

Effect of cetirizine on mast cell-mediator release and cellular traffic during the cutaneous late-phase reaction

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A new H₁ antihistamine, cetirizine, was studied to determine its effects on mediators and cellular infiltration during the cutaneous late-phase response (LPR). Ten ragweed-allergic subjects, who had previously demonstrated a cutaneous LPR, were examined in a double-blind, crossover study. Either cetirizine, 20 mg, or placebo was administered orally once daily for 2 days before and the morning of placement of a skin chamber overlying an unroofed heat/suction-induced blister to which was added antigen or buffer. Skin test erythema was significantly reduced by cetirizine at 15 minutes, 2 hours, and 4 hours by 56%, 40%, and 39%, respectively (all, $p \leq 0.01$), but by 6 and at 8 hours, the cutaneous erythema was not significantly lessened. Histamine release was not altered by cetirizine treatment, but prostaglandin D₂ (PGD₂) production, which peaked at 3 to 5 hours, was clearly reduced by cetirizine treatment, being lower at all time points during the reaction; this was significant by analysis of variance ($p \leq 0.04$). The inhibition was most marked during the fifth hour of the reaction when there was a 50% suppression of the PGD₂ level by cetirizine (0.193 ng/ml to 0.075 ng/ml [$p \leq 0.03$]). The most dramatic effect of cetirizine was attenuation of the inflammatory cell migration into the chamber. Eosinophil infiltration was decreased by about 75% during hours 6, 7, and 8 ($p \leq 0.04$), whereas the number of neutrophils was reduced by the same magnitude at the same times ($p \leq 0.04$). Basophils appeared to enter the chamber together with the eosinophils and were decreased by 64% during hours 6 through 8, but not significantly due to small numbers. We conclude that cetirizine influences the pathogenesis of the LPR by causing a significant reduction in both the inflammatory cell infiltrate and PGD₂ production. These effects apparently are not related to its anti-H₁ activity. (J ALLERGY CLIN IMMUNOL 1989;83:905-12.)

Cetirizine, a new H₁ antagonist, is the major human metabolite of hydroxyzine, being formed by oxidation of the primary alcoholic side chain to a carboxylic acid moiety.¹ Hydroxyzine is widely and preferentially

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Supported by National Institutes of Health Grants AI7290 and AI8270.

The opinions expressed are those of the authors and do not necessarily represent the opinions of the Department of Defense or the United States Air Force.

Received for publication May 31, 1988.

Accepted for publication Oct. 28, 1988.

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Publication No. 748 from the O'Neill Laboratories of The Good Samaritan Hospital, Baltimore, Md.

*Recipient of a Pfizer Biomedical Research Award.

Abbreviations used

Ag:	Antigen
LPR:	Late-phase response
LTB ₄ :	Leukotriene B ₄
OCD:	Optimal challenge dose
PGD ₂ :	Prostaglandin D ₂
PNU:	Protein nitrogen unit
RW:	Ragweed
RLH:	Ringer's lactate with 250 µg/ml of heparin

used to treat skin disorders, allergic and inflammatory.² It has been demonstrated to be somewhat more effective than other H₁ antagonists in these conditions, although the mechanism of this increased efficacy has not been identified.³ Ting et al.⁴ reported that the parent antihistamine, hydroxyzine, inhibits the release of histamine from human skin mast cells in vivo, whereas Juhlin et al.⁵ reported that cetirizine signifi-

TABLE I. Individual skin test reactivity tabulated with the corresponding OCD and the final amount of antigen that was placed into the skin

Subject	OCD (PNU)	Skin test	Challenge dose (PNU)
		(wheal times erythema) (mm)	
1	100	15 × 57	1000
2	100	18 × 66	1000
3	100	16 × 65	1000
4	100	15 × 40	1000
5	100	16 × 44	1000
6	100	14 × 62	1000
7	10	16 × 45	100
8	100	13 × 53	1000
9	100	22 × 62	1000
10	10	20 × 62	100

cantly decreases the wheal-and-flare reaction in patients with idiopathic and cold urticaria induced by several stimuli, including histamine and 48/80. More interesting was the observation by Fadel et al.⁶ that cetirizine blocks the migration of eosinophils onto a skin window 24 hours after Ag challenge, a phenomenon not explained by H₁ blockade.

