

Modulation of eicosanoid and histamine release from human dispersed lung cells by terfenadine

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Terfenadine is an H_1 -blocker that may have antiallergic properties. A study was carried out to examine the ability of terfenadine to inhibit the release of histamine and arachidonic-acid-derived mediators from human lung cells. Cells were dispersed from fresh human lung tissue obtained from four accident victims whose hearts were donated for transplantation and four lung cancer resections. Cells were dispersed by enzymatic digestion with type XIV protease and chymopapain, and this resulted in a cell population containing approximately 5% mast cells. The remaining cells were mainly macrophages. The cells were challenged with anti-IgE at a 1/1000 dilution. Cells were challenged without terfenadine and after a preincubation of 0.1, 1, and 10 μmol terfenadine. The release of PGD_2 and LTC_4/D_4 was assessed with an EIA. Histamine was assayed by RIA with a monoclonal antibody against acylated histamine.

A release of both eicosanoids and histamine was observed in all experiments. An inhibition of eicosanoids was observed at both 1 and 10 μmol terfenadine (median percentage of inhibition of PGD_2 : 38.00 ± 15.65 and 56.00 ± 13.12 ; median percentage of inhibition of LTC_4/D_4 : 37.5 ± 19.80 and 52.5 ± 26.8). On the other hand, histamine release was not blocked by terfenadine.

Terfenadine inhibits, in a dose-dependent manner, the release of eicosanoids after challenge of dispersed lung cells by anti-IgE, and this effect may have some clinical relevance.

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H_1 -blockers are extensively used in the treatment of allergic rhinitis and may have some potential in the treatment of asthma (34, 36, 41). The mechanism of action of H_1 -blockers is still incompletely known since they have been shown to possess properties unrelated to the blockage of histamine at its receptor level. In particular, terfenadine has been shown *in vivo* (2, 12, 23, 32) or *in vitro* (30) to block the release of mediators from basophils and mast cells.

Mediators of the allergic reaction are released by a variety of cells. Histamine is specific for basophils and mast cells (10). PGD_2 is the major prostanoid of mast cells (24), but other cell types release only smaller amounts of PGD_2 (17). $\text{LTC}_4/\text{D}_4/\text{E}_4$ are released by several cell types, including mast cells, monocytes and eosinophils (4, 11, 17, 25). All these cells are present in the lung and lipid mediators; moreover, histamine can be released from human lung dispersed cells stimulated by several stimuli including anti-IgE antibodies (18–20, 27, 37, 43).

This study has investigated whether terfenadine, a potent H_1 -blocker, can inhibit the cyclooxygenase or lipoxygenase pathways of arachidonic acid metabolism as well as histamine release from human dispersed lung cells stimulated by anti-IgE antibodies.

Material and methods

Cell dispersion

Macroscopically normal human lung was obtained from patients aged 45–70 years who had undergone surgery for bronchial carcinoma (four patients) or subjects who had donated their hearts for transplantation (four subjects). Apparently, all subjects were smokers. The dispersion was performed according to the method of Holgate et al. (15). The lung sample was dissected free of visible bronchi and chopped finely with scissors into pieces of approximately 2 mm^3 . Excess blood was removed by washing in HEPES buffered salt solution (HBSS; NaCl

137 mmol, NaH_2PO_4 0.4 mmol, KCl 2.7 mmol, glucose 5.6 mmol, *N*-2'-hydroxyethylepiperazine-*N'*-2-ethanesulfonic acid (HEPES) 10 mmol, and gelatin 0.1%, adjusted to pH 7.4). Cell disaggregation was achieved by enzymatic digestion in four volumes of HBSS containing 2 mg/ml of protease type XIV (Sigma Co., St Louis, MO, USA) and 0.5 mg/ml of chymopapain (Sigma Co.) for two sequential 45-min periods. The cell dispersion was performed at 37°C, and during this time the fragments were agitated by drawing them into, and expelling them from, a 20-ml syringe.

