

Effect of desloratadine and loratadine on rhinovirus-induced intercellular adhesion molecule 1 upregulation and promoter activation in respiratory epithelial cells

Alberto Papi, MD,^{a,b} Nikolaos G. Papadopoulos, PhD,^a Luminita A. Stanciu, PhD,^{a,c}
Klaus Degitz, MD,^d Stephen T. Holgate, MD,^a and Sebastian L. Johnston, PhD^{a,c}

Southampton and London, United Kingdom, Ferrara, Italy, and Munich, Germany

Background: Rhinoviruses have been recently associated with the majority of asthma exacerbations for which current therapy is inadequate. Intercellular adhesion molecule 1 (ICAM-1) has a central role in airway inflammation in asthma, and it is the receptor for 90% of rhinoviruses. Rhinovirus infection of airway epithelium induces ICAM-1. Desloratadine and loratadine are compounds belonging to the new class of H₁-receptor blockers. Anti-inflammatory properties of antihistamines have been recently documented, although the underlying molecular mechanisms are not completely defined.

Objective: We have investigated the effects of desloratadine and loratadine on rhinovirus-induced ICAM-1 expression, mRNA upregulation, and promoter activation.

Methods: Cultured primary bronchial or transformed (A549) respiratory epithelial cells were pretreated with desloratadine and loratadine for 16 hours and infected with rhinovirus type 16 for 8 hours. ICAM-1 surface expression was evaluated with flow cytometry, and ICAM-1 mRNA was evaluated with specific RT-PCR. In A549 cells promoter activation was evaluated with a chloramphenicol acetyltransferase assay, and binding activity of nuclear factor κ B in nuclear extracts was evaluated with an electrophoretic mobility shift assay.

Results: Desloratadine and loratadine (0.1–10 μ mol/L) inhibited rhinovirus-induced ICAM-1 upregulation in both primary bronchial or transformed (A549) respiratory epithelial cells. In A549 cells the 2 compounds showed a dose-dependent inhibition with similar efficacy (inhibitory concentration of 50%, 1 μ mol/L). Desloratadine and loratadine also inhibited ICAM-1 mRNA induction caused by rhinovirus infection in a dose-dependent manner, and they completely inhibited rhinovirus-induced ICAM-1 promoter activation. Desloratadine also inhibited rhinovirus-induced nuclear factor κ B activation. Desloratadine and loratadine had no direct effect on rhinovirus infectivity and replication in cultured epithelial cells.

Conclusion: These effects are unlikely to be mediated by H₁-receptor antagonism and suggest a novel mechanism of action that may be important for the therapeutic control of virus-induced asthma exacerbations. (*J Allergy Clin Immunol* 2001;108:221–8.)

Key words: Asthma, rhinovirus, intercellular adhesion molecule 1, histamine H₁ receptor blockers

Intercellular adhesion molecule 1 (ICAM-1) is a cell surface glycoprotein involved in leukocyte trafficking and accumulation and more recently recognized as an important costimulatory molecule in antigen presentation.^{1,2} ICAM-1 is also the cellular receptor for the major group of rhinoviruses, which includes 90% of rhinovirus serotypes.^{3,4} We have previously shown that rhinovirus infection induces ICAM-1 upregulation in bronchial and lung epithelial cells by activating ICAM-1 promoter activity through a nuclear factor κ B (NF- κ B)-dependent mechanism.⁵

Rhinoviruses are the major cause of the common cold, the most common acute infectious disease in human subjects, and the complications of otitis, sinusitis, and bronchitis.^{6–8} We and others have recently demonstrated that rhinoviruses are also associated with the majority of asthma exacerbations.^{9–11}

Although the precise mechanisms underlying virus-induced asthma exacerbations are not clear, it is currently believed that rhinoviruses induce asthma exacerbations by directly infecting the lower airway epithelium^{12,13} and inducing expression and release of a number of proinflammatory mediators^{14,15} that recruit and activate inflammatory cells, resulting in lower airway inflammation.^{16,17}

Inhaled steroids are the mainstay of asthma treatment, but they are poorly effective in both the treatment and the prevention of virus-induced asthma exacerbations.^{18,19}

Histamine H₁-receptor antagonists have a long history of clinical efficacy in a variety of allergic-inflammatory disorders. Desloratadine and loratadine are new-generation antihistamines that lack central nervous system and cardiovascular side effects. Desloratadine and loratadine dose dependently inhibit histamine-induced ICAM-1 upregulation.²⁰ Recent studies have demonstrated that in addition to their classic antihistaminic effects,^{21–23} desloratadine and loratadine possess a number of anti-inflammatory activities that are unlikely to be related to histamine receptor antagonism.^{24,25} However, the molecular mechanisms underlying these anti-inflammatory actions are unknown.