To assess further whether cetirizine has activities other than histamine antagonism, we used a skin-chamber model to study the effects of cetirizine in a double-blinded Ag challenge study. The parameters observed were visible skin test responses, the appearance of preformed (histamine) and newly generated (PGD₂) mast cell mediators, and the appearance of eosinophils and neutrophils over time after Ag challenge. We observed that cetirizine had effects apparently unrelated to its histamine antagonism. These included inhibition of the production of the cyclooxygenase product, PGD₂, and a decrease in the number of inflammatory cells that attend the late-phase reaction.

MATERIAL AND METHODS

Ten atopic subjects (six male and four female subjects), 18 to 45 years of age (median age, 29), were studied. They gave written informed consent and previously had demonstrated a cutaneous LPR to RW extract. Intradermal skin tests with RW extract (saline diluent with 0.03% human albumin, Hollister-Stier Laboratories, Spokane, Wash.) in concentrations ranging from 0.1 PNU/ml to 1000 PNU/ml were used to define the OCD as the concentration of Ag required to produce a 4+ cutaneous reaction, i.e., a wheal with a diameter >10 mm with pseudopods or >15 mm without pseudopods, and having >45 mm of erythema (Table I). Cutaneous erythema was also measured at 15

minutes, and 2, 4, 6, and 8 hours. At the time of the study, all subjects were out of the pollen season that caused their symptoms.

Study design

Subjects received either 20 mg of cetirizine or placebo each morning for 3 consecutive days in a randomized, crossover design. There was a 7-day washout period between the initial treatment and the crossover. On the second day of treatment, a blister was created on the volar forearm by placing a hollow plate heated to 40° C over the precleaned skin. Continuous suction (60 mm Hg) was applied for 45 to 60 minutes until a complete 5 mm blister was formed. All blisters were formed 12 hours before skin-chamber placement to allow any inflammatory mediators related to trauma to return to baseline.

Skin-chamber collections

On the morning of the third dose of study medication, the blisters formed 12 hours previously were aseptically unroofed. Sterile aluminum chambers, with entry and exit ports, were placed over the unroofed blister and taped in place. A cutaneous adhesive (Skin-Bond, Largo, Fla.) was used to secure an adequate seal with the skin. Five hundred microliters of RW extract in RLH, representing 10 times the OCD, were placed into the chamber, and an equal amount of RLH alone was placed into the control chamber. The chamber contents were removed hourly for 8 hours and replaced with 500 µl of RLH. The chamber fluid from each sample was spun at 1000 rpm in microcentrifuge tubes for 2 minutes, and the supernatant was saved for measurement of PGD₂ and histamine. The pellet was resuspended in 500 µl of RLH and gently agitated, after which 10 µl was placed on a Neubauer hemocytometer (American Optical, Buffalo, N.Y.) for counting.

Cell counts

Cell counts were done on at least 100 nucleated cells in nine large squares. The cell suspension was then placed in a Cytospin centrifuge (Shandon Southern Instruments, Inc., Sewichley, Pa.) and spun at 600 rpm for 6 minutes. Paired slides were stained with Diff-Quick (American Scientific Products, McGraw Park, Ill.), a modified Wright-Giemsa stain, and 0.5 % alcian blue (pH, 1.0) for 60 minutes. After rinsing with distilled water, slides were counter stained with 0.1% safranin in 1% acetic acid. For Wright-Giemsa stained cells, 200 to 300 cells were counted. For alcian blue-stained cells, either the total number of cells on the slide or 1000 cells were counted.⁷ All cell counts reported represent the net influx of cells into the Ag-challenged chamber. The absolute number of cells in the control chamber was subtracted from the number of cells in the Ag-challenged chamber for each respective time point that was examined.

