

Comparison of effects of topical levocabastine and nedocromil sodium on the early response in a conjunctival provocation test with allergen

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Background: Multiple ocular challenges or seasonal trials have demonstrated the efficacy of levocabastine and nedocromil sodium in the treatment of allergic conjunctivitis.

Objective: This study was designed to compare the protective effect of levocabastine eye drops with that of nedocromil in a conjunctival provocation test with allergen.

Methods: Twenty-four patients with allergic conjunctivitis to grass pollen were recruited. After a preliminary provocation to determine conjunctival reaction threshold (erythema of at least 50% of the conjunctiva with ocular itching), patients were randomized to receive either topical levocabastine (0.05%) or nedocromil (2%) 15 minutes before provocation. Erythema and pruritus intensity were recorded at each concentration of allergen up to the reaction threshold.

Results: The allergen concentration level necessary to reach reaction threshold was increased ($p < 0.001$) after treatment with both drugs. Comparison between screening and each treatment indicated that the shift in allergen concentration was significantly greater after levocabastine treatment than after nedocromil treatment ($p = 0.019$). Conjunctival itching (symptom score) and erythema (percent conjunctival surface) were also better controlled by levocabastine than by nedocromil during provocation ($p < 0.05$).

Conclusion: In a provocation test with allergen, levocabastine and nedocromil were both effective in increasing the conjunctival tolerance to allergen, with better protection provided by levocabastine. (*J Allergy Clin Immunol* 1996;98:1045-50.)

Key words: Levocabastine, nedocromil sodium, allergic conjunctivitis, allergen challenge, provocation test

Local treatment of allergic conjunctivitis has been based for years on vasoconstrictors, on sodium cromoglycate, and more recently on medications such as ketorolac and lodoxamide. Levocabastine, a long-acting antihistamine, and nedocromil sodium have been progressively developed as first-line therapy, though they have never been compared objectively in a conjunctival provocation test with allergen.

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Abbreviations used

BU: Biologic units

PBS: Phosphate-buffered saline

Levocabastine is the first long-acting and selective H₁-receptor antagonist developed in topical application for the treatment of allergic conjunctivitis and rhinitis.^{1,2} Several clinical seasonal trials and ocular provocation challenges with allergen have demonstrated the efficacy of levocabastine in the treatment of allergic conjunctivitis compared with either placebo²⁻⁴ or sodium cromoglycate.⁵⁻⁸ Levocabastine was shown to be rapidly effective with an onset of action within 10 minutes of conjunctival application of histamine.⁹⁻¹¹ Moreover, a single dose of levocabastine administered

TABLE I. Patient data

No. of patients (M/F)	24 (13/11)
Age (yr)*	25.4 $\pm$ 4.8 (19-35)
Duration of conjunctivitis (yr)*	15.5 $\pm$ 7.0 (4-29)
Ocular symptoms†	
Mild	5
Moderate	12
Severe	7
Other allergic manifestations†	
Rhinitis	24
Asthma	11
Previous treatment†	
None	4
Oral	13
Topical	0
Oral + topical	7

*Mean $\pm$ SD (range).

†Number of patients.

15 minutes before allergen challenge better inhibited itching, hyperemia, and swelling than sodium cromoglycate administered either as a single dose before provocation test or four times daily for 2 weeks.^{4,5} Topical nedocromil has been largely used in the treatment of allergic rhinitis^{12,13} and conjunctivitis and has provided good relief of itching and conjunctival erythema compared with placebo.^{14,16} Its in vitro mechanisms of action seem to depend on chloride channel inhibition, which results in a variety of immune modulatory effects.¹⁷ So far, no clinical data on the effect of nedocromil in conjunctival challenges with allergen are available.

The aim of this double-blind, crossover trial was to compare the protective effect of levocabastine and nedocromil eye drops in an ocular provocation test with allergen. Levocabastine and nedocromil were both effective in increasing the conjunctival tolerance to grass pollen extract, and protection induced by levocabastine in our experimental setting was superior to that obtained with nedocromil.

