

Inhibitory activity of loratadine and descarboethoxyloratadine on expression of ICAM-1 and HLA-DR by nasal epithelial cells

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Nasal epithelial cells represent the first barrier against noxious agents and allergens. In allergic rhinitis, these cells are activated and histamine may be involved in this activation. Loratadine and one of its active metabolites, descarboethoxyloratadine, were studied for their ability to reduce the activation of nasal epithelial cells by histamine. Nasal turbinates or polyps were removed during surgery from 19 subjects, and nasal epithelial cells were recovered after enzymatic digestion. The *in vitro* activation of epithelial cells with histamine using an optimal dose (1 μ M) and an optimal time (24 h) of incubation was studied, and the effect of loratadine or descarboethoxyloratadine (10 μ M) was investigated. The expression of membrane markers (intercellular adhesion molecule-1 (ICAM-1) and a human leukocyte class II antigen (HLA-DR) was assessed by immunocytochemical analysis using an alkaline-antialkaline phosphatase (APAAP) system. The spontaneous expression of both markers was not significantly different in cells recovered from nasal turbinates or polyps, and there was a highly significant increase in the numbers of cells expressing ICAM-1 and HLA-DR following incubation with histamine. Loratadine or descarboethoxyloratadine significantly blocked these effects. This study shows a new possible antiallergic effect of H_1 -blockers and suggests that their effects on epithelial cells may be relevant *in vivo*.

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The nasal epithelial barrier is the first part of the body to interact with inhaled noxious agents and allergens. In allergic rhinitis, in contrast to asthma, epithelial cells are not shed (3, 11–13), but recent studies have shown that they are activated (Canonica et al., unpublished results). The activation of nasal epithelial cells may be induced directly by viruses and pollutants (2, 5), and possibly indirectly by cytokines (1) and histamine (as has been recently observed for bronchial epithelial cells (21)). The activation of airways epithelial cells may be measured by the release of proinflammatory mediators (6) or by the expression of surface markers such as class II antigens (HLA-DR) or intercellular adhesion molecule-1 (ICAM-1) (21, 22).

Loratadine (8, 18) is an H_1 -blocker possessing antiallergic properties (4, 14, 20) which may act to

reduce histamine-induced activation of epithelial cells. When administered orally, loratadine is rapidly degraded, and one of its major metabolites is descarboethoxyloratadine (15).

A study was undertaken to assess the inhibitory effect of loratadine and descarboethoxyloratadine on the activation of nasal epithelial cells by histamine.

Material and methods

Subjects

Nineteen nonallergic patients ranging in age from 27 to 49 years underwent a biopsy during nasal surgery. The absence of allergy was ascertained by a negative history (both personal and familial) and negative skin prick tests to the 30 most common allergens in the region. Seven subjects underwent polypectomy,

and the other 12 underwent removal of nasal turbinates.

The study was performed after obtaining informed consent and fulfilled all the criteria set by the ethical committee of the hospital.

Investigations

Recovery of nasal epithelial cells. The epithelial cells were recovered by enzymatic and mechanical dispersion. Biopsy specimens were washed three times with RPMI (Gibco, Paisley, UK) supplemented with antibiotics (penicillin 50 U and streptomycin 50 µg/ml). The specimens were then incubated for 2 h with 1 mg/ml protease XIV (Sigma, St Louis, MO, USA). Cell dispersion was performed by gentle aspiration with a 1-ml micropipette. After centrifugation, the cells were washed three times with 1 ml of RPMI medium supplemented with 10% fetal calf serum to block the enzymatic activity. Cells were then resuspended in 1 ml of Ham-F12 medium (Gibco), supplemented with epidermal growth factor (25 ng/ml; Sigma), hydrocortisone (1 µg/ml; Sigma), transferrin (5 µg/ml; Sigma), insulin (5 µg/ml; Sigma), gentamycin (60 µg/ml), and fungizone (2 µg/ml). Macrophages were removed by adherence to plastic in a humidified incubator of 95% air and 5% CO₂ for 1 h at 37°C.

