

Short communication

Pharmacologic heterogeneity of human lung and colon cells: effect of terfenadine and cetirizine

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H₁-blockers may have antiallergic properties which cause the blocking of histaminic release, and the effect of these drugs may differ according to the phenotype of mast cells. This study examined the ability of terfenadine and cetirizine to inhibit the release of arachidonic acid-derived mediators from human lung and colon cells. Dispersed cells were challenged with anti-IgE in the presence or absence of 10 µM of terfenadine or cetirizine, and the release of prostaglandin (PG)D₂ and leukotriene (LT)C₄/D₄ was assessed by enzyme immunoassay (EIA). Terfenadine caused significant inhibition of both PGD₂ and LTC₄/D₄ (49 ± 9 and 29 ± 19%, respectively) from human lung cells but had a less marked effect on PGD₂ release from human colon cells (21 ± 9% for PGD₂ and 18 ± 9% for LTC₄/D₄). In contrast, although cetirizine caused significant inhibition of both mediators measured in lung cells (38 ± 16% for PGD₂ and 34 ± 19% for LTC₄), it did not cause any significant inhibition of either mediator from human colon cells. These findings suggest that H₁-antagonists may have additional properties, and the differential effects of cetirizine on lung and colon tissue may indicate differences in mast cell phenotype.

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The mechanisms of action of H₁-blockers are still not fully understood (because they have been shown to possess properties unrelated to the blockage of histamine at its receptor level), and they have been ascribed to "antiallergic" properties (23, 24, 26). In particular, terfenadine has been shown to block the release of mediators from basophils and mast cells both *in vivo* (2, 9) and *in vitro* (4, 20), and cetirizine can block the release of leukotriene (LT)C₄/D₄ in nasal secretions (21) after allergen challenge, as well as inhibit the influx of eosinophils into the skin (19), but not into the nose (14). Thus, there may be an organ-specific component in the antiallergic properties of H₁-blockers.

Mast cells may be of different phenotypes, and it has been observed that cells obtained from lung and colon differ in their sensitivity to formaldehyde, their levels of chymase, and their response to drugs such as nedocromil sodium and development of tachyphylaxis to sodium cromoglycate (11, 22).

Mediators of the allergic reaction are released by various cells. Prostaglandin (PG)D₂ is the major prostanoid produced by mast cells (16), while other cell types release smaller amounts of PGD₂. LTC₄/D₄/E₄ are released by several cell types including mast cells, monocytes, and eosinophils (3, 8, 17). All these cells are present in the lung and colon mucosa and may be activated by several stimuli including anti-IgE antibodies (12, 13, 18).

This study investigated whether terfenadine and cetirizine, potent H₁-blockers, can inhibit the cyclooxygenase or lipoxygenase pathways of arachidonic acid metabolism from human dispersed lung or colonic cells after stimulation with anti-IgE antibodies.

Material and methods

Cell dispersion

Macroscopically normal human lung was obtained from six patients aged 45–70 years who had under-

gone surgery for bronchial carcinoma. All the subjects were smokers. Macroscopically normal human colon was obtained from six patients aged 42–63 years who had undergone surgery for colonic carcinoma (two patients) or nonneoplastic disease (four patients).

The dispersion of cells was done under identical conditions in both types of tissue by the method of Holgate et al. (10). Cell disaggregation was achieved by enzymatic digestion in four volumes of HBSS (Gibco, Paisley, UK) containing 2 mg/ml of protease type XIV (Sigma, St Louis, MO, USA) and 0.5 mg/ml of chymopapain (Sigma) for two sequential 45-min periods. The cell dispersion was done at 37°C while the fragments were agitated.

At the end of the digestion period, the cells were filtered to separate them from any undigested tissue. The cells were then washed in HBSS, and aliquots were taken for differential cell counts with Kimura's stain (15) and for preparing fixed preparations with a cytocentrifuge (Shandon Southern Products, Runcorn, Cheshire, UK) in order to confirm the differential cell counts with May-Grünwald Giemsa stain.

Before challenge, cells were passively sensitized by incubation for 45 min at 37°C with atopic serum (10%, v/v).

Inhibition studies

An optimal concentration of H₁-blockers was selected according to data previously published (4). Human dispersed lung or colon cells, at a concentration of 0.5×10^6 mast cells per ml, were preincubated with terfenadine (10 µM), cetirizine (10 µM),

or DMSO (0.1%) (the solvent used to dissolve the drugs) for 20 min before challenge with 1:1000 ε-chain specific anti-IgE (Immunotech, Luminy, France) for 45 min at 37°C.

Analysis of mediators

The supernatants from all experiments were stored at -20°C as duplicate aliquots. Quantification of PGD₂ and LTC₄/D₄ was done by enzyme immunoassay (EIA) with commercially available kits (Stallergènes, Fresnes, France).

Statistical analysis and data presentation

Results are presented as medians and range of *n* experiments. Significance of difference was determined by Wilcoxon's W-test.

Results

Challenge of the cells with anti-IgE resulted in the release of all of the mediators measured in all patients, as shown in Tables 1 and 2. The percentage of mast cells present in each preparation is also shown in Tables 1 and 2. Neither at base line nor after anti-IgE challenge was there a significant difference between lung and colon cells in the median release of either eicosanoid (Tables 1 and 2). The coefficient of variation for measurement of replicate samples was <6%.