METHODS

Patients

Twenty-five patients (13 men and 12 women) with a history of grass pollen conjunctivitis were screened for inclusion in the study. Hospital review board approval was obtained, and volunteers gave their written, informed consent. Inclusion criteria were: (1) age between 18 and 60 years; (2) positive history of allergic conjunctivitis during the last pollen season, confirmed by positive skin prick test results and elevated specific IgE levels for grass pollen (>1.0 kU/L), as titrated by Phadezym CAP (Pharmacia, Uppsala, Sweden); (3) positive con-

junctival reaction threshold of 10,000 biologic units (BU)/ml or less during the screening phase. The following groups of patients were excluded from the study: (1) pregnant and lactating women or women not using adequate contraception; (2) patients using any medication that might affect the test parameters (e.g., oral or topical antihistamines, steroids, antidepressants with antiallergic properties); (3) those with any significant illness that could be expected to interfere with the study; (4) those with decompensated asthma (peak flow rate $<20\%$ of normal value); (5) those wearing contact lenses during the course of the study; (6) those with hypersensitivity to one of the investigated drugs; (7) those with signs or symptoms of allergic, viral, or infectious conjunctivitis at the time of the study; (8) patients undergoing specific immunotherapy. The first patient was challenged on December 2, 1994, and the last one on February 21, 1995, a period of the year during which Switzerland is free of grass pollen.

Allergen

The same batch of standardized allergen (a mixture of 5 grass pollens; ALK, Horsholm, Denmark) was used throughout the study. Allergen extract was reconstituted in phosphate-buffered saline (PBS), pH 7.4, at 100,000 BU/ml and stored in 100 μ l aliquots at -70° C. Serial dilutions of allergen in PBS were prepared from single aliquots immediately before the provocation test (100, 320, 1000, 3,200, 10,000, 32,000, and 100,000 BU/ml).

Study protocol

In this double-blind crossover study at the first visit, each volunteer's allergy history and previous therapy were recorded; and a preliminary provocation test (screening phase) with allergen was performed to determine the allergen concentration at reaction threshold, defined as the dose of allergen less than or equal to 10,000 BU/ml necessary to induce erythema extending over at least 50% of the conjunctival surface with ocular itching. A score of 1 or greater was necessary for inclusion in the study (score, 0 to 3: 0 = absent; 1 = mild; 2 = moderate; 3 = severe). First, 1 drop of the allergen diluent (PBS) was instilled into each eye, followed every 10 minutes by increasing concentrations of allergen extract, until the reaction threshold was reached, as evaluated by a single observer. The allergen was applied to each eye alternately, starting with the right eye. One patient was excluded because of an allergen concentration at reaction threshold greater than 10,000 BU/ml. A washout period of at least 1 week was interposed between the three challenges. In the following visits, subjects ($n = 24$) were randomized to receive 1 drop in each eye of either topical levocabastine (0.5 mg/ml) or topical nedocromil (20 mg/ml) 15 minutes before starting the provocation test. One drop per eye for each medication corresponds to the usual dose recommended by the manufacturer per application. Reaction threshold was determined by the same inves-

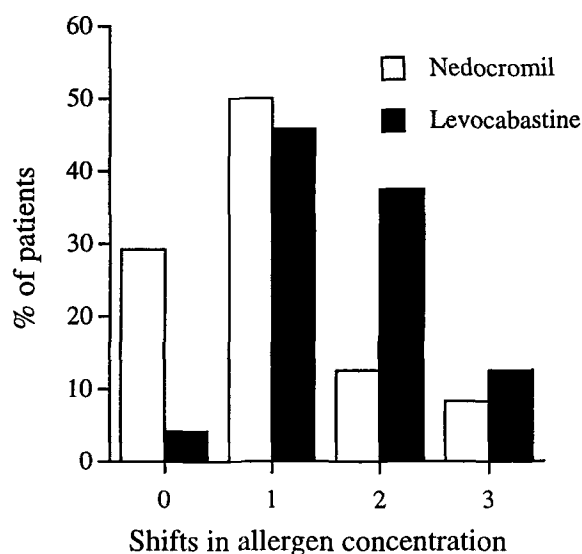


FIG. 1. Shifts in allergen concentration for each patient after levocabastine or nedocromil treatment compared with screening phase. Results are expressed as number of shifts in allergen concentration. Levocabastine versus nedocromil $p = 0.019$ (chi square trend).

tigator who had undertaken the screening phase in a double-blind fashion. Because medications could be recognized by their coloration, handling of the randomized medication and instillation into the eye were performed by an independent investigator. The provocation test always began with instillation of diluent drops (PBS) 15 minutes later. The surface area of the conjunctival erythema and the severity of pruritus were recorded before provocation, 15 minutes after instillation of the medication, 10 minutes after instillation of the diluent, and 10 minutes after provocation with each allergen concentration. Volunteers were challenged with increasing allergen concentrations (1 drop) until the reaction threshold was reached, but not with more than 100,000 BU/ml.

