

Effect of loratadine on nitrogen dioxide-induced changes in electrical resistance and release of inflammatory mediators from cultured human bronchial epithelial cells

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Background: Recent studies have demonstrated that some antihistamines can attenuate histamine-induced release of inflammatory mediators from bronchial epithelial cells. **Objective:** The purpose of study was to test the hypothesis that loratadine may influence pollution-induced inflammation of the airways by modulating epithelial membrane integrity and the synthesis and/or release of inflammatory mediators from airway epithelial cells. **Methods:** We have cultured human bronchial epithelial cell (HBEC) cultures from surgical explants and investigated the effect of loratadine on NO₂-induced changes in both electrical resistance of HBEC cultures and release of IL-8, RANTES, and soluble intercellular adhesion molecule-1 (sICAM-1) from these cells after exposure for 6 hours to either air or 400 ppb NO₂. **Results:** Exposure for 6 hours to NO₂ significantly decreased the electrical resistance of HBEC cultures by 18.1% from baseline ($P < .05$). Incubation with 0.25 to 25 $\mu\text{mol/L}$ loratadine did not alter the NO₂-induced decrease in the electrical resistance of HBEC cultures. NO₂ also significantly increased the release of IL-8 from a control value of 52.5 pg/ μg cellular protein to 81.9 pg/ μg cellular protein ($P < .05$), RANTES from a control value of 0.023 pg/ μg cellular protein to 0.062 pg/ μg cellular protein ($P < .05$), and sICAM-1 from a control value of 7.7 pg/ μg cellular protein to 16.3 pg/ μg cellular protein ($P < .05$). The NO₂-induced release of all 3 mediators was significantly attenuated by incubation of HBECs with 25 $\mu\text{mol/L}$ loratadine. Incubation with 2.5 $\mu\text{mol/L}$ loratadine also significantly attenuated the NO₂-induced release of RANTES and sICAM-1, but not IL-8.

Conclusions: These results suggest that loratadine has the potential to reduce airway inflammation by modulating the release of inflammatory cytokines from airway epithelial cells. (*J Allergy Clin Immunol* 1999;104:93-9.)

Key words: Loratadine, nitrogen dioxide electrical resistance, inflammatory mediators, bronchial epithelial cells

Studies that investigated the pathophysiologic effects resulting from exposure to pollutants such as NO₂ and ozone have demonstrated that these lead to marked histologic changes and inflammation in the airways.^{1,2} Although the precise mechanisms underlying pollution-induced airway damage and inflammation are not clear, there is increasing evidence that airway epithelial cells, the primary target for air pollutants, may play an important role in the cause of airway diseases because they express and synthesize a large variety of proinflammatory mediators, which directly or indirectly influence the activity of eosinophils, neutrophils, mast cells, macrophages, and lymphocytes.^{3,4} Studies of human bronchial epithelial cells exposed to 400 to 800 ppb NO₂ in vitro have demonstrated that this agent leads to increased epithelial permeability, decreased ciliary activity, and increased release of proinflammatory mediators, including leukotriene C₄ (LTC₄), GM-CSF, TNF- α , and IL-8.^{5,6}

Some studies have demonstrated that histamine can also induce the expression and release of proinflammatory mediators, such as IL-6, fibronectin, intercellular adhesion molecule-1 (ICAM-1) and HLA-DR, from human bronchial epithelial cells,^{7,8} suggesting that antihistamines may have a role in the management of airway inflammation. Although studies of the new generation of nonsedating H₁-receptor antagonists have demonstrated that these compounds have marked clinical efficacy and antiinflammatory properties,⁹ the precise mechanisms underlying their anti-inflammatory actions are not clearly understood.

