

The effects of cetirizine on the adhesion of human eosinophils and neutrophils to cultured human umbilical vein endothelial cells

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There is increasing evidence that eosinophils are important effector cells in allergic inflammation. Accumulations of eosinophils and eosinophil-derived products are seen to occur in the skin, lungs, and nasal airways of atopic individuals after antigenic challenge.

Cetirizine is a carboxylated analogue of hydroxyzine, which has potent and specific H_1 receptor blocking activity. In addition, it inhibits eosinophil accumulation in the skin challenged with anti-IgE antibodies or platelet activating factor.^{1, 2}

Since a critical initial step in the recruitment of eosinophils into target tissues is the interaction of the granulocyte with the endothelial cell surface, we have assessed the capacity of cetirizine to modulate eosinophil adhesion in cultured human umbilical vein endothelial cells.

METHODS

Experiments were performed to assess the effects of cetirizine on the adherence of eosinophils or neutrophils (unstimulated and stimulated by *N*-formyl-methionyl-leucyl-phenylalanine (FMLP), 10^{-6} mol/L for 15 minutes) to unstimulated endothelial cells and endothelial cells activated by IL-1 (10 U/ml) for 6 hours. Leukocytes were preincubated with increasing concentrations of cetirizine (0.01 μ g/ml to 100 μ g/ml) for 15 minutes before addition to the adhesion assay, which was performed in the presence of the same concentration of cetirizine.

Eosinophils and neutrophils were isolated to greater than 90% purity from peripheral blood of volunteers by discontinuous metrizamide density gradient centrifugation and radiolabeled as previously described.³

Endothelial cell monolayers were prepared as previously

Abbreviations used

FMLP:	<i>N</i> -formyl-methionyl-leucylphenylalanine
HBSS:	Hanks' balanced salt solution
HEPES:	<i>N</i> -2-hydroxyethylpiperazine- <i>N</i> -2-ethanesulfonic [acid]
IL-1:	Interleukin-1
ICAM-1:	Intercellular adhesion molecule-1
ELAM-1:	Endothelial leucocyte adhesion molecule-1
VCAM-1:	Vascular cell adhesion molecule-1
VLA-4:	Very late antigen-4

described.³ After washing the confluent monolayers, chromium 51-labeled neutrophils or eosinophils (2×10^5 in 0.2 ml HBSS with 30 mmol/L HEPES) were added per well. After incubation for 30 minutes at 37° C in 5% CO₂, the microwells were washed twice with 0.2 ml warm assay medium. Nonidet P-40 (BDH, Poole, Dorset, U.K.) (0.2 ml, 1% solution) was then added to each well, and the plate was incubated for at least 10 minutes to lyse the adherent eosinophils or neutrophils.

The percentage of leukocytes bound to endothelial cells in the adhesion assay was calculated as follows:

% leukocytes bound = (CPM in 0.1 ml lysate $\div$ CPM in 0.1 ml of original leukocyte suspension) $\times$ 100.

Statistical analysis was performed by use of a two-tailed, paired *t* test to compare the results obtained for each of the cetirizine concentrations with the baseline values. The Bonferroni correction was used where multiple comparisons were made.

RESULTS

When either endothelial cells were stimulated by 10 U/ml IL-1 or eosinophils were stimulated by 10^{-6} mol/L FMLP, the percent adhesion was increased over basal levels of unstimulated cells. The hyperadhesion of eosinophils to stimulated endothelial cells was significantly decreased by 100 μ g/ml cetirizine ($p = 0.003$) (Table I). The hyperadhesion of FMLP-stimulated eosinophils to unstimulated endothelial

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Supported by a grant from UCB (Belgium).