From ^aUniversity Medicine, University of Southampton, Southampton; ^bthe Research Center on Asthma and COPD, University of Ferrara; ^cthe Department of Respiratory Medicine, National Heart and Lung Institute, Imperial College School of Medicine at St Mary's, London; and ^dthe Department of Dermatology, Ludwig-Maximilians University, Munich.

Supported by the National Asthma Campaign Grant no. 332; the University of Ferrara, Italy; the Deutsche Forschungsgemeinschaft Grant no. 405/5 (K.D.); and Schering-Plough, USA.

Received for publication October 25, 2000; revised April 16, 2001; accepted for publication April 23, 2001.

Reprint requests: Alberto Papi, MD, Research Centre on Asthma and COPD, Via Savonarola, 9, 44100 Ferrara, Italy.

Copyright © 2001 by Mosby, Inc.

0091-6749/2001 \$35.00 + 0 1/83/116861

doi:10.1067/mai.2001.116861

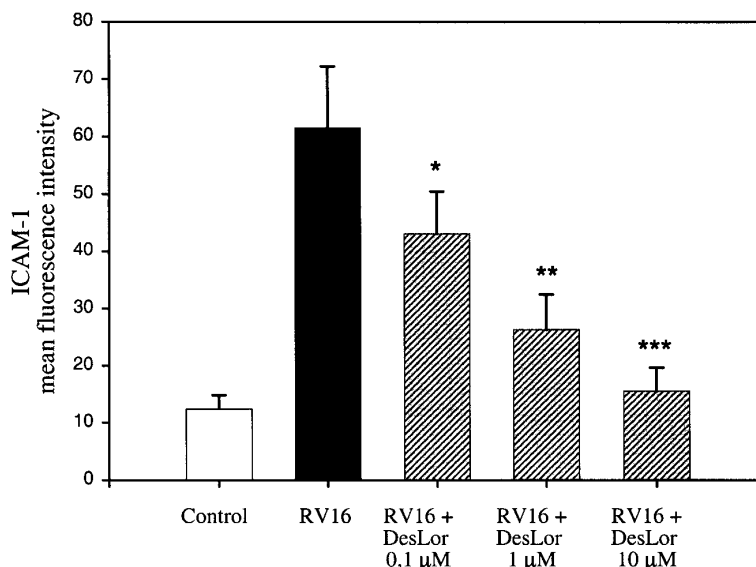


FIG 1. Effects of desloratadine on rhinovirus 16-induced ICAM-1 surface expression on A549 pulmonary epithelial cells. ICAM-1 expression was measured by using flow cytometry. Data are means $\pm$ SEM of at least 4 separate experiments. * $P < .05$, ** $P < .01$, and *** $P < .001$ versus samples infected but not pretreated with antihistamines. *RV*, Rhinovirus; *DesLor*, desloratadine.

Abbreviations used

APRT:	Adenine phosphoribosyltransferase
CAT:	Chloramphenicol acetyltransferase
DMSO:	Dimethyl sulfoxide
ICAM-1:	Intercellular adhesion molecule 1
NF- κ B:	Nuclear factor κ B
TCID ₅₀ :	Tissue culture infective dose 50%

To search for a therapeutic intervention for virus-induced asthma and to further investigate the anti-inflammatory mechanism of action, we studied whether desloratadine and loratadine prevent rhinovirus-induced ICAM-1 protein upregulation in respiratory epithelial cells and their effects on ICAM-1 mRNA expression and promoter activation.

METHODS

Cell culture

Ohio HeLa cells were obtained from the Medical Research Council Common Cold Unit (Salisbury, United Kingdom), and A549 cells (type II respiratory cell line) were obtained from the American Type Culture Collection (Rockville, Md).

Primary human bronchial epithelial cells were obtained by means of bronchial brushing from normal volunteers, as previously described.⁵ These cells are more than 95% cytokeratin 18 immunoreactive, as assessed by using immunofluorescence microscopy. Cells were split weekly and cultured as described elsewhere.⁵

Virus stocks

Rhinovirus type 16 (a major group rhinovirus) was used for all studies described. Viral stocks were prepared by infection of sensitive cell monolayers (Ohio HeLa), as described elsewhere.⁵ The tissue culture infective dose of 50% (TCID₅₀) per milliliter values were determined as previously described.²⁶ Virus at a multiplicity of infection of 1 was used for all the experiments.