Measurement of mediators

For measurement of PGD₂, 200 µl of blister fluid was precipitated immediately after collection in 1600 µl of high-

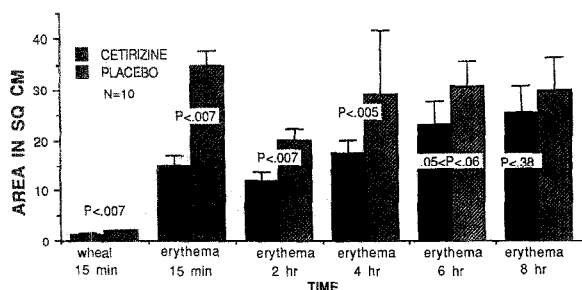


FIG. 1. Skin test response to 0.02 ml of RW Ag at the doses equal to the previously determined OCD. The results are expressed as the mean area in square centimeters ($\pm$ SE).

performance liquid chromatography-grade ethyl alcohol. The tubes were centrifuged at 5000 rpm for 5 minutes, and the supernatants that contained the prostanoids were transferred to separate tubes to be evaporated in an evaporating centrifuge for 3 hours. The specimens were then stored at -70°C . The specimens from each subject at the cetirizine and placebo visits were assayed for PGD_2 in parallel. A competitive radioimmunoassay with a sensitivity of 40 pg/ml, as described previously, was used for PGD_2 measurements.^{8,9}

Raw blister fluid and the hourly chamber collections for histamine assay were frozen immediately after removal of the cells and stored at -70°C . The histamine levels from the subjects at the drug and placebo visits were also assayed in parallel with a previously described radioenzymatic assay with a sensitivity of 0.10 ng/ml. This radioenzymatic assay is based on the conversion of histamine to [^3H]-methylhistamine in the presence of histamine-*N*-methyltransferase and [^3H]-S-adenosyl-L-methionine.¹⁰

As with the cell counts, all values reported for both histamine and PGD_2 represent the mediator production with the control value being subtracted from the respective Ag for each of the time points.

Skin testing

Simultaneous with the introduction of RW Ag into the skin chamber, each subject was skin tested with 0.02 ml of the RW concentration that had previously been determined to cause a +4 reaction followed by a cutaneous LPR. The surface area of erythema was calculated at 2, 4, 6, and 8 hours with a composite diameter derived by averaging the long and short axis of cutaneous erythema. The opposite arm was consistently used for placing both skin tests and introducing Ag into the chamber during the subsequent visit to avoid interference induced by a local refractoriness.

Statistical analysis

Nonparametric analysis was used for comparison of drug versus placebo parameters, since they did not necessarily follow a Gaussian distribution. The Wilcoxon signed-rank test was applied between the paired visits with the Statview program (Brainpower, Inc., Calebasas, Calif.). A *z* value

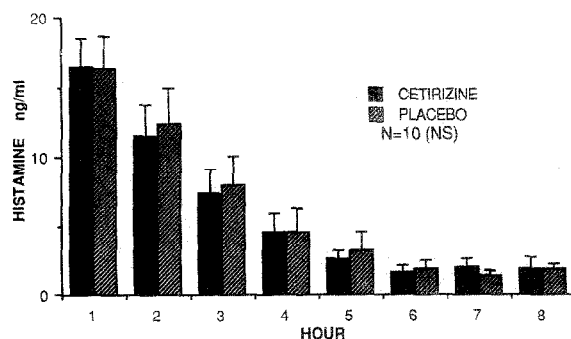


FIG. 2. Hourly histamine levels in skin chamber fluids after initial placement of 10 times the OCD of RW Ag in the chambers for 60 minutes. The results are expressed as mean nanograms per milliliter ($\pm$ SE).

was determined and the two-tailed probability was obtained from a table of probabilities.

RESULTS

The erythema was significantly less in patients treated with cetirizine at 15 minutes and for at least 4 hours thereafter, although the magnitude of the difference between placebo- and cetirizine-treated patients decreased from 60% to <40% during this time (Fig. 1). The wheal was also inhibited during the acute reaction by about 30%. The induration at later times was difficult to quantify and was not measured. Our subjective impression, however, was that the induration was decreased by the antihistamine with a time course similar to that observed with respect to erythema.

The time course of histamine release observed during the 8 hours after Ag challenge was similar to that described previously and did not differ whether the patients were receiving placebo or drug (Fig. 2).¹¹ In five of the study subjects, enough raw blister fluid could be collected to be measured for histamine with the average being 1.74 ng/ml ($\text{SE} \pm 0.58$), representing a 12-hour accumulation. The highest histamine levels were found during the first hour; i.e., about 16 ng/ml in both groups. The histamine levels fell thereafter, and by 5 hours, levels were not significantly above control levels. The control values for histamine ranged from 0.33 ng/ml during the first hour to 1.27 ng/ml at 8 hours.

