

Suppressive effect of loratadine on allergen-induced histamine release in the nose

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It has been speculated whether the recently developed non-sedating antihistamines may possess other properties than merely being antagonists at the H_1 -receptors. To investigate this suggestion 12 patients with strictly seasonal allergic rhinitis participated in a double-blind placebo controlled randomized cross-over study outside the pollen season. At steady state levels of 10 mg loratadine, a new non-sedating antihistamine, the patients were challenged with methacholine. This was followed by a nasal challenge with increasing doses of allergen. 24 h later the patients were rechallenged nasally with the same methacholine dose as the day before. The volume of the methacholine-induced nasal secretion was measured and the response to allergen was determined by scoring technique. In returned nasal lavage fluid the levels of histamine and TAME-esterase activity were measured. It was found that loratadine significantly reduced the immediate allergic nasal symptoms compared with placebo ($P < 0.01$). Loratadine also reduced the allergen-induced release of histamine into the nasal cavity after the strongest allergen dose, from 9.6 ± 1.5 (mean $\pm$ SEM) to 6.4 ± 1.4 ng/ml ($P < 0.05$). A similar decrease in the TAME-esterase activity after treatment with loratadine was observed. The TAME-esterase activity decreased from $11.6 \cdot 10^3 \pm 2.47 \cdot 10^3$ to $5.60 \cdot 10^3 \pm 1.45 \cdot 10^3$ CMP ($P < 0.05$). There were no significant changes between the active and placebo treatments regarding the methacholine-induced secretory response. This was true for the initial methacholine challenge as well as the secretory response 24 h later. This study shows that loratadine is an effective drug in the suppression of immediate allergic nasal symptoms. One of the beneficial effects of this drug could be a reduction in allergen-induced histamine release. A finding which further implicates that antihistamines may possess other effects than just a blocking of the H_1 -receptor.

Key words: allergic rhinitis; histamine; lavage; methacholine; non-specific reactivity.

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Allergic rhinitis is considered a type 1 allergic reaction where the induction of mediator release from mast cells and basophils requires the cross linking of two or more IgE molecules at the cell membrane with one molecule of antigen or ligand (13). This leads to the release of inflammatory, so-called preformed, granular-associated mediators and newly synthesized,

membrane-derived mediators. These mediators are responsible for the symptoms of the immediate allergic reaction. Histamine can mimic all the symptoms associated with allergic rhinitis. It acts directly on vascular histamine receptors, this being the main cause of nasal blockage, and indirectly via reflexes which then account for sneezing and hypersecretion (17). The good clinical effect of antihistamines in the treatment of rhinitis supports that histamine is an important

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mediator. Histamine, however, can not induce the increase in nasal reactivity to specific and non-specific stimuli (11). This increase in reactivity has previously been possible to demonstrate in the majority of a hay-fever patient population after an initial strong nasal allergen challenge (1, 14). Further, histamine cannot recruit the inflammatory cells, especially the eosinophils to the inflammatory site. These two phenomena are more likely to be associated with the post-allergen induced inflammatory event.

Antihistaminic drugs have been used in the treatment of allergic disorders for more than 40 years. During recent years, however, some studies have indicated that antihistamines may possess other properties than just being H_1 -receptor antagonists. For instance, it has been shown that one of the newer developed antihistamines can reduce the allergen-induced histamine release *in vivo* (18). Another antihistamine could inhibit the increased response to a non-specific stimuli in the nose (15), as well as inhibit the eosinophil chemotaxis in the skin (10).

Loratadine is a new non-sedating antihistamine with long-term activity and weak affinity for acetylcholine receptors (10). This study was performed in order to evaluate whether this drug also possesses pharmacological properties which are not directly related to a blocking of the H_1 -receptor. We therefore monitored the symptoms, mediator release and methacholine-induced non-specific secretory response after a nasal allergen challenge in a placebo controlled fashion.

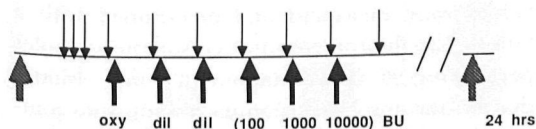


Fig. 1. A schematic figure outlining the challenge procedure. After an initial methacholine challenge followed a nasal lavage procedure. Arrows above the line indicate the lavages. After three initial lavages in a row, lavages followed every 10 min. The challenges are indicated below the line. 24 h later a final methacholine challenge was undertaken. Methacholine, diluent and allergen challenges were performed in the same nasal cavity.

