

Seasonal rhinitis and azelastine: Long- or short-term treatment?

Giorgio Ciprandi, MD, Vittorio Ricca, MD, Giovanni Passalacqua, MD,
Tiziana Truffelli, MD, Claudia Bertolini, MD, Nicola Fiorino, BS,
Anna Maria Riccio, BS, Marcello Bagnasco, MD, and Giorgio Walter Canonica, MD
Genoa, Italy

Background: Azelastine is a topical antihistamine, clinically demonstrated to be effective in allergic rhinitis.

Objective: We evaluated the clinical efficacy and the antiallergic activity of azelastine nasal spray, administered 0.56 mg per day, 0.28 mg per day, or on demand over a 3-month period during natural allergen exposure, in a double-blind, placebo-controlled fashion.

Methods: Thirty patients, sensitized to grass or *Parietaria* pollen, were allocated to three treatment groups: those receiving the standard dosage (0.14 mg/nostril two times a day), half the dosage (0.07 mg/nostril two times a day), or placebo daily for 3 months. All patients were allowed to take additional doses of azelastine when needed. Evaluation parameters were as follows: clinical symptoms recorded on a diary card, number of additional, on-demand azelastine puffs, nasal inflammatory cell count, intercellular adhesion molecule-1 expression on nasal epithelial cells, and pollen count.

Results: This study showed the following: (1) the half dose (0.28 mg/day) and the standard dose (0.56 mg/day) were equally effective in reducing clinical symptoms ($p = \text{NS}$), although the standard dosage required fewer additional puffs during times of peak pollen counts ($p < 0.05$); (2) both dosages were able to reduce the allergic inflammation ($p < 0.05$ vs placebo); and (3) on-demand use achieved acceptable clinical control but did not significantly reduce allergic inflammation.

Conclusion: Continuous treatment was more effective than on-demand use as assessed by both clinical evaluation and antiinflammatory action. (J Allergy Clin Immunol 1997;99:301-7.)

Key words: Azelastine, allergic inflammation, seasonal rhinitis

Allergic rhinitis is the most common immune-mediated disease. The allergic reaction is not only an immediate short-lived phenomenon but also comprises a more prolonged process, characterized by a complex inflammatory response. Both clinical and experimental evi-

Abbreviations used

APAAP:	Alkaline phosphatase-anti-alkaline phosphatase
ICAM-1:	Intercellular adhesion molecule-1
LFA-1:	Leukocyte function-associated molecule-1
mAb:	Monoclonal antibody
PBS:	Phosphate-buffered saline

dence support a relationship between IgE-mediated reactions and the resulting immune-mediated inflammatory process.¹

A hallmark of the allergic reaction is eosinophil infiltration. Eosinophils release basic proteins (e.g., eosinophil cationic protein) that can damage the epithelium. The phenomena leading to eosinophil infiltration are being debated. Eosinophils migrate from the bloodstream through the endothelium into the matrix, finally reaching the tissue site of the inflammatory reaction (i.e., the mucosa).¹ The mechanisms involved in the genesis of this phenomenon include chemotactic factors, cytokines, and, above all, adhesion molecules. The latter are intimately involved in the mechanisms of recognition and intercellular contact.¹

Because of these considerations, we focused on the adhesion molecule intercellular adhesion molecule-1 (ICAM-1; CD54). It belongs to the immunoglobulin superfamily and is constitutively expressed on various types of cells (B and T cells, fibroblasts, and monocytes). In addition, it is the natural ligand of the integrin, leukocyte function associated molecule-1 (LFA-1) (CD11a/CD18-Mac 1), also expressed on eosinophils. We found that epithelial cells (normally not expressing ICAM-1) expressed ICAM-1 after specific allergen challenge.^{2,3} The epithelial origin of ICAM-1 is confirmed by the presence of both messenger RNA in epithelial cells (detected by in situ hybridization) and soluble ICAM-1 in the supernatants of epithelial cell lines.⁴ In fact, ICAM-1 may be shed from the cell surface and is therefore detected in biologic fluids (such as nasal lavage fluids).⁵ Surprisingly, ICAM-1 expression on epithelial cells on allergen stimulation is rapid (as early as 30 minutes after challenge) and specific: it occurs only in allergic patients and not in healthy subjects.³ We have ob-

From the Allergy and Clinical Immunology Service, Department of Internal Medicine, Genoa University, Genoa.