Flow cytometric analysis of ICAM-1 surface expression

Flow cytometry was used to quantify the level of ICAM-1 surface expression on resting and rhinovirus-infected A549 cells or primary bronchial epithelial cells in the presence or absence of desloratadine or loratadine (0.1–10 μ mol/L). Briefly, 2×10^5 cells were cultured in 12-well plates. When cells were confluent, desloratadine, loratadine (supplied by Schering-Plough), or solvent alone (dimethyl sulfoxide [DMSO], final concentration of 0.1% vol/vol) was added, and incubation was continued for various lengths of time (1–48 hours) before 8-hour rhinovirus type 16 or sham infection. On the basis of preliminary results, dose-response studies were carried out at 16 hours before incubation. The optimal time of infection for the detection of rhinovirus-induced ICAM-1 upregulation was adopted according to previous studies.⁵ Primary bronchial epithelial cells were pretreated with 1 μ mol/L desloratadine, 1 μ mol/L loratadine, or solvent alone. Cells were thereafter harvested (cell dissociation solution, Sigma), incubated with saturating amounts of FITC antihuman ICAM-1 antibody (Serotec) or isotype-specific control antibody, and analyzed for fluorescence by using single-color flow cytometry on a FACScan analyzer (Becton Dickinson), as described elsewhere.⁵ Mean fluorescence intensity was measured and calculated after subtraction of background staining. The effects of desloratadine and loratadine were calculated, and the inhibitory concentration of 50% was evaluated for both desloratadine and loratadine. The effects of 16-hour desloratadine (0.1–10 μ mol/L) pretreatment on IFN- γ (100 U/mL)-induced ICAM-1 upregulation 8 hours after stimulation were also evaluated.

RT-PCR analysis of ICAM-1 mRNA

RT-PCR was used to evaluate ICAM-1 mRNA on resting and rhinovirus-infected cells in the presence or absence of desloratadine or loratadine. Desloratadine, loratadine, or solvent alone (DMSO, 0.1% vol/vol) was added, and incubation was continued for 16 hours before 8-hour exposure to rhinovirus type 16 or medium alone. Dose-response studies were performed on A549 cells by using concentrations ranging from 0.1 to 10 μ mol/L. Primary bronchial

epithelial cells were pretreated with 1 $\mu\text{mol/L}$ desloratadine or solvent alone. Cells were harvested, and RNA was extracted and used for a quantitative estimation of ICAM-1 mRNA expression by means of specific RT-PCR, as previously described.⁵ PCR products (10 μL) were electrophoresed through 1.5% agarose gel that was subsequently stained in ethidium bromide and photographed under UV light. Densitometry was performed by using a scanning densitometer (Epson scanner), and densitometric analysis was obtained with an appropriate program (Phoretic, Biomerea LTD).

In parallel, PCR for adenine phosphoribosyltransferase (APRT) mRNA (GCTGCGTGCTCATCCGAAAG and CCT-TAAGCGAGGTCAGCTCC primers, 40 cycles, annealing temperature of 56°C, expected amplification products of 246 bp) was performed in each sample to evaluate uniformity of loading and of RT RNA capable of amplification. These methods were previously shown to give linear quantification of input mRNA and have been shown to give good quantification of mRNA in the experimental conditions here adopted.^{5,27}

ICAM-1 promoter activation

Reporter gene construct. An ICAM-1 promoter-chloramphenicol acetyltransferase (CAT) construct containing the full-length sequence (–1160 bp) of the ICAM-1 5'-flanking region linked to the coding region of the CAT reporter gene was used for transfection.²⁸

Cell transfection and CAT assay. A549 cells were transfected with the reporter construct (10 μg) by using the calcium phosphate coprecipitation technique, as previously described.⁵ Transfected cells were cultured in 10% MEM overnight. One micromole per liter of desloratadine, loratadine, or solvent alone (DMSO, 0.1% vol/vol) was added, and incubation was continued for 16 hours before the addition of rhinovirus 16 or medium alone. Twenty-four hours later, cells were harvested, proteins were extracted, and protein-equivalent aliquots were assayed for CAT activity.⁵ Acetylated and unacetylated forms of chloramphenicol were resolved by means of thin-layer chromatography, visualized by means of autoradiography, and measured on a scintillation counter. CAT activity was expressed as the percentage conversion of chloramphenicol into its acetylated derivatives.

Electrophoretic mobility shift assay

Preparation of nuclear extracts. Confluent A549 cells were pretreated for 16 hours with 1 $\mu\text{mol/L}$ desloratadine or solvent alone before the addition of rhinovirus 16 or medium alone for 30 minutes. The optimal time of infection for the evaluation of NF- κB activation was adopted according to previous studies. Nuclear extracts were obtained as previously described.⁵

Oligonucleotide probes. NF- κB and AP-1 consensus double-stranded oligonucleotides were obtained commercially (Promega).

Oligonucleotides were labeled and incubated with 5 μg of nuclear protein, as previously reported.⁵ Fifty-fold molar excess unlabelled oligonucleotides were used for cold competition. Complexes were resolved on 5% nondenaturing polyacrylamide gels. Dried gels were autoradiographed at –70°C overnight.

