

Short communication

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

Background: Mizolastine is a potent and selective H_1 -receptor antagonist with antiallergic properties; in in-vitro animal models, mizolastine was shown to inhibit 5-lipoxygenase activity and to decrease the release of leukotrienes (LT) and tumor necrosis factor- α (TNF- α). This study investigated the effects of three concentrations of mizolastine (0.1, 1.0, 10 μ M) on the release of LT (LTB_4 and LTC_4/D_4) and prostaglandin D_2 (PGD_2) after stimulation by anti-IgE, and on the spontaneous release of cytokines (TNF- α and granulocyte/macrophage-colony-stimulating factor [GM-CSF]), from dispersed cells obtained from surgically resected nasal polyps of patients with nasal polyposis.

Methods: Cells from nasal polyps were obtained using enzymatic dispersion. For experiments involving the measurement of LT and PGD_2 , the cells were preincubated with mizolastine or its dissolution vehicle for 20 min prior to challenge with 10 μ g/ml ϵ -chain specific anti-IgE for 45 min at 37°C; for the cytokine release, cells were incubated with mizolastine or its dissolution vehicle for 24 h. LT and PGD_2 were measured by enzyme immunoassay (EIA) and cytokines by enzyme-linked immunosorbent assay (ELISA) using commercially available kits.

Results: Mizolastine inhibited significantly and in a dose-dependent manner the release of LTB_4 and TNF- α at all concentrations, LTC_4/D_4 at 10 μ M, and GM-CSF from 1 μ M; no effect was observed on the release of PGD_2 .

Conclusion: Mizolastine inhibits the release of LT, TNF- α and GM-CSF in this *in vitro* model, which mimics closely the inflammatory cells of allergic rhinitis.

N. Carayol¹, L. Crampette²,
B. Mainprice³, Paul Ben-Soussen⁴,
M. Verrecchia⁴, J. Bousquet¹,
B. Lebel¹

¹Clinique des Maladies Respiratoires and INSERM U454, Hôpital Arnaud de Villeneuve; ²Service d'ORL, Hôpital Arnaud de Villeneuve, Center Hospitalier Universitaire; ³Medical-Bio-Medical, Montpellier; ⁴Sanofi-Synthelabo, Clinical Research Department, Chilly Mazarin, France

Key words: cytokines; leukotrienes; mizolastine; nasal polyps.

Prof. Jean Bousquet
INSERM U454
Hôpital Arnaud de Villeneuve
Avenue du Doyen G. Giraud
34295 Montpellier Cedex 5
France

Accepted for publication 17 June 2002

H_1 -blockers remain a first-line treatment in allergic rhinitis (1). The mechanism of action of these drugs requires further elucidation since they were shown to possess properties unrelated to the blockage of histamine at its receptor level ascribed to "antiallergic properties" (2).

Mizolastine is a potent and selective antagonist of H_1 histamine receptors indicated in allergic rhinitis and in chronic urticaria; the recommended daily dose is 10 mg. The pharmacological activity of mizolastine is due to a selective blockade of H_1 histamine receptors (3), and to an inhibition of mastocyte histamine release, and of the recruitment of inflammatory cells (eosinophils and neutrophils) (4,5). Moreover, in in-vitro animal models, mizolastine was shown to inhibit 5-lipoxygenase (5-LO) activity and to decrease the release of leukotrienes (LT) (6,7); it also inhibits release of tumor necrosis factor- α (TNF- α) (8). This inhibitory effect on the 5-LO pathway could explain the inhibition of the rat-paw inflammatory edema induced by arachidonic acid (9). Nasal polyps are characterized by the presence of hyperplastic, swollen mucosa and arise from the ethmoid sinuses and may have variable etiologies. Epithelial cells, fibroblasts,

macrophages, mast cells, eosinophils, and immunocompetent cells are among the cells recovered from nasal polyps (10, 11). Cells dispersed from nasal polyps represent an interesting model to study in the pharmacologic evaluation of antiallergic drugs since they are derived from inflamed tissue and are primed or stimulated by comparison to cells obtained from the nasal mucosa or the blood in normal subjects (12). Nasal polyp cells release eicosanoids (13, 14), cytokines (15, 16), and chemokines (17); the release of histamine is, however, less constant than that of other mediators (18–20). The model of nasal polyps was used previously to study the *in vitro* antiallergic activities of terfenadine (21), ebastine (22), and cyclosporin A (23). It was found that the inhibition of mediator release from nasal polyps was correlated to the *ex vivo* effect of the same drug (22).

