

Nedocromil sodium and levocabastine reduce the symptoms of conjunctival allergen challenge by different mechanisms

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Background: Nedocromil sodium and levocabastine are widely used for the treatment of ocular allergy, but their mechanisms of action are unclear.

Objective: We sought to compare the efficacy and mechanisms of action of nedocromil sodium and levocabastine in reducing conjunctival symptoms after ocular allergen challenge.

Methods: We performed a double-blind, placebo-controlled study in which 48 subjects were randomized to 3 groups to receive nedocromil sodium (2%), levocabastine (0.05%), or placebo eye drops twice daily for 2 weeks before ocular challenge with 10 μ L of ryegrass extract. Symptoms and tear histamine and PGD₂ concentrations were determined before challenge and at 10, 20, 30, 60, 180, and 360 minutes after challenge. Bulbar biopsy specimens were taken at 6 and 24 hours after challenge to assess conjunctival inflammatory cell numbers, adhesion molecule expression, and mast cell-associated IL-4, IL-5, IL-6, IL-13, and TNF- α levels.

Results: Both drugs significantly reduced total symptom scores ($P < .05$) at all times after challenge compared with placebo.

Itching, hyperemia, and lacrimation were most affected.

Nedocromil sodium treatment reduced tear concentrations of histamine (by 77%) and PGD₂ (by 70%) at 30 minutes after challenge (both $P < .05$). In biopsy specimens nedocromil sodium reduced the number of 3H4-positive mast cells (purportedly the secreted form of IL-4) by 49% at 6 hours and 59% at 24 hours (both $P < .05$). Levocabastine reduced intercellular adhesion molecule 1 expression by 52% at 6 hours and 45% at 24 hours (both $P < .05$).

Conclusion: Nedocromil sodium and levocabastine both reduced the conjunctival symptoms after ocular allergen challenge but appeared to work by different mechanisms.

Nedocromil sodium reduced mast cell function, whereas levocabastine appeared to have primarily antihistaminic actions, although it also reduced the expression of intercellular adhesion molecule 1. (J Allergy Clin Immunol 2001;108:449-54.)

Key words: Nedocromil sodium, levocabastine, conjunctival challenge, seasonal allergic conjunctivitis, mast cells, histamine, prostaglandin D₂, intercellular adhesion molecule 1

Seasonal allergic conjunctivitis (SAC) is a common, prevalent, and clinically significant type I hypersensitivity response¹ in which the mast cell is considered to play a pivotal role in causing signs and symptoms, including ocular itching, hyperemia, lacrimation, and chemosis.² This hypothesis is supported by the observations of increased levels of histamine, tryptase, PGD₂, and leukotriene C₄ in the tears of both patients with active SAC and of patients with SAC out of the pollen season after conjunctival allergen challenge.^{3,4} Biopsy studies of atopic individuals who have undergone ocular challenge with allergen have shown that this model mimics active SAC in that there is upregulation of the adhesion molecules E-selectin and intercellular adhesion molecule (ICAM) and a cellular infiltrate of mast cells, neutrophils, eosinophils, macrophages, and T cells.⁴⁻⁶

Both nedocromil sodium and levocabastine are effective in attenuating ocular symptoms of active SAC and conjunctival allergen challenge.⁷⁻¹¹ The primary mechanism of action of nedocromil sodium is considered to be mast cell stabilization, although the drug has also been shown to possess anti-inflammatory properties⁷ and an ability to modulate sensory nerve function.^{12,13} Levocabastine is a topically applied histamine H₁-receptor antagonist or antihistamine. Besides inhibiting the effects of histamine, this group of drugs has also been suggested to have anti-inflammatory effects and to downregulate adhesion protein expression.^{14,15}

This study compares the efficacy of these 2 drugs after ocular allergen challenge of atopic individuals and examines their mechanisms of action by assessing their effects on mast cell mediator release and cytokine expression, on the expression of adhesion proteins, and on the influx into the tissue of eosinophils and neutrophils.

METHODS

Forty-eight volunteers (23 men and 25 women; age range, 21-49 years) were enrolled in this double-blind, placebo-controlled allergen challenge study. Subjects were recruited if they were atopic and had a history of SAC but were excluded if they were contact lens

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Abbreviations used

ICAM-1: Intercellular adhesion molecule 1
SAC: Seasonal allergic conjunctivitis
VCAM-1: Vascular cell adhesion molecule 1

users, had perennial allergic conjunctivitis, or had used topical or oral antihistamines, steroids, nedocromil sodium, or sodium cromoglycate 4 weeks before starting the study. Ethical approval was granted by Southampton and South West Joint Research Ethics Committee, and the study was conducted in concordance with the tenets of the Declaration of Helsinki.

Study design

All subjects attended the eye clinic at Southampton General Hospital (United Kingdom) on 3 occasions out of the hay fever season between October 1, 1998, and March 31, 1999.