Rhinovirus replication

Rhinovirus replication in the presence of either desloratadine or loratadine was evaluated by performing titration assays, as previously described,²⁷ in the presence or absence of 10 $\mu\text{mol/L}$ desloratadine or loratadine for 16 hours before infection. The cells were incubated in 4% MEM at 37°C in 5% CO_2 for 5 days, fixed in methanol, and stained with 0.1% crystal violet. The cytopathic effect was evaluated by means of visual assessment and assessment of the continuity of the monolayer. For each experiment, TCID₅₀ per milliliter values were calculated in the samples with and without antihistamines.

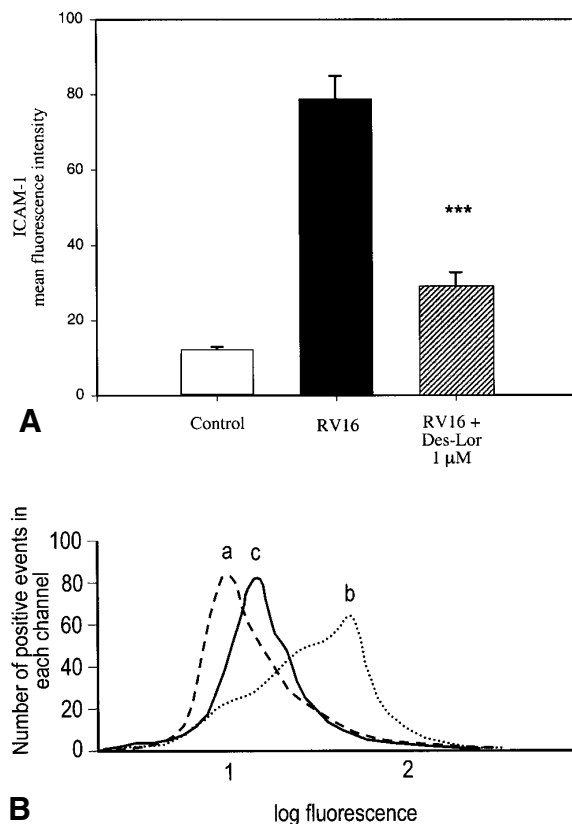


FIG 2. Effects of desloratadine on rhinovirus 16-induced ICAM-1 surface expression on primary bronchial epithelial cells. ICAM-1 expression was measured by using flow cytometry, and values were normalized as percentage of rhinovirus-induced ICAM-1 upregulation in control nonpretreated samples. **A**, Data are mean $\pm$ SEM of at least 4 separate experiments. *** $P < .001$ versus samples infected but not pretreated with antihistamines. **B**, Representative histogram. ICAM-1 levels are referred to resting conditions (a), rhinovirus infection (b), and desloratadine pretreatment followed by rhinovirus infection (c). RV, Rhinovirus; Des-Lor, desloratadine.

Statistical analysis

Data were expressed as means $\pm$ SEM, and comparison between groups was performed with paired and unpaired Student *t* tests. The Bonferroni correction was performed when indicated. All experiments were carried out at least 4 times.

RESULTS

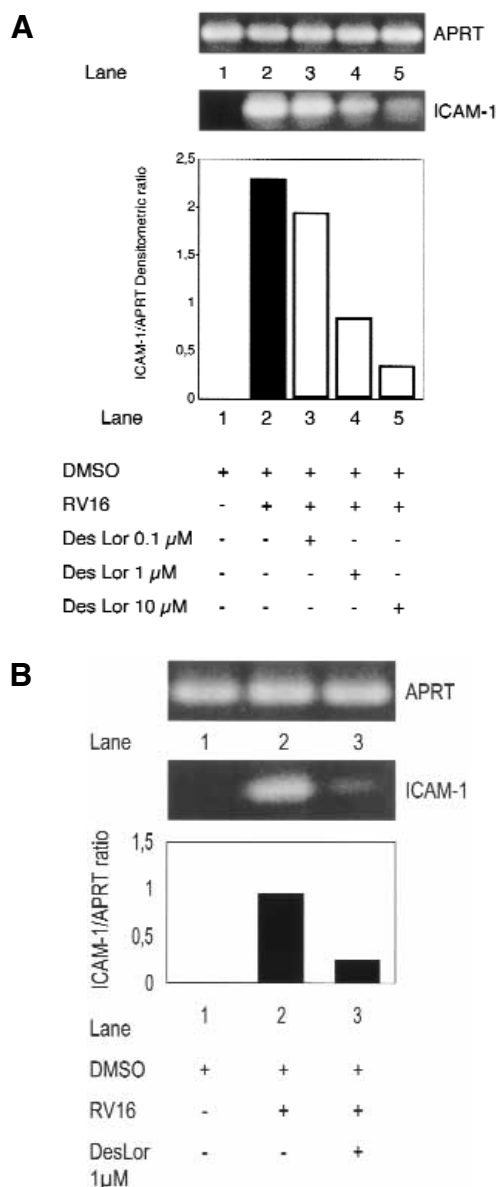
ICAM-1 surface expression on A549 respiratory epithelial cells

ICAM-1 was constitutively expressed on A549 cells (mean fluorescence intensity, 12.3 ± 2.5), and its basal expression was not affected by vehicle, desloratadine, or loratadine at any of the tested concentrations (data not shown). Preliminary studies indicated that desloratadine or loratadine effects on rhinovirus-induced ICAM-1 upregulation were evident after 4 hours of preincubation and were maximal after 8 to 24 hours of pretreatment (data not shown). On the basis of these data, a preincubation period of 16 hours was used in all subsequent studies.

