

Short communication

Inhibition of mediator and cytokine release from dispersed nasal polyp cells by terfenadine

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The mechanism of action of H₁-blockers requires elucidation because they may possess properties unrelated to the blockage of histamine at its receptor level. A study was performed with enzymatically dispersed cells obtained from nasal polyps to examine the effect of terfenadine (0.1–10 µmol) on the release of leukotrienes (LT) (LTC₄/D₄ and LTB₄) after stimulation by anti-IgE, and on the spontaneous release of cytokines (granulocyte/macrophage-colony stimulating factor [GM-CSF] and tumor necrosis factor-alpha [TNF-α]) released from cells cultured for 6 h. Terfenadine inhibited significantly, and in a dose-dependent manner, the release of LTC₄/D₄, LTB₄, TNF-α, and GM-CSF. IC₅₀ values were determined for LTC₄/D₄ (8 µmol), LTB₄ (9.9 µmol), TNF-α (6.1 µmol), and GM-CSF (4 µmol). Terfenadine was found to possess new antiallergic properties with a novel *in vitro* model which mimics more closely inflammatory cells of allergic rhinitis or asthma.

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H₁-blockers remain the treatment of choice for allergic rhinitis. The mechanism of action of these drugs requires elucidation because 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* (1–4) and *in vitro* (5–7) to block the release of mediators from basophils, mast cells, and eosinophils.

Nasal polyps are characterized by the presence of hyperplastic, swollen mucosa, arise from the ethmoid sinuses, and may have various causes (8). Cells dispersed from nasal polyps are an interesting model to evaluate antiallergic and antiasthmatic drugs because they are derived from inflamed tissue and are primed or stimulated, unlike cells obtained from the nasal mucosa or the blood in normal subjects (9–11). A vast array of mediators involved in the allergic reaction are released by airways cells. Polyp-dispersed cells may produce mediators such as PAF (12), eicosanoids (13), histamine (14), and cytokines (15). However, the release of histamine is less constant than that of other mediators (14).

Using dispersed cells obtained from surgically resected nasal polyps, a study was performed to examine the effect of terfenadine on the release of leukotrienes (LT) (LTC₄/D₄ and LTB₄) after stimulation by anti-IgE and on the spontaneous release of cytokines (granulocyte/macrophage-colony stimulating factor [GM-CSF] and tumor necrosis factor-alpha [TNF-α]) by cells cultured for 6 h.

Material and methods

Cell recovery

Nasal polyps were obtained, after polypectomy, from 10 patients (seven males, three females), ranging in age from 16 to 60 years, with nasal polyposis. All of these patients were suffering from nonallergic rhinitis, and none of them had aspirin intolerance or cystic fibrosis. None of these patients had received corticosteroids for the previous 2 weeks. Cell disaggregation was achieved by enzymatic digestion by a method previously described in detail (6). Briefly, finely chopped tissue was incubated for

2 h in the presence of 0.75 mg/ml of hyaluronidase (Sigma Co, St Louis, MO, USA), 1.5 mg/ml of collagenase (Sigma), and 2 mg/ml of protease type XIV (Sigma) at 37°C in a shaking water bath. At the end of the digestion period, the cells were filtered sequentially to separate them from any undigested tissue and washed.

Characterization and viability of cells

The cells were placed on slides prepared by cyto-centrifugation and stained with May-Grünwald and Giemsa to characterize macrophages, eosinophils, neutrophils, and lymphocytes. Viability was assessed before stimulation and at the end of the incubation period by measuring the levels of lactate dehydrogenase (LDH) in the supernatant by colorimetric determination with a commercially available kit (Sigma).

Inhibition studies

The cells were resuspended at a concentration of 1×10^6 cells/ml in RPMI 1640. For eicosanoid release, cells were preincubated with terfenadine (0.1, 1, and 10 μ mol) or the vehicle used to dissolve terfenadine (dimethyl sulfoxide at a final concentration of 0.1%) for 20 min before challenge with 1:1000 ϵ -chain specific anti-IgE (Immunotech, Luminy, France) for 45 min at 37°C. For cytokine release, cells were incubated with terfenadine or dimethyl sulfoxide for 6 h.

Measurement of mediators

LTC₄/D₄ and LTB₄ were measured with commercially available enzyme immunoassay kits (Stallergènes, Fresnes, France, and Cayman Chemical Company, Ann Arbor, MI, USA, respectively). TNF- α and GM-CSF were measured by the enzyme-linked immunosorbent assay (ELISA) with commercially available kits (Medgenix Diagnostics, Fleurus, Belgium).