Supported partially by P.F. CNR FATMA SP2 grant to G. W. C. (n 5710); by CNR target project "Ingegneria genetica" P.F. 99.00018 grant to M. B. and by the ARMIA (Associazione Ricerca Malattie Immunologiche e Allergiche) foundation.

Received for publication Feb. 21, 1996; revised July 18, 1996; accepted for publication July 22, 1996.

Reprint requests: Giorgio W. Canonica, MD, Dipartimento di Medicina Interna, Allergy and Clinical Immunology Service, Viale Benedetto XV 6, 16132 Genoa, Italy.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/76690

served this phenomenon on both nasal and conjunctival cells in patients with pollen allergy outside the pollen season (i.e., in the absence of natural exposure to the antigen),^{2, 6} and the relevance of these data has also been confirmed by evaluating ICAM-1 expression after natural exposure to the allergens (both pollens and house dust mites).^{7, 8} The close relationship between inflammation and nonspecific hyperreactivity is confirmed by the occurrence, on histamine and hyperosmotic solution challenge, of a nonspecific conjunctival hyperreactivity only in patients sensitive to house dust mites and not in those experiencing hay fever outside the pollen season. These findings underscore the importance of inflammation in the genesis of nonspecific hyperreactivity.^{9, 10}

For these reasons, it would be of interest to assess the preventive and therapeutic effects of nasal azelastine, a new topical antihistamine, on allergic inflammation resulting from pollen allergy. In particular, the assessment of ICAM-1 expression on epithelial cells appears to be useful for better defining the mechanism of action of the drug. This new experimental approach is supported both by the above-mentioned considerations and by additional recent studies by our group concerning the *in vitro* activity of budesonide on epithelial ICAM-1 expression.¹¹

We therefore designed a study aimed at evaluating the antiinflammatory activity of azelastine in patients with pollen allergy and at assessing the preventive effect of azelastine at different doses during natural allergen exposure.

METHODS

Patients

Thirty patients (17 men and 13 women; mean age, 33.2 years; range, 18 to 53 years) who had allergic rhinoconjunctivitis without asthma and required symptomatic treatment during the last 2 years were enrolled in the study. All patients were sensitized only to grass or *Parietaria*, as confirmed by skin prick test responses and RAST responses. The protocol was approved by the Ethical Committee of our department, and informed consent was required before enrollment. Pregnant and nursing women were ineligible for the study; women of childbearing age were included only if they were using appropriate methods of contraception. Patients with an upper airway disease, with anatomic nasal alterations, or with systemic disease were excluded, as were patients receiving specific immunotherapy. No drugs were allowed for 1 month before the study.

Study design

The study was designed as a double-blind, randomized, three parallel group, placebo-controlled study. Thirty patients were enrolled: they were assigned to the following three groups: (1) patients receiving the standard dose of azelastine nasal spray, one puff (0.14 mg) per nostril two times a day, as recommended by the manufacturer (ASTA MEDICA); (2) patients receiving the half dose of azelastine nasal spray, one puff (0.07 mg) per nostril two times a day; and (3) patients receiving placebo nasal spray (the diluent of the commercial product), one puff per

nostril two times a day. In addition, all patients could take additional azelastine puffs (0.14 mg) on demand. The treatment started at the beginning of pollen season and lasted until the end of the season (3 months).

The evaluated parameters were (1) nasal symptom score on diary cards, (2) number of additional azelastine spray puffs required, (3) inflammatory markers in nasal scraping (i.e., number of neutrophils and eosinophils and level of ICAM-1 expression on nasal epithelial cells), and (4) pollen count.

The symptoms of rhinitis were recorded daily on a proper diary card. The considered symptoms were nasal itching, obstruction, sneezing, and rhinorrhea. These symptoms were scored on an arbitrary 4-point scale (0 = absent, 1 = mild, 2 = moderate, and 3 = severe), according to our previous studies.^{2, 3} Patients also recorded the number of additional azelastine sprays used.