Statistical analysis

Chi square trend for ordered categories was used to analyze the shifts in allergen concentration. Mean values were compared by using Student's t test for independent samples. In appropriate cases (i.e., intragroup comparisons before and after), Student's t test for paired data was used.

RESULTS

Twenty-five atopic volunteers were recruited for the screening phase. Twenty-four were included in the study and completed the trial. One was excluded because of an allergen concentration at reaction threshold (erythema $\geq 50\%$ of conjunctival surface with ocular itching) greater than 10,000

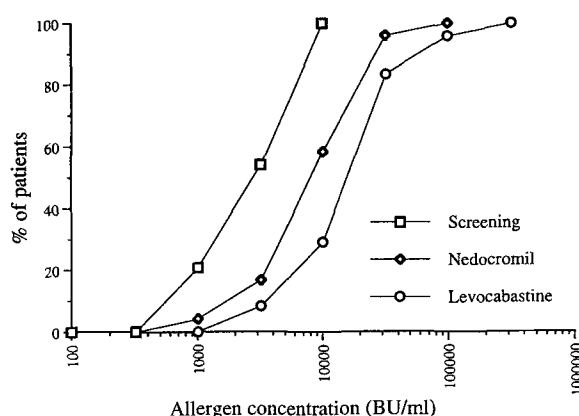


FIG. 2. Cumulative percent of patients reaching reaction threshold at each allergen concentration level.

BU/ml. Patients' characteristics are described in Table I. Duration of atopic conjunctivitis to grass pollen was 15.5 years (range, 4 to 29 years). Results of grass pollen skin prick tests and specific IgE determinations, as measured by Phadezym-CAP (mean $\pm$ SD, 34.86 ± 31.96 kU/L), were positive in all patients, as a condition for eligibility.

The number of shifts in allergen concentration necessary to reach the reaction threshold was defined as the primary end point of the trial. In volunteers treated with nedocromil for 15 minutes before allergen challenge, there was no shift in allergen concentration in seven patients (29%), whereas only one volunteer (4%) in the levocabastine treatment phase did not show a shift in allergen concentration (Fig. 1). The dose of allergen that could be administered after levocabastine or nedocromil treatment to reach the threshold was improved respectively by: one shift in 11 (46%) and 12 (50%) volunteers, two shifts in 9 (38%) and 3 (13%) volunteers, and three shifts in 3 (13%) and 2 (8%) volunteers. Compared with the screening phase, both drugs allowed a significant increase in the tolerated dose of allergen expressed as shift in allergen concentration (chi square trend, $p < 0.001$). However, comparison between treatments indicated that the number of shifts in allergen concentration was significantly greater after levocabastine treatment than after nedocromil treatment (chi square trend, $p = 0.019$).

Compared with the screening phase, the protection provided by both drugs is indeed indicated by a shift in the cumulative patient-response curves to the right (Fig. 2). Detailed noncumulative analysis showed that with levocabastine treatment, 29% of the volunteers reached reaction threshold at an allergen concentration of 10,000 BU/ml or less,

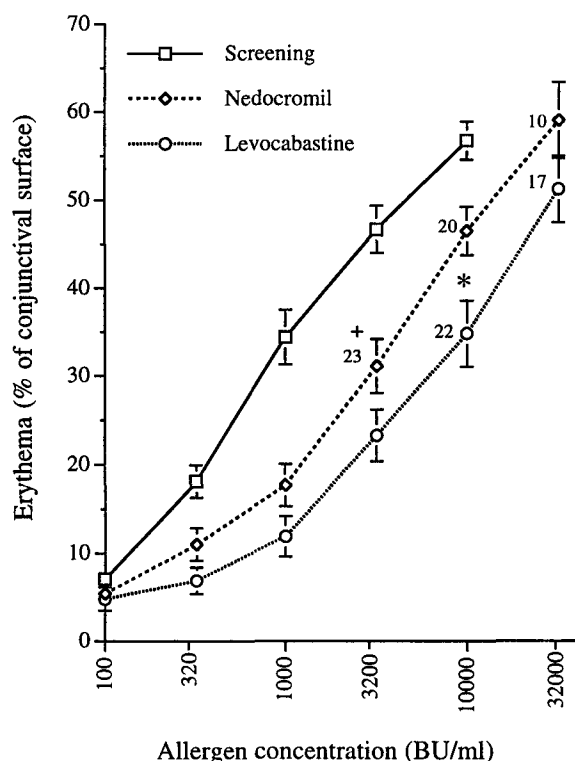


FIG. 3. Mean erythema (percent) at different allergen concentrations. *Levocabastine versus nedocromil $p < 0.05$ (Student's t test) + number of patients when less than 24.

