

- reactions to food additives: the aspartame experience. *Am J Clin Nutr* 1986;43:464-9.
8. Witt J. The stability of aspartame and its conversion products in wet beverage systems. Presented at Proceedings International Aspartame Workshop. November 17-21, 1986; Marbella, Spain.
 9. Fleiss JL. The analysis of data from matched samples. In: Fleiss JL, ed. *Statistical methods for rates and proportions*. New York: John Wiley and Sons, 1981:112-9.
 10. Miettinen OS. The matched pairs design in the case of all-or-none responses. *Biometrics* 1968;24:339-52.
 11. Butchko HH, Kotsonis FN. Acceptable daily intake vs. actual intake: the aspartame example. *J Am Coll Nutr* 1991;10:258-66.
 12. Garriga MM, Berkebile C, Metcalfe DD. A combined single-blind, double-blind, placebo-controlled study to determine the reproducibility of hypersensitivity reactions to aspartame. *J ALLERGY CLIN IMMUNOL* 1991;87:821-7.

Comparison of azelastine and chlorpheniramine in the treatment of mastocytosis

Beth S. Friedman, MD, Maria L. Santiago, BS, Carole Berkebile, RN, and Dean D. Metcalfe, MD Bethesda, Md.

Background: Azelastine, a novel antiallergic medication, was compared with chlorpheniramine maleate for efficacy and safety in the treatment of systemic mastocytosis.

Methods: Fifteen subjects with mastocytosis participated in a double-blind, randomized, three-period, crossover trial, which compared an azelastine regimen of 4 mg or 8 mg every 12 hours with a chlorpheniramine regimen of 12 mg every 12 hours. Response to therapy was assessed by daily symptom scores, extinction dilution skin tests, plasma histamine levels, and global evaluations.

Results: Subjects' mean wheal area responses provoked by histamine or morphine sulfate were significantly lowered by azelastine when compared with chlorpheniramine. Plasma histamine levels in subjects receiving azelastine or chlorpheniramine were not significantly different. There were no significant differences between azelastine and chlorpheniramine in individual symptom scores or global evaluations except that azelastine at both doses significantly relieved pruritus and at 4 mg significantly relieved abdominal pain and that chlorpheniramine was associated with less fatigue in comparison to azelastine at 8 mg.

Conclusions: It thus appears that azelastine is superior to chlorpheniramine in suppressing skin responses to histamine and morphine sulfate and in suppressing pruritus in patients with mastocytosis. However, when all parameters are considered, neither drug is clearly superior for the treatment of patients with mastocytosis. (*J ALLERGY CLIN IMMUNOL* 1993;92:520-6.)

Key words: Mastocytosis, antihistamines, mast cells, histamine, clinical trial

Mastocytosis is a disease of unknown etiology characterized by the presence of excessive numbers of mast cells in the skin and internal organs such as the bone marrow, gastrointestinal tract,

liver, and spleen.¹ Subjects with this disorder frequently have manifestations related to the presence of excess mast cells and release of mast cell-derived mediators. The most common signs and symptoms include pruritus, flushing, urticaria, bone pain, abdominal pain, diarrhea, and occasionally malabsorption. Treatment of this disease is directed at controlling the mast cell mediator-induced signs and symptoms by either stabilizing the mast cell membrane (with cromolyn sodium) or blocking the effects of histamine with H₁ and H₂ histamine antagonists (e.g., chlorpheniramine

From Mast Cell Physiology Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda.

Received for publication Dec. 10, 1992; revised Apr. 13, 1993; accepted for publication Apr. 16, 1993.

Reprint requests: Dean D. Metcalfe, MD, Building 10, Room 11C210, National Institutes of Health, Bethesda, MD 20892.