Viability and characterization of origin of the cells. The epithelial origin of cells was characterized on slides prepared by cytocentrifugation using immunohistochemistry with a highly specific monoclonal antibody (mAb) anticytokeratin (MNF-116, Dako, Versailles, France) (16) and the APAAP system (9) (Dako, Versailles, France). Viability was assessed on an aliquot of cells before stimulation and at the end of the incubation period by exclusion of trypan blue stain.

Immunostaining of epithelial cells. HLA-DR and ICAM-1 expression was studied with the APAAP system. mAbs against ICAM-1 (10) (Immunotech, Luminy, France) and class II antigens (19) (Dako, Versailles, France) were used as first antibodies in the immunostaining assay. Their specificity and the optimal concentration had been previously characterized with a positive control represented by peripheral blood mononuclear cells stimulated for 36 h with phytohemagglutinin-A (1 µg/ml) and interferon-gamma (300 U/ml) and fixed after cytocentrifugation. Control slides were incubated without the primary specific mAb or with an irrelevant antibody used at the same dilution. All the slides were stored at -20°C, and levamisole (10 l) was added to the substrate solution to inhibit the endogenous alkaline phosphatase activity (9). Under these two condi-

tions, no staining due to endogenous alkaline phosphatase activity was observed. A rabbit antimouse IgG (Dako, Versailles, France) and a mouse anti-rabbit monoclonal antibody-APAAP complex were used as secondary and tertiary antibodies, respectively (9). Cells were counted by two independent observers. A very high correlation was obtained for both observers. Results were expressed as the percentage of cells stained.

Stimulation of epithelial cells by histamine and inhibition by loratadine and descarboethoxyloratadine. Cells were suspended at a concentration of 10⁵ viable cells per ml. The optimal dose of histamine (1 µM) and the optimal time of incubation (24 h) were used according to a previous study (21). Loratadine dissolved in dimethylsulfoxide (DMSO) (final concentration 0.2% v/v) and its active metabolite, descarboethoxyloratadine, dissolved in saline (15) were used at a concentration of 10 µM and added at the same time as histamine. A control with the final concentration of DMSO was used when loratadine was studied. The number of cells recovered did not permit the performance of tests using both loratadine and descarboethoxyloratadine during the same experimental series.

Statistical analysis

Statistical analysis was performed by means of non-parametric tests. The Wilcoxon W test was used for paired comparisons and the Mann-Whitney U test for unpaired comparisons.

Results

Characterization of the cells

The percentage of nasal epithelial cells, as ascertained using an anticytokeratin mAb, was significantly greater in turbinate specimens than in polyps (Table 1). The viability of these cells was always over 48% and was significantly higher in cells recovered from nasal polyps.

Table 1. Global results

	Turbinate 12	Polyps 7	P value
<i>n</i>			
Spontaneous expression			
Cytokeratin	95.1 (94.0 to 96.2)	45 (42.3 to 48.8)	<0.0001
Viability	66.0 (64.7 to 71.7)	78.0 (74.4 to 81.3)	<0.002
HLA-DR	0 (-0.3 to 3.9)	5.5 (-1.3 to 18.3)	NS
ICAM-1	0 (-2.2 to 16.1)	10 (3.8 to 16.8)	NS

P value by Mann-Whitney U test.

Results expressed in median percentage of cells and 95% confidence interval.

Activation of epithelial cells by histamine

There was no significant (Mann-Whitney U test) difference between nasal turbinates and nasal polyps in the baseline values and the histamine-induced activation (Table 1; Fig. 1). There was a significant increase in cells expressing ICAM-1 or HLA-DR after histamine incubation from both types of tissue ($P < 0.03$ to $P < 0.003$, Wilcoxon W test). DMSO slightly and nonsignificantly reduced the expression of membrane markers.