This study was performed using dispersed cells obtained from surgically resected nasal polyps to examine the effects of mizolastine on the release of LT (LTC_4/D_4 and LTB_4) and PGD_2 following stimulation by anti-IgE and on the spontaneous release of cytokines (TNF- α and granulocyte/macrophage-colony-stimulating factor [GM-CSF]) from cells cultured for up to 24 h.

Material and methods

Cell recovery

A total of 28 nasal polyps were obtained, after polypectomy, from 10 patients (7 men, 3 women), ranging in age from 19 to 58 years, with nasal polyposis. All of these patients were suffering from nonallergic chronic rhinitis and none of them had aspirin-intolerance or cystic fibrosis. None of these patients had received corticosteroids or antihistamines for the previous 2 weeks. The dispersion of polyp cells was carried out as previously described in detail (24).

Characterization and viability of cells

The origin of cells was characterized on slides prepared by cyto-centrifugation. The epithelial origin of cells was characterized using immunohistochemistry with a highly specific monoclonal antibody (mAb) anticytokeratin (MNF-116, Dako, Versailles, France) (25) and an alkaline phosphatase, antialkaline phosphatase system; the other cell types (macrophages, eosinophils, neutrophils, and lymphocytes) were characterized with May Grunwald Giemsa. Viability was assessed before stimulation and at the end of the incubation period by exclusion of Trypan Blue stain.

Inhibition studies

Mizolastine was dissolved in dimethylsulfoxide (DMSO) to give concentrations of 0.1, 1.0 and 10 μ M; the final concentration of DMSO was 0.1%. In previous studies (21, 23), we studied the effect of DMSO as dissolution vehicle at various concentrations and we retained 0.1% as final concentration because it had no effect on polyp cells. The cells were resuspended at a concentration of 1×10^6 cells/ml in Roswell Park Memorial Institute (RPMI). For experiments involving the measurement of eicosanoids, the cells were preincubated with mizolastine (0.1, 1.0 and 10 μ M) or medium containing DMSO for 20 min prior to challenge with 10 μ g/ml ϵ -chain specific anti-IgE (Dako, Versailles, France) for 45 min at 37°C. For experiments involving cytokine release, cells were incubated with mizolastine or DMSO for 24 h.

Measurement of mediators

Mediators were measured using commercially available kits. LT and PGD₂ were measured using enzyme immunoassay (EIA) kits (Stallergènes, Fresnes, France and Cayman Chemicals, Ann Arbor, MI, respectively). TNF- α and granulocyte/macrophage stimulating factor (GM-CSF) were measured using enzyme-linked immunosorbent assay (ELISA) kits (Genzyme, Cambridge, MA).

Statistical analyses

Statistical analyses were performed using a variant of Friedman's nonparametric test (26); if the overall $P < 0.05$, the three comparisons (each concentration vs. anti-IgE for eicosanoids release or each concentration vs. spontaneous release for cytokine release) were conducted without correction. The tests were two-sided with $\alpha = 5\%$.

Results

Characteristics of cells

Epithelial cells composed $41\% \pm 9\%$ (mean $\pm$ standard deviation) of the cell population. Mononuclear cells

(lymphocytes and macrophages) ($40 \pm 8\%$), mast cells ($4 \pm 2\%$), neutrophils ($4 \pm 3\%$), and eosinophils ($11 \pm 10\%$) were the other cell types recovered. Mizolastine did not affect cell viability as assessed by the exclusion of Trypan Blue Stain after challenge with anti-IgE and after 24 h.

Eicosanoid release

Of the 10 polyps tested in this series of experiments, challenge with anti-IgE elicited release (taken as being at least three times the baseline value) of LTB₄ in eight samples, LTC₄/LTD₄ in nine samples, and PGD₂ in seven samples. In cases where the release of eicosanoid in response to stimulation with anti-IgE did not reach the level of three times the spontaneous release, activation was deemed not to have occurred and the results for these samples were not used for further analysis. Mizolastine significantly inhibited the release of LTB₄ at all concentrations; the release of LTC₄/D₄ was significantly inhibited at a concentration of 10 μ M but there was a trend ($P = 0.06$) at 1 μ M; mizolastine did not significantly affect the levels of PGD₂ (Table 1).

Cytokine release

Mizolastine caused a significant inhibition of TNF- α at all concentrations; the release of GM-CSF was significantly inhibited at concentrations from 1 μ M but there was a trend ($P = 0.09$) at 0.1 μ M (Table 2).

Discussion

Mizolastine is a H₁-blocker that was previously shown to inhibit the release of LT and TNF- α in in-vitro animal studies (7, 8); it is also able to reduce the release of these mediators from human nasal polyp cells.