1/1/47920

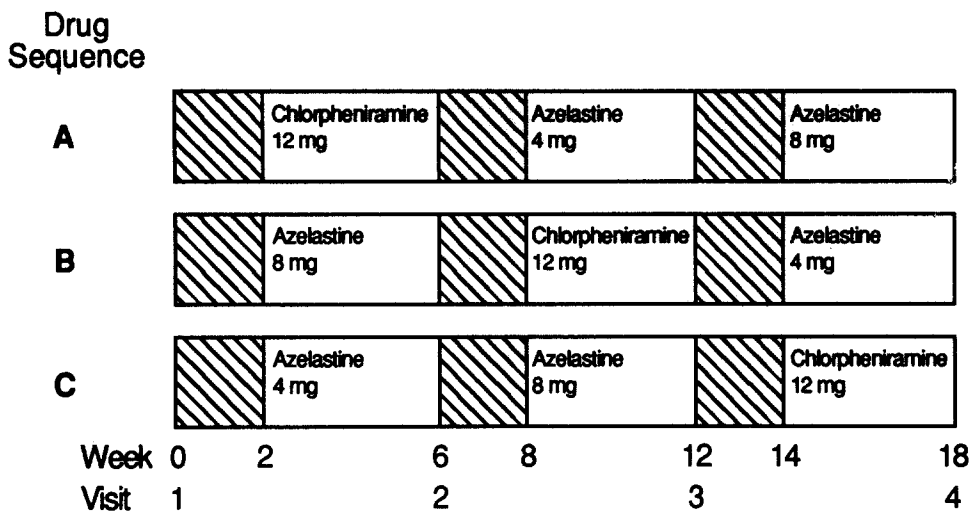


FIG. 1. Randomization and medication schemes.

maleate, cimetidine).² However, despite high doses of antihistamines, subjects often continue to have symptoms, resulting in part from the inability to block the effects of high levels of histamine and the presence of other mast cell mediators.

Azelastine is a novel antiallergic medication with activity in the upper and lower airways. In addition to its antihistamine activity, it has also been shown to inhibit the release of histamine and other allergic mediators from mast cells and basophilic leukocytes.^{3, 4} Pretreatment with azelastine blocks passive cutaneous anaphylaxis in rats and skin test reactivity in human beings.^{5, 6} This study was designed to determine whether these additional properties of azelastine (mast cell stabilization and inhibition of mediator effects) would provide a therapeutic benefit in subjects with symptomatic mastocytosis.

METHODS

Fifteen subjects were enrolled in this double-blind, three-period, crossover study under a protocol approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board after informed consent had been obtained. At the initial screening visit, subjects underwent a physical examination, laboratory safety tests (blood chemistry, hematology, urinalysis, and plasma histamine evaluation), and extinction dilution skin tests with histamine and morphine sulfate. Subjects were skin tested 72 hours or more after withdrawal of antihistamines, and no subject had been receiving a long-acting antihistamine. Fifteen subjects who had histologic evidence of mastocytosis, symptoms, and plasma histamine levels above the upper limit of normal (617 pg/mL) were selected.⁷ Subjects were randomized, according to a computer-generated code, to one of three drug sequences: azelastine hydro-

chloride (Astelin) 4.4 mg,* azelastine hydrochloride 8.8 mg, or chlorpheniramine (Chlor-Trimeton) 12 mg (Fig. 1). Three bottles were given to each subject according to the randomization scheme for each period; one bottle contained drug and two contained placebo. Subjects were then instructed to take one tablet from each of their three bottles every 12 hours and to assess their symptoms daily. After each 4-week study period, a washout period ensued in which no study drug was taken (cross-hatched area, Fig. 1). Subjects recorded the primary manifestations of their disease daily in a diary. Symptoms included pruritus, flushing, fatigue, abdominal pain, bone pain, headache, and number of stools; symptoms were scored on a semiquantitative scale of 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe).

On the twenty-eighth day of each study period, subjects underwent a physical examination, laboratory safety tests, plasma histamine determination, and extinction dilution skin tests with both histamine (0.05 to 100 mg/mL) and morphine sulfate (0.5 to 1000 mg/mL). These skin tests were performed during the baseline period and on study day 28 (last day) of each of the three periods (5 hours after dosing). Areas of wheal and flare were calculated with a computerized morphometric system.⁸ Plasma histamine levels were determined by a modification of a single-isotope enzymatic assay involving extraction of H₁ methylhistamine into chloroform and isolation of the H₁ methylhistamine by thin-layer chromatography.⁹

For skin test measurements, plasma histamine levels, symptom scores, and global evaluations, the paired Student's *t* test was used to compare means (or least squares means of data) before study drug administration (baseline) for each treatment and between administration of study drugs.