When the lung cells were preincubated with terfenadine for 20 min before challenge, release of PGD₂ or LTC₄/D₄ was significantly reduced (Table 1). Similar results were obtained with this

Table 1. Effect of terfenadine and cetirizine on eicosanoid release from human dispersed lung cells challenged with anti-IgE for 45 min at 37°C. Global results from human lung cells

Subjects	1	2	3	4	5	6	P value vs max. release	Median (range)
PGD₂								
Release (ng/million cells)								
Base line	2.5	1.4	1.6	4.7	3.8	4.2		3.15 (1.4–4.7)
Max. release	33	21	29	59	29	51		31 (21–59)
Inhibition of release (%)								
Terf. 10 µM	42	38	60	59	51	47	<0.03	
Cet. 10 µM	30	47	28	40	65	19	<0.03	
LTC₄/D₄								
Release (ng/million cells)								
Base line	0.4	1.1	1.2	0.9	0.02	0.6		0.75 (0.02–1.2)
Max. release	3.7	5	4.8	5.3	3.4	5.8		4.9 (3.4–5.8)
Inhibition of release (%)								
Terf. 10 µM	33	29	0	27	59	28	<0.05	
Cet. 10 µM	37	12	14	32	60	50	<0.03	
% Mast cells	3	5	4	6	5	7		5 (3–7)

Statistical analysis by Wilcoxon's W test.

Table 2. Effect of terfenadine and cetirizine on eicosanoid release from human dispersed colon cells challenged with anti-IgE for 45 min at 37°C. Global results from human colon cells

Subjects	1	2	3	4	5	6	P value vs max. release	Median (range)
PGD₂								
Release (ng/million cells)								
Base line	8.45	4.3	1	5	6.2	3.7		4.65 (1–8.45)
Max. release	24	21	38	42	48	35		36.5 (21–48)
Inhibition of release (%)								
Terf. 10 µM	25	29	12	30	18	9	<0.03	
Cet. 10 µM	38	0	4	7	3	5	NSD	
LTC₄/D₄								
Release (ng/million cells)								
Base line	0.3	0.9	1	1.2	0.4	0.5		0.7 (0.3–1.2)
Max. release	3.8	3.3	4.2	4.7	4.6	6.8		4.4 (3.3–6.8)
Inhibition of release (%)								
Terf. 10 µM	3	12.5	22	20	52	0	<0.05	
Cet. 10 µM	0	0	12.5	13	67	3	NSD	
% Mast cells	2	6	4	3	8	4		4 (2–9)

Statistical analysis by Wilcoxon's W test.

compound on cells obtained from human colon, although the levels of inhibition were slightly lower (Table 2). In contrast, although cetirizine caused significant inhibition of both PGD₂ and LTC₄/D₄ from human lung cells, no significant inhibition of either mediator was found in human colon cells (Tables 1 and 2).

Discussion

In this study we have shown that PGD₂ and LTC₄/D₄ were released from human dispersed lung cells in response to IgE-dependent challenge, and that terfenadine and cetirizine inhibit the release of cyclooxygenase and lipoxygenase metabolites of arachidonic acid from human lung cells, while terfenadine (but not cetirizine) inhibits the release of cyclooxygenase and lipoxygenase metabolites of arachidonic acid from human colon cells. Moreover, we did not observe a difference in the levels of eicosanoids released from the two different anatomic sites. In all cases, a passive sensitization step was used to reduce differences which may exist between atopic and nonatopic patients.

Although it is possible that the eicosanoids measured have a cellular provenance other than mast cells, we believe that this is unlikely in this case because 1) the major source of PGD₂ in the lung is the mast cell, and 2) previous experiments using the same mode of stimulation have shown that the ratio between histamine release and LTC₄ release remained constant when the mast cell purity varied between 2 and 70% (Campbell, unpublished observations).

Mast cells have been implicated in the pathogen-

esis of inflammatory diseases because of their ability to synthesize and release inflammatory mediators in response to the IgE-mediated stimuli. In response to challenge with IgE-mediated stimuli, mast cells are probably the main cell type involved. However, it is clear that cells in the lung or in the colon do not act in isolation but rather interact in a complex mediator network, and that factors that affect the responses of other cell types may be as important as those which directly influence the inflammatory reaction. A number of mediators can both cause pathologic effects and influence cell activation, and the release of mediators from unfractionated lung or colon cells usually represents a network of cell, mediator, and cytokine interactions rather than a single-cell-type phenomenon.

Terfenadine was previously found to be a potent inhibitor of PGD₂ and LTC₄/D₄ but had no effect on histamine on human lung cells (4); therefore, the inhibition of histamine release was not measured in this model. Terfenadine significantly reduced the release of both eicosanoids from colon mast cells, whereas cetirizine was found to block significantly only the release of PGD₂ from colon cells. Although it is possible that the different effects of cetirizine on lung and colon mast cells may be related to mast cell heterogeneity between these two anatomic sites, we cannot be sure of this because the percentage purity of these cells is under 10%. We did not do any experiments with purified cells because these are difficult to obtain in sufficient number. However, in response to challenge with IgE-mediated stimuli, mast cells are probably the primary cell type involved because the number and affinity of IgE receptors on these cells are approximately 100 times

higher than of those present on other cell types in the lung (25).

This study provides new evidence that H₁-blockers have many properties other than a simple antireceptor effect and that their antiallergic properties may differ according to the anatomic site. H₁-blockers were found to block mediator release *in vitro* (6, 27) and *in vivo* (5, 7), but the clinical relevance of these findings needs to be clarified (1). Although it is clear that a concentration of 10 μ M is never reached *in vivo*, we believe that these results may have physiologic relevance because the concentration of IgE used for challenge is also supraphysiologic, and therefore increased levels of a potential therapeutic compound would be required to cause inhibition.

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