MATERIAL AND METHODS

Study design

The study was performed as a double-blind, placebo-controlled, randomized cross-over study in 12 patients with strictly seasonal allergic rhinitis. The nasal challenge procedure included an initial methacholine challenge followed by an allergen challenge increasing 10-fold. The patients were rechallenged 24 h later using the same methacholine dose from the previous day.

Patients

Informed consent was obtained from a total of 11 men (aged 18 to 36 years) and one woman (aged 24 years). They all had a positive skin prick test to birch (*Betula verrucosa*) and/or timothy (*Phleum pratense*) pollen extract. The patients had previously shown a positive nasal response to a challenge with timothy or birch pollen extract and had also demonstrated an increase in nasal non-specific methacholine-induced reactivity 24 h after an initial strong nasal allergen challenge (14, 15). The study was performed outside the relevant pollen season and was conducted according to the declaration of Helsinki, and approved by the Ethics Committee at the University of Lund.

Investigational drugs

Loratadine, supplied as capsules of 10 mg, was given once daily for 5 days prior to the challenge procedure and continued up to the rechallenge with methacholine. The challenges were performed 2 h after the last given dose. Placebo capsules, identical in appearance and taste to loratadine, were used as reference drug. No medication other than the study drug was allowed during the trial and the patients were free from any medication for at least 3 weeks prior to the challenge procedure. A 3-week wash-out period was instituted between each challenge.

The treatment order was done according to a randomization scheme.

Nasal provocation test

The challenge protocol is outlined in Fig. 1. On the first day an initial methacholine challenge, was performed using a mechanical nasal pump spray delivering 200 µl of fluid into the nasal cavity. An isotonic solution containing 30 mg/ml of methacholine-chloride was utilized, thereby producing an effective dose of 6 mg methacholine. Thereafter a nasal challenge with allergen and succeeding nasal lavages with saline solution was undertaken. The procedure involved prechallenge lavages with saline solution to reduce the cell-free mediators that are known to be present in the nose. 100 µg of oxymethazoline was thereafter sprayed into each nasal cavity to prevent mucosal congestion. It has been shown previously that this dose of oxymethazoline does not affect mediator release (19). This was then followed by two challenges of diluent (the same as the pollen extracts) in order to establish a baseline. Thereafter three serial challenges were performed with pollen allergen increasing 10-fold. The doses used were 10^2 , 10^3 , and 10^4 Biological Units (BU) of birch or timothy pollen extracts (Pharmalgen®, Pharmacia, Uppsala, Sweden). 24 h later the patients were rechallenged with the same methacholine dose as the day before. Thereafter the lavage fluid was chilled and centrifuged at 3000 rpm for 8 min. The lavage supernatant was divided into aliquots and immediately frozen for later analysis of histamine and TAME-esterase activity. All challenges, except for the oxymethazoline challenge, were performed in the right nasal cavity. The lavages were performed in both cavities.

Recording of nasal symptoms

A detailed symptom score sheet was used during the challenge procedure. A five-point scale from 0 to 4 was used for the symptoms of secretion and blockage. The number of sneezes was counted.

Methacholine-induced secretory response

After thoroughly clearing the nasal cavity by nose-blowing a challenge with methacholine was made using a nasal pump spray as described above. Specific volume measurements of the secretions obtained after each methacholine challenge were made with the aid of a plastic funnel connected to a syringe according to the technique described by Borum (6). The nasal secretions were measured for 10 min after each methacholine challenge. Both methacholine challenges were performed in the right nasal cavity.

Histamine measurement

The histamine levels in the returned lavage fluid were measured using a glass fibre-based fluorometric method as previously described (3). 25 µl of the aliquots was pipetted into a 96 microtitre plate coated with a glass fibre matrix which selectively binds histamine (22). After 1 h of incubation at 37°C the plate was rinsed twice automatically using a SLT immuno-washer with deionized water. Interfering substances from the nasal lavage fluid were further dissolved by adding a detergent to the wells for 30 min at 37°C followed by another rinse. Subsequently, histamine bound to glass fibres was resolved by adding a mixture of 75 µl OPT reagents (10 mg o-phalldialdehyde dissolved in 2 ml methanol alkalized by 60 µl 0.05 N NaOH to each well). 10 min later the histamine -o-phalaldehyde reaction was stopped by adding 75 µl 0.6% HCl04. Histamine fluorescence values were measured and determined with a Gilson 122 fluorometer and Gilson autosampler performing 96 determinations in 5 min. Immediately, the absolute histamine values were computed using software for MS-Dos compatible personal computers.