*Azelastine hydrochloride 4.4 mg and 8.8 mg are equivalent to 4.0 and 8.0 mg of the base, respectively.

TABLE I. Subject symptom demographics

Patient No.	Age (yr)	Sex	Pruritus	Flushing	Abdominal pain	Fatigue	Bone pain	Headache	Diarrhea	Bone marrow mast cells
1	52	F	+	+	+	—	+	—	—	Increased
2	65	F	+	+	+	+	+	+	+	Increased
3	44	M	+	—	+	—	+	+	+	Increased
4	41	F	+	+	+	+	+	+	+	Increased
5	51	F	+	+	+	+	+	+	+	Increased
6	58	M	+	+	—	—	—	—	—	Increased
7	40	M	+	+	—	—	—	—	—	Increased
8	31	F	+	+	+	+	+	+	+	Increased
9	31	F	+	+	—	+	+	+	+	Increased
10	26	F	—	+	+	+	+	+	—	Normal
11	25	F	+	+	+	+	+	—	—	ND
12	51	F	+	+	+	+	+	+	+	Increased
13	57	M	+	+	—	—	—	—	—	Increased
14	65	F	+	+	+	—	+	—	—	Increased
15	29	M	+	—	—	—	—	—	—	ND

ND, Not done.

TABLE II. Mean wheal area response (mm²) to concentrations of histamine after treatment with azelastine and chlorpheniramine

Histamine (μg/ml)	Treatment							
	AZ 4 mg			AZ 8 mg			CL 12 mg	
	Mean change from baseline	p Value		Mean change from baseline	p Value		Mean change from baseline	p Value
		BL	CL		BL	CL		BL
0.05	— 1.5	NS	0.0001	— 1.6	NS	0.0001	— 2.2	0.09
0.165	— 7.8	0.05	NS	— 7.8	0.05	NS	— 2.2	NS
0.5	— 9.4	0.05	NS	— 9.9	NS	NS	— 6.2	NS
1.65	— 8.8	0.06	NS	— 4.4	NS	NS	— 4.0	NS
5.0	— 14.5	0.003	0.04	— 10.8	0.03	NS	— 6.9	NS
16.5	— 14.0	0.004	0.08	— 17.1	0.004	0.02	— 8.0	0.07
50.0	— 27.6	0.0002	0.02	— 29.5	0.0003	0.01	— 13.1	0.03
100.0	— 53.8	0.0001	0.002	— 60.6	0.0001	0.0004	— 32.3	0.004

AZ, Azelastine; CL, chlorpheniramine; BL, Baseline; NS, not significant ($p > 0.1$).

RESULTS

Baseline demographics and subject disposition

Fifteen subjects with mastocytosis (5 men, 10 women) age 24 to 65 years (mean = 44.1 years) were enrolled in the study. All had urticaria pigmentosa as documented by skin biopsy specimens. Twelve subjects had increased numbers of bone marrow mast cells as determined by bone marrow biopsy. All indicated that they had at

least one of seven symptoms associated with mastocytosis, which were assessed before and during the study by means of daily symptom scores (Table I). Treatment group sequences were comparable with respect to age, race, weight, and wheal and flare responses to histamine and morphine sulfate.

Twelve of the 15 subjects completed the protocol for all three study periods. Subject 8 was discontinued after 9 days of period 2 because of

exacerbation of mastocytosis, which was considered to be unrelated to the study. Subjects 7 and 11 discontinued study drugs because of a moderate to severe bitter, metallic taste. (Subject 7 completed all of period 2, 2 days of period 1 [azelastine 8 mg], and 2 days of period 3 [azelastine 4 mg]; subject 11 completed periods 1 and 2 and dropped out on study day 12 of period 3 [azelastine 8 mg].)

Efficacy

Skin tests. Concentration-related decreases from baseline in the wheal responses (in square millimeters) to histamine challenges were observed over the concentration range evaluated (0.05 to 100 $\mu\text{g/ml}$) for both the azelastine 4 mg and 8 mg doses. These decreases ranged from -1.5 to -53.8 mm^2 for the 4 mg dose and from -1.6 to -60.6 mm^2 for the 8 mg dose and were statistically significant, with concentrations of histamine as low as 0.165 $\mu\text{g/ml}$ (Table II). Decreases from baseline in wheal responses with chlorpheniramine were also dependent on the concentration of histamine and ranged from -2.2 to -32.3 mm^2 , which were statistically significant at the two highest concentrations of histamine (50 and 100 $\mu\text{g/ml}$). The differences in the magnitude of the response (decreases in wheal diameter) between azelastine and chlorpheniramine were statistically significant beginning with the 5 $\mu\text{g/ml}$ concentration of histamine (Table II). Similar findings were observed with the flare response to histamine. The absolute changes in wheal and flare diameters are displayed in Fig. 2, A and B, respectively.