Although the H_1 antagonist did not affect the production of the preformed mediator, it had a clear effect on the generation of a newly synthesized product, PGD_2 . In six of the 10 patients studied, cetirizine dramatically decreased the PGD_2 levels at virtually every time point (Fig. 3, A-C), whereas in four subjects, the drug appeared to have no effect (Fig. 3, D).

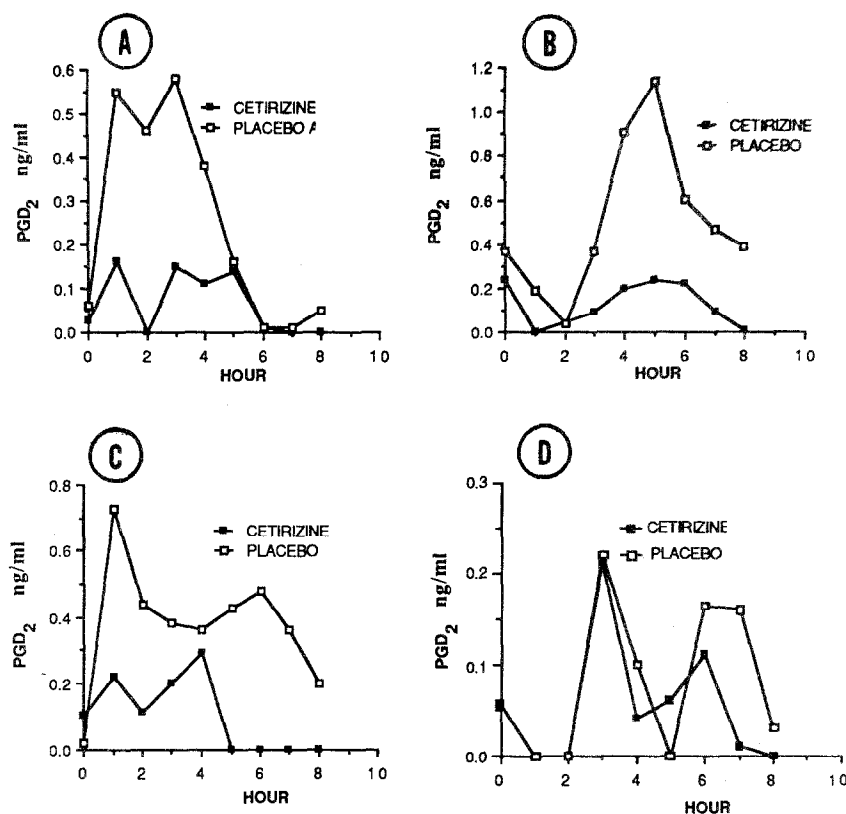


FIG. 3. A-C, Individual PGD₂ data from four representative patients challenged with RW Ag. The results demonstrate attenuation of PGD₂ generation by cetirizine that occurred in 60% of subjects. **D,** The remaining 40% of the subjects demonstrated no inhibition of PGD₂ by the drug. Note that all subjects maintained the same pattern of release in response to the Ag, whether or not they received cetirizine.

The patterns of PGD₂ generation, unique for each individual patient, were duplicated on both cetirizine-treatment and placebo-treatment days, although they were much lower (in six of 10 cases) with the drug. There was no tendency for histamine release to be less while patients were receiving the drug in those patients in whom the PGD₂ generation was blunted.

Averaging the PGD₂ data, suppression of PGD₂ generation by cetirizine is noted (Fig. 4). The level of this prostaglandin in chamber fluids was reduced at every time point from 1 to 8 hours, which, by analysis of variance, is significant ($p \leq 0.04$). The control PGD₂ values, with no Ag stimulation, ranged from 0.07 ng/ml at baseline to a high of 0.17 ng/ml during the second hour. As mentioned previously, all PGD₂ values presented represent the control values subtracted from the corresponding Ag-stimulation chamber. When the data from each individual time point are examined, the PGD₂ levels are significantly lower in the second, fourth, and fifth hours during cetirizine treatment because of the marked interindividual vari-

ation. When the overall pattern is observed, it appears that the effect of cetirizine is to markedly blunt both the magnitude and duration of PGD₂ production. Thus, in the presence of cetirizine, the level of PGD₂ at 5 hours is clearly lower than that observed at the peak of the reaction at 3 hours, whereas in placebo-treated individuals, the third to the fifth hours are essentially the same and a decrease in PGD₂ levels is not observed until 6 hours.