TAME-assay

Tame-esterase (N-alpha-tosyl-L-arginine-methyl-esterase) activity was measured in the super-

natants using a radiochemical technique, essentially the same as the one described by Imanari et al. (12). This method is based on the liberation of [^3H]-labelled methanol from the syn-

thetic substrate TAME and has previously been described in detail (19).

Statistics

The statistical analysis was performed on a microcomputer (Macintosh, Apple Computer, Cupertino, CA, USA) with the aid of a statistical software package (Statview 512+, by Brainpower Inc, Calabasas, CA, USA). Students t-test for paired data was used to compare symptoms, histamine and TAME-esterase activity levels obtained after placebo and active treatments. A variance of analysis was used to evaluate any differences between the methacholine values day 1, day 2, active, and placebo treatment.

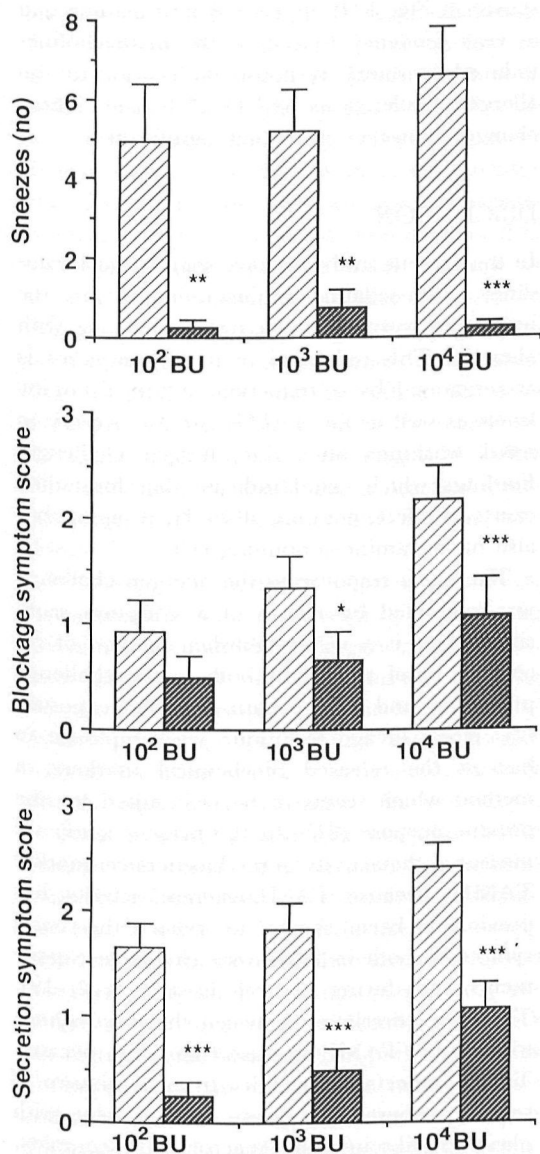


Fig. 2. A composite figure showing the nasal response after pretreatment with placebo or loratadine to an allergen challenge with three increasing doses (BU = Biological Units). The values are given as mean $\pm$ SEM. The upper panel shows the number of sneezes. The middle panel the blockage symptom score, and in the lower panel the symptoms of secretion. ■ treatment with placebo, ▨ treatment with loratadine. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