For morphine sulfate challenges, concentration-related changes in wheal diameter ranged from -0.1 to -46.7 mm^2 for the azelastine 4 mg dose and from 1.7 to -54.6 mm^2 for the 8 mg dose. The decreases were statistically significant from baseline areas beginning with the 50 $\mu\text{g/ml}$ concentration of morphine sulfate (Table III). In contrast, chlorpheniramine had no consistent (9.9 to -8.1 mm^2) effect on wheal diameters (none of the changes were statistically significant) as a result of this challenge. The differences in response between at least one dose of azelastine and chlorpheniramine were statistically significant beginning with the 50 $\mu\text{g/ml}$ concentration of morphine sulfate. The absolute changes in wheal diameter are displayed in Fig. 3, A.

Concentration-dependent decreases in the flare response to morphine sulfate were observed with both treatments (azelastine 4 and 8 mg, chlorpheniramine). However, the decreases with azelastine were statistically significantly greater than the chlorpheniramine decreases (from 16.5 to 1000 $\mu\text{g/ml}$ of morphine sulfate) and more than 200% greater at some concentrations (Fig. 3, B).

Histamine plasma levels. Mean plasma concentrations of histamine decreased from baseline by 0.43 and 0.29 ng/ml for the azelastine 4 mg and 8 mg doses and

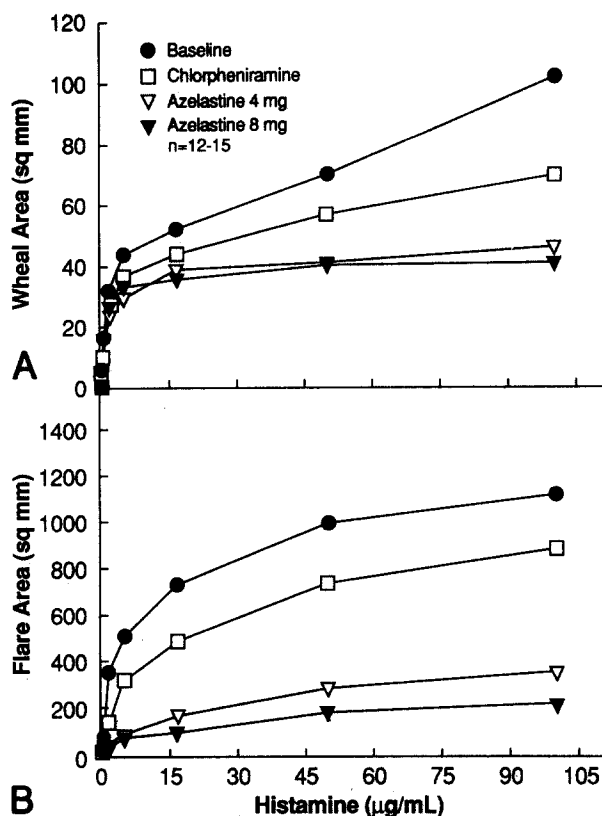


FIG. 2. Mean wheal area (panel A) or flare area (panel B) after intradermal administration of histamine.

by 0.63 ng/ml for chlorpheniramine dose (Fig. 4). The mean decrease for chlorpheniramine was statistically significant ($p < 0.005$) from baseline but not when compared with either dose of azelastine.

Symptom scores. Overall symptom scores indicated that there was no overall difference between chlorpheniramine and azelastine in alleviation of the majority of the symptoms associated with mastocytosis (Fig. 5). Azelastine did significantly relieve pruritus ($p < 0.05$) at both doses, and at 4 mg it significantly relieved abdominal pain ($p < 0.01$) when compared with chlorpheniramine. In comparison with azelastine 8 mg, chlorpheniramine was associated with less fatigue ($p < 0.05$). Overall global evaluations by both subjects and the physician were not statistically significantly different between any of the treatment periods (data not shown).