Inhibitory activity of loratadine and descarboethoxylopratadine

The addition of loratadine ($10 \mu\text{M}$) or descarboethoxylopratadine ($10 \mu\text{M}$) inhibited histamine-induced increase in HLA-DR and ICAM-1 expression (Figs. 2 and 3; $P < 0.04$ to $P < 0.004$, Wilcoxon W test).

Discussion

This study shows that histamine stimulates the expression of HLA-DR and ICAM-1 molecules on nasal epithelial cells. Moreover, it was found that loratadine, an H_1 -blocker, and one of its active metabolites, descarboethoxylopratadine, block the activation of epithelial cells by histamine, suggesting a new mechanism of action for H_1 -blockers.

The recovery of epithelial cells was derived from studies carried out with lung parenchyma (7), and this technique is similar to that of the recently published study of Altman et al. (1). Cells recovered from turbinates were mostly of epithelial origin, but a mixed cell population was recovered from polyps. This difference is related to the sampling procedure, because, for turbinates, the biopsy was purposely superficial. Since a mixed cell population was recovered from polyps, for the immunocytochemistry study we counted only ciliated epithelial cells for both turbinates and polyps. The viability of cells was greater in specimens from polyps for an unknown

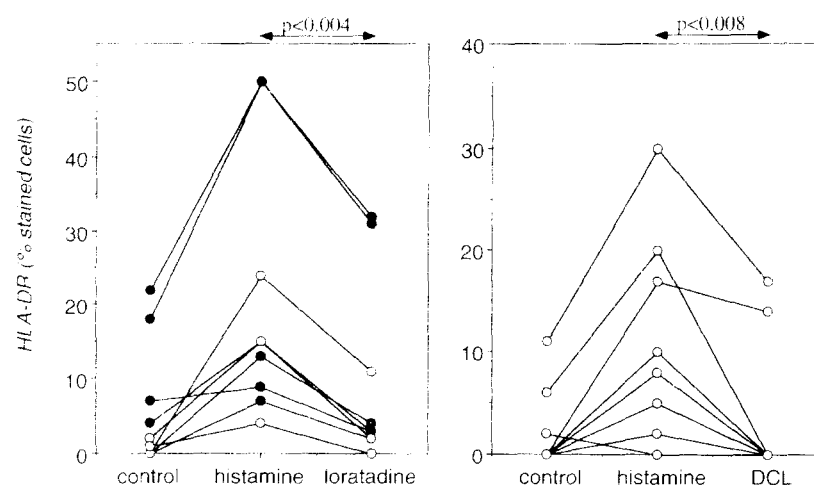


Fig. 2. Expression of HLA-DR by turbinate (○) and polyp (●) cells stimulated by histamine and inhibition by loratadine or descarboethoxylopratadine (DCL). Statistical analysis by Wilcoxon W test.

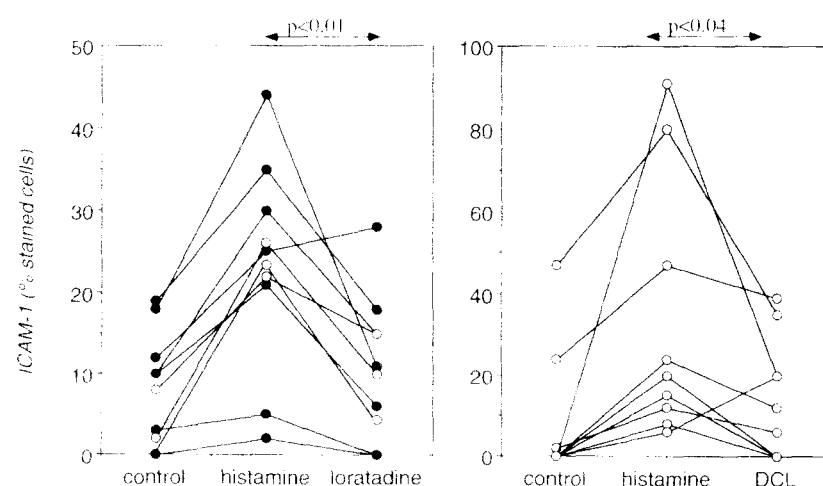


Fig. 3. Expression of ICAM-1 by turbinate (○) and polyp (●) cells stimulated by histamine and inhibition by loratadine or descarboethoxylopratadine (DCL). Statistical analysis by Wilcoxon W test.

reason. Although nasal epithelial cells were recovered by two different sampling procedures, the expression of ICAM-1 and HLA-DR was similar and nonsignificantly different both at baseline and after histamine incubation.