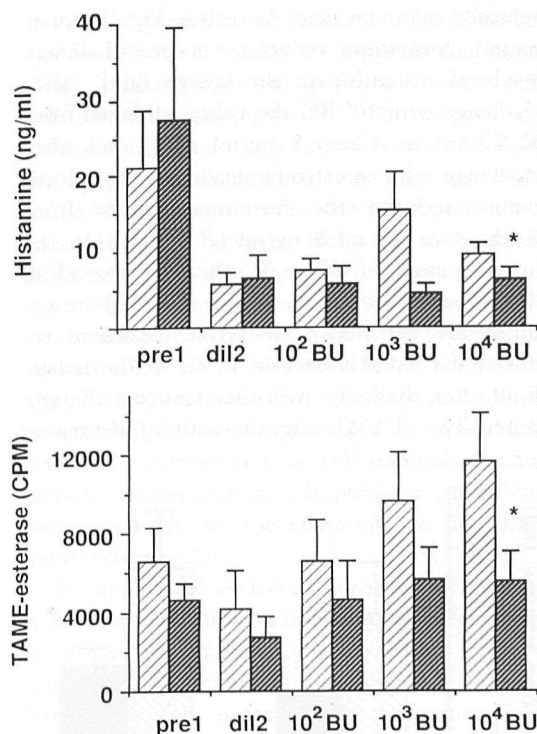


Fig. 3. In the upper panel the histamine values in nasal lavage fluid after treatment with placebo or loratadine prior to and after three increasing doses of allergen (BU = Biological Units). In the lower panel the corresponding TAME-esterase activity levels. The values are given as mean $\pm$ SEM). ■ treatment with placebo, ▨ treatment with loratadine. * $P < 0.05$.

RESULTS

All patients completed the study. Of the 12 patients five were challenged with birch pollen extracts and seven with timothy pollen extract. All values are given as mean $\pm$ SEM. As seen in Fig. 2 loratadine reduced the immediate allergic nasal reaction compared with placebo. The number of sneezes decreased from 5.6 ± 1.4 to 0.3 ± 0.2 ($P < 0.01$) for lowest allergen dose and from 5.2 ± 1.1 to 0.8 ± 0.5 (10^3 BU) ($P < 0.01$) and 6.6 ± 1.2 to 0.3 ± 0.1 ($P < 0.001$) (10^4 BU). Similar decreases were also observed for the symptoms of nasal blockage and secretion symptom score. The levels of histamine in the initial lavages both after active and placebo treatment were fairly high and the active treatment did not influence these levels. The initial raised histamine levels were reduced after the repeated saline lavages. As seen in Fig. 3 (upper panel), loratadine reduced the post-challenge levels of histamine in the lavage fluid. After challenge with 10^4 BU the values changed from 15.3 ± 5.0 to 4.5 ± 1.3 ng/ml (NS) and after challenge with the strongest allergen dose loratadine reduced the histamine values from 9.6 ± 1.4 to 6.4 ± 1.4 ng/ml ($P < 0.05$). In the lower panel of Fig. 3, the corresponding TAME-esterase values in the returned lavage fluids are depicted. The active treatment reduced the TAME-esterase levels in the lavage fluid after challenge with the strongest allergen dose. The TAME-esterase activity decreased

from $11.6 \cdot 10^3 \pm 2.4 \cdot 10^3$ to $5.6 \cdot 10^3 \pm 1.4 \cdot 10^3$ CPM ($P < 0.05$). No significant differences between the methacholine-induced secretory response after active or placebo treatment on day 1 and 24 h later could be disclosed. This is shown in Fig. 4. It appears as if loratadine had a weak tendency to reduce the methacholine-induced secretory response both prior to the allergen challenge as well as 24 h later. These changes, however, were not significant.

DISCUSSION

In the present study we have shown that loratadine, a non-sedating antihistamine reduces the immediate response to a nasal challenge with allergen. This reduction of nasal symptoms is accompanied by a reduction of the histamine levels as well as the TAME-esterase activity in nasal washings after the allergen challenge. Findings which could indicate that loratadine exerts its effect, not only at the H_1 -receptor, but also on histamine-containing cells.

The nasal response to the allergen challenge was evaluated by means of a symptom score sheet. This is a well-established method of recording nasal symptoms both in the challenge procedure and during natural pollen exposure (2). Nasal lavage technique was employed to harvest the released biochemical markers; a method which seems to be well suited for the present purpose (21). In the present study we measured the activity of the biochemical marker TAME, because TAME-esterase activity has previously been shown to reflect the nasal symptoms both in laboratory challenge experiments and during natural disease (1, 2, 19). The close correlation between the nasal symptoms and TAME-esterase may be because TAME reflects the activity from a mixture of sources, where a majority is consistent with plasma kallikrein- α_2 -macroglobulin complex, and mast cell tryptase and glandular kallikrein are responsible for the remaining part (5). We used a glass fibre-based method to measure histamine. Recently, it was reported that this method is highly reproducible and correlates well with the radioimmunoassays of histamine (3). This method is inexpensive and time-sav