DISCUSSION

Several properties of azelastine indicate that it might have some therapeutic value in relieving the symptoms associated with mastocytosis. One report demonstrated by in vitro competitive radioligand binding assays that azelastine has a higher affinity for human lung histamine H_1 receptors compared with ketotifen, hydroxyzine, and chlorpheniramine.¹⁰ There is also in vitro evidence for

TABLE III. Mean wheal area response (mm²) to concentrations of morphine sulfate after treatment with azelastine and chlorpheniramine

Morphine sulfate (μ g/ml)	Treatment							
	AZ 4 mg			AZ 8 mg			CL 12 mg	
	Mean change from baseline	<i>p</i> Value		Mean change from baseline	<i>p</i> Value		Mean change from baseline	<i>p</i> Value
		BL	CL		BL	CL		
0.5	0	—	0.0001	0	—	0.0001	— 1.3	NS
1.65	0	—	NS	0	—	NS	0.3	NS
5.0	— 0.1	NS	0.06	1.7	NS	NS	9.4	NS
16.5	— 0.8	NS	NS	— 4.2	NS	NS	— 3.2	NS
50.0	— 17.1	0.05	0.05	— 14.8	0.02	0.08	9.9	NS
165.0	— 25.0	0.007	0.008	— 21.4	0.0003	0.04	— 1.4	NS
500.0	— 38.1	0.0007	0.0005	— 36.2	0.001	0.0006	— 7.2	NS
1000.0	— 46.7	0.0001	0.001	— 54.6	0.0002	0.0001	— 8.1	NS

AZ, Azelastine; CL, Chlor-Trimeton; BL, baseline; NS, not significant ($p > 0.01$).

its mast cell stabilizing activity. In this study it was demonstrated that a reduction in the inhibition of histamine release was observed with increasing calcium ion concentration, suggesting that azelastine may inhibit histamine release from mast cells by blocking calcium ion channels.¹⁰ Its antihistamine activity has also been demonstrated clinically by a placebo-controlled trial in which azelastine at 8 mg per day significantly decreased skin reactivity to histamine, codeine, and antigen.⁶ For perspective, however, it should be noted that chlorpheniramine inhibits antigen-induced histamine release from human peripheral leukocytes¹¹ and human lung fragments.¹²

In addition, azelastine appears to be safe and relatively well tolerated, with the primary side effects being bitter or metallic taste and drowsiness.¹³ No clinically significant changes in blood pressure, heart rate, or hematologic, hepatic, or renal laboratory tests have been demonstrated.¹⁴ On the basis of azelastine's safety, tolerability, and efficacy as an antihistamine and its stabilizing properties in cells involved in the inflammatory process, the effects of azelastine on both biochemical and clinical features of mastocytosis were investigated and compared with those of chlorpheniramine, an H₁ histamine antagonist. A previous double-blind clinical comparison of the mast cell stabilizer cromolyn sodium versus chlorpheniramine and cimetidine (an H₂ histamine blocker) indicated that none of these treatment

regimens was superior in alleviating the symptoms of mastocytosis.²

The present study found no statistically significant difference in overall clinical effects when azelastine was compared with chlorpheniramine in the treatment of mastocytosis (Fig. 5). Although overall global evaluations by subjects and physicians and total subject symptoms scores indicate that azelastine was the preferred drug, this preference was not statistically significant. Azelastine was, however, determined to be significantly better at relieving pruritus than chlorpheniramine (Fig. 5). The scores that indicate that chlorpheniramine significantly relieved fatigue in comparison with azelastine are probably an indication that azelastine itself caused fatigue in concordance with results of previous clinical studies of azelastine.^{13, 14}

When azelastine was compared with chlorpheniramine, neither was found to be superior in reducing plasma histamine concentrations (Fig. 4). Similar results were seen in the trial of chlorpheniramine and cimetidine versus cromolyn sodium.² There was some indication that chlorpheniramine may have, on average, lowered plasma histamine levels, which is in agreement with *in vitro* data that chlorpheniramine may inhibit mast cell histamine release.¹² Azelastine was found to be statistically significantly better at reducing wheal and flare response to intradermal histamine and morphine sulfate when compared with chlor-