Statistical analyses

Statistical analyses were performed with Wilcoxon's W test. The results of inhibition are given for net release for eicosanoids and for maximal release for cytokines.

Results

Characteristics of cells

Epithelial cells composed $43 \pm 13\%$ (mean $\pm$ standard deviation) of the cell population, as shown by an anticytokeratin antibody. Mononuclear cells (lymphocytes and macrophages) ($47 \pm 11\%$), neu-

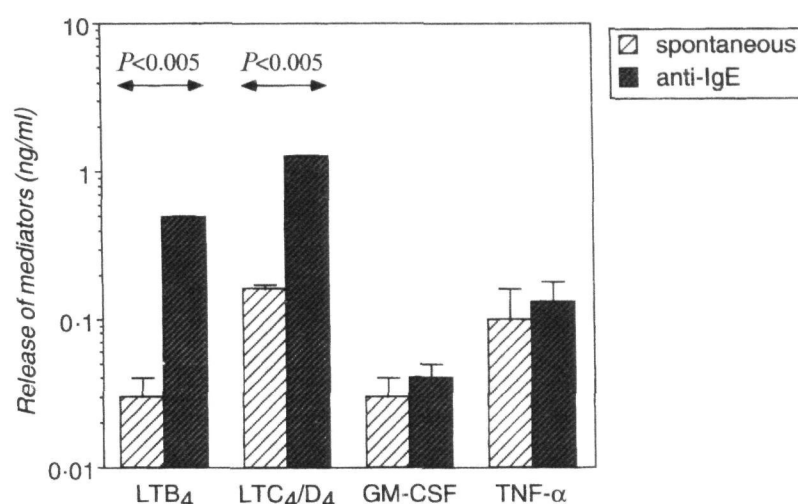


Fig. 1. Release of leukotrienes and cytokines from nasal polyp dispersed cells (results expressed in means $\pm$ SD; statistical analysis by Wilcoxon's W test).

trophils ($3 \pm 2\%$), and eosinophils ($7 \pm 11\%$) were the other cell types recovered.

Release of mediators

Anti-IgE induced a significant increase in the release of the two leukotrienes studied (Fig. 1). On the other hand, anti-IgE did not induce an increased release of cytokines. Thus, inhibition levels were studied on anti-IgE-stimulated cells for eicosanoids and on spontaneous release over 6 h for cytokines.

Inhibition of mediator release

LDH release was not increased when cells were stimulated with terfenadine. Terfenadine inhibited significantly, and in a dose-dependent manner, the release of LTC₄/D₄ ($P < 0.005$ for a dose of 10 μ mol, Wilcoxon's W test), LTB₄ ($P < 0.01$ for a dose of 10 μ mol), TNF- α ($P < 0.005$ for a dose of 10 μ mol and $P < 0.02$ for a dose of 1 μ mol), and GM-CSF ($P < 0.01$ for a dose of 10 μ mol and $P < 0.02$ for a dose of 1 μ mol) (Fig. 2). The calculated IC₅₀ values were determined for LTC₄/D₄ (8 μ mol), LTB₄ (9.9 μ mol), TNF- α (6.1 μ mol), and GM-CSF (4 μ mol).

Discussion

Terfenadine, an H₁-blocker which was shown to reduce the release of inflammatory mediators from blood, lung, skin, and colon cells is also able to reduce the release of leukotrienes and cytokines from nasal polyp cells.

Cells present in the airways submucosa play an important role in rhinitis and asthma, but technical difficulties have prevented an in-depth study of these cells in the disease state. Lung tissue cells are usually obtained from patients suffering from lung carcinoma where the microenvironment is likely to differ