At visit 1, all volunteers gave written informed consent, underwent a skin prick test to ryegrass (*Lolium perenne*), and provided a blood sample for a RAST. Subjects were included in the study if they had a positive skin prick test response (wheal diameter of at least 3 mm larger than that produced by the diluent control) and a positive RAST result (concentration of IgE specific for ryegrass >0.35 kU/L).

At visit 2, the subjects were examined to confirm that they were free of any ocular disease, including signs of perennial allergy, and randomized to 3 groups: 12 to receive placebo eye drops; 14 to receive nedocromil sodium; and 22 to receive levocabastine. Nonresponders were excluded. One eye of each subject was then challenged with 10 μ L of ryegrass extract (500 or 1000 SU) and the other with 0.9% NaCl (saline solution control). Signs and symptoms were assessed, and tear fluid was collected for histamine and PGD₂ assay, as described below. Subjects were then given eye drops of nedocromil sodium (2%), levocabastine (0.05%), or placebo and were requested to instill one drop into each eye twice daily for 2 weeks before visit 3, including the morning of the visit. Two weeks of treatment was chosen because a previous study suggested that this time was necessary for nedocromil sodium to achieve maximal efficacy.¹⁶

At visit 3, allergen challenge, evaluation of the signs and symptoms, and tear collection was repeated. In addition, a biopsy specimen was taken from the upper bulbar conjunctiva at 6 hours in half the subjects and at 24 hours in the other half for histologic evaluation.

Evaluation of signs and symptoms

Ocular itching, hyperemia, lacrimation, and chemosis were scored blind by a single individual (P. A.) before challenge and at 10, 20, 30, 60, 180, and 360 minutes after challenge using a grading system modified from Abelson et al.¹⁷

The following scoring systems were used to quantify the signs and symptoms. Itching was assessed by asking the patients to grade the sensation of itching on a scale of 0 to 4, with 0 representing no itching, 1 representing intermittent itching in the inner corner of the eye, 2 representing mild continuous itching that did not require rubbing, 3 representing continuous itching that required rubbing, and 4 representing severe itching that required rubbing. Hyperemia (redness) was scored on a scale of 0 to 4, with 0 representing no redness, 1 representing mild dilatation of the conjunctival blood vessels, 2 representing moderate and patchy areas of hyperemia, 3 representing evenly distributed marked hyperemia, and 4 representing extreme dilation of episcleral vessels. Lacrimation (tear formation) was scored on a scale of 0 to 3, with 0 representing no tear formation, 1 representing slight tear formation, 2 representing marked tear formation necessitating frequent wiping of the eyes, and 3 representing tears rolling down the cheeks. Chemosis (edema) was

assessed on a scale of 0 to 4, with 0 representing no conjunctival swelling, 1 representing mild conjunctival elevation, 2 representing patchy areas of elevation separating the conjunctiva from the deeper layers, 3 representing confluent areas of elevation separating the conjunctiva from the deeper layers, and 4 representing gross swelling of the conjunctiva. The maximum total symptom score for any individual was 15.

Tear collection and mediator assay

Tear fluid was collected from the eye before challenge and at 10, 20, 30, 60, 180, and 360 minutes after challenge by using sterile glass microcapillary pipettes (Camlab Ltd) placed in the inferior fornix of the eye for 1 to 2 minutes. Once collected, tear fluid was aliquoted into 10- μ L volumes, placed immediately into Eppendorf tubes, and stored at -20°C to await analysis. Histamine was measured by using a specific RIA with a detection limit of 0.2 nmol/L (Immunotech International). PGD₂ was measured as its methoxime derivative by using a commercial immunoassay with a detection limit of 3 pmol (Cayman Chemical Co). Both histamine and PGD₂ are expressed as absolute amounts having been corrected for total tear volumes.

Conjunctival biopsy and immunohistochemistry

Consistent with our previous studies,^{18,19} bulbar conjunctival specimens were taken with scissors 6 or 24 hours after challenge from beneath the upper fornix after achievement of local anaesthesia (topical benoxinate 0.4%). The specimens were placed in acetone containing the enzyme inhibitors iodoacetamide (20 mmol/L) and phenylmethylsulfonyl fluoride (2 mmol/L) and fixed overnight at -20°C before processing into glycolmethacrylate (JB4 kit, Park Science Ltd).²⁰ Sequential 2- μ m sections were cut and stained immunocytochemically for mast cell tryptase (AA121) and for neutrophils (antineutrophil elastase, Dako Ltd), eosinophils (EG2, Pharmacia & Upjohn), the stored and secretory forms of IL-4 (antibodies 4D9 and 3H4,¹⁹ AMS Biotechnology), IL-5 (MAB5, a gift from P. Hissey, Glaxo, United Kingdom), IL-6 (antibody 104B11, a gift from P. Hissey, Glaxo, United Kingdom), IL-13 (MAB213, R&D systems Europe Ltd), or TNF- α (R&D systems Europe Ltd). Biotinylated mouse anti-mouse F(ab)2 (Dako) was used as the secondary antibody, followed by streptavidin biotin peroxidase and incubation with aminoethylcarbazole as a substrate. Cell counting was carried out as previously described.^{18,22,23} Briefly, immunocytochemically positive nucleated cells were counted, and their numbers were expressed as cells per square millimeter, with the area of conjunctival submucosa having been measured by using semiautomated image analysis with Colourvision 1.7.4 software (Improvision). Colocalization studies were performed on adjacent sections by using the camera lucida (Leica Ltd).