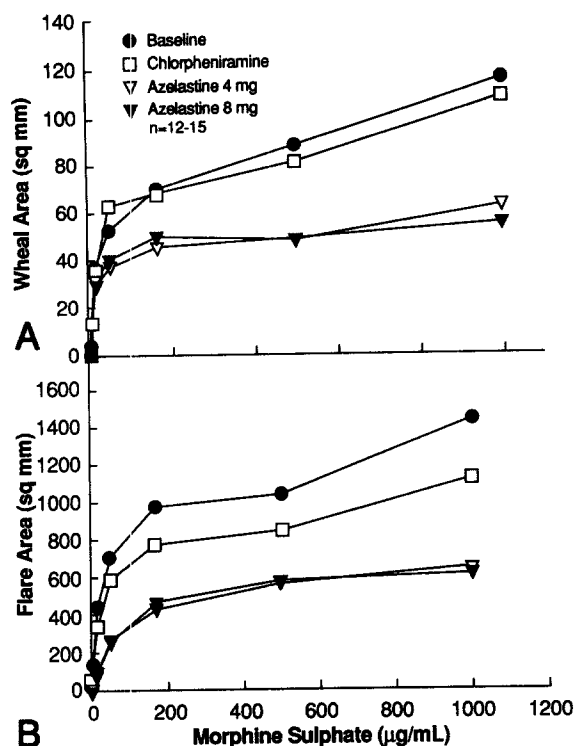


FIG. 3. Mean wheal area (panel A) or flare area (panel B) after intradermal administration of morphine sulfate.

pheniramine (Tables II and III, Figs. 2 and 3). Thus although azelastine's mast cell stabilizing and antihistamine activities cause a significant decrease in skin reactivity, this was not reflected in symptom scores other than that for pruritus. This would seem to indicate that the symptoms of mastocytosis involve more complex or more amplified mechanisms than those that occur in skin wheal and flare responses. For instance, much of the histamine release may be from mast cell turnover. However, this hypothesis has not been tested, and indeed, it is difficult to imagine how mast cell turnover may be assessed. For instance, simple measurement of histamine levels in serum would not distinguish histamine released by activated mast cells from that released by dying mast cells.

The results of this investigation thus indicate that azelastine and chlorpheniramine are equally effective in the treatment of mastocytosis. Chlorpheniramine is a well-studied antihistamine, and azelastine was found to be only somewhat better in relieving pruritus. Considering these points, in practice chlorpheniramine may be the more useful of these two alternative antihistamines in the management of the symptoms of mastocytosis.

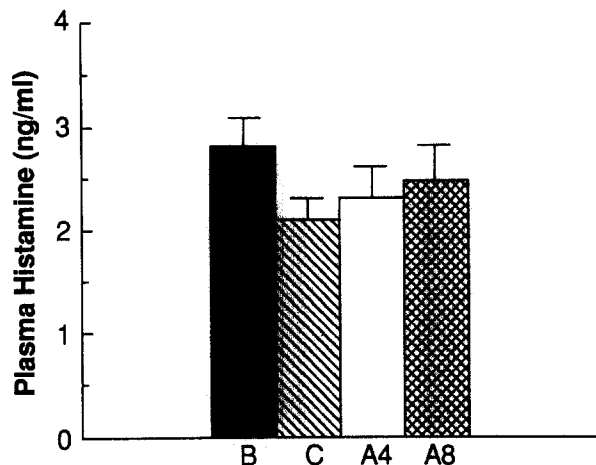


FIG. 4. Plasma histamine levels (mean $\pm$ SEM). B, Before study; C, chlorpheniramine 12 mg; A4, azelastine 4 mg; A8, azelastine 8 mg.