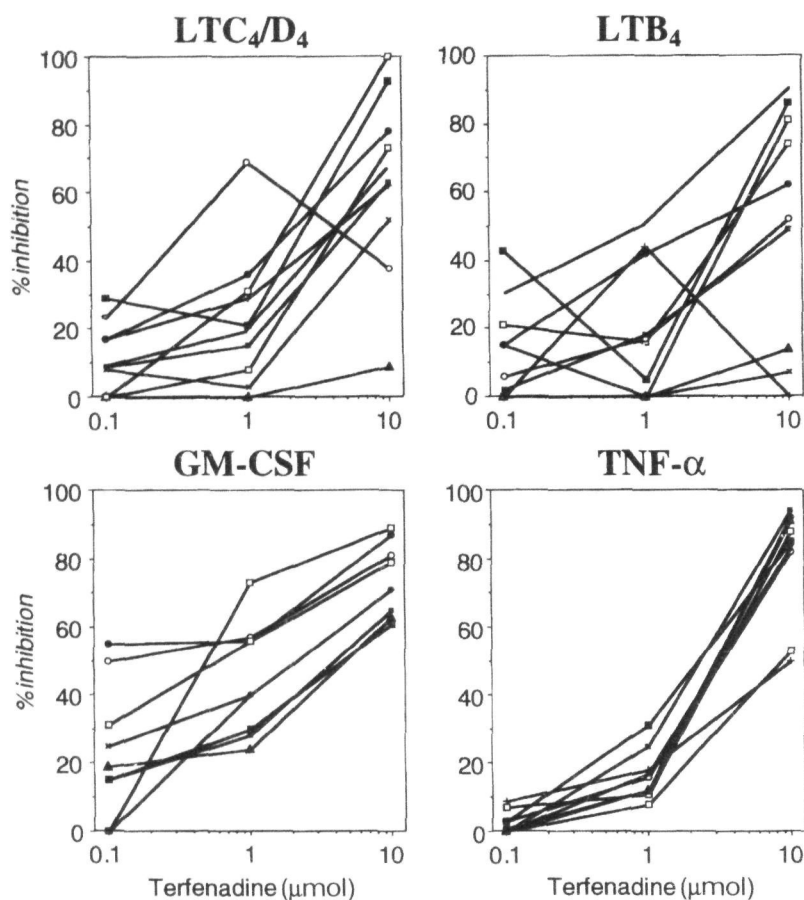


Fig. 2. Inhibition of leukotriene and cytokine release from nasal polyp dispersed cells.

substantially from that of rhinitis or asthma. In the present study, it was decided to use dispersed nasal polyp cells because polyps represent an inflamed tissue that can be recovered in large quantities, and these cells are already primed in a manner similar to the inflammation of rhinitis or asthma (12–15). As expected, the cell population included epithelial cells, mononuclear cells, eosinophils, and neutrophils. However, there was a large degree of heterogeneity among polyps in the cell content. The release of LDH was also measured as an index of cell mortality, this enzyme being released by dead or dying cells. Terfenadine has been previously found to block the *in vitro* release of leukotrienes from peripheral blood and tissue cells (5–7). Although the tissue levels of terfenadine or its metabolites after treatment have never been established, it is clear that a concentration of 10 μmol is never reached *in vivo*. However, we believe that these results may have physiologic relevance because the concentration of IgE used for challenge is also supraphysiologic, and one would therefore expect that increased levels of a potentially therapeutic compound would be required to cause inhibition. Therefore, this study extends previous results obtained with other tissues and provides new evidence that H₁-blockers have many other properties besides a single antireceptor activity. Since terfenadine blocked similarly the release of both LTC₄/D₄ and LTB₄, it may be postulated that terfenadine may have an inhibitory effect on 5-lipoxygenase,

but more studies are needed to validate this hypothesis, and an indirect effect – for example, via histamine – cannot be ruled out.

Apart from azelastine (16, 17), no other H₁-blocker has been found to decrease the release of cytokines in an *in vitro* study. The inhibition reported in this study was potent and even more pronounced than that of inflammatory mediators. GM-CSF, a hematopoietic growth factor involved in eosinophilic and macrophagic inflammation, is released in nasal secretions during allergen challenge (18). GM-CSF inhibition may be of importance since it has been found that long-term treatment with terfenadine started before the pollen season is more effective than treatment introduced during the season when symptoms are established (19). Although the cellular provenance of TNF-α in this study is unclear, it is known that eosinophils in nasal polyps express and release TNF-α (20). The inhibition of the secretion of this cytokine by terfenadine may also be of importance in understanding the antiallergic effects of the drug.

These anti-inflammatory effects of terfenadine may be related to the therapeutic activity of the drug, but the concentration used *in vitro* appears to be superior to the blood concentration of the product. However, the tissue levels of terfenadine or its metabolite have never been demonstrated. Thus, this study should be supplemented by an *in vivo* study to clarify the effect of the drug.

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