TABLE I. Pretreatment with desloratadine and loratadine inhibits rhinovirus induction of ICAM-1 mRNA expression in A549 cells

Conditions	ICAM-1 mRNA expression (ICAM-1/APRT densitometric ratio; arbitrary units)	P value	Conditions	ICAM-1 mRNA expression (ICAM-1/APRT densitometric ratio; arbitrary units)	P value
Control	0		Control	0	
Rhinovirus 16	2.54 ± 0.24		Rhinovirus 16	2.48 ± 0.18	
Desloratadine, 0.1 μmol/L	1.89 ± 0.13	*	Loratadine, 0.1 μmol/L	2.05 ± 0.09	
Desloratadine, 1 μmol/L	0.95 ± 0.17	†	Loratadine, 1 μmol/L	1.07 ± 0.11	†
Desloratadine, 10 μmol/L	0.4 ± 0.13	†	Loratadine, 10 μmol/L	0.5 ± 0.08	†

Data are expressed as means ± SEM.

P* < .05 and †*P* < .001 versus samples infected but not pretreated with antihistamines.FIG 3.** Effect of desloratadine on rhinovirus 16-induced ICAM-1 mRNA expression in respiratory epithelial cells. Representative ethidium bromide-stained gel electrophoreses of products of RT-PCR for APRT (housekeeping gene) and ICAM-1 RT-PCR in A549 respiratory epithelial cells (**A**) and primary bronchial epithelial cells (**B**). RV, Rhinovirus; DesLor, desloratadine.

According to previous reports, 8-hour rhinovirus 16 infection in the presence of vehicle alone significantly increased ICAM-1 surface epithelial expression to 4.7 ± 0.9 -fold over that of control uninfected cells (*P* < .01). Pretreatment with either desloratadine or loratadine inhibited rhinovirus ICAM-1 induction in a dose-response manner, with an inhibitory concentration of 50% of $1.11 \mu\text{mol/L}$ for desloratadine and $1.08 \mu\text{mol/L}$ for loratadine. Desloratadine, but not loratadine, produced a significant inhibition at a concentration as low as $0.1 \mu\text{mol/L}$ (Fig 1). Experiments conducted on A549 cells stimulated for 8 hours with IFN- γ showed that IFN- γ increased ICAM-1 surface epithelial expression to 7.1 ± 1.6 -fold over control nonstimulated cells (*P* < .01). Desloratadine only partially inhibited IFN- γ -induced ICAM-1 upregulation at the highest concentration used (mean fluorescence intensity, 10.5 ± 2.8 in control samples; mean fluorescence intensity, 88.3 ± 14.1 and 61.3 ± 11.6 in IFN- γ -stimulated and IFN- γ -stimulated and desloratadine-pretreated samples, respectively; *P* < .05).

ICAM-1 surface expression on primary bronchial epithelial cells

ICAM-1 was constitutively expressed on primary bronchial epithelial cells (mean fluorescence intensity, 11.2 ± 2.4), and its basal expression was not affected by vehicle, $1 \mu\text{mol/L}$ desloratadine, or $1 \mu\text{mol/L}$ loratadine (mean fluorescence intensity, 10.7 ± 1.1 , 12.1 ± 1.7 , and 10.9 ± 1.4 , respectively). As previously reported,⁵ 8-hour rhinovirus 16 infection in the presence of the vehicle alone significantly increased ICAM-1 surface epithelial expression to 6.8 ± 1.7 -fold over that of control uninfected cells (*P* < .01). As observed in Fig 2, pretreatment of primary bronchial epithelial cells with $1 \mu\text{mol/L}$ desloratadine significantly inhibited rhinovirus-induced ICAM-1 upregulation to a similar degree to that observed in the A549 cell line. Similar results were obtained with $1 \mu\text{mol/L}$ loratadine (data not shown).