All patients made four clinic visits: at the beginning of the study and every month thereafter. At each visit a nasal scraping was taken. Pollen counts was performed by a pollen-trap device, as described previously.¹²

Cytologic assessment

Nasal scrapings were obtained as described in previous reports.^{3, 12} In brief, after the scraping was taken, the cotton tip of the swab was immersed in a plastic tray with phosphate-buffered saline (PBS) and transferred to a 10 ml polypropylene tube. The recovered fluids were centrifuged at 1400 g for 10 minutes, and each pellet was resuspended in PBS (2 ml). Cell suspensions were filtered to reduce the quantity of mucus, and cytospin slides were prepared by using standard techniques.

Slides were stained with May-Grünwald/Giemsa stain to differentiate between eosinophils, neutrophils, and epithelial cells and were viewed with an optic microscope (Leitz Laborlux Microscope; magnification, 250×). The number of inflammatory cells (neutrophils and eosinophils) was expressed as the mean of 10 microscope fields. Slides were examined by two investigators in a blinded fashion.

Immunocytochemistry

An immunoenzymatic alkaline phosphatase-monoclonal anti-alkaline phosphatase (APAAP) complex procedure modified from the method of Cordell et al.¹³ was used.

Specimens were air-dried at room temperature for 30 minutes and treated with a 1:50 dilution of purified anti-CD54 monoclonal antibody (mAb) (IgG₁, mg/ml; 84H10, Immunotech, Marseilles, France) or with a 1:100 dilution of anti-cytokeratin mAb (aCK19, IgG₁; DAKO, Milan, Italy) as a marker for epithelial cells. After washing in PBS, pH 7.6, samples were incubated with rabbit anti-mouse Ig, followed with the APAAP complex. Specimens were then incubated in substrate solution containing basic new fuchsin, Naphthol AS-BI phosphate, and levamisole as an inhibitor of endogenous alkaline phosphatase (Sigma, St. Louis, Mo.). In control samples, either mAb or anti-mouse Ig was omitted. As negative isotype control for CD54 staining on epithelial cells, an anti-T lymphocyte (anti-CD3) mAb (OKT3, IgG₁; Ortho Diagnostic, Raritan, N.J.) (1:20 dilution) was used. The dilutions were established on the basis of previous titration experiments. All preparations were counterstained with Carazzi's hematoxylin and examined by two investigators in a blinded manner.