Similar sequential sections were immunostained for E-selectin (Cymbus Bioscience Ltd), ICAM-1 (a gift from Rothelin), or vascular cell adhesion molecule 1 (VCAM-1; Serotech Ltd) and expressed as a percentage of the total number of blood vessels. Endothelial length was assessed with antibody EN4 for endothelium to evaluate adhesion protein expression.²⁴

Two negative controls, omission of the primary antibody and addition of an isotype IgG₁ immunoglobulin (Sigma Chemical Co) used at the highest concentration of primary antibody, were included in the immunostaining regimen. All slides remained coded throughout the counting and colocalization.

Statistical analysis

Because the data were not normally distributed, results are expressed as medians with ranges, and comparisons between treatment groups were made by using the Mann-Whitney *U* test. A *P* value of less than .05 was considered to be statistically significant.

RESULTS

Signs and symptoms

There were no statistically significant differences between the groups with respect to the total symptom score or the scores of the individual signs and symptoms before allergen challenge (all scores = 0) or before treatment.

Administration of nedocromil sodium or levocabastine eye drops for 2 weeks significantly ($P < .05$) reduced the total symptom score in comparison with placebo at all time points (Fig 1). At 10 and 20 minutes, the total symptom score in the levocabastine group was significantly ($P < .05$) lower than that in the nedocromil sodium group.

The median scores for itching, hyperemia, and tear formation are shown in Fig 2. Itching and hyperemia scores in the nedocromil sodium and levocabastine groups were significantly ($P < .05$) lower at all times after challenge compared with placebo. Tear formation was significantly ($P < .05$) reduced by both drugs at 10, 20, and 30 minutes. Negligible chemosis was observed within 6 hours of challenge (data not shown).

Mediators in tears

Histamine and PGD₂ concentrations in tears are shown in Fig 3. Histamine levels peaked at 30 minutes and again at 360 minutes, the first of which was reduced by 77% ($P < .05$) by nedocromil sodium. Levocabastine did not significantly inhibit either peak. In contrast, there was only one peak of PGD₂ at 30 minutes, which was inhibited by 70% ($P < .05$) by nedocromil sodium. Levocabastine had no significant inhibitory effect on PGD₂ levels. Eosinophil cationic protein levels rose later than those of histamine and PGD₂, with baseline levels and those at 60, 180, and 360 minutes being a median of 0.01 pmol (range, 0-0.49 pmol), 0.45 pmol (range, 0-1.2 pmol), 1.08 pmol (range, 0.17-1.78 pmol), and 1.29 pmol (range, 0.62-1.65 pmol), respectively. Drug treatment did not reduce eosinophil cationic protein levels significantly, with those at 360 minutes being a median of 1.26 pmol (range, 0.39-1.73 pmol) and 1.13 pmol (range, 0.72-1.96 pmol), respectively, in the nedocromil sodium- and levocabastine-treated groups.

Biopsy studies

In bulbar biopsy specimens taken 6 or 24 hours after challenge, there were no statistically significant differences in the median numbers of mast cells, neutrophils, or eosinophils after drug treatment. The numbers of cells present in the placebo group at 6 hours were a median of 27.4 cells/mm² (range, 17.2-37.8 cells/mm²), 7.4 cells/mm² (range, 6.1-8.1 cells/mm²), 2.7 cells/mm² (range, 0-6.3 cells/mm²), and 1.9 cells/mm² (range, 0-3.7 cells/mm²), and those in 24-hour biopsy specimens were 22.9 cells/mm² (range, 15.7-29.1 cells/mm²), 7.8 cells/mm² (range, 6.4-8.5 cells/mm²), 0 cells/mm² (range, 0-1.4 cells/mm²), and 0.5 cells/mm² (range, 0-1.4 cells/mm²), respectively.