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1/1/38401

TABLE I. Effect of cetirizine on adherence of granulocytes to endothelial cells

		% adhesion $\pm$ SD		
Cetirizine (μ g/ml)		A*	B†	C‡
Eosinophils	0.0	8.80 $\pm$ 2.07 (9)	16.72 $\pm$ 2.76 (6)	16.02 $\pm$ 3.47 (5)
	0.1	9.67 $\pm$ 2.36 (8)	18.42 $\pm$ 4.91 (5)	17.12 $\pm$ 5.74 (5)
	1.0	9.67 $\pm$ 2.96 (9)	17.80 $\pm$ 6.61 (6)	17.16 $\pm$ 6.09 (5)
	10.0	9.60 $\pm$ 3.35 (9)	18.28 $\pm$ 6.29 (6)	17.30 $\pm$ 5.39 (5)
	100.0	7.59 $\pm$ 2.39 (9)	*12.62 $\pm$ 3.83 (6)	†11.78 $\pm$ 3.72 (5)
*p = 0.003, †p = 0.018				
Neutrophils	0.0	7.06 $\pm$ 2.39 (7)	18.16 $\pm$ 6.53 (7)	22.48 $\pm$ 1.26 (4)
	0.1	9.03 $\pm$ 1.94 (6)	20.00 $\pm$ 8.65 (6)	21.85 $\pm$ 6.26 (4)
	1.0	8.36 $\pm$ 2.27 (7)	20.96 $\pm$ 8.46 (7)	23.18 $\pm$ 5.96 (4)
	10.0	7.84 $\pm$ 1.72 (7)	19.11 $\pm$ 8.91 (7)	24.33 $\pm$ 4.87 (4)
	100.0	5.20 $\pm$ 3.34 (6)	16.78 $\pm$ 7.82 (5)	22.07 $\pm$ 6.85 (3)

*A, neither cell type stimulated.

†B, endothelial cells were stimulated with 10 U/ml IL-1.

‡C, granulocytes stimulated with 10^{-6} mol/L FMLP (numbers in parentheses denote number of experiments).

cells was also significantly decreased by 100 μ g/ml cetirizine ($p = 0.018$) (Table I).

Stimulation of either endothelial cells with 10 U/ml IL-1 or neutrophils with 10^{-6} mol/L FMLP resulted in an increase of neutrophil adhesion over basal levels of unstimulated cells. Cetirizine (0.01 μ g/ml to 100 μ g/ml) did not produce any significant inhibition of this enhanced adhesion when either the neutrophils or the endothelial cells were stimulated (Table I).

Cetirizine (100 μ g/ml) had no toxic effect on cell viability, as assessed by trypan blue exclusion, under identical conditions to the adhesion assay. With unstimulated eosinophils, viability was $96.0\% \pm 3.5\%$ and $94.0\% \pm 3.5\%$ (mean $\pm$ SD, $n = 3$) in the absence and presence of cetirizine, respectively, and with unstimulated neutrophils was $88.0\% \pm 6.9\%$ and $78.7\% \pm 3.5\%$ (mean $\pm$ SD, $n = 3$) in the absence and presence of cetirizine, respectively. The viability of stimulated eosinophils was $96.7\% \pm 2.1\%$ and $94.3\% \pm 5.5\%$ (mean $\pm$ SD, $n = 3$) in the absence and presence of cetirizine, respectively, and with stimulated neutrophils was $86.3\% \pm 4.9\%$ and $80.7\% \pm 4.9\%$ (mean $\pm$ SD, $n = 3$) in the absence and presence of cetirizine, respectively.

DISCUSSION

The results demonstrate that cetirizine (100 μ g/ml) significantly inhibited the adhesion of eosinophils to endothelial cells when adherence is enhanced by either FMLP or IL-1 stimulation. However, the concentration of drug required to give significant inhibition of cell adherence was much higher than the plasma level concentrations reached during usual treatment. In contrast, cetirizine had no effect on neutrophil adhesion.

These results indicate that the antiadhesive properties of cetirizine are selective for the eosinophil; the mechanism, however, is not known.