54% between 10,000 and 32,000 BU/ml, and 13% between 32,000 and 100,000 BU/ml compared with 58%, 38%, and 4%, respectively, after nedocromil treatment (chi square trend, $p = 0.039$).

Two secondary end points were also considered: erythema, as a percent of conjunctival surface, and intensity of pruritus scored from 0 to 3. Both medications reduced erythema (Fig. 3) and pruritus (Fig. 4) when compared with the screening phase. However, in comparison with nedocromil, levocabastine reduced pruritus only at doses between 1000 and 10,000 BU/ml and erythema only at 10,000 BU/ml (Student's t test, $p < 0.05$).

The incidence of side effects was similar with both medications. The most frequent complaint was ocular irritation after application of eye drops. Baseline erythema was 5% before treatment in 25% of the volunteers in the levocabastine group and in 42% of those in the nedocromil group (Fisher's exact test, $p = 0.18$). Erythema increased significantly after the application of either medication (Student's t test $p < 0.05$). After levocabastine treatment, there was a 5% increase in seven volunteers, a 10% increase in one volunteer, and a

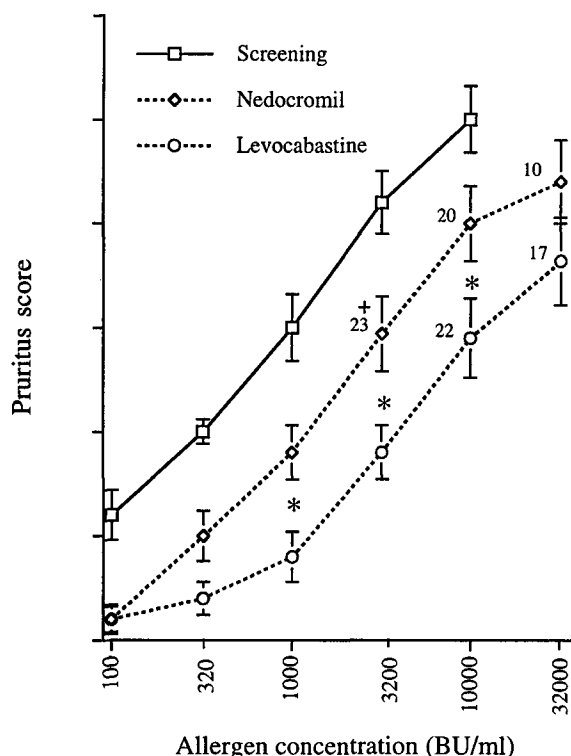


FIG. 4. Mean pruritus score (range, 1 to 3) at different allergen concentrations. *Levocabastine versus nedocromil $p < 0.05$ (Student's t test) + number of patients when less than 24.

30% increase in one volunteer. The corresponding values after nedocromil were 17%, 13%, and 0, respectively. However, these figures were not significantly different between the two treatment groups (Student's t test, $p > 0.1$).

The results of this double-blind, crossover trial demonstrate that the allergen concentration necessary to reach reaction threshold was increased after treatment with either medication, with a significantly better protection provided by levocabastine. Moreover, levocabastine was more potent than nedocromil in limiting pruritus and erythema.

DISCUSSION

The aim of this ocular provocation test with allergen was to compare the protective effect of levocabastine and nedocromil eye drops. The number of shifts in allergen concentration over threshold, as an expression of the improved tolerance to allergen, was defined as the primary end point of the trial; and the severity of erythema and pruritus were defined as secondary objectives. Reaction threshold was reached when erythema covered 50% or more of the conjunctival surface and was

associated with pruritus. Our data show that either drug significantly elevated patient tolerance to allergen compared with the screening phase, with a greater shift in allergen concentration after levocabastine treatment than after nedocromil treatment. Furthermore, the severity of pruritus and, to a lesser extent, of erythema were also significantly lower after levocabastine treatment than after nedocromil treatment.