Studies of loratadine, a long-acting, selective, peripheral H₁-receptor antagonist,¹⁰⁻¹² have demonstrated that in addition to its classical antihistaminic effect, loratadine can attenuate platelet-activating factor-induced eosinophil chemotaxis and superoxide generation,¹³ attenuate IgE- and calcium ionophore-induced leucocyte histamine release from basophils,¹⁴ inhibit leukotrienes

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Abbreviations used

ECP:	Eosinophil cationic protein
HBEC:	Human bronchial epithelial cell
ICAM-1:	Intercellular adhesion molecule-1
LTC ₄ :	Leukotriene C ₄
NEC:	Nasal epithelial cell
SF-1 medium:	Serum-free supplemented medium
sICAM-1:	Soluble intercellular adhesion molecule-1

and PGD₂ release from mast cells,¹⁵ and inhibit the expression of ICAM-1 on endothelial cells.¹⁶ Studies with nasal epithelial cells have demonstrated that loratadine can also attenuate histamine-induced increase in the numbers of cells expressing ICAM-1 and HLA-DR.¹⁷

In this study, we have hypothesized that loratadine may influence pollution-induced inflammation of the airways by modulating epithelial membrane integrity and the synthesis and/or release of inflammatory mediators from airway epithelial cells. To test this hypothesis, we have cultured human bronchial epithelial cells and investigated effects of loratadine on the electrical resistance and release of IL-8, RANTES, and soluble ICAM-1 (sICAM-1) from these cells after exposure for 6 hours to 400 ppb NO₂.

METHODS

All chemicals and reagents were of tissue culture grade and, unless stated otherwise, were obtained from the Sigma Chemical Co (Poole, England).

Bronchial tissue

Bronchial tissue was obtained from 8 patients who underwent lobectomy or pneumonectomy for lung cancer at St Bartholomew's Hospital in London. All these patients were heavy smokers, with no known allergies to any of the common allergens; the mean age was 54.8 years (range, 28 to 64 years). After resection, only tissue that appeared macroscopically free of tumor and was deemed to be normal by the operating surgeon was placed into ice-cold Medium 199 (Northumbria Biologicals Ltd, Cramlington, UK) and brought back to the laboratory within 0.5 to 1 hour of resection for processing for tissue culture.

Isolation, culture, and identification of human bronchial epithelial cells

Human bronchial epithelial cells (HBECs) were cultured by an explant cell culture technique that we developed in our laboratory and that has been described fully elsewhere.¹⁸ Briefly, the epithelium was dissected away carefully from the underlying tissue and cut further into smaller sections of approximately 1-2 mm³ in size. All the sections were gently washed 3 times in prewarmed and pre-gassed Medium 199 containing 1% (vol/vol) antibiotics and antimycotic solution (Sigma); then 2 to 3 sections were explanted into either untreated 35-mm diameter plastic culture dishes (Falcon Primaria; Becton Dickinson Ltd, Oxford, England) or into 9-mm diameter Falcon Cell Culture Inserts, with 0.45 µm pore-sized microporous membranes (Becton Dickinson Ltd). The explants were incubated at 37°C in a 5% carbon dioxide in air atmosphere in sterilized culture medium containing 2.5 mL Nu-serum IV (Flow Laboratories, Scotland, UK), 250 µg bovine pancreatic insulin, 250 µg of human transferrin, 36 µg of hydrocortisone, 1 mg L-glutamine,

and 1 mL of antibiotic and antimycotic solution in 100 mL of Medium 199. Explants cultured in dishes were incubated in 2.0 mL of culture medium and observed for cell outgrowth over a period of 2 to 3 weeks, until the cells had grown to confluence. For explants incubated in cell culture inserts, 0.5 mL and 0.4 mL of medium were added into the insert and the insert well, respectively, and treated as the cultures established in dishes were treated.

The purity and identity of the cells were checked in randomly selected cultures and confirmed by (1) light microscopy, (2) electron microscopy, and (3) indirect immunoperoxidase staining techniques, with specific mAbs directed toward cytokeratin.¹⁸

Exposure of HBEC cultures to NO₂

HBEC cultures were exposed to NO₂ in an airtight polycarbonate chamber with a capacity of 5.5 L, as described fully elsewhere.⁶ Briefly, a gas mixture containing 400 ppb NO₂ was generated by diluting a 20 ppm NO₂ in air Spectraseal grade stock (BOC Special Gases, London, UK) with a 5% carbon dioxide in air gas mix by using a gas blender (Model QC 101; Quantitec Ltd, Milton Keynes, UK). The concentration of NO₂ in the final gas mix to be investigated was assessed and confirmed by the use of an NO₂ monitor (Model TGM 555; CEA Instruments Inc, Quantitec Ltd, Milton Keynes, UK), and the cultures were exposed to 400 ppb NO₂ for 6 hours at a flow rate of 1.5 L/min at 37°C in a humidified tissue culture cabinet. Appropriate controls were prepared by exposing cultures to a 5% carbon dioxide in air atmosphere for 6 hours under similar culture conditions.