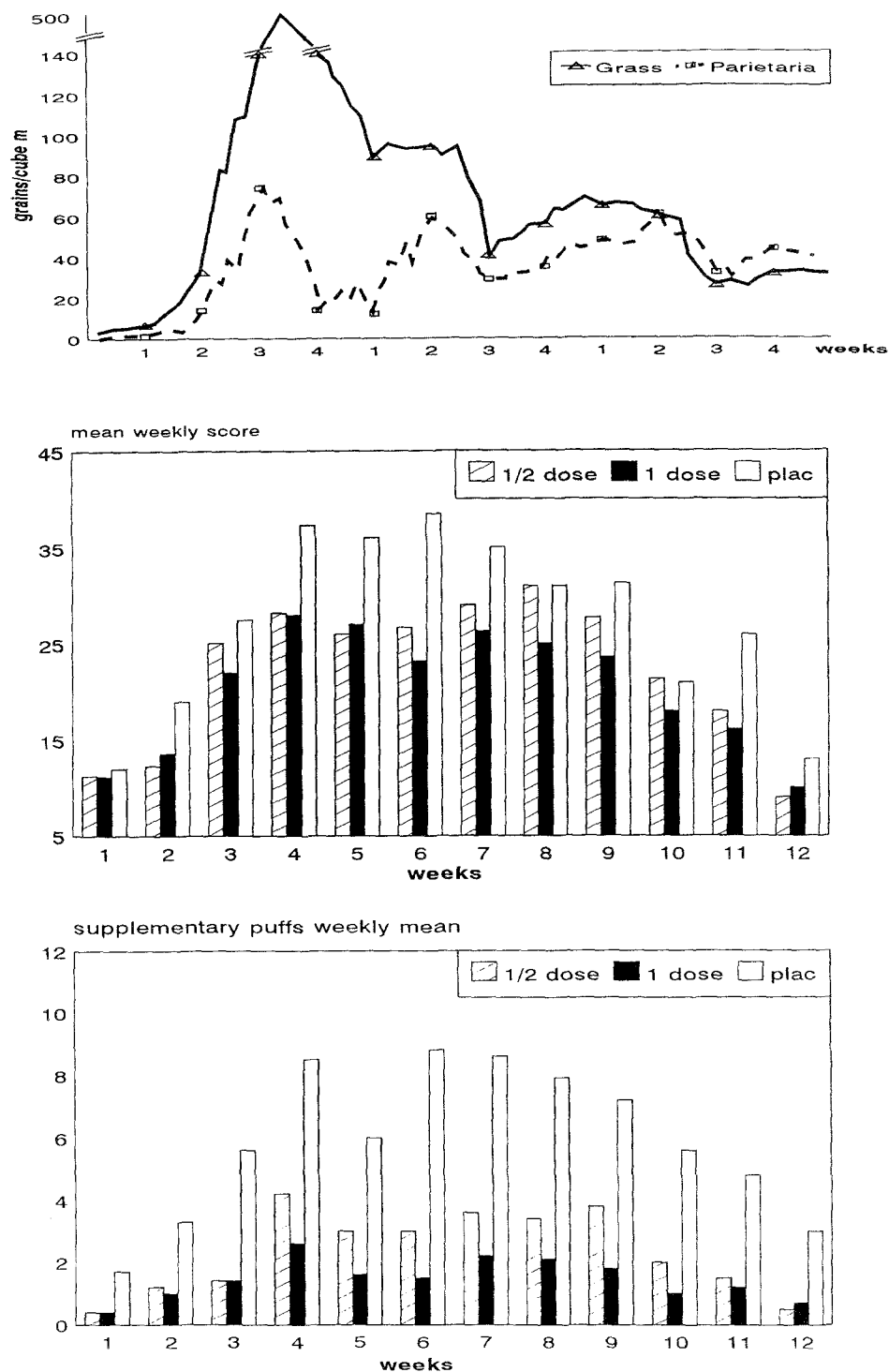


FIG. 1. Pollen count, clinical scores, and additional azelastine doses. **Upper panel,** Pollen count; **middle panel,** mean weekly score; **lower panel,** mean weekly supplementary doses.

CD54 expression on epithelial cells was expressed according to a 5-point rating scale (0 = no positive cells, 1 = mild positivity on 25% of epithelial cells, 2 = mild positivity on 75% of epithelial cells, 3 = intense positivity on 75% of epithelial cells, 4 = very intense positivity on all epithelial cells) as described previously.^{2, 3, 6-10, 12}

Statistical analysis

Because the parameters were not normally distributed, the Wilcoxon and Mann-Whitney nonparametric tests were used for intragroup and intergroup comparisons, respectively. A global comparison was also performed by Friedman's nonparametric analysis of variance.

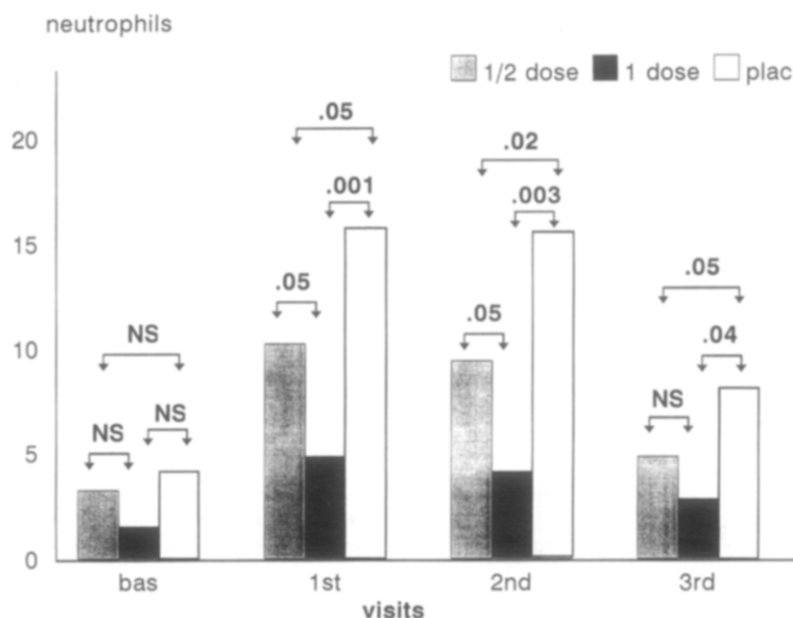


FIG. 2. Neutrophil count in nasal scraping in the three treatment groups at monthly visits. Intergroup comparison *p* values (Mann-Whitney test) are indicated above the bars.