ICAM-1 mRNA analysis

Uniform processing was confirmed by means of APRT RT-PCR performed in parallel. Densities of APRT products showed less than 10% variability among all samples, and ICAM-1 product densities were normalized according to the APRT values. Control A549 cells expressed very low levels of ICAM-1 mRNA. Rhinovirus 16

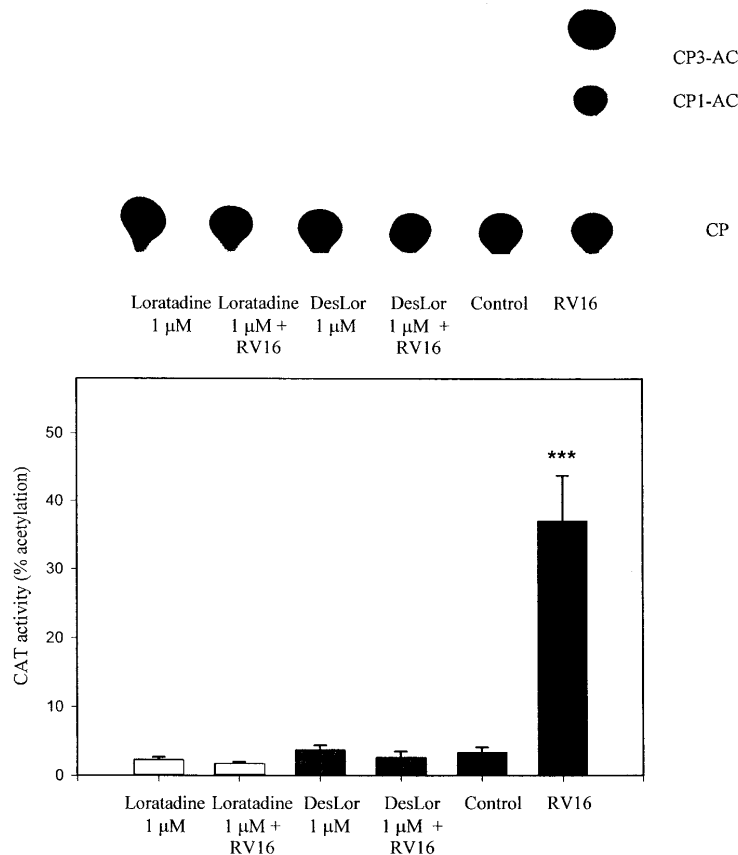


FIG 4. Inhibition by desloratadine and loratadine of rhinovirus 16-induced ICAM-1 promoter activation in A549 pulmonary epithelial cells. *Top*, A representative autoradiograph of CAT assay. *Bottom*, ICAM-1 promoter activation expressed as percentage of chloramphenicol acetylation. Data are means $\pm$ SEM of at least 5 separate experiments. *** $P < .001$ versus control uninfected cells. *RV*, Rhinovirus; *DesLor*, desloratadine; *CP*, unacetylated chloramphenicol; *CP1-AC* and *CP3-AC*, acetylated forms of chloramphenicol.

strongly induced ICAM-1 mRNA expression after 8-hour infection. Desloratadine and loratadine inhibited rhinovirus induction of ICAM-1 mRNA in a dose-dependent manner (Table I). A representative experiment is depicted in Fig 3, A, in which a clear increase in ICAM-1 mRNA expression was detected after rhinovirus 16 infection (*lane 2*), whereas pretreatment (desloratadine: 0.1 μ mol/L, *lane 3*; 1 μ mol/L, *lane 4*; and 10 μ mol/L, *lane 5*) resulted in dose-dependent almost complete inhibition of rhinovirus-induced ICAM-1 mRNA induction. Similar results were obtained with loratadine pretreatment (data not shown). Similar inhibition by desloratadine of rhinovirus-induced ICAM-1 upregulation was also observed in primary bronchial epithelial cells (ICAM-1/APRT densitometric ratio of 0 in control samples; ICAM-1/APRT densitometric ratio of 1.27 ± 0.18 in rhinovirus 16-infected and 0.28 ± 0.11 in rhinovirus 16-infected and 1 μ mol/L desloratadine-pretreated samples, respectively; $P < .01$; Fig 3, B).

ICAM-1 promoter activation

As previously demonstrated and as shown in Fig 4, *lanes 5 and 6*, rhinovirus 16 induced ICAM-1 promoter activity compared with that of media-inoculated cells

(acetylation of $3.1\% \pm 0.6\%$ and $35.4\% \pm 7.1\%$ for control and infected cells, respectively; $P < .001$). Similarly, the presence of vehicle alone did not modify the rhinovirus-induced ICAM-1 promoter activation, as previously described.²⁷ Loratadine, 1 μ mol/L (Fig 4, *lanes 1 and 2*), or desloratadine, 1 μ mol/L (Fig 4, *lanes 3 and 4*), pretreatment completely abrogated rhinovirus induction of ICAM-1 promoter activity. These results confirm that desloratadine and loratadine completely inhibit rhinovirus-induced ICAM-1 promoter activation.