Effect of NO₂ with or without loratadine on changes in the electrical resistance of confluent HBEC cultures

Changes in permeability of HBEC cultures were investigated by measuring changes in the electrical resistance of cell cultures established in cell culture inserts. Before the investigation, HBECs were grown to confluence, and the explants were removed. The epithelial cells in the inserts were allowed to grow over the area of the insert membrane left barren on removal of the explant and were used further when completely confluent. Immediately before exposure to NO₂, the cultures were washed gently with fresh culture medium and incubated for 30 minutes in the presence of 0.025 µCi ¹⁴C-BSA. At the end of this incubation, the medium in each insert well was collected and analyzed for total radioactivity by liquid scintillation counting in a scintillation counter (LS6500; Beckman-RIC Ltd, High Wycombe, UK). When the total radioactivity passing through the culture was found to be less than 0.5% of the total ¹⁴C-BSA added to the inserts at the beginning of the experiment, the culture was deemed to be fully confluent, and the experiment proceeded further. In this manner, sets of at least 6 separate cultures, each from different individuals, were exposed for 6 hours to 5% carbon dioxide in air or 400 ppb NO₂ in the absence or presence of 0.25 µmol/L, 2.5 µmol/L, and 25 µmol/L loratadine in SF-1 serum-free supplemented medium, which was prepared by mixing 0.5 mL SF-1 serum-free supplement (Northumbria Biologicals Ltd, Cramlington, UK) and 1.0 mL antibiotic and antimycotic solution in 100 mL of Medium 199. Before exposure to NO₂, electrical resistance was measured immediately and again after incubation for 6 hours, with a micro volt-ohm meter (EVOM; World Precision Instruments, Owslebury, UK).

Effect of NO₂ with or without loratadine on release of IL-8, RANTES, and sICAM-1 from HBEC cultures

Confluent HBEC cultures (2 to 3 weeks old) established in cell culture dishes were used for these experiments. Before exposure to

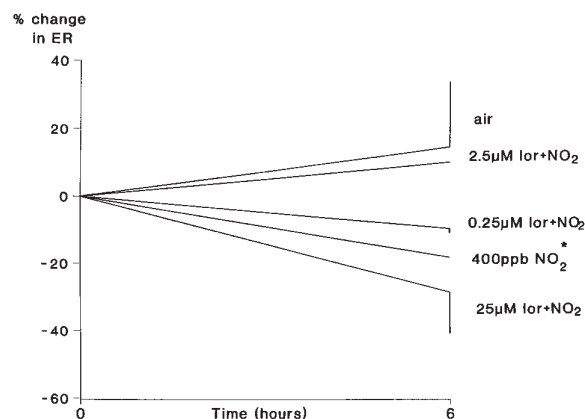


FIG 1. Effect of exposure for 6 hours to air or 400 ppb $\text{NO}_2 \pm 0.25$ to 25 $\mu\text{mol/L}$ loratadine (*lor*) on the electrical resistance (*ER*) of HBEC cultures in vitro. Results are calculated as percent change from baseline in each culture and expressed as median and interquartile values ($n = 6$; * $P < .05$ air vs NO_2).