Histamine was chosen because of its important role in rhinitis and because histamine receptors are present on airway epithelial cells (17). In rhinitis, an infiltration of activated mast cells is often observed between epithelial cells (3, 11, 13), suggesting possible interactions between these cells. We used only one concentration of histamine due to the low number of epithelial cells recovered and because of data obtained from a previous study on bronchial epithelial cells in which concentration-response and time-response curves were established (21).

We studied two membrane markers, HLA-DR and ICAM-1, because 1) their expression in rhinitis was shown to be increased compared to normal subjects (Canonica, unpublished data), 2) there is a highly significant correlation between the severity of asthma and the number of epithelial cells expressing these two membrane markers (22), and 3) histamine

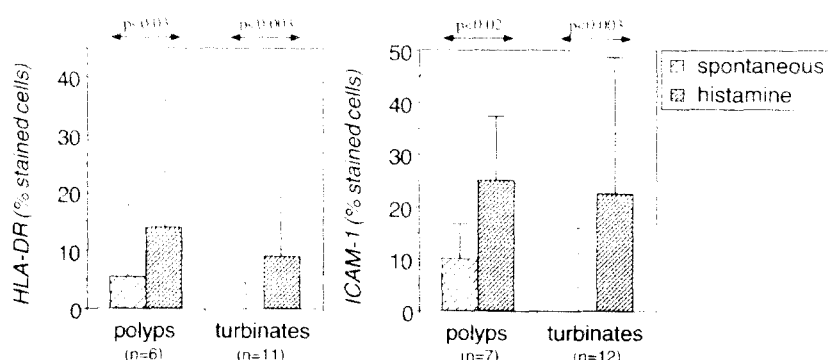


Fig. 1. Expression of surface markers before and after stimulation by histamine. Results are expressed as medians and 95% confidence interval. Statistical analysis by Wilcoxon W test.

significantly increases their expression on bronchial epithelial cells (21). The results of this study confirm a previous study on bronchial epithelial cells showing the effect of histamine on the surface markers of nasal epithelial cells (22), and suggesting that interactions between mast cells and histamine are also present in the nasal epithelium. The effect of the increased expression of these membrane markers remains to be elucidated, but it may contribute, at least for ICAM-1, to induce the adhesion between inflammatory cells bearing the lymphocyte-function-associated antigen-1 and epithelial cells (1), leading to an increased number of inflammatory cells in the epithelial layer.

Loratadine or descarboethoxyloratadine reduced the activation of epithelial cells by histamine in most experiments. 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 since the concentration of IgE used for challenge is also supraphysiologic, and it would therefore be expected that increased levels of a potential therapeutic compound would be required to cause inhibition. The significance of these results may be of relevance for the treatment of rhinitis since nasal epithelial cells are likely to play an important role in the pathogenesis of this disease. For many years, epithelial cells were considered to have the simple role of a barrier which was involved in mucous secretion and removal of noxious agents by cilia. However, it is likely that these cells may be involved in the inflammation and remodeling of the airways. Thus, the decreased activation of epithelial cells by loratadine and descarboethoxyloratadine may be of importance in the regulation of inflammation in the nose.

The new effect of loratadine and descarboethoxyloratadine on the activation of epithelial cells by a stimulus compatible with the physiologic situation during rhinitis may be of importance in the understanding of the mechanisms of action of H_1 -blockers.

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