Effect of desloratadine on rhinovirus-induced NF- κ B activation

An electrophoretic mobility shift assay was undertaken to investigate whether desloratadine could affect rhinovirus-induced increased binding activity of NF- κ B in nuclei extracted from infected A549 cells. As previously documented and as shown in Fig 5, *lane 3*, 2 protein-DNA complexes were induced in nuclear extracts from rhinovirus-infected A549 cells compared with uninfected cells (Fig 5, *lane 2*). Competition experiments (Fig 5, *lanes 4 and 5*) confirmed the specificity of the NF- κ B binding. Desloratadine, 1 μ mol/L (Fig 5, *lane 6*), clearly reduced rhinovirus induction of the NF- κ B binding.

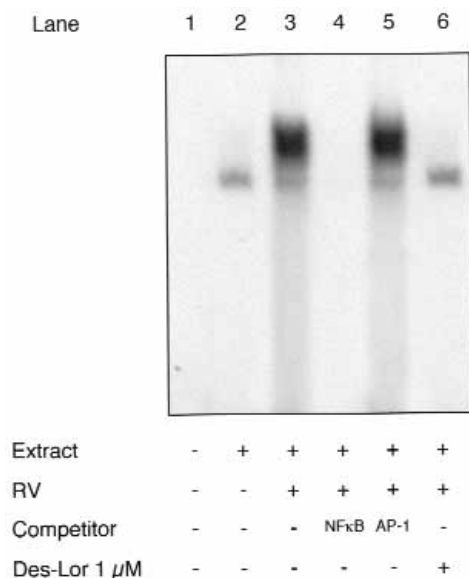


FIG 5. Inhibition by desloratadine of rhinovirus-induced binding of NF- κ B. Representative autoradiograph of 2 separate electrophoretic mobility shift assays of nuclei lysates prepared from uninfected (lane 2) or rhinovirus 16-infected (lane 3) A549 cells in absence or presence of 1 μ mol/L desloratadine (lane 6). Cold competition with NF- κ B (lane 4) and irrelevant (AP-1; lane 5) consensus competitor was performed to confirm the NF- κ B specificity of the signal. Clear inhibition of rhinovirus induction of NF- κ B binding activity by desloratadine is seen. RV, Rhinovirus; Des-Lor, desloratadine.

Effect of desloratadine and loratadine on rhinovirus replication and infectivity

Titration assays were performed to assess replication in a sensitive epithelial cell line to evaluate whether desloratadine and loratadine inhibit rhinovirus replication. Ohio HeLa cells were inoculated with rhinovirus 16 at a multiplicity of infection of 1 in the presence or absence of 10 μ mol/L desloratadine or 10 μ mol/L loratadine for 16 hours before infection. These concentrations were chosen because they were the highest used in the earlier studies. At 5 days, there was no difference in virus yields expressed as TCID₅₀ per milliliter mean values between desloratadine- or loratadine-pretreated and control samples. Furthermore, no difference was found in the progression of cytopathic effect, as assessed by visual inspection on a daily basis (data not shown). These data demonstrate that desloratadine and loratadine have no inhibitory effect on rhinovirus replication in a sensitive cell line.

DISCUSSION

ICAM-1, the receptor of 90% of rhinoviruses, is an adhesion protein that has a central role in inflammatory cell recruitment to the lower airway in asthma and after rhinovirus infection. We have previously demonstrated that rhinovirus infection of respiratory epithelial cells increases cell surface expression of ICAM-1 through a

mechanism that is critically NF- κ B dependent. In the present study we investigated the effects of the histamine H₁-receptor blockers desloratadine and loratadine on rhinovirus-induced ICAM-1 expression. We demonstrated that desloratadine and loratadine inhibited rhinovirus induction of surface ICAM-1 on both primary bronchial epithelial cells and the type II respiratory epithelial cell line A549. Desloratadine and loratadine exerted dose-dependent inhibition of rhinovirus-induced ICAM-1 mRNA expression and completely suppressed rhinovirus-induced ICAM-1 promoter activation. Finally, we demonstrated that desloratadine and loratadine did not inhibit rhinovirus replication in a sensitive cell line.

Despite the wide use of antihistamines in the symptomatic treatment of allergic rhinitis, comparatively few studies have examined their anti-inflammatory effects. Vignola et al²⁰ have also demonstrated that desloratadine and loratadine significantly attenuated the histamine-induced increase in the number of nasal epithelial cells expressing ICAM-1. Our finding that desloratadine and loratadine inhibit rhinovirus-induced ICAM-1 upregulation suggests that this class of antihistaminic compounds may have properties useful for the anti-inflammatory treatment of airway diseases, especially rhinovirus-induced asthma exacerbations, that are unrelated to histamine antagonism. Indeed, other authors have demonstrated that desloratadine, loratadine, and other antihistamines possess anti-inflammatory properties,²⁹⁻³² suggesting that these drugs may exert anti-inflammatory activities by inhibiting the release of preformed and de novo synthesized mediators. We have extended these findings by demonstrating that desloratadine and loratadine protect against ICAM-1 upregulation in a system in which histamine is not present or involved.^{33,34} The fact that desloratadine and loratadine exert a similar inhibitory effect on ICAM-1 expression on both bronchial epithelial cells and lung-originated epithelial cells (A549) suggests that both cell types might be targets for such compounds in clinical settings.