NO_2 , the explants were removed, and all cultures were incubated for 24 hours in SF-1 medium at 37°C in 5% carbon dioxide in an air atmosphere. After this initial incubation, the cultures were gently washed 3 times with fresh prewarmed and pregassed SF-1 medium, and sets of at least 6 cultures, each from different individuals, were exposed for 6 hours to 5% carbon dioxide in air or 400 ppb NO_2 in the absence or presence of 0.25 $\mu\text{mol/L}$, 2.5 $\mu\text{mol/L}$, and 25 $\mu\text{mol/L}$ loratadine, as described earlier. At the end of 6 hours of exposure, the cells were incubated for a further 18 hours in 5% carbon dioxide in air. At the end of this incubation, the medium was collected, and the cells were gently washed with 1 mL of fresh ice-cold Medium 199. The wash and the culture medium were pooled and stored at -70°C until analysis for IL-8, RANTES, and sICAM-1 with commercially available ELISA kits (R&D Systems). The cells were scraped off into 1.0-mL 1 mol/L NaOH and stored at -20°C until analysis for cellular protein, according to the method of Lowry et al.¹⁹ All results were expressed as picograms of mediator per microgram of cellular protein.

Statistical analysis

All data were tested for skewing and analyzed further with non-parametric statistical tests. First, the Kruskal-Wallis 1-way ANOVA by rank was applied to test for the significance of any differences between all the sets of treated cultures. Paired data were then analyzed with the Wilcoxon signed rank test, and the Mann-Whitney *U* test was applied to assess differences between individual treatment groups. Bonferroni's correction was used for multiple comparisons, and all *P* values of less than .05 were considered to be significant. The data were presented as median and interquartile (Q1 and Q3) values. All statistical tests were performed with a standard computer package (Minitab Data Analysis Software Release 6.1.1, 1987; Minitab Inc, State College, Pa).

RESULTS

Effect of NO_2 with or without loratadine on the electrical resistance of HBEC cultures

Light microscopic examination of epithelial cell cultures exposed for 6 hours to 400 ppb NO_2 in the absence or presence of loratadine demonstrated that these agents

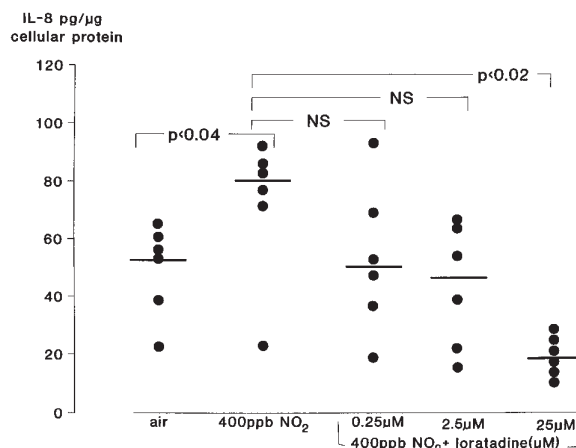


FIG 2. Effect of exposure for 6 hours to air or 400 ppb $\text{NO}_2 \pm 0.25$ to 25 $\mu\text{mol/L}$ loratadine on release of IL-8 from HBEC cultures in vitro. Results are expressed as median and interquartile values ($n = 6$ at each time point).

did not lead to any gross cellular damage or detachment of the cells at the concentrations studied.

Measurement of the electrical resistance across the HBEC cultures demonstrated that this was significantly decreased by a median value of 18.1% from baseline (Q1, -30.85% and Q3, 4.85% decrease in electrical resistance; $P = .02$) by exposure for 6 hours to 400 ppb NO_2 , when compared with control cultures exposed to air. Incubation of HBEC cultures with 0.25 to 25 $\mu\text{mol/L}$ loratadine did not significantly alter the NO_2 -induced decrease in the electrical resistance of HBEC cultures (Fig 1).

Effect of NO_2 with or without loratadine on NO_2 -induced release of IL-8, RANTES, and sICAM-1 from HBEC cultures

Analysis of IL-8 released into the culture medium demonstrated that this was significantly increased by exposure of HBEC cultures for 6 hours to 400 ppb NO_2 , from 52.52 pg IL-8/ μg cellular protein (Q1, 34.37 pg IL-8/ μg cellular protein; Q3, 62.43 pg IL-8/ μg cellular protein) to 81.9 pg IL-8/ μg cellular protein (Q1, 60.7 pg IL-8/ μg cellular protein; Q3, 88.9 pg IL-8/ μg cellular protein; $P < .04$) after 24-hour incubation. Incubation of HBECs with 25 $\mu\text{mol/L}$ loratadine significantly decreased the NO_2 -induced release of this cytokine to 19.08 pg/ μg cellular protein (Q1, 12.18 pg IL-8/ μg cellular protein; Q3, 22 pg IL-8/ μg cellular protein; $P < .02$) after 24-hour incubation. In contrast, 0.25 to 2.5 $\mu\text{mol/L}$ loratadine did not affect the NO_2 -induced release of this cytokine (Fig 2).