Cetirizine also changed the infiltration of inflammatory cells into the skin chamber after Ag challenge. In the control (placebo-treated) individuals, eosinophil numbers began to increase at 6 hours when there were approximately 300,000 eosinophils per milliliter, and this continued until the end of the experiment, at 8 hours, when there were more than a million eosinophils per milliliter. Cetirizine pretreatment decreased eosinophil infiltration by 80% ($p \leq 0.04$) during the 3-hour period when increased numbers were observed in the control patients (Fig. 5, A). When single hourly measurements were observed there was a statistically

significant decrease of eosinophil numbers only during the eighth hour ($p \leq 0.03$), again because of the marked interindividual variation.

The effect of cetirizine on neutrophil infiltration was similar. Significant accumulation of neutrophils occurred by 5 hours when there was not a difference between the two groups (Fig. 5, B). However, during hours 6, 7, and 8, the cetirizine-treated individuals had less than half the number of neutrophils, and at 8 hours, there was a 75% reduction. When the pooled data are observed for hours 6, 7, and 8, there is a significant decrease in neutrophil infiltration ($p \leq 0.05$), although because of the marked variation from patient to patient, this became significant in each individual hour only at the eighth hour ($p \leq 0.02$).

Basophil numbers were lower than numbers of the other two cell types. The pattern, however, appears to be the same with the basophil numbers being lower at hours 6, 7, and 8 when the individuals were treated with cetirizine (Fig. 5, C). By the eighth hour, there were only one-fourth as many basophils in the treated individuals as in the control individuals. However, because of the low numbers, this decrease did not reach statistical significance.

DISCUSSION

Antihistamines were introduced about 50 years ago and, since that time, have been in widespread use for diseases accompanied by the real or supposed release of histamine.^{12, 13} Although there is no question that these drugs are quite specific at inhibiting the effects of histamine *in vitro*, their mechanism of action in man is not clear, but it is assumed that their effects are due to antagonism of H_1 receptors.¹⁴⁻¹⁶ Based on the tissue concentrations of antihistamines used clinically and on the affinity of histamine and its antagonists for the histamine receptor, there is reason to believe that receptor blockade may not be their only mechanism of action. It has been speculated that locally, *in vivo* concentrations of histamine may reach high micromolar levels. Some years ago we carried out a study in primates in which we induced a wheal-and-flare reaction with either *Ascaris* Ag or histamine and then attempted to block these reactions with either antihistamines or drugs that block histamine release (theophylline and β -adrenergic agonists).¹⁷ The antihistamine blocked the response to injected histamine but not the Ag-induced wheal-and-flare reaction, whereas the antirelease drugs had the reverse effect, i.e., blocked the Ag-induced skin reactions but did not block the response to histamine. Clinically, the efficacy of antihistamines in pruritic diseases and in allergic rhinitis is clear, even though their mechanism

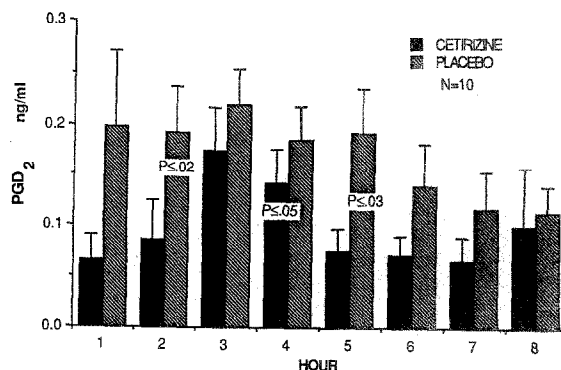


FIG. 4. Hourly PGD_2 production in response to 10 times the OCD of RW Ag in the skin chamber for 60 minutes. The results are expressed as mean nanograms per milliliter ($\pm$ SE).

of action is not. For example, while we were studying the antiallergic effects of the antihistamine, chlorpheniramine, we used as controls both placebo and phenobarbital.¹⁸ Both drugs were clinically more effective than placebo, but phenobarbital was less sedative than the antihistamine. Given these observations, it appears fair to conclude that the mechanism of action of antihistamines *in vivo* in man is not clear and that further studies on this subject are needed.