This study shows once more that H₁-blockers have properties other than a strict blockage of the H₁-histamine receptor. Moreover, these so-called "antiallergic" effects differ between the different molecules using the same in-vitro model (21, 22), showing that the observed effect is not related to H₁-blockage.

In this study, mizolastine significantly inhibited the release of LTB₄ and, to a lesser extent, that of LTC₄/D₄; as mizolastine had no effect on the release of PGD₂, the inhibitory effect on the release of LT seems to be related to a primary effect on the 5-LO pathway, that is in close agreement with the inhibitory effect of mizolastine on 5-LO activity observed in animal studies (6, 7, 9). Mizolastine also significantly inhibited the release of TNF- α and GM-CSF, which are important mediators of the inflammatory response. The inhibition of the release of these mediators seems to be dose-dependent. The mean steady-state peak plasma concentration of mizolastine 10 mg once daily in human is 259 ng/ml, i.e. 0.6 μ M,

Table 1. Effect of mizolastine on anti-IgE-induced eicosanoid release (values expressed in pg/ml)

	Spontaneous release	Anti-IgE	Anti-IgE + mizolastine 0.1 μ M	Anti-IgE + mizolastine 1.0 μ M	Anti-IgE (10 μ g/ml) + mizolastine 10 μ M
LTB ₄ (8 polyps)					
Mean $\pm$ SD	95 $\pm$ 87	993 $\pm$ 571	827 $\pm$ 449	764 $\pm$ 429	562 $\pm$ 385
Median (min–max)	59 (35–285)	789 (365–1880)	716 (190–1550)	597 (294–1470)	449 (21–1260)
P value*	–	–	0.0002	0.00003	< 0.00001
LTC ₄ /D ₄ (9 polyps)					
Mean $\pm$ SD	123 $\pm$ 90	1930 $\pm$ 1520	2080 $\pm$ 1840	1740 $\pm$ 1460	1310 $\pm$ 910
Median (min–max)	100 (30–310)	1400 (750–4700)	1200 (800–6100)	1100 (600–4600)	900 (570–3500)
P value*	–	–	0.71	0.06	0.0008
PGD ₂ (7 polyps)					
Mean $\pm$ SD	32 $\pm$ 8	223 $\pm$ 140	216 $\pm$ 136	206 $\pm$ 142	334 $\pm$ 235
Median (min–max)	31 (21–45)	169 (90–442)	265 (35–383)	221 (50–446)	414 (74–620)
P value*	–	–	ND**	ND**	ND**

* Versus anti-IgE; ** ND: not done as the overall P value > 0.05.

Table 2. Effect of mizolastine on spontaneous cytokine release (values expressed in pg/ml)

	Spontaneous release	Mizolastine		
		0.1 μ M	1.0 μ M	10 μ M
TNF- α (10 polyps)				
Mean $\pm$ SD	3.2 $\pm$ 4.2	1.7 $\pm$ 2.4	1.5 $\pm$ 2.2	1.5 $\pm$ 2.2
Median (min–max)	2.0 (0.7–14.8)	1.0 (0.5–8.3)	0.9 (0.3–7.6)	0.6 (0.4–7.7)
P value*	–	0.00007	< 0.00001	< 0.00001
GM-CSF (10 polyps)				
Mean $\pm$ SD	31.4 $\pm$ 41.4	25.9 $\pm$ 35.2	21.8 $\pm$ 27.9	18.2 $\pm$ 24.3
Median (min–max)	11.5 (0.6–109.2)	7.6 (0.4–97.3)	7.6 (0.5–68.4)	5.80 (0.4–59.4)
P value*	–	0.09	0.015	0.00001

* Versus spontaneous release.

falling in the range of concentrations that significantly inhibited the release of LTB₄ (0.1 μ M), TNF- α (0.1 μ M), and GM-CSF (1 μ M); therefore, the inhibitory effect of mizolastine on the release of these mediators may be related to its therapeutic activity. The concentration that significantly inhibited the release of LTC₄/D₄ (10 μ M) seems high to be reached *in vivo*; however, the inhibitory effect of mizolastine on the release of this mediator may be clinically relevant since 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 inhi-

bition. As LT and pro-inflammatory cytokines (in particular TNF- α and GM-CSF) play an important role in allergic rhinitis, with a primary role relating to the late-phase reaction and to nasal congestion (27–29), these inhibitory effects on the release of these mediators could explain the efficacy of mizolastine in improving nasal obstruction in patients with perennial allergic rhinitis (30).

In conclusion, in addition to its H₁-blocking activity, mizolastine also inhibits the release of LT, TNF- α and GM-CSF in this in-vitro model, which mimics closely inflammatory cells of allergic rhinitis.

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