Analysis of RANTES demonstrated that release of this cytokine was also significantly increased after exposure for 6 hours to NO_2 , from a control value of 0.023 pg RANTES/ μg cellular protein (Q1, 0.019 pg RANTES/ μg cellular protein; Q3, 0.059 pg RANTES/ μg cellular protein) to 0.062 pg RANTES/ μg cellular protein (Q1, 0.049

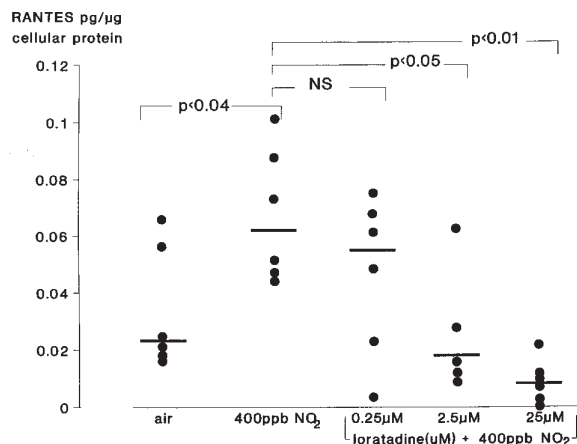


FIG 3. Effect of exposure for 6 hours to air or 400 ppb NO₂ ± 0.25 to 25 µmol/L loratadine on release of RANTES from HBEC cultures in vitro. Results are expressed as median and interquartile values (n = 6 at each time point).

pg RANTES/µg cellular protein; Q3, 0.090 pg RANTES/µg cellular protein; $P < .04$) after 24-hour incubation. Incubation of HBEC cultures in the presence of 2.5 µmol/L loratadine and 25 µmol/L loratadine significantly inhibited the NO₂-induced release of RANTES to 0.017 pg RANTES/µg cellular protein (Q1, 0.012 pg RANTES/µg cellular protein; Q3, 0.037 pg RANTES/µg cellular protein; $P < .04$) and 0.008 pg RANTES/µg cellular protein (Q1, 0.007 pg RANTES/µg cellular protein; Q3, 0.011 pg RANTES/µg cellular protein; $P < .01$), respectively, after 24 hours (Fig 3).

Analysis of sICAM-1 demonstrated that the release of this adhesion molecule from HBEC cultures was also significantly increased by exposure for 6 hours to 400 ppb NO₂, from a control value of 7.7 pg sICAM-1/µg cellular protein (Q1, 4.31 pg sICAM-1/µg cellular protein; Q3, 14.35 pg sICAM-1/µg cellular protein) to 16.33 pg sICAM-1/µg cellular protein (Q1, 10.85 pg sICAM-1/µg cellular protein; Q3, 23.87 pg sICAM-1/µg cellular protein; $P < .04$) after 24-hour incubation, as was the case for release of both IL-8 and RANTES. Incubation of the HBEC cultures with 0.25 to 25 µmol/L loratadine significantly attenuated the NO₂-induced release of sICAM-1 from 16.33 pg sICAM-1/µg cellular protein to 8.26 pg sICAM-1/µg cellular protein (Q1, 44.81 pg sICAM-1/µg cellular protein; Q3, 10.66 pg sICAM-1/µg cellular protein; $P < .03$), 7.27 pg sICAM-1/µg cellular protein (Q1, 5.2 pg sICAM-1/µg cellular protein; Q3, 9.59 pg sICAM-1/µg cellular protein; $P < .04$) and 0.95 pg sICAM-1/µg cellular protein (Q1, 0.07 pg sICAM-1/µg cellular protein; Q3, 1.65 pg sICAM-1/µg cellular protein; $P < .01$), respectively (Fig 4).