That antihistamines have effects in addition to histamine antagonism has been appreciated since 1960 when Mota and DaSilva¹⁹ demonstrated that certain classes of antihistamines at high concentrations were histamine releasers, whereas the same drugs at lower concentrations decreased Ag-induced mediator release in certain animal models. Fifteen years later, we repeated these studies using human basophils and found that most classes of antihistamines would inhibit release at 0.1 to 1 mmol/L concentrations, with the phenothiazine antihistamines being about tenfold more active.²⁰ Although these concentrations are not usually obtained clinically, we have found that topically applied phenothiazine-like antihistamines inhibit nasal mast cell-mediator release *in vivo* in man.^{21, 22} In this context, Ting et al.⁴ demonstrated that hydroxyzine, the parent compound of cetirizine, decreased histamine release in a skin chamber. Using a rat model, Lemanske et al.²³ demonstrated that diphenhydramine decreased both the immediate and the cutaneous LPR to anti-IgE and further demonstrated that this effect was even greater if combined with an H_2 antihistamine. Smith et al.²⁴ examined the effect of chlorpheniramine on the immediate and cutaneous LPR in human volunteers. They were unable to demonstrate an inhibition of the cutaneous LPR with an H_1 antihistamine alone, but when both an H_1 and an

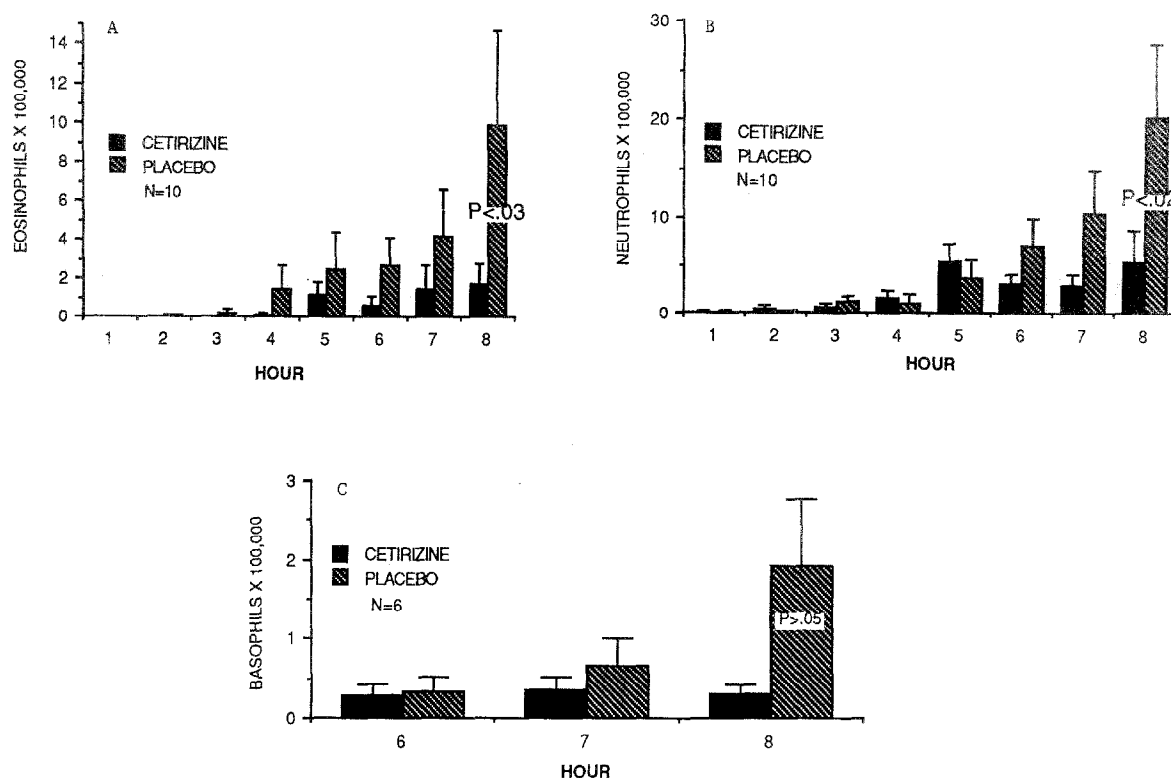


FIG. 5. **A**, Absolute numbers of eosinophils in the skin chamber in response to Ag challenge expressed as mean ($\pm$ SE). **B**, Absolute number of neutrophils in the skin chamber after Ag challenge expressed as mean ($\pm$ SE). **C**, Absolute number of basophils in the skin chamber expressed as mean ($\pm$ SE).