TABLE I. *p* values of intergroup comparison by week of treatment

Week	Azelastine supplementary doses (weekly mean)			Symptom score (weekly mean)		
	Full dose vs half dose	Full dose vs placebo	Half dose vs placebo	Full dose vs half dose	Full dose vs placebo	Half dose vs placebo
I	NS	NS	NS	NS	NS	NS
II	NS	0.03	0.05	NS	NS	NS
III	NS	0.02	0.02	NS	NS	NS
IV	NS	0.001	0.05	NS	0.05	NS
V	NS	0.01	NS	NS	0.05	0.05
VI	NS	0.001	0.01	NS	0.01	0.05
VII	NS	0.005	0.03	NS	NS	NS
VIII	NS	0.003	0.03	NS	NS	NS
IX	NS	0.01	NS	NS	NS	NS
X	NS	0.006	0.03	NS	NS	NS
XI	NS	0.03	NS	NS	0.03	0.01
XII	NS	NS	NS	NS	NS	NS
Friedman's	Full-dose vs half dose: <i>p</i> = NS			Friedman's	Full dose vs half dose: <i>p</i> = NS	
ANOVA:	Full dose vs placebo: <i>p</i> = 0.001			ANOVA:	Full dose vs placebo: <i>p</i> = NS	
	Half dose vs placebo: <i>p</i> = 0.001				Half dose vs placebo: <i>p</i> = NS	

ANOVA, Analysis of variance.

RESULTS

All patients completed the treatment. Baseline comparisons among the three groups showed no significant differences among the evaluated parameters.

The median weekly pollen counts were sufficient to induce symptoms, according to our previous studies (Fig. 1).^{2,6,12}

The mean weekly nasal symptom score analysis did not reveal a significant difference between the group treated with the standard dose of azelastine (0.56 mg/day) and the group receiving the half standard dose (0.28

mg/day), even during pollen peaks (Fig. 1 and Table I). However, both groups receiving active treatment had lower mean weekly nasal symptom scores in comparison with the placebo group at pollen peaks; in particular, the full-dose group showed a more pronounced difference (Fig. 1 and Table I).

The analysis of additional azelastine puffs taken by patients showed no difference between the two active treatment groups (Fig. 1 and Table I), whereas there was a significant difference for the placebo group in comparison with active treatment groups.

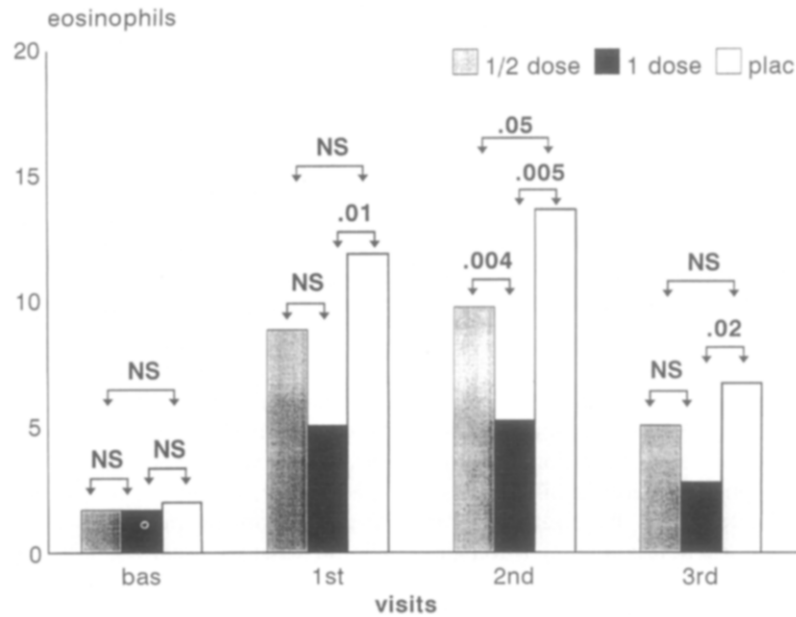


FIG. 3. Eosinophil count in nasal scraping in the three treatment groups at monthly visits. Intergroup comparison *p* values (Mann-Whitney test) are indicated above the bars.