Analysis of differences between constitutive release of the mediators after air exposure and after exposure to NO₂ with loratadine demonstrated that release of IL-8 ($P < .02$), RANTES ($P < .02$), and sICAM-1 ($P < .01$) was significantly decreased in the presence of the highest concentration of 25 µmol/L loratadine that was investigated.

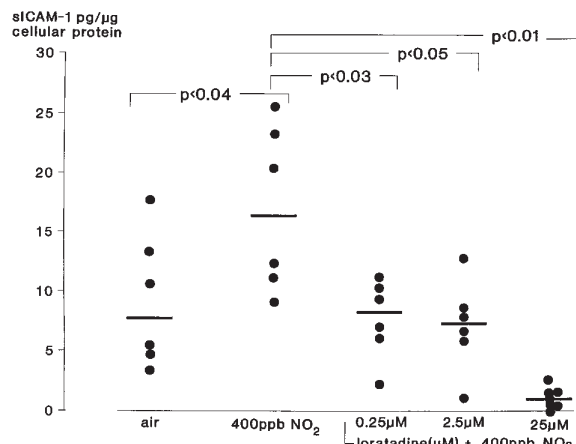


FIG 4. Effect of exposure for 6 hours to air or 400 ppb NO₂ ± 0.25 to 25 µmol/L loratadine on release of sICAM-1 from HBEC cultures in vitro. Results are expressed as median and interquartile values (n = 6 at each time point).

DISCUSSION

These studies have demonstrated that exposure for 6 hours to 400 ppb NO₂ decreases the electrical resistance of HBEC cultures and also induces the release of proinflammatory mediators, such as IL-8, RANTES, and sICAM-1 from these cultures. Additionally, our studies have demonstrated that the NO₂-induced release of the proinflammatory mediators is attenuated by the incubation of HBEC cultures with 0.25 to 25 µmol/L of loratadine in vitro. To our knowledge, this is the first report about such an effect of loratadine on NO₂-induced changes in HBEC and is of particular significance because of the therapeutic potential of loratadine, and likely other antihistamines, in the reduction of airway inflammation and damage in individuals susceptible to the effects of air pollutants.

These findings of detrimental effects of NO₂ on HBEC function confirm our previous findings.^{5,6} Additionally, the findings of the anti-inflammatory effects of loratadine also complement the findings of others, who have investigated the effects of anti-inflammatory drugs on pollutant-induced changes in these cells. Wang et al²⁰ have demonstrated that pretreatment with fluticasone propionate significantly decreased NO₂ plus allergen-induced eosinophil cationic protein (ECP) release in nasal lavage fluid of patients with allergic rhinitis, compared with placebo. Rusznak et al²¹ have demonstrated that incubation with the nonsteroidal drug nedocromil sodium significantly attenuated ozone-induced release of IL-8, GM-CSF, TNF-α, and sICAM-1 from HBEC cultures established from surgical explants.

Despite the wide usage of antihistamines in the symptomatic treatment of allergic rhinitis, comparatively few studies have examined their anti-inflammatory effects. Our finding that loratadine attenuates NO₂-induced release of proinflammatory mediators from these cells suggests that this predominantly antihistaminic agent may generally be useful for its anti-inflammatory prop-