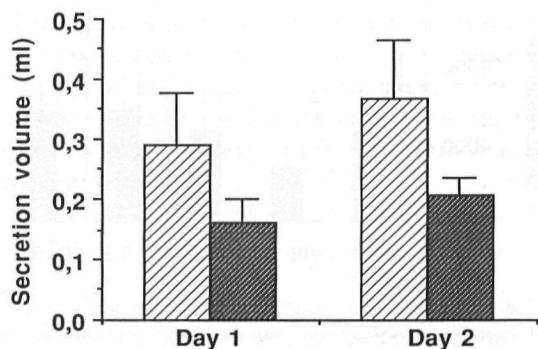


Fig. 4. The secretion volume induced by methacholine performed prior to and 24 h after a nasal allergen challenge after treatment with placebo (hatched bars) or loratadine (solid black bars). No significant differences were found.

ing, especially when many samples are analysed.

To study any effect of loratadine on the allergen-induced non-specific nasal reactivity, a methacholine challenge was added to the protocol prior to and 24 h after the allergen challenge. We measured the response to methacholine according to the technique by Borum (6), a technique with a fairly high degree of reproducibility. Furthermore, methacholine is known, in the present dose, to produce a monosymptomatic purely glandular response, without any sneezes or other symptoms. This was also confirmed in the present study since no nasal blockage or sneezes were induced. It has also been shown that even repeated nasal methacholine challenges do not alter the responsiveness of the nasal mucosa and do not induce any influx of eosinophils (14). The test for a change in reactivity, the actual rechallenge, was performed 24 h after the initial allergen challenge, a practical time-point which also eliminates any diurnal variability.

We found, in similarity to previous studies, that loratadine is an effective drug for reducing the symptoms following allergen exposure (8). In the present study we also found that the drug did not alter the response to the initial methacholine challenge as similar methacholine-induced secretion volumes were obtained after placebo and active treatment. In the figure, however, it would appear that loratadine had a tendency to reduce the methacholine-induced secretion volume both on the initial challenge day and 24 h later. These reductions, however, were not significant and therefore difficult to interpret. Whether the change in secretory response is due to a weak anticholinergic effect can therefore not be decided. In previous studies we have found an increase in methacholine-induced secretion volume 24 h after the allergen challenge (14, 15). Although the patients before entering the study, had shown an increase in methacholine-induced secretion volume 24 h after a nasal allergen challenge, this could not be demonstrated after the placebo treatment. This could be because the inclusion test was performed some months before the study started, and it can only be speculated as to whether

or not the response had changed between the inclusion time-point and study start. Nevertheless, we cannot interpret the effect of loratadine on allergen-induced increase in non-specific nasal reactivity since there was no increase. This is in contrast to another study where we demonstrated an effect of two different non-sedating antihistamines on allergen-induced increase in non-specific reactivity. (15).

In the nasal challenge protocol we could confirm previous findings of the initial high levels of histamine (3) and these levels were reduced to a baseline by repeated washings. Further, loratadine did not decrease this increased histamine level prior to the allergen challenge, but reduced the increased levels after allergen challenge. Terfenadine, another non-sedating antihistamine, has also been reported to reduce allergen-induced histamine levels in nasal washings after 1 week of pretreatment (18), and loratadine has previously been shown to suppress the histamine release from rat peritoneal mast cells stimulated by ionophore (16). The origin of the released histamine can not be evaluated from the present results. Whether the histamine comes from bacteria, mast cells/basophils is not clear. It seems, however, likely that the released histamine primarily comes from mast cells, in harmony with other findings (9). Furthermore, it can not be concluded whether the suppression of loratadine on the histamine levels is due to a direct mast cell stabilizing effect or an indirect neural reflex activity. It could be speculated whether the effect is mast cell-stabilizing, since another antihistaminic compound reduced the neural reflexes, such as sneezing, but not histamine release (20).

In conclusion, we have shown that loratadine, a new non-sedating antihistamine, reduces the levels of released histamine to a nasal allergen challenge. This suggests that this antihistaminic compound may possess other properties than just a competition at the H_1 -receptor level.

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