Vascular endothelial cells express adhesion molecules on activation with appropriate stimuli. Because the recruitment of leukocytes into the site of inflammation must initially involve an interaction with the endothelial cell, adhesion mechanisms of endothelial cells for leukocytes may be a critical determinant for the time-course and magnitude of the cellular infiltrate. Recent work has shown that ICAM-1 and ELAM-1 both bind neutrophils and eosinophils,³ whereas VCAM-1 binds eosinophils preferentially over neutrophils.^{4,6} There is evidence that VLA-4/VCAM-1 interaction between leukocytes and endothelial cells may be selective for the eosinophil and not for the neutrophil.^{4,5,7} This raises the possibility that drugs such as cetirizine might affect the function or expression of these adhesion molecules. The capacity of cetirizine to inhibit adhesion and the accumulation of eosinophils in vivo may be an important property in the efficacy of this drug in allergic disease.

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Persistent neutrophil activation in mild asthma

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Neutrophils appear in asthmatic airways in association with attacks provoked by diverse stimuli. However, their contribution to the pathophysiology of the stable patient is uncertain and was the focus of the present study. Serial measurements were made of plasma lactoferrin and myeloperoxidase levels in asthmatic patients and controls.

SUBJECTS AND METHODS

Seventeen patients with mild disease were studied. None had ever used any form of corticosteroid medication. The diagnosis was confirmed in each by the presence of (a) significant airway obstruction ($FEV_1 < 70\%$ predicted) and β -2-adrenergic bronchodilator aerosol reversibility (at least 20%) or (b) a positive exercise bronchoprovocation test (at least 20% fall in PEFR). Seven controls were studied, each with a negative history for asthma and atopy, and with normal pulmonary function and a negative exercise test.

The subjects were studied under standardized conditions between 3:30 PM and 8 AM. Medications were discontinued from the morning of the study. Blood was sampled every 4 hours through an indwelling cannula beginning at 4 PM, and measurements of PEFR were made at 4 PM and 4 AM.

Plasma lactoferrin levels were measured by an ELISA developed in our laboratory with use of rabbit polyclonal monospecific antibodies to purified breast milk lactoferrin.¹ Myeloperoxidase levels were measured by a colorimetric method based on the reduction of dimethoxybenzidine (O-dianisidine HCl; Sigma Chemical Co., St. Louis, Mo.), optical density was measured at 460 nm.²

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Abbreviations used

FEV₁: Forced expiratory volume in 1 second
PEFR: Peak expiratory flow rate
ELISA: Enzyme linked immunoassay
BAL: Bronchoalveolar lavage

The neutrophil is the major source of lactoferrin and myeloperoxidase, with plasma levels critically dependent on neutrophil count. To take into account variations in neutrophil count, results were expressed as lactoferrin:neutrophil and myeloperoxidase:neutrophil ratios by dividing plasma levels by simultaneously measured neutrophil counts.

The Mann-Whitney U test was used for pairwise comparisons, and Spearman's Rank test was used for correlations. A cluster analysis divided the asthmatic patients into those with and without a significant fall in PEFR from 4 PM to 4 AM, termed "dippers" and "nondippers," respectively. Time-weighted averages were calculated to provide estimates of overall values.

RESULTS

The lactoferrin:neutrophil ratio was significantly higher in asthmatic than nonasthmatic subjects at all times (except 8 AM) despite similar neutrophil counts (Table I, A). However, no correlation was observed between the lactoferrin:neutrophil ratios or the time-weighted average and the percent fall in PEFR in the asthmatic subjects.

The dividing point between the dippers and nondippers was a 16% fall in PEFR. Although the lactoferrin:neutrophil ratios of the dippers were higher at most times than those of the nondippers, the differences did not attain significance (Table I, B).

No difference occurred between asthmatic and nonasthmatic subjects in the myeloperoxidase:neutrophil ratios. Plasma myeloperoxidase levels were uniformly