Analysis of the percentage of mast cells staining positive for cytokines (Table I) revealed a reduction in 3H4

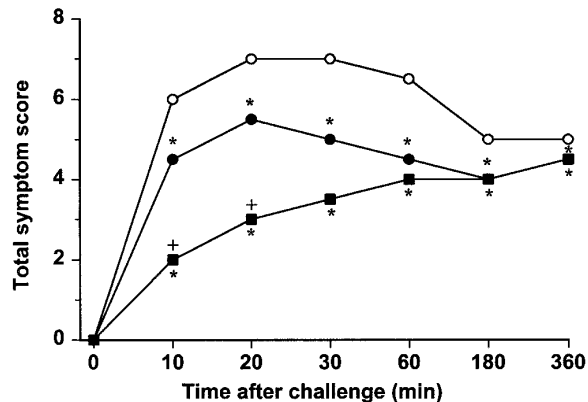


FIG 1. Total symptom scores after ocular allergen challenge of atopic subjects treated with nedocromil sodium (2%; filled circles, $n = 14$), levocabastine (0.05%; filled squares, $n = 22$), or placebo (open circles, $n = 12$). The severity of ocular itching, hyperemia, lacrimation, and chemosis were summed to give a maximum total symptom score of 15. Data are presented as medians (ranges have been omitted for clarity). *Statistically significant ($P < .05$) from placebo; +statistically significant ($P < .05$) from nedocromil sodium.

immunoreactivity in the nedocromil sodium group of 49% at 6 hours and 59% at 24 hours (both $P < .05$). Antibody 3H4 has been suggested to identify the secretory form of IL-4 associated with activated mast cells.^{19,25} There were no significant differences from placebo levels in the percentages of mast cells immunoreactive to antibody 4D9, a marker of the stored form of IL-4.^{19,25} Similarly, there were no statistically significant differences between placebo- and drug-treated groups for mast cell-immunoreactive IL-5, IL-6, IL-13, and TNF- α (Table I).

The effects of treatment on adhesion protein expression are shown in Fig 4. The percentage of blood vessels staining for ICAM-1 was lower by 52% at 6 hours and 45% at 24 hours in subjects treated with levocabastine compared with those given placebo (both $P < .05$). In contrast, nedocromil sodium did not reduce ICAM-1 expression. Neither nedocromil sodium nor levocabastine had significant effects on the expression of E-selectin or VCAM-1.

DISCUSSION

This double-blind study showed that 14 days of topical treatment with either nedocromil sodium or levocabastine was significantly effective in preventing the development of itching, hyperemia, and lacrimation after ocular challenge with allergen. Their mechanisms of action, however, appeared to be different. Nedocromil sodium reduced the concentrations of histamine and PGD₂ in the tears and the percentage of 3H4-positive mast cells. In contrast, the antihistaminic action of levocabastine was presumably largely responsible for the relief of symptoms because there was no reduction of either histamine or PGD₂ concentrations in the tears. This drug did, however, reduce the expression of ICAM-1 on conjunctival blood vessels.

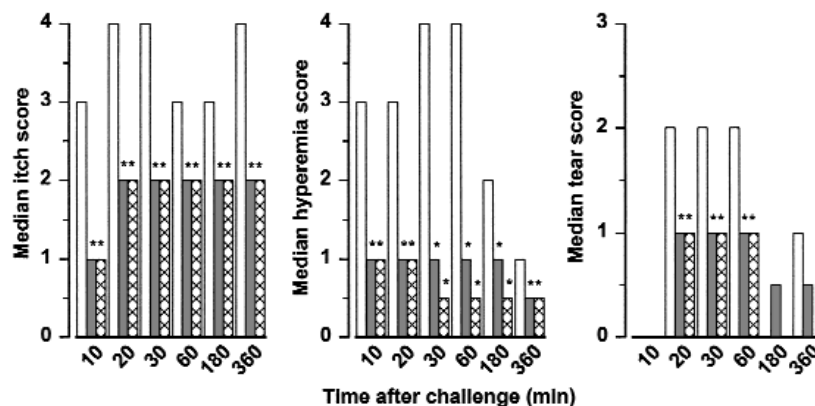


FIG 2. Itching, hyperemia, and lacrimation scores after ocular allergen challenge of atopic subject treated with nedocromil sodium (2%; filled columns, $n = 14$), levocabastine (0.05%; hatched columns, $n = 22$), or placebo (open columns, $n = 12$). Data are presented as medians (ranges have been omitted for clarity). *Statistically significant ($P < .05$) from placebo.

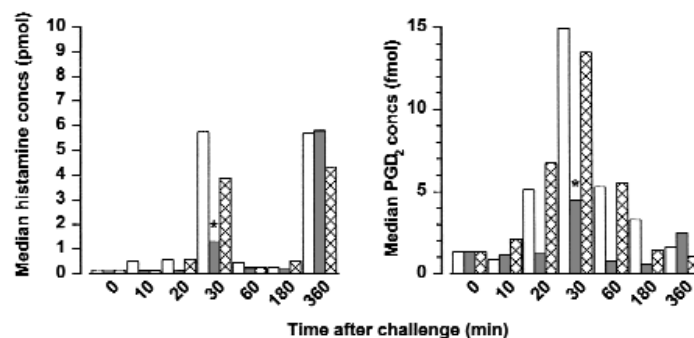


FIG 3. Concentrations of histamine, PGD₂, and eosinophil cationic protein in tears after ocular allergen challenge of atopic subjects treated with nedocromil sodium (2%; filled columns, $n = 14$), levocabastine (0.05%; hatched columns, $n = 22$), or placebo (open columns, $n = 12$). Data are presented as medians (ranges have been omitted for clarity). *Statistically significant ($P < .05$) from placebo.

