after placebo ($P = \text{NS}$). A significant reduction in basophil histamine release stimulated by IgE-dependent and IgE-independent agents was observed after treatment with loratadine. Median anti-IgE-induced histamine release from basophils was 30% (range 1.7–73.3) after treatment with loratadine, and 41.9% (range 27.8–79.2) after placebo ($P < 0.05$). Active treatment exerted an inhibitory effect also on basophil histamine release induced by fMLP (median 16.4%, range 1.7–31.9, vs 24.6%, range 14.3–50.0, after placebo, $P < 0.05$) and Ca^{2+} ionophore A23187 (median 36.7%, range 1.7–52.8, vs 61.9%, range 16.1–94.4, after placebo, $P < 0.05$) (Fig. 2).

Discussion

The results of this study indicate that 1-week treatment with loratadine (10 mg daily p.o.) can reduce early allergen-induced nasal obstruction, rhinorrhea, and itching, and histamine release into nasal airways. Furthermore, *ex vivo* basophil histamine release induced by anti-IgE, fMLP, and Ca^{2+} ionophore A23187 is significantly reduced after treatment with loratadine. The drug was well tolerated, and no adverse effects were reported by the patients included in the study.

The clinical efficacy of loratadine in the treatment of seasonal allergic rhinitis has been previously documented by several studies (3, 4, 6); our results indicate that loratadine is active also in reducing nasal symptoms and histamine release induced by a perennial allergen, such as

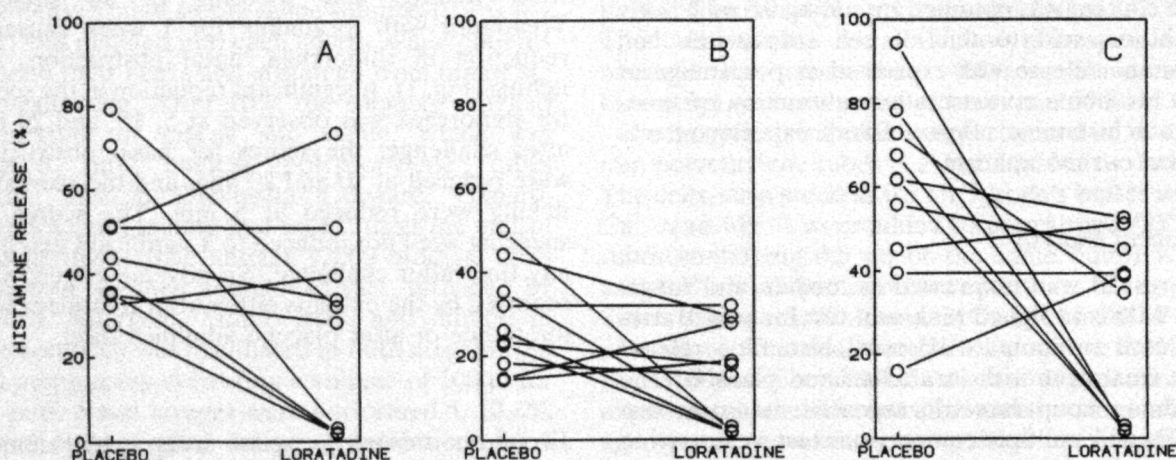


Fig. 2. Basophil histamine release induced by 10 µg/ml anti-IgE (panel A), 1 µM fMLP (panel B), and 1 µM Ca^{2+} ionophore A23187 (panel C) in 10 patients with allergic rhinitis after administration of placebo for 1 week and after treatment with loratadine for 1 week. Active treatment determined statistically significant ($P < 0.05$) reduction of histamine release induced by either IgE-dependent (anti-IgE) or IgE-independent (fMLP, Ca^{2+} ionophore A23187) agents. Each line represents one subject.

D. pteronyssinus. The activity of loratadine may be due in part to its characteristics of H_1 -antagonist and in part to its inhibitory effect on mediator release from nasal mast cells. As observed previously by Bousquet et al. (12) and Andersson et al. (13), treatment with loratadine can suppress antigen-induced histamine release in the nose. *In vitro* studies have clearly shown that loratadine can inhibit histamine release from rat and human lung mast cells (6–8). Therefore, the inhibition of antigen-induced histamine release in the nose is likely to be the result of a direct effect on mast cells.

The most interesting feature emerging from our data is that oral treatment with loratadine also inhibits basophil histamine release in patients with allergic rhinitis. Plasma concentrations of loratadine after administration of a 10-mg tablet (the usual daily dosage) range from 2 to 10 nM (20). *In vitro* experiments have shown that loratadine, at concentrations in the micromolar range, can inhibit IgE-mediated and IgE-independent histamine release from human basophils (9–11). However, the experimental conditions *in vitro* do not reproduce exactly the situation *in vivo*: this study demonstrates that 1-week oral treatment with loratadine downregulates *in vivo* and *ex vivo* IgE-mediated histamine release. The mechanism of action of loratadine is not fully understood, but its effect on histamine release induced by agents which activate basophils by different mechanisms suggests that it interferes with a common biochemical step that leads to histamine release.

Anti-IgE, fMLP, and Ca^{2+} ionophore A23187 have different mechanisms of action, but they all cause an increase in intracellular Ca^{2+} concentration. Anti-IgE provokes histamine release by membrane-IgE cross-linking, followed by Ca^{2+} transmembrane influx, intracellular Ca^{2+} mobilization, and activation of two different pathways, one protein kinase C-dependent and the other protein kinase C-independent (21, 22). The peptide fMLP binds to a membrane receptor, which is different from IgE and C5a receptors and activates a pertussis-toxin-sensitive G protein; furthermore, it causes an increase in cytoplasmic Ca^{2+} concentration in human basophils, without activating protein kinase C (22–24). The Ca^{2+} ionophore A23187 is a lipid-soluble antibiotic which carries Ca^{2+} ions across the basophil membrane, bypassing membrane receptors and Ca^{2+} channels (25). Kreutner et al. demonstrated that loratadine reduces ^{45}Ca uptake by rat peritoneal mast cells stimulated with concanavalin A (6). It is known that some antihistaminic and antiallergic drugs can inhibit histamine release, probably by inducing membrane stabilization (26); therefore, it is conceivable that the inhibitory effect of loratadine on basophil

histamine release is related to its effects on membrane stabilization, transmembrane Ca^{2+} flux, and intracellular Ca^{2+} increase.

Whatever the mechanism of action, the capacity to inhibit basophil histamine release at concentrations attained with usual therapeutic dosages renders loratadine a drug of particular interest. Basophils play an important role in allergic disorders of respiratory airways, notably in the late antigen-induced reactions and allergic inflammation (27, 28); furthermore, basophils have been implicated in allergic skin diseases and in allograft rejection (29, 30). The demonstration that loratadine can modulate *ex vivo* basophil response to IgE-mediated and IgE-independent stimuli provides further evidence that this drug has an antiallergic effect that goes beyond a simple H_1 -antagonism and could be utilized in various allergic and inflammatory disorders.

Acknowledgment

This research was funded in part by Schering-Plough S.p.A., Milan, Italy.

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