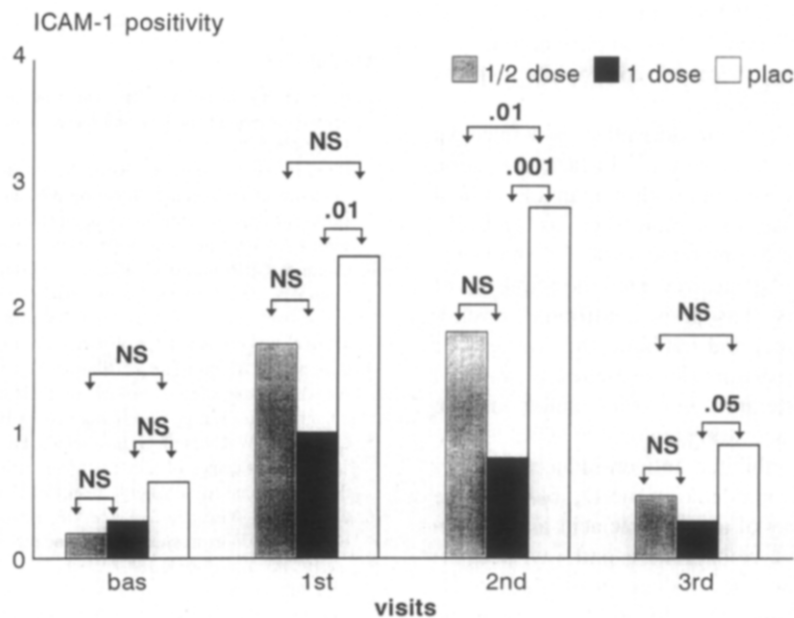


FIG. 4. ICAM-1 positivity on nasal epithelial cells in the three treatment groups at monthly visits. Intergroup comparison *p* values (Mann-Whitney test) are indicated above the bars.

An analysis of inflammatory parameters revealed the following:

1. Number of neutrophils: The standard-dose group had the lowest neutrophil count when compared with the other groups. The half-dose group also had significantly lower counts in comparison with the placebo group (Fig. 2).
2. Number of eosinophils: Again, the standard-dose

group had the lowest level of eosinophil infiltration. In addition, the difference between the standard-dose group and the half-dose group was statistically significant only at one observation point (after a prolonged exposure to high pollen levels, at which point the inflammation was more extensive. The placebo-treated group showed statistically higher eosinophil infiltration, mainly when compared with the standard-dose group (Fig. 3).

- ICAM-1 expression on nasal epithelial cells: There was no statistical difference in ICAM-1 expression between the two active treatment groups. On the other hand, the placebo-treated group had a significantly higher level of ICAM-1 expression in comparison with other groups, mainly with the standard-dose group (Fig. 4).

The treatment was well tolerated. Three patients in the standard-dose group and two patients in the half-dose group reported a transient alteration in taste.

DISCUSSION

It is now widely accepted that an inflammatory reaction sustains allergic rhinitis. After activation of mast cells, mediators are released, and infiltration with inflammatory cells (neutrophils and eosinophils) occurs. Thus an eosinophilic infiltrate may be regarded as a hallmark of allergic reactions. Several pathophysiologic mechanisms are involved in eosinophil recruitment: cytokines that are released (e.g., IL-3, IL-4, IL-5, and IL-8) act as chemoattractants and activate adhesion molecules.¹

On their surface, eosinophils express LFA-1, which is the counterreceptor of ICAM-1. Epithelial cells do not constitutively express ICAM-1 but do so early after allergen exposure. This may, at least in part, account for the adhesion process, because eosinophils express LFA-1 and Mac-1 on activation.²⁻⁴

