

Inhibitory effect of levocabastine on allergen-induced increase of nasal reactivity to histamine and cell influx

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For evaluation of the effect of levocabastine pretreatment on allergen-induced rhinitis symptoms, changes in nasal washings, and nasal responsiveness to histamine, 12 asymptomatic patients with documented allergic rhinitis participated in a single-blind, placebo-controlled study. Eight-day treatment with levocabastine (twice in each nostril, four times a day) caused significant reduction in nasal symptoms and inflammatory cell influx after allergen challenge, as compared with placebo administration. Levocabastine inhibited increased nasal reactivity to histamine induced by allergen provocation, as controlled by rhinitis symptoms and albumin level in nasal washings. These data reveal a high effectiveness of levocabastine in the prevention of allergen-induced rhinitis symptoms. Moreover, its inhibitory effect on inflammatory cell influx and hyperresponsiveness to histamine suggest that levocabastine is more than a simple H_1 -receptor antagonist.

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Levocabastine is a new topical antiallergic agent with documented high specificity and potency for H_1 -receptor blockage (9). Recent studies have shown its effectiveness in relieving the symptoms of rhinitis to be higher than that of sodium cromoglycate (5). Since their effect was similar to that obtained with topical steroids (8), the question arises whether these benefits are the result only of H_1 -receptor blocking or of other antihistaminic properties of the medicine. In the light of previous findings that some antihistamines inhibit processes other than H_1 -receptor-dependent features of allergic diseases, such as allergen-induced influx of eosinophils (1) or changes in nonspecific reactivity (4), the present study was undertaken to answer the question of whether it is the highly potent H_1 -receptor blocker that possesses these properties. The aim of the study was to assess the efficacy of levocabastine on allergen-induced rhinitis symptoms and changes in nasal reactivity to histamine and influx of eosinophils into nasal lavage fluid.

Material and methods

Study design

The study was designed as a single-blind, placebo-controlled examination. A cell number and albumin

level in saline washings were determined, and nasal reactivity to histamine was evaluated 20 h before and 2 h after provocation with allergen in patients treated with placebo. Then, 1 month later, the patients underwent the same challenge procedure after an 8-d pretreatment with levocabastine (Fig. 1).

Patients

Twelve nonsmoking patients (10 men and 2 women) between 21 and 28 years of age were studied after their consent had been obtained. All subjects were defined as having allergic rhinitis with previously documented rhinitic response to provocation with mites. All were skin-prick-test (Beecham-Bencard) and RAST (Phadebas-Pharmacia) positive to *Der-matophagoides pteronyssinus*. None of the volunteers complained of rhinitis symptoms at the time of challenge. None had received any treatment for at least 1 month before the study, and no drug except levocabastine was allowed during the trial.

Study protocol

The study protocol is outlined in Fig. 1. Levocabastine (0.5 mg/ml solution, Janssen Laboratories, Belgium) or saline solution was vaporized twice in each

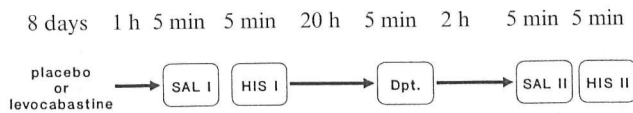


Fig. 1. Schematic figure outlining nasal washings and provocation procedure used. After 8-d treatment with placebo or levocabastine, saline washings (SAL I) and histamine provocation (HIS I) were performed. Then allergen challenge (Dpt.) was performed, and 2 h later, saline washings (SAL II) and histamine provocation (HIS II) were repeated.

nostril four times a day (total dose amounted to 0.4 mg/ml). After 8-d treatment with placebo or levocabastine, the cell number and albumin level in saline washings were determined, and nasal reactivity to histamine was evaluated (1 h after the last dose of medicine). Twenty hours later, the provocation with allergen was performed. Two hours after the allergen challenge, the saline washings and histamine provocation were repeated. There were 1-month intervals between consecutive specific provocations.

Nasal lavage and provocation challenge procedure

The "nasal pool" method (2) was used to perform nasal washings with saline and provocation with histamine (50 mg/l, histamine dihydrochloride BDH Ltd, Poole, UK) and allergen (*D. pteronyssinus* extract, 2000 BU, Pharmedgen). The modified "nasal pool" device (10-ml syringe closely fitting nostril) filled with 6 ml saline or provocation agent was inserted in the nasal cavity for 5 min and then withdrawn. All washings and provocations were performed at only one side of the same cavity. A 5-s single saline irrigation was performed before each provocation.