The efficacy of both nedocromil sodium and levocabastine in relieving the ocular symptoms after challenge confirms previous studies in both the allergen challenge model and in active SAC.^{7-11,26-28}

Allergen challenge provoked 2 increases in the concentration of histamine in the tears, with the first at 30 minutes and the second at 6 hours, a finding that confirms previous studies.^{4,17,29} The first increase, being accompanied by the generation of PGD₂, is consistent with histamine being released as a result of mast cell degranulation.³⁰ However, the second peak at the time of onset of the late-phase response was not accompanied by PGD₂ generation. The source of histamine at this time is likely to be basophil influx into the site of allergic inflammation.⁴ Treatment with nedocromil sodium resulted in a 77% reduction in histamine release and a 70% reduction in PGD₂ generation into the tears 30 minutes after challenge. These observations are consistent with the mast cell-stabilizing effect of the drug.³¹ The comparatively strong mast cell-stabilizing effect of nedocromil sodium in the eye compared with in the lung in asthma⁷ has 2 possible explanations. The first is that the mast cells of the conjunctiva are very sensitive to the effects of the cromone stabilizers cromolyn sodium and nedocromil sodium. Indeed, there is a wide variability of the sensitivity of mast cells from different tissues to

these drugs, which does not follow protease expression.³² For example, mediator release from the MC_{CT} mast cell subtype of the intestine is inhibited well by nedocromil sodium, that from the MC_T of the lung is inhibited less well, and that of the skin is entirely refractory to the effects of the drug.³¹ The second explanation relates to the concentration of the drug at its site of action. In asthma, a single puff of nedocromil sodium delivers 1 mg of drug. Even assuming 100% bioavailability (in reality it is closer to 15%), distribution of the drug over the entire surface of the airways would result in extremely low concentrations at its site of action. In contrast, a single 300- μ L drop of 2% nedocromil sodium (total dose, approximately 7 mg) onto the relatively small surface of the conjunctiva would result in much higher concentrations at the site of action.

The second increase in histamine, which was apparent at 6 hours after challenge, was not inhibited by nedocromil sodium. This is consistent with the failure of the drug to inhibit histamine release from human basophils.³³

In addition to generating mediators that cause the symptoms of the early phase of the allergic response, they also synthesize and store cytokines, including IL-4, IL-5, IL-6, IL-13, and TNF- α ,^{19,34} which are capable of initiating the late-phase response and allergic inflammation. Of particular interest in this study was IL-4, a cytokine that

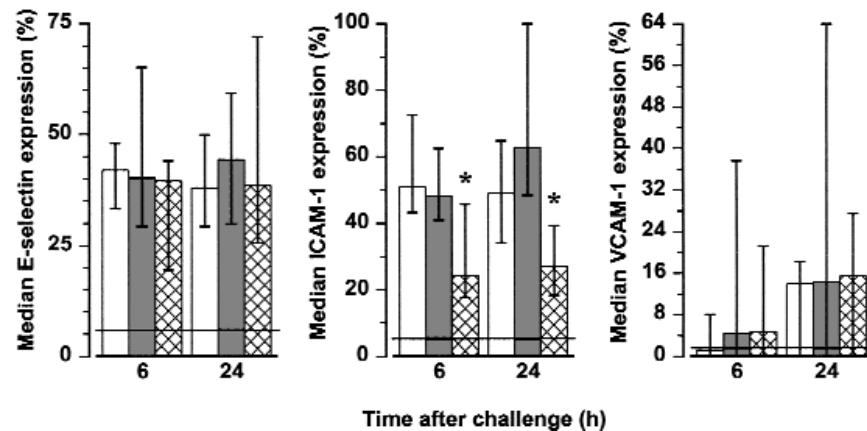


FIG 4. Expression of E-selectin, ICAM-1, and VCAM-1 in bulbar biopsy specimens taken 6 or 24 hours after ocular allergen challenge of atopic subjects treated with nedocromil sodium (2%; filled columns, each $n = 7$), levocabastine (0.05%; hatched columns, each $n = 11$), or placebo (open columns, each $n = 6$). Data are presented as medians (with ranges). *Statistically significant ($P < .05$) from placebo. Horizontal line represents median expression of adhesion proteins in unchallenged atopic individuals (data from Bacon et al⁴).