Azelastine, a phthalazinone derivative, is a selective H₁ blocker with multiple activities.¹⁴ In fact, azelastine inhibits histamine release from both human and animal mast cells in response to stimuli such as anti-IgE, calcium ionophores, and compound 48/80.¹⁵⁻¹⁸ In vitro and in vivo studies, azelastine prevented the activation of inflammatory cells (e.g., basophils, neutrophils, eosinophils, and macrophages) and inhibited the synthesis or release of different mediators (leukotrienes, Na-p-Tosyl L arginine methyl esterase, superoxide anion, oxygen free radicals, platelet activating factor).^{15, 16} Oral azelastine, in a single dose, inhibited antigen-induced production of leukotriene C₄ and leukotriene D₄, blocking the 5-lipoxygenase pathway of arachidonic acid metabolism without affecting the cyclooxygenase pathway, because in vivo azelastine does not reduce prostaglandin D₂ levels on allergen challenge.¹⁹ In addition, azelastine exerts a corticosteroid-sparing effect in the management of bronchial asthma.²⁰

We have recently demonstrated the antiallergic activity of azelastine both at the nasal level and at the conjunctival level. Azelastine reduced (1) symptoms, (2) cytologic parameters (i.e., numbers of neutrophils and eosinophils), (3) mediator levels (i.e., eosinophil cationic protein recovered by nasal lavage), and (4) ICAM-1 expression on epithelial cells during both early- and late-phase reactions (manuscript provisionally accepted).

Taking previous results into consideration, we designed this study to optimize the strategy for treatment of pollen allergy. The data from this study support the

following references. (1) Continuous treatment during pollen season is better than symptomatic treatment (i.e., on demand) with respect to symptoms and allergic inflammation and (2) A half-dose of azelastine (0.28 mg/day) may be sufficient to achieve this target. In fact, the on-demand use of azelastine nasal spray does not allow optimal control of nasal symptoms and, overall, a reduction of allergic inflammation. A previous study with azelastine²¹ did not document any change in the eosinophilic infiltrate. Another study with cetirizine failed to demonstrate an effect of the drug on eosinophil recruitment at the cutaneous level.²² It is possible that these differing results are due to the differences in experimental models: the cetirizine study examined cutaneous reactions, and in the azelastine study, the observation time was 6 weeks.

Moreover, we have recently demonstrated that azelastine eye drops provide more symptomatic relief from clinical changes occurring after allergen-specific conjunctival challenge when azelastine has been administered before the challenge than when it was administered after the challenge (manuscript submitted for publication). In conclusion, continuous preventive treatment with azelastine achieves significant results in the treatment of patients with pollen allergy.

REFERENCES

- Bochner BS, Schleimer RP. The role of adhesion molecules in human eosinophil and basophil recruitment. *J Allergy Clin Immunol* 1993;94:427-38.
- Ciprandi G, Pronzato C, Ricca V, Bagnasco M, Canonica GW. Evidence of intercellular adhesion molecule-1 expression on nasal epithelial cells in acute rhinoconjunctivitis caused by pollen exposure. *J Allergy Clin Immunol* 1994;94:738-46.
- Ciprandi G, Pronzato C, Ricca V, Passalacqua G, Bagnasco M, Canonica GW. Allergen-specific challenge induces intracellular adhesion molecule-1 (ICAM-1 or CD54) on nasal epithelial cells in allergic subjects. *Am J Respir Crit Care Med* 1994;150:1653-9.
- Canonica GW, Pronzato C, Fiorino N, Ciprandi G, Bagnasco M. Adhesion molecules: A review of their clinical roles in allergic inflammation. *Allergy Clin Immunol News* 1993;5:80-4.
- Canonica GW, Ciprandi G, Buscaglia S, Pesce GP, Bagnasco M. The adhesion molecules of allergic inflammation: Recent insights into their functional roles. *Allergy* 1994;49:135-41.
- Ciprandi G, Buscaglia S, Pesce GP, Bagnasco M, Canonica GW. ICAM-1/CD54 expression on conjunctival epithelium during pollen season. *Allergy* 1995;50:184-7.
- Ciprandi G, Buscaglia S, Pesce GP, Villaggio B, Bagnasco M, Canonica GW. Allergic subjects express intercellular adhesion molecules-1 (ICAM-1 or CD54) on epithelial cells of conjunctiva after allergen challenge. *J Allergy Clin Immunol* 1993;91:783-92.
- Ciprandi G, Buscaglia S, Pesce GP, Pronzato C, Ricca V, Parmiani S, et al. Minimal persistent inflammation is present at mucosal level in patients with asymptomatic rhinitis and mite allergy. *J Allergy Clin Immunol* 1995;96:971-9.
- Ciprandi G, Buscaglia S, Pesce GP, Bagnasco M, Canonica GW. Ocular challenge and hyperresponsiveness to histamine in patients with allergic conjunctivitis. *J Allergy Clin Immunol* 1993;91:1227-30.
- Ciprandi G, Buscaglia S, Pesce GP, Lotti R, Rolando M, Bagnasco M, Canonica GW. Effects of conjunctival hyperosmolar challenge in allergic subjects and normal controls. *Int Arch Allergy Appl Immunol* 1994;104:92-6.
- Scordamaglia A, Paolieri F, Battifora M, Milanese M, Riccio AM, Bagnasco M, et al. Budesonide in vitro activity on a human