Clinical score and processing of nasal washings

The degree of mucosal edema and itching was evaluated by patients, while the physician rated the sneezing and degree of rhinorrhea. Total symptoms scores ranged from 0 to 8, representing the sum of scores for sneezing (no sneezes, 0 points; 1–3 sneezes, 1 point; and > 3 sneezes, 2 points), rhinorrhea (none, 0 points; mild, 1 point; and abundant, 2 points), mucosal edema (none, 0 points; mild, 1 point; and nasal block, 2 points), and itching (none, 0 point; itchy eyes, 1 point; and itchy eyes and throat, 2 points).

Centrifugation at room temperature (10 min at 1000 rpm) of saline or allergen washings was performed to separate cell pellet and supernatant, and then sol and gel phases were pipetted. The cell pellets were washed in sterile phosphate-buffered saline (PBS, Sigma) and then resuspended in 1 ml RPMI (Sigma) to obtain cell counts. A Fuchs Rosenthal

chamber was used to allow determination of the number of each cell type in 1 ml of recovered fluid. After the cells (Hansel's method) (3) were stained, the total cell count and number of eosinophils, neutrophils, and metachromatic cells were determined (the term "metachromatic cells" includes basophils and mast cells). Cell viability ranged from 93 to 99%, as estimated in selected samples by trypan blue exclusion. Biochemical assays were performed on the sol phase. Albumin concentration was measured by the single radial immunodiffusion method (range of assay 20–2000 mg/ml).

Statistics

The Wilcoxon matched-pairs (6), signed-rank test was used to determine the significance of the symptom score increase and the albumin level and cell number elevation. The data were expressed as the mean value $\pm$ SEM. The results obtained after placebo treatment were compared with those after treatment with levocabastine by the Mann-Whitney U-test. The value below 0.05 was considered to be statistically significant.

Results

All of the 12 patients who completed the study revealed positive response to allergen challenge, as assessed by rhinitis symptoms, changes in nasal lavage fluid, and response to histamine provocation. However, the treatment with levocabastine affected all determined parameters.

Symptom registration

Nasal challenge with allergen caused rhinitis symptoms in all patients. The symptoms included sneezing, mucosal edema, rhinorrhea, and itching, and their score amounted to 6.3 points (SEM $\pm$ 1.6). Four weeks later, after levocabastine pretreatment, nasal provocation with allergen at the same dose did not cause sneezing, itching, and rhinorrhea (mean value 1.1, SEM $\pm$ 0.8), although five patients complained of mucosal edema (Fig. 2).

Cellular findings

As shown in Fig. 3, the allergen induced a cell influx. The total cell count increased from 194×10^3 /ml, SEM $\pm$ 76, to 354×10^3 /ml, SEM $\pm$ 99.2, $P < 0.05$, and, respectively, the number of eosinophils increased from 21×10^3 /ml, SEM $\pm$ 6.3, to 63×10^3 /ml, SEM $\pm$ 17.2, $P < 0.05$, and neutrophils from 43×10^3 /ml, SEM $\pm$ 1.3, to 81×10^3 /ml, SEM $\pm$ 24.1, $P < 0.05$. Treatment with a topical antihistamine significantly reduced the total cell count (86×10^3 /ml,

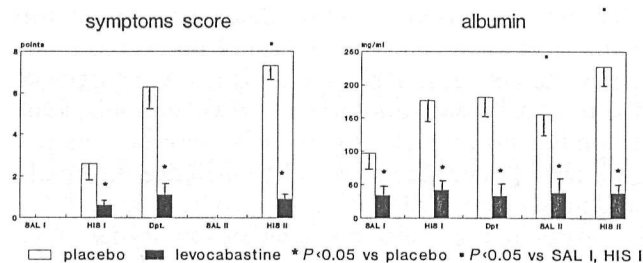


Fig. 2. Effect of placebo and levocabastine pretreatment on composite nasal symptoms score and albumin level in nasal lavage fluid after washings with saline, histamine, and allergen (mean $\pm$ SEM).

SEM $\pm$ 23), the number of eosinophils (9.3×10^3 /ml, SEM $\pm$ 5.3), neutrophils (12.3×10^3 /ml, SEM $\pm$ 4.2), and metachromatic cells (17.2×10^2 /ml, SEM $\pm$ 11). There were no signs of an increase in total cell count (101×10^3 /ml, SEM $\pm$ 35.2) and number of eosinophils (12.4×10^3 /ml, SEM $\pm$ 4.9), neutrophils (14.2×10^3 /ml, SEM $\pm$ 3.9), and metachromatic cells (15×10^2 /ml, SEM $\pm$ 8.2) after allergen challenge.

Provocation with allergen caused a twofold increase of albumin level in allergen and saline washings obtained 2 h later, as compared with base line (from 97 mg/ml, SEM $\pm$ 22, to 182 mg, SEM $\pm$ 23.1 and 156 mg/ml, SEM $\pm$ 45, respectively). Eight-day pretreatment with levocabastine reduced the albumin level, and no differences were observed between base line (34 mg/ml, SEM $\pm$ 9.2), allergen (33 mg/ml, SEM $\pm$ 12.1), and saline washings obtained 2 h later (38 mg, SEM $\pm$ 13.5).