TABLE I. Numbers of cytokine-positive mast cells per square millimeter in bulbar biopsy specimens taken 6 and 24 hours after challenge after 2 of weeks topical treatment with nedocromil sodium (2%), levocabastine (0.05%), or placebo

Cytokine	Drug treatment					
	Placebo		Nedocromil sodium		Levocabastine	
	6 h	24 h	6 h	24 h	6 h	24 h
IL-4 (3H4)	34.0 (24.1-40.2)	34.7 (29.1-39.4)	17.2 (12.6-35.2)*	14.3 (10.1-19.5)*	34.9 (22.1-47.1)	37.4 (24.7-49.8)
IL-4 (4D9)	22.7 (17.2-27.1)	26.7 (18.4-31.4)	32.4 (29.6-49.7)	33.4 (23.2-37.4)	23.5 (18.3-32.1)	27.1 (19.1-32.0)
IL-5	1.6 (0-3.5)	0.9 (0-2.3)	1.6 (0-2.9)	0 (0-2.1)	0.7 (0-7.2)	1.3 (0-7.6)
IL-6	4.2 (0-7.1)	2.6 (0-6.9)	0 (0-14.7)	2.6(0-8.7)	0 (0-11)	0 (0-14.3)
IL-13	2.1 (1.0-4.9)	0.5 (0-3.2)	2.9 (1.4-5.3)	1.4 (0-2.2)	3.1 (1.5-3.4)	1.4 (0-1.9)
TNF- α	13.8 (9.7-15.9)	13.9 (8.0-15.8)	13.5 (5.1-17.6)	9.1 (6.4-17.6)	14.3 (7.4-20.2)	12.1 (7.4-16.3)

Values are given as median (range).

* $P < .05$ compared with placebo.

has numerous proallergic effects, including augmentation of IgE synthesis by B lymphocytes,³⁵ induction of the expression of Fc ϵ RI on mast cells and dendritic cells,^{36,37} and stimulation of VCAM-1 expression on endothelial cells.³⁸ In mast cells IL-4 may exist in 2 forms, a granule-associated stored form detectable by antibody 4D9, which is found in both resting and activated mast cells, and a secretory form, detectable by antibody 3H4, which appears as a membrane-associated ring in activated mast cells only.^{19,25} That nedocromil sodium reduced the expression only of 3H4-immunoreactive IL-4 is consistent with its ability to reduce mast cell activation.

Levocabastine effectively inhibited the ocular symptoms after topical allergen challenge of the eye but did not reduce the release of histamine and PGD₂ or 3H4 immunoreactivity, indicating that it did not have mast cell- or basophil-stabilizing properties. This confirms a report from an in vitro study in which levocabastine failed to suppress histamine release from human basophils.³⁹ Thus the suppression of symptoms is likely to be due to the antihistaminic action of levocabastine.^{9,10,27,28}

Levocabastine did, however, reduce the expression of ICAM-1 on conjunctival blood vessels. This confirms

previous findings with levocabastine and other antihistamines.^{11,15,40,41} The mechanism of the suppression of ICAM-1 expression has been suggested to involve the transcription factor nuclear factor κ B, activation of which through the histamine H₁-receptor stimulation^{42,43} may be reduced by antihistamines.^{44,45} However, a reduction of nuclear factor κ B activation is unlikely to be the sole effect of antihistamines in suppressing ICAM-1 expression because this transcription factor is also involved in the expression of E-selectin and VCAM-1,⁴⁶ neither of which were reduced in this study.

In conclusion, this study showed that 14 days of topical treatment with either nedocromil sodium or levocabastine reduced the development of itching, hyperemia, and lacrimation after ocular challenge with allergen. Nedocromil sodium appeared to work primarily as a mast cell stabilizer and levocabastine as an antihistamine, although the latter did reduce the expression of ICAM-1 on conjunctival blood vessels. The similarity between the actions of these 2 drugs in this study and one in active SAC suggest that the ocular allergen challenge model is a valid model for the evaluation of drugs for the treatment of allergic ocular disease.