- continuous bronchial cell line: Expression of ICAM-1. *Allergy, Suppl.* 1995;26:50:266.
12. Ciprandi G, Pronzato C, Ricca V, Varese P, Del Giacco GS, Canonica GW. Terfenadine exerts antiallergic activity reducing ICAM-1 expression on nasal epithelial cells in patients with pollen allergy. *Clin Exp Allergy* 1995;25:871-8.
13. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, McDonald S, et al. Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984;32:219-45.
14. McTavish D and Sorkin EM. Azelastine: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential. *Drugs* 1984;38:778-800.
15. Chand N, Pillar J, Diamantis W, Sofia RD. Inhibition of IgE mediated allergic histamine release from rat peritoneal mast cells by azelastine and selected antiallergic drugs. *Agents Actions* 1985;16:318-22.
16. Fields DAS, Pillar J, Diamantis W, Perhach JL Jr, Sofia RD, Johnstone AJ, et al. Inhibition by azelastine of non allergic histamine release from rat peritoneal mast cells. *J Allergy Clin Immunol* 1984;73:400-3.
17. Fisher B, Schmutzler W. Inhibition by azelastine of immunologically induced histamine release from isolated guinea pig mast cells. *Arzneimittelforschung* 1981;31:1193-5.
18. Katayama S, Tsunoda H, Sakuma Y, Kai H, Tanaka I, Fakura O, et al. Effect of azelastine on the release and action of leukotriene C4 and D4. *Int Arch Allergy Immunol* 1987;83:284-9.
19. Shin MH, Baroody F, Proud D, Kagey-Sobotka A, Lichtenstein LM, Naclerio RM. The effect of azelastine on the early allergic response. *Clin Exp Allergy* 1992;22:289-95.
20. Busse WW, Middleton E, Storms W, Dockhorn J, Chu TJ, Grossman J, et al. Corticosteroid-sparing effect of Azelastine in the management of bronchial asthma. *Am J Resp Crit Care Med* 1996;153:122-7.
21. Pelucchi A, Chiapparino A, Mastopasqua B, Marazzini L, Hernandez A, Foresi A. Effects of intranasal azelastine and beclomethasone dipropionate on nasal symptoms, nasal cytology and bronchial responsiveness to metacholine in allergic rhinitis in response to grass pollens. *J Allergy Clin Immunol* 1995;95:515-23.
22. Taborda Barata L, Jacobson M, Walker S, Njuki F, Ying S, Randev P, et al. Effect of cetirizine and prednisolone on cellular infiltration and cytokine mRNA expression during allergen-induced late cutaneous responses. *Clin Exp Allergy* 1996;26:68-78.

Bound volumes available to subscribers

Bound volumes of The Journal of Allergy and Clinical Immunology are available to subscribers (only) for the 1997 issues from the Publisher, at a cost of \$88.50 for domestic, \$116.10 for Canadian, and \$108.50 for international subscribers for Vol. 99 (January-June) and Vol. 100 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. Copies are shipped within 30 days after publication of the last issue in the volume. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318; phone 1 (800) 453-4351 or (314) 453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.