Nasal reactivity to histamine

During placebo pretreatment, the first provocation with histamine caused short-term sneezing and itch-

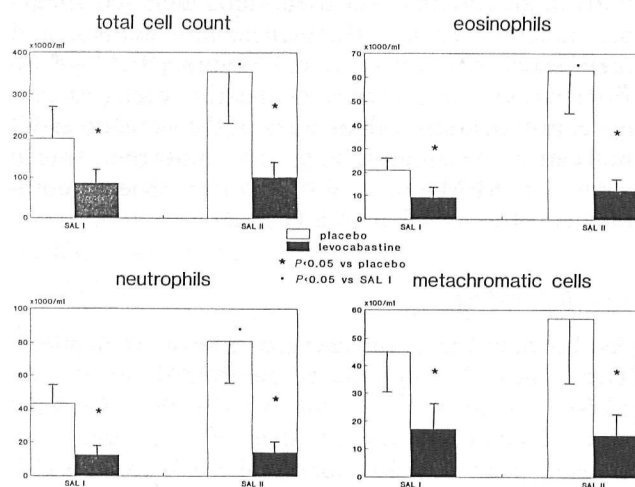


Fig. 3. Total cell count and number of eosinophils, neutrophils, and metachromatic cells in nasal washings 20 h before and 2 h after allergen challenge and pretreatment with placebo or levocabastine (mean $\pm$ SEM).

ing in five of the 12 patients; total symptom score ranged from 0 to 4 points (mean 2.6, SEM $\pm$ 1.0). Rechallenge with histamine after provocation with allergen induced sneezing, itching, rhinorrhea, and mucosal edema in all patients (mean 7.3, SEM $\pm$ 0.8). Albumin level in histamine washings obtained after provocation with allergen was significantly higher than base-line histamine washings (respectively, 177 mg/ml, SEM $\pm$ 32 and 227 mg/ml, SEM $\pm$ 36, $P < 0.05$).

Topical pretreatment with levocabastine inhibited symptomatic response to histamine and reduced albumin level in histamine washings (Fig. 2). No differences were observed between consecutive histamine-induced symptom scores (respectively, 0.6 points, SEM $\pm$ 0.6 and 0.9, SEM $\pm$ 0.4) or between albumin levels (respectively, 42 mg/ml, SEM $\pm$ 12 and 37 mg/ml, SEM $\pm$ 15.2).

Discussion

During allergic reaction in the nose, a series of biochemical reactions and cellular responses follows, associated with stimulation of irritant nerves and glandular secretion, increased vascular permeability, and attraction of inflammatory cells. This study mimicked antigen-induced rhinitis symptoms, changes in nasal washings, and nonspecific reactivity to evaluate the efficacy of a topical antihistamine. After placebo pretreatment, the provocation with allergen induced rhinitis symptoms, caused an influx of inflammatory cells, such as eosinophils and neutrophils, and enhanced nasal reactivity to histamine, confirming previous reports (10). However, pretreatment with levocabastine not only inhibited allergen-induced sneezing, itching, and vascular permeability, supporting the thesis that H_1 -receptor-dependent mechanisms are involved, but also inhibited hyperresponsiveness to histamine, which was observed 2 h later. Since the mechanisms of antigen-provoked hyperresponsiveness to histamine may include changes in the epithelial permeability to provoking agents, interactions between mediators inducing inflammation, and alteration in neuronal regulation of responses, the question still remains of which mechanisms are affected by topical H_1 -receptor-blocking agents. Although similar data were reported previously for terfenadine and cetirizine, further studies with other than histaminic, nonspecific stimuli are required to confirm this property of levocabastine that may be disguised by its antihistaminic potency. Another explanation for levocabastine's efficacy in reduction of nasal hyperresponsiveness is its observed inhibitory effect in allergen-induced inflammatory cell influx. Since during allergic reaction mediators other than histamine, such as leukotrienes, PAF-acether, and prostaglandin PGD_2 , with docu-

mented chemotactic activity are released (7), and taking into account that antihistamines are not reported to inhibit eosinophil influx in the nose, our findings would indicate the involvement of more than only H_1 -receptor-blocking properties of levocabastine.

In conclusion, our results demonstrate beneficial effects of pretreatment with levocabastine in the prevention of antigen-induced features of rhinitis. Since some of these effects, such as the inhibition of inflammatory cell influx, suggest that the drug has properties other than H_1 -receptor-blocking properties, further studies are necessary to confirm the findings.

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