At the end of the digestion period the cells were sequentially 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 (22) and for preparing fixed preparations with a cytocentrifuge (Shandon Southern Products, Runcorn, Cheshire, UK) in order to confirm differential cell counts by May-Grundwal Giemsa.

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

Inhibition studies

Human dispersed lung cells were preincubated with terfenadine (0.1, 1, and 10 μmol) or the vehicle used to dissolve terfenadine (0.1% ethanol) 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. Control experiments showed that sulfidopeptide leukotrienes were stable for up to 7 months under these conditions. Quantification of PGD_2 and LTC_4 was performed by enzymeimmunoassay (EIA) according to the method of Maclouf et al. (26); acetylcholinesterase from electric eel was the enzyme used in the assay (35) (Stallergenes, Fresnes, France). The assays were highly specific for the eicosanoids tested and their sensitivity was 3 pg/ml and 15 pg/ml, respectively. Histamine was measured by radioimmunoassay using a monoclonal antibody against acylated-histamine (29) (Immunotech, Luminy, France). Total histamine content was assayed after neutralization of perchloric acid in the supernatant by potassium hydroxide before acylation.

The release of LTC_4/D_4 and PGD_2 was expressed in ng/ml. For histamine, the results are given in percentage of net histamine release.

Statistical analysis and data presentation

Results are presented as medians $\pm$ SD of *n* experiments. The significance of difference was evaluated by Wilcoxon W-test.

Results

Cell population recovered

Dispersion of human lung tissue resulted in a cell population consisting of $60 \pm 7\%$ macrophages/monocytes, $6 \pm 1\%$ mast cells, $28 \pm 3\%$ lymphocytes, $1 \pm 0.5\%$ neutrophils, and $5 \pm 3\%$ epithelial cells.

Mediator inhibition studies

Challenge of the cells with anti-IgE resulted in the release of all mediators measured in all patients, as shown in Table 1. There was no difference between cells obtained from lung transplant subjects and cancer resections.

When the cells were preincubated with terfenadine for 20 min before challenge or at the time of the challenge, the release of histamine was not significantly affected (Table 1). In contrast, release of the cyclooxygenase product PGD_2 and the lipoxygenase products $\text{LTC}_4/\text{LTD}_4$ was significantly reduced (Table 1, Fig. 1).

Discussion

In this study we have shown that histamine, PGD_2 , and LTC_4/D_4 were released from human dispersed lung cells in response to IgE-dependent challenge and that terfenadine inhibits the release of cyclooxygenase and lipoxygenase metabolites of arachidonic acid but not of histamine. Histamine was expressed as percentage of release following measurement of total histamine in the cells; however, since arachidonic acid metabolites are newly generated, these were measured only in the supernatants.

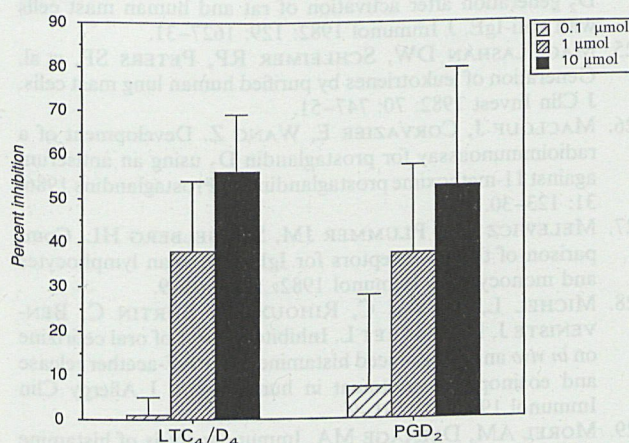
Mast cells have been implicated in the pathogenesis of inflammatory diseases such as rhinitis and asthma because of their ability to synthesize and release inflammatory mediators in response to IgE-mediated stimuli. In response to challenge with IgE-mediated stimuli, mast cells are probably the primary cell type involved since the number and affinity of IgE receptors on these cells is approximately 100-fold higher than those present on other cells in the lung (39), and cells bearing the low-affinity receptor for IgE are usually triggered by antigen-IgE complex. However, it is clear that cells in the lung 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