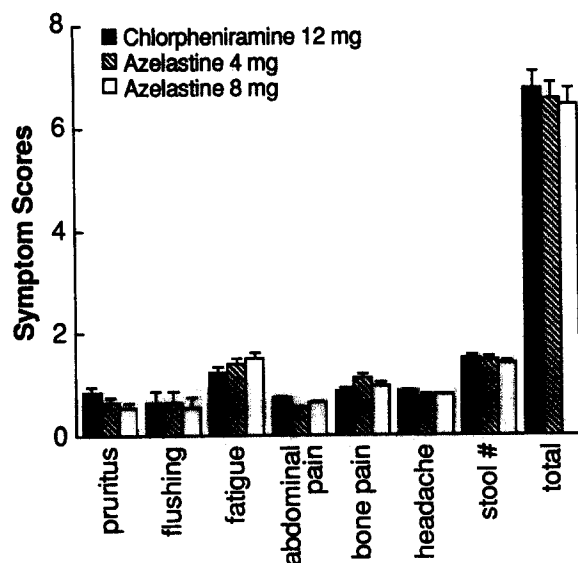


FIG. 5. Symptom scores (least square means) for seven symptoms and total scores. Symptom scores: 0 = none, 1 = mild, 2 = moderate, 3 = severe. Total is additive total of all scores.

We thank Mrs. Belinda Richardson for her assistance in the preparation of this report and Dr. J. Perhach, Dr. J. Howard, and Dr. R. K. Dix from Wallace Laboratories for their aid and counsel in the performance of this study.

REFERENCES

- Friedman BS, Metcalfe DD. Mastocytosis. In: Tauber AI, Wintrob BU, Simon AS, eds. *Biochemistry of the Acute Allergic Reactions: Fifth International Symposium*. New York: Alan R. Liss, 1989:163-73.

2. Frieri M, Alling DW, Metcalfe DD. Comparison of the therapeutic efficacy of cromolyn sodium with that of combined chlorpheniramine and cimetidine in systemic mastocytosis: results of a double-blind clinical trial. *Am J Med* 1985;78:9-14.
3. 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.
4. Chand N, Pillar J, Diamantis W, Perhach JL Jr, Sofia RD. Inhibition of calcium ionophore (A23187)-stimulated histamine release from rat peritoneal mast cells by azelastine: implications for its mode of action. *Eur J Pharmacol* 1983;96:227-33.
5. Diamantis W, Chand N, Harrison JE, Rooney SM, Sofia RD. Inhibition of IgE-mediated passive cutaneous anaphylaxis by azelastine in rat [Abstract]. *J ALLERGY CLIN IMMUNOL* 1984;73:184.
6. Atkins P, Merton H, Karpink P, Weliky I, Zweiman B. Azelastine inhibition of skin test reactivity in humans [Abstract]. *J ALLERGY CLIN IMMUNOL* 1985;75:167.
7. Friedman BS, Steinberg S, Meggs WJ, et al. Analysis of plasma histamine levels in patients with mast cell disorders. *Am J Med* 1989;87:649-54.
8. Keffer JM, Bressler RB, Wright R, Kaliner MA, Metcalfe DD. Analysis of the wheal-and-flare reactions that follow the intradermal injection of histamine and morphine in adults with recurrent, unexplained anaphylaxis and systemic mastocytosis. *J ALLERGY CLIN IMMUNOL* 1989;83:595-601.
9. Dyer J, Warren K, Merlin S, Metcalfe DD, Kaliner MA. Measurement of plasma histamine: description of an improved method and normal values. *J ALLERGY CLIN IMMUNOL* 1982;70:82-7.
10. Little M, Ecklund P, Casale T. Azelastine's therapeutic action in asthma [Abstract]. *J ALLERGY CLIN IMMUNOL* 1988;81:278.
11. Lichtenstein LM, Gillespie E. The effects of the H1 and H2 antihistamines on "allergic" histamine release and its inhibition by histamine. *J Pharmacol Exp Ther* 1975;192:441-50.
12. Church MK, Gradidge CF. Inhibition of histamine release from human lung *in vitro* by antihistamines and related drugs. *Br J Pharmacol* 1980;69:663-7.
13. Weiler JM, Donnelly A, Campbell BH, et al. Multicenter, double-blind, multiple-dose parallel-groups efficacy and safety trial of azelastine, chlorpheniramine, and placebo in the treatment of spring allergic rhinitis. *J ALLERGY CLIN IMMUNOL* 1988;82:801-11.
14. Ollier S, Gould CAL, Davies RJ. The effect of single and multiple dose therapy with azelastine on the immediate asthmatic response to allergen provocation testing. *J ALLERGY CLIN IMMUNOL* 1986;78:358-64.