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REFERENCES

1. Weeke ER. Epidemiology of hay fever and perennial allergic rhinitis. *Monogr Allergy* 1987;21:1-20.
2. McGill JI, Holgate ST, Church MK, Anderson DF, Bacon A. Allergic eye disease mechanisms. *Br J Ophthalmol* 1998;82:1203-14.
3. Proud D, Sweet J, Stein P, Settiple RA, Kagey-Sobotka A, Friedlander MH, et al. Inflammatory mediator release on conjunctival challenge of allergic subjects with allergen. *J Allergy Clin Immunol* 1990;85:896-905.
4. Bacon AS, Ahluwalia P, Irani AM, Schwartz LB, Holgate ST, Church MK, et al. Tear and conjunctival changes during the allergen-induced early and late phase responses. *J Allergy Clin Immunol* 2000;106:948-54.
5. Anderson DF, MacLeod JD, Baddeley SM, Bacon AS, McGill JI, Holgate ST, et al. Seasonal allergic conjunctivitis is accompanied by increased mast cell numbers in the absence of leukocyte infiltration. *Clin Exp Allergy* 1997;27:1060-6.
6. Fries JW, Williams AJ, Atkins RC, Newman W, Lipscomb MF, Collins T. Expression of VCAM-1 and E-selectin in an in vivo model of endothelial activation. *Am J Pathol* 1993;143:725-37.
7. Brogden RN, Sorkin EM. Nedocromil sodium: An updated review of its pharmacological properties and therapeutic efficacy in asthma. *Drugs* 1993;45:693-715.
8. Bleecker ER, Mason PL, Moore WC. Clinical effects of nedocromil sodium on allergen-related mechanisms. *J Allergy Clin Immunol* 1996;98:S118-23.
9. Dechant KL, Goa KL. Levocabastine. A review of its pharmacological properties and therapeutic potential as a topical antihistamine in allergic rhinitis and conjunctivitis. *Drugs* 1991;41:202-24.
10. Noble S, McTavish D. Levocabastine. An update of its pharmacology, clinical efficacy and tolerability in the topical treatment of allergic rhinitis and conjunctivitis. *Drugs* 1995;50:1032-49.
11. Ciprandi G, Cerqueti PM, Sacca S, Cilli P, Canonica GW. Levocabastine versus cromolyn sodium in the treatment of pollen-induced conjunctivitis. *Ann Allergy* 1990;65:156-8.
12. Jackson DM, Pollard CE, Roberts SM. The effect of nedocromil sodium on the isolated rabbit vagus nerve. *Eur J Pharmacol* 1992;221:175-7.
13. Chung KF. Effects of nedocromil sodium on airway neurogenic mechanisms. *J Allergy Clin Immunol* 1996;98:S112-6.
14. Pazdrak K, Gorski P, Ruta U. Inhibitory effect of levocabastine on allergen-induced increase of nasal reactivity to histamine and cell influx. *Allergy* 1993;48:598-601.
15. Buscaglia S, Paolieri F, Catrullo A, Fiorino N, Riccio AM, Pesce G, et al. Topical ocular levocabastine reduces ICAM-1 expression on epithelial cells both in vivo and in vitro. *Clin Exp Allergy* 1996;26:1188-96.
16. Butrus S, Greiner JV, Discepolo M, Finegold I. Comparison of the clinical efficacy and comfort of olopatadine hydrochloride 0.1% ophthalmic solution and nedocromil sodium 2% ophthalmic solution in the human conjunctival allergen challenge model. *Clin Ther* 2000;22:1462-72.
17. Abelson MB, Chambers WA, Smith LM. Conjunctival allergen challenge. A clinical approach to studying allergic conjunctivitis. *Arch Ophthalmol* 1990;108:84-8.
18. Bacon AS, McGill JI, Anderson DF, Baddeley S, Lightman SL, Holgate ST. Adhesion molecules and relationship to leukocyte levels in allergic eye disease. *Invest Ophthalmol Vis Sci* 1998;39:322-30.
19. MacLeod JD, Anderson DF, Baddeley SM, Holgate ST, McGill JI, Roche WR. Immunolocalization of cytokines to mast cells in normal and allergic conjunctiva. *Clin Exp Allergy* 1997;27:1328-34.
20. Britten KM, Howarth PH, Roche WR. Immunohistochemistry on resin sections: a comparison of resin embedding techniques for small mucosa biopsies. *Biotech Histochem* 1993;68:271-80.
21. Walls AF, Bennett AR, McBride HM, Glennie MJ, Holgate ST, Church MK. Production and characterization of monoclonal antibodies specific for human mast cell tryptase. *Clin Exp Allergy* 1990;20:581-9.
22. Morgan SJ, Williams JH, Walls AF, Church MK, Holgate ST, McGill JI. Mast cell numbers and staining characteristics in the normal and allergic human conjunctiva. *J Allergy Clin Immunol* 1991;87:111-6.
23. Baddeley SM, Bacon AS, McGill JI, Lightman SL, Holgate ST, Roche WR. Mast cell distribution and neutral protease expression in acute and chronic allergic conjunctivitis. *Clin Exp Allergy* 1995;25:41-50.
24. Montefort S, Gratzou C, Goulding D, Polosa R, Haskard DO, Howarth PH, et al. Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules 6 hours after local allergen challenge of sensitized asthmatic airways. *J Clin Invest* 1994;93:1411-21.
25. Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST, et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects: the mast cell as a source of IL-4, IL-5 and IL-6 in human allergic mucosa inflammation. *J Immunol* 1993;151:3853-65.
26. Blumenthal M, Casale T, Dockhorn R, Jamoszuk I, Kaiser H, Smith R, et al. Efficacy and safety of nedocromil sodium ophthalmic solution in the treatment of seasonal allergic conjunctivitis. *Am J Ophthalmol* 1992;113:56-63.
27. Kremer B, Tundermann A, Goldschmidt O. Onset of action, effectiveness and tolerance of levocabastine and nedocromil in topical therapy of seasonal allergic rhinoconjunctivitis. The Deutsche Rhinitis-Studiengruppe. *Arzneimittelforschung* 1998;48:924-30.
28. Janssens M. Efficacy of levocabastine in conjunctival provocation studies. *Doc Ophthalmol* 1992;82:341-51.
29. Helleboid L, Khatami M, Wei ZG, Rockey JH. Histamine and prostacyclin. Primary and secondary release in allergic conjunctivitis. *Invest Ophthalmol Vis Sci* 1991;32:2281-9.
30. Robinson C, Benyon RC, Holgate ST, Church MK. The IgE- and calcium-dependent release of eicosanoids and histamine from human cutaneous mast cells. *J Invest Dermatol* 1989;93:397-404.
31. Okayama Y, Benyon RC, Rees PH, Lowman MA, Hillier K, Church MK. Inhibition profiles of sodium cromoglycate and nedocromil sodium on mediator release from mast cells of human skin, lung, tonsil, adenoid and intestine. *Clin Exp Allergy* 1992;22:401-9.
32. Church MK, Benyon RC, Rees PH, Lowman MA, Campbell AM, Robinson C, et al. Functional heterogeneity of human mast cells. In: Galli SJ, Austen KF, editors. *Mast cell and basophil differentiation and function in health and disease*. New York: Raven Press; 1989. p. 161-70.
33. Amon U, Dieckmann D, Nitschke N, Wehrhahn C, Wolff HH. Role of basophilic leukocytes in inflammatory skin diseases. *Hautarzt* 1995;46:234-9.
34. Church MK, Okayama Y, Bradding P. The role of the mast cell in acute and chronic allergic inflammation. In: Chignard M, Pretolani M, Renesto P, Vargaftig BB, editors. *Cells and cytokines in lung inflammation*. New York: New York Academy of Sciences; 1994. p. 13-21.
35. Del Prete G, Maggi E, Parronchi P, Chretien I, Tiri A, Macchia D, et al. IL-4 is an essential cofactor for the IgE synthesis induced in vitro by human T-cell clones and their supernatants. *J Immunol* 1988;140:4193-8.
36. Toru H, Ra C, Nonoyama S, Suzuki K, Yata J, Nakahata T. Induction of the high-affinity IgE receptor (Fc epsilon RI) on human mast cells by IL-4. *Int Immunol* 1996;8:1367-73.
37. Geiger E, Magerstaedt R, Wessendorf JH, Kraft S, Hanau D, Bieber T. IL-4 induces the intracellular expression of the alpha chain of the high-affinity receptor for IgE in in vitro-generated dendritic cells. *J Allergy Clin Immunol* 2000;105:150-6.
38. Hickey MJ, Granger DN, Kubes P. Molecular mechanisms underlying IL-4-induced leukocyte recruitment in vivo: a critical role for the alpha 4 integrin. *J Immunol* 1999;163:3441-8.
39. Garred IM, Graaft VT, Van Toorenbergen AW, Van Wijk RG, Boegheim JP, Saxena PR, et al. Selective effect of levocabastine on histamine receptor and histamine release from human leukocytes and guinea pig isolated tissue. *Ann Allergy Asthma Immunol* 1996;76:327-34.
40. Ciprandi G, Buscaglia S, Pesce G, Passalacqua G, Rihoux JP, Bagnasco M, et al. Cetirizine reduces inflammatory cell recruitment and ICAM-1 (or CD54) expression on conjunctival epithelium in both early- and late-phase reactions after allergen-specific challenge. *J Allergy Clin Immunol* 1995;95:612-21.
41. Ciprandi G, Catrullo A, Cerqueti P, Tosca M, Fiorino N, Canonica GW. Loratadine reduces the expression of ICAM-1. *Allergy* 1998;53:545-6.
42. Aoki Y, Qiu D, Zhao GH, Kao PN. Leukotriene B4 mediates histamine induction of NF-kappaB and IL-8 in human bronchial epithelial cells. *Am J Physiol* 1998;274:L1030-9.
43. Bakker RA, Schoonus S, Smit M, Timmerman H, Leurs R. Constitutive activation of NF-B by the histamine H1 receptor is mediated via G subunits. *Proceedings of the International Sendai Histamine Symposium*; 2000; Sendai, Japan.
44. Yoneda K, Yamamoto T, Ueta E, Osaki T. Suppression by azelastine hydrochloride of NF-kappa B activation involved in generation of cytokines and nitric oxide. *Jpn J Pharmacol* 1997;73:145-53.
45. Rihoux JP, Michel L, Arnold R, Konig W. Hypothetical mechanisms of action of an H1-antihistamine in asthma. *Int Arch Allergy Immunol* 1999;118:380-3.
46. Spiecker M, Darius H, Liao JK. A functional role of I kappa B-epsilon in endothelial cell activation. *J Immunol* 2000;164:3316-22.