H₂ were combined, they were able to demonstrate a decrease in the LPR. Ketotifen has also been demonstrated to have a slight suppressive effect on the cutaneous LPR not found with clemastine.²⁵ It is worth noting that cetirizine has the same effects as other antihistamines *in vitro*, inhibiting histamine release from peripheral blood basophils, albeit at rather high concentrations. It is as effective in inhibiting histamine release in human skin mast cells as the phenothiazine class of antihistamines (unpublished data).

How can the observations presented in this article be explained? The first effect of cetirizine, to decrease both the immediate skin reaction and the erythema that occurs during the first several hours after Ag challenge, is not different from what has been demonstrated with multiple other antihistamines.^{26, 27} This may represent H₁ receptor antagonism. By contrast, although we did not reproduce the observations of Ting et al.⁴ regarding inhibition of histamine release by hydroxyzine, we did demonstrate that cetirizine blocks the release of PGD₂. Although there are other possibilities, it remains likely that the PGD₂ produced in this situation is of mast cell origin and that cetirizine may be acting to prevent release of a mediator that is synthesized after the allergic stimulus is applied.²⁸⁻³⁰

It would not be unexpected to find inhibition of a product of arachidonic acid metabolism without the inhibition of histamine release, since several drugs have been demonstrated to be more effective in altering arachidonic acid metabolism than affecting histamine release.³¹ We may, in this experimental protocol, simply have been at a dose range in which the first effect was observed without the latter.

The other effects of cetirizine are more unusual. It causes inhibition of both eosinophil and neutrophil infiltration during the late-phase reaction in the skin. This inhibition of the influx of inflammatory cells into the Ag-challenged skin chamber corroborates the work by Fadel et al.⁶ using a modified Rebuck skin-window technique. Our experiments suggest that with larger numbers and a longer period of study, a decreased infiltration of basophils might also be demonstrated. In attempting to analyze this effect of cetirizine, we are hampered by a lack of knowledge concerning the mechanisms that promote and direct inflammatory cells to the site of an allergic reaction. In many IgE-mediated allergic reactions, there can be a 5% to 10% local eosinophil infiltration when there is a <1% peripheral eosinophilia. The eosinophils are clearly being selectively attracted to the site of Ag

challenge, but our knowledge of the mechanisms that control this phenomenon is incomplete. Although there are many chemical substances that cause eosinophil chemotaxis, the question of whether there is a selective eosinophil chemotactic substance is controversial, but the eosinophil chemotactic factor of anaphylaxis tetrapeptide (a family of acidic peptides with molecular weights ranging from 360 to 1000) described several years ago appears to be inactive.³²⁻³⁵ Other factors including platelet-activating factor, LTB₄, and monohydroxyeicosatetraenoic acids have been demonstrated to be chemotactic for eosinophils, but they all lack selectivity.³⁶⁻³⁸ Therefore, the mechanism of selective eosinophil tissue accumulation at the site of allergic reactions is not fully understood, and we do not yet have an adequate model to examine this aspect of the activity of cetirizine. Given the clear-cut in vivo effects of cetirizine, it may indeed be possible to use this drug to study many aspects of the complex processes that lead to inflammatory cell infiltration, including cell margination, endothelial cell adherence, chemokinesis, and chemotaxis itself. It may well be that a drug that blocks inflammatory cell infiltration in vivo will provide an insight into more basic pathogenetic mechanisms.

Thus, further studies with cetirizine are indicated. Whether the effects that are observed with this H₁ antagonist are characteristic only of this particular class of antihistamine or pertain to antihistamines in general remains to be determined.

We are appreciative of the expert technical assistance of Ms. Valerie Alexander and the assistance of Linda Rae Freidhoff with the statistical analysis.

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