erties in airway disease. Indeed, other authors have also demonstrated that loratadine and other antihistamines possess anti-inflammatory properties. Lippert et al²² have investigated the effects of loratadine on phorbol myristate acetate and Ca-ionophore-stimulated release of cytokines from human mast and basophilic cell lines and demonstrated that loratadine significantly inhibited the release of IL-6 and IL-8 from these cells, in a dose-dependent manner, at all concentrations between 10^{-5} mol/L to 10^{-14} mol/L. Similarly, Vignola et al¹⁷ have demonstrated that 10 μ mol/L loratadine and its metabolite descarboethoxyloratadine significantly attenuated histamine-induced increase in the number of nasal epithelial cells expressing ICAM-1 and human leukocyte class II antigen (HLA-DR) in vitro. In a separate study, these authors have also demonstrated that incubation of HBEC with pyrilamine can significantly attenuate a histamine-induced increase in epithelial expression of ICAM-1 and HLA-DR and release of fibronectin.⁸ Molet et al²³ have demonstrated that loratadine and descarboethoxyloratadine significantly attenuated histamine-induced expression of P-selectin and release of IL-6 and IL-8 from human endothelial cells in vitro. Recently, Genovese et al²⁴ have demonstrated that incubation of purified basophils and human lung/skin mast cells with loratadine, before incubation with anti-Fc ϵ RI, inhibited the release of histamine, LTC₄, PGD₂, and tryptase from these cells, suggesting that these drugs may impart anti-inflammatory activities by inhibiting the release of preformed and de novo-synthesized mediators from human Fc ϵ RI⁺ cells. More recently, Abdelaziz et al²⁵ demonstrated that incubation of nasal epithelial cell (NEC) cultures with fexofenadine, the main active metabolite of terfenadine, significantly blocked eosinophil-induced decrease in the electrical resistance of these NEC cultures. Furthermore, these authors found that fexofenadine also significantly inhibited the eosinophil-induced increase in the release of RANTES, GM-CSF, and sICAM-1 from the NEC cultures and also attenuated the epithelial cell-conditioned medium-induced chemotaxis and adhesion of eosinophils to endothelial cell cultures. Contrary to our findings and those of others, however, Amsellem et al²⁶ could not demonstrate significant inhibition of either constitutive or TNF- α -induced release of IL-8 or GM-CSF from HBECs treated with 0.3 to 30 μ mol/L loratadine or ceterizine.

Recently, several studies have confirmed the anti-inflammatory properties of antihistamines in vivo. Greiff et al²⁷ have investigated the effect of treatment with 20 mg loratadine every day for 5 days on allergen-induced changes in the levels of nasal mast-cell tryptase and α_2 -macroglobulin in individuals with allergic rhinitis and demonstrated that the release of both of these proteins was significantly decreased after treatment with loratadine, as compared with matched placebo. The differences in the release of α_2 -macroglobulin suggest that this drug may modulate the permeability of the microvasculature in the nasal airways. Similarly, Baroody et al²⁸ have demonstrated that treatment with loratadine and terfenadine

resulted in significant reductions in allergen-induced sneezing and the levels of histamine, kinins, albumin, and N- α -tosyl-L-arginine methyl esterase activity in nasal lavage fluid of allergic volunteers after allergen challenge. Furthermore, Ciprandi et al³⁰ have investigated the effect of topical azelastine²⁹ and oral terfenadine on specific allergen (*Parietaria judaica*)-induced changes in the nasal airways of individuals with seasonal allergic rhinitis and demonstrated that these agents significantly decreased the airway inflammatory cell infiltrate, serum, and nasal lavage ECP levels and ICAM-1 expression on nasal epithelial cells of these individuals. More recently, these authors have also demonstrated that oral loratadine treatment (10 mg/day) for 2 weeks, during the pollen season, significantly reduced eosinophil and metachromatic cell infiltration and ICAM-1 expression on nasal epithelial cells obtained by nasal cell scrapings.³¹ The levels of ECP, eosinophil peroxidase, and histamine in nasal lavage fluid were also reduced.

Although the anti-inflammatory effects of antihistamines have now been well documented, the specific mechanisms underlying these effects are not well understood. Some studies have demonstrated that antihistamines can inhibit Ca²⁺-influx into cells.³²⁻³⁴ Kleine-Tebbe et al¹⁴ have found that loratadine can inhibit anti-IgE and allergen- and calcium ionophore-induced histamine release from human basophils. Collectively, these studies suggest that these drugs may modulate the activity of genes encoding for proinflammatory mediators by influencing the signal transduction pathways primed by changes in cytosolic Ca²⁺.³⁵⁻³⁹ More recently, Yoneda et al⁴⁰ have demonstrated that azelastine hydrochloride blocked the synthesis of IL-1 β , IL-6, GM-CSF, and TNF- α , the expression of mRNA for these cytokines, and activation of NF- κ B in human peripheral blood leucocytes and monocytes and suggested that azelastine exerts its effect through inhibition of NF- κ B activation.