Seasonal studies have two major drawbacks: the natural variability in environmental exposure to pollen and an estimated 70% placebo effect from eye drops.^{6, 7, 15} The conjunctival provocation test is a reproducible, quantitative, and safe method, which has been largely validated in the diagnosis of allergic conjunctivitis or in the evaluation of the protective effect of different ocular medications. Though a theoretical risk of mucosal desensitization might exist after repeated provocation tests, even those carried out at weekly intervals,^{4, 18} our analysis of the different treatment phases did not disclose any altered reactivity of the conjunctiva to allergen over time (chi square trend, $p > 0.1$). The relevance of ocular challenge compared with seasonal exposure can be questioned, but unfortunately, no quantitative data are currently available for comparison. However, it is interesting to note that among patients with the most severe seasonal symptoms, the provocation test was often experienced as equally or less potent than seasonal exposure. In this regard, either medication, compared with the screening phase or with each other, already significantly protected challenged volunteers against moderately elevated concentrations of allergen (1000 BU/ml to 10,000 BU/ml) both in intensity of pruritus or erythema.

If current results represent the first comparison by conjunctival provocation tests of the protective effect of levocabastine versus nedocromil, several provocation tests and seasonal trials have previously demonstrated the superiority of levocabastine over sodium cromoglycate.⁵⁻⁸ In clinical seasonal studies on atopic conjunctivitis, the protective effect obtained from nedocromil was equal to that obtained from sodium cromoglycate, except during the peak pollen season in which nedocromil was more effective. This might be related to the greater potency of nedocromil in inhibiting the release of various mediators from mast cells, as demonstrated *in vitro*.^{14, 15} Until now, there were no available data on the protective effect conferred by nedocromil in studies based on the provocation test with allergen in patients.

In this study medication was applied 15 minutes

before challenge and strongly decreased conjunctival reactivity to allergen, after both levocabastine and nedocromil treatment, though with a superior effect of levocabastine over nedocromil. The early onset of action of levocabastine has been documented. Stokes et al.¹¹ found a reduction of the conjunctival inflammation, already 10 minutes after levocabastine instillation, in a conjunctival provocation test with histamine. Though there are no available data from provocation test, the immediate effect of nedocromil in asthma has been studied in the guinea pig by bronchial provocation tests with allergen.¹⁹ Nedocromil was able to inhibit both the early and late inflammatory phase after allergen challenge performed only 15 minutes after nedocromil inhalation, suggesting an early protective effect. Though data are more difficult to interpret during the pollen season, a meta-analysis of seven studies with nedocromil performed in a total of 295 patients with conjunctivitis showed a relief of symptoms within 15 minutes in 49% of patients and within the first hour in 74% of patients after treatment with nedocromil eye drops for about 1 week.¹⁶ These data thus do not suggest that the superior protection obtained with the antihistamine levocabastine was related to its earlier onset of action. The protective effect might instead depend on the specific mechanisms of action of the concerned medications. The spectrum of action of nedocromil on the events leading to allergic symptoms differs considerably from that of topical antihistamines. Nedocromil, through the inhibition of chloride channels, appears to exert its action at multiple cell activation levels. In contrast, levocabastine has a narrower range of action as a potent, highly specific H_1 -receptor antagonist, resulting in pruritus inhibition and partial limitation of the vascular component of the allergic reaction.³ Furthermore, one cannot exclude that the effects of both medications on the late-phase reaction may also differ, which has not been examined here. In an animal model of asthma, nedocromil was shown to block both the immediate and late responses to antigen.^{19, 20} In patients with allergic asthma, nedocromil, administered 15 minutes before allergen challenge, inhibited the early and late reactions, with a greater protective effect on late reactions.²¹ Though few studies have investigated the effect of antihistamines on the late phase, recently in allergen conjunctival challenges, several H_1 -receptor antagonists appeared to provide a protective effect on both clinical and cellular late phases.^{22, 23}

In conclusion, in this first study comparing the

effects of levocabastine and nedocromil eye drops on the early response, both drugs were superior to placebo. However, levocabastine was more effective than nedocromil eye drops in increasing the conjunctival tolerance to allergen and in inhibiting pruritus and, to a lesser extent, erythema. Levocabastine thus appears to be a well tolerated topical medication and a powerful alternative to systemic antihistamines for the relief of acute symptoms of allergic conjunctivitis or for prophylaxis.

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