Table 1. Global results

Subjects	1	2	3	4	5	6	7	8	P value vs max. release
PGD₂									
Release (ng/million cells)									
Base line	5	4.4	3.5	2.8	1.6	4.7	3.8	4.2	
Max. release	48	24.8	44	35	29	59	29	51	
Inhibition of release (%)									
Terf. 0.1 μ mol	0	0	12	0	4	3	2	0	NS
Terf. 1 μ mol	39	2	41	58	31	32	37	39	P<0.01
Terf. 10 μ mol	53	44	86	63	60	59	51	47	P<0.01
LTC₄/D₄									
Release (ng/million cells)									
Base line	1.1	1.4	1.8	0.5	1.2	0.9	0.02	0.6	
Max. release	5	5.4	6.5	4.6	4.8	5.3	3.4	5.8	
Inhibition of release (%)									
Terf. 0.1 μ mol	5	0	60	0	0	19	0	11	NS
Terf. 1 μ mol	34	0	28	45	41	68	30	46	P<0.02
Terf. 10 μ mol	63	55	50	87	0	27	59	28	P<0.02
Histamine									
Release (% total histamine)									
Base line	9.8	6.2	4.9	8.0	7.3	4.2	7.4	8.3	
Max. release	34.0	50.1	37.8	39.6	36.2	31.3	19.4	48.7	
Inhibition of release (%)									
Terf. 0.1 μ mol	0	6	0	9	0	0	7	0	NS
Terf. 1 μ mol	0	0	0	0	0	18	2	0	NS
Terf. 10 μ mol	0	0	6	0	12	0	0	6	NS

Subjects 4–8=cancer.

Statistical analysis by Wilcoxon W-test.

Fig. 1. Median inhibition of LTC₄/D₄ and PGD₂ release by the three concentrations of terfenadine.

those which directly influence the inflammatory reaction (21). A number of mediators are capable of both causing pathologic effects and influencing cell activation. For example, both PGD₂ and LTC₄ can cause bronchoconstriction, but LTC₄ also causes increased polymorphonuclear leukocyte adherence and PGD₂ causes chemokinesis (13, 14, 16). Thus, the release of mediators from unfractionated lung cells usually represents a network of cell, mediator, and cytokine interactions rather than a single cell type phenomenon. The release of mediators from

human dispersed lung mast cells observed in the present study was similar to that of previous experiments (18–20, 27, 37, 43), and there was no apparent difference between cells obtained from cancer resection and those of transplant subjects.

Terfenadine was found to be a potent inhibitor of PGD₂ and LTC₄ but had no effect on histamine in this model. This latter result does not accord with previous data on the release of inflammatory mediators in nasal secretions after allergen challenge which showed that both histamine and PGD₂ release were decreased by terfenadine (2, 32). The differences between these *in vivo* studies and our study may derive from the heterogeneity of mast cells between the nose and the lung, from a different cellular environment, and also from the difference in the model used. There was no evidence of substrate shunting, a phenomenon reported by a number of researchers, although several other groups have not observed this action (1, 7, 8, 28, 31, 38, 40).

This study provides further evidence that H₁-blockers have many other properties than the single antireceptor activity found by other groups (8, 33). Other H₁-blockers were found to block mediator release *in vivo* (7, 28, 31) and *in vitro* (8, 30, 42). Other drugs used in the treatment of asthma can block mediator release from pulmonary mast cells. Sodium cromoglycate inhibits the release of histamine and of mediators derived from arachidonic acid

at a similar concentration (5). β_2 -agonists are effective inhibitors of the release of histamine and mediators derived from arachidonic acid but at a much lower dose (6), whereas their *in vivo* antiallergic activity is, at best, moderate for the classical drugs (9). Thus, the clinical relevance of these findings needs to be clarified for H_1 -blockers (3).

It was shown that both major pathways of the degradation of arachidonic acid were inhibited by terfenadine, whereas histamine release was unaffected. These findings may explain some of the activities of H_1 -blockers and suggest that these drugs possess properties other than strict receptor antagonism.

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