Such mechanisms could certainly account for the decrease in NO₂-induced mediator release effected by loratadine in our studies. Studies of other irritant air pollutants with potent oxidizing properties, namely ozone and diesel exhaust particles, have demonstrated that these lead to increased generation of superoxide and hydroxyl radicals and inhibition of the activities of superoxide dismutase, glutathione peroxidase, and catalase in epithelial lining fluid; this suggested that the resultant increase in oxygen radical load leads to increased lung injury and death.^{2,21,41} Studies of animals exposed for prolonged periods to high doses of ozone have suggested that the increased generation of free oxy and lipid radicals may lead to activation of specific transcription factors and therefore to increased gene expression, a result of imbalance of intracellular Ca²⁺.^{2,42} In vitro studies of epithelial cell lines have demonstrated that ozone increases the total mRNA for cytokines, such as IL-1 β , IL-6, IL-8, and TNF- α , and that this may itself be modulated by transcription factors such as NF- κ B.⁴³

However, although it was not possible to investigate the direct effect of loratadine on mediator release in the

present study due to the unavailability of suitably large numbers of confluent cultures, the demonstration that the combination with NO₂ and the highest concentration of 25 µmol/L loratadine significantly decreased release of all mediators to below their constitutive levels observed in control cultures exposed to air raises the possibility that loratadine may indeed influence the function of genes expressing these mediators. In view of the findings of others for antihistamines, however, it is tempting to speculate that loratadine may act by influencing calcium flux into the epithelial cells, subsequently modulating the signal transduction pathways primed by cytosolic Ca²⁺.

In summary, these studies indicate that concentrations of NO₂, which can be found at the road side in heavy traffic or in enclosed spaces where gas is used for cooking, may induce airway inflammation through damage and release of proinflammatory mediators from airway epithelial cells. Furthermore, the adverse effects of NO₂ at the epithelial cells may be inhibited by the treatment of the cells with loratadine. Although the immediate clinical relevance of this finding remains unclear because serum concentrations (2 to 10 nmol/L) attainable at therapeutic doses^{12,22} *in vivo* are much lower than those used *in vitro*, this study nevertheless gives important information on the putative mechanisms underlying the anti-inflammatory effects of loratadine, and indeed other second-generation H₁-histamine receptor antagonists that have been proved to be effective at nanomolar concentrations *in vitro*, in the airways. Consequently, this study gives important pointers for the development of similar, highly efficacious, and safe anti-inflammatory alternatives to corticosteroids for use in the management of inflammatory airway disease. Also, their potential for use as either prophylactic or nonprophylactic rescue medication, in combination with corticosteroids, in the management of severe airway disease cannot be ruled out. It is likely that when these drugs are applied topically, the local concentrations attained will be comparatively higher than those attained through the systemic route.

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Correction

The following correction applies to an abstract that appeared in volume 103, number 1, part 2, p S98, 1999, of the Journal.

373 Oral Allergy Syndrome (OAS) in 8 Pediatric Patients. *Jacob*

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The oral allergy syndrome (OAS) is a term applied to many clinical symptoms exclusively confined to the oropharynx and occur after direct contact with many kinds of foods, especially fresh fruits and vegetables.

Objective: Describe the clinical and laboratorial findings of 8 patients with food allergy and OAS followed in Allergy and Immunology Pediatric Unit.

Methodology: The authors carried out a prospective study in 42 patients with food allergy underwent to epidemiological, clinical and laboratorial evaluations.

Results: Eight patients (4F:4M) presented OAS due to cow's milk (6/8), egg (1/8) and corn (1/8). The clinical findings included: rapid onset of pruritus and angioedema of the lips, tongue, and throat. Three patients presented sensations of tightness in the throat with hoarseness and systemic anaphylaxis. These patients received epinephrine and anti-histamines in emergency room. The mainly laboratorial findings were: hypereosinophilia (average= 632 cel/mm³), heightened IgE levels (average= 451 UI/l) and RAST class 3 or 4 for the triggers foods.

Conclusion: The authors alert the physician about OAS as manifestation of food allergy and our results confirm the role of cow's milk in children with OAS in our country.