The inhibitory effect of desloratadine on rhinovirus induction of ICAM-1 was detectable at 4 hours of preincubation, which is similar to observations for other anti-inflammatory compounds in the same experimental conditions.²⁷ The choice of a preincubation period of 16 hours was governed by maximal inhibition being observed between 8 and 24 hours and by previous investigators using long incubation periods.²⁰ There are several possible reasons why long preincubation times may be required for maximal effect, including both intracellular and extracellular pharmacokinetic steps (drug uptake into the cells, drug activation, metabolism, and effects on molecular targets). Unfortunately, detailed information on these aspects of the mechanisms of action of loratadine and desloratadine are not available.

Recently, Yoneda et al³⁵ demonstrated a suppressive effect exerted by another antihistaminic compound, azelastine hydrochloride, on cytokine production and mRNA in unstimulated conditions. At variance with our findings, this study was conducted in a cell culture sys-

tem (ie, human peripheral blood leukocytes and monocytes) in which histamine production cannot be excluded.^{33,34} Interestingly, cetirizine, another H₁ receptor, has recently been shown to reduce histamine-independent chemokine (IL-8) release from lung epithelial cells; however, this compound did not inhibit respiratory syncytial virus induction of IL-8.³⁶

The ICAM-1 promoter contains several potential transcription factor binding sites, several of which have been implicated in induction of ICAM-1 gene transcription in response to various proinflammatory stimuli.^{28,37} In particular, we have recently reported that the -187/-178 NF- κ B site on the ICAM-1 promoter is necessary and sufficient for rhinovirus induction of ICAM-1 promoter activity.⁵ The negative modulation by an antihistamine of virus-induced gene promoter activation has never been described before. The above data strongly suggest that this inhibition is occurring through inhibition of NF- κ B activation. To indirectly investigate this hypothesis, we studied the effect of desloratadine on ICAM-1 upregulation induced by a stimulus that, at the molecular level, acts through a pathway more dependent on transcription factors other than NF- κ B, namely IFN- γ .^{38,39} The fact that desloratadine only partially inhibited IFN- γ -induced ICAM-1 upregulation indirectly supports our hypothesis that inhibition of virus-induced ICAM-1 by desloratadine occurs through inhibition of NF- κ B activation. This interpretation is supported by the data in Fig 5 indicating that desloratadine does inhibit rhinovirus induction of binding activity to consensus NF- κ B sequences, which indicates that this compound is effective in reducing rhinovirus-induced NF- κ B activation. The precise molecular mechanisms of action of desloratadine in inhibiting virus-induced inflammation clearly require further investigation.

The control experiments performed on a rhinovirus sensitive cell line (Ohio HeLa) permitted evaluation of the effect of the drugs on rhinovirus replication. Although there is no evidence, to our knowledge, that rhinovirus infection is inhibited by antihistamines, we wished to confirm that the observed effects were not simply related to desloratadine and loratadine inhibition of rhinovirus replication. We chose to perform these experiments in Ohio HeLa cells to examine the effects of the tested drugs independently from the upregulatory effect of rhinovirus on its own receptor (ICAM-1), which is observed in respiratory epithelial cells. Indeed, ICAM-1 surface expression decreases on rhinovirus-infected Ohio HeLa cells in parallel with the appearance and the severity of cytopathic effects (data not shown). These experiments demonstrated that the highest concentrations of desloratadine and loratadine used in the respiratory epithelial cell experiments had no effect on rhinovirus replication and infectivity in this sensitive cell line. These data suggest that desloratadine and loratadine are unlikely to affect rhinovirus replication in respiratory epithelium independently of their effect on ICAM-1 expression.

Given the known role of ICAM-1 in inflammatory cell recruitment and activation at sites of inflammation, the lymphocyte and eosinophil infiltration observed during

experimental rhinovirus infection, its role as rhinovirus receptor, and the fact that rhinovirus infection upregulates respiratory epithelial ICAM-1 expression, ICAM-1 is likely to play a critical role in promoting lower airways inflammation in virus-induced asthma. Our demonstration that desloratadine and loratadine inhibit this induction through a novel mechanism indicates that desloratadine and loratadine are attractive options for the treatment of virus-induced asthma exacerbations.

We thank Dr Stephen Wright Caughman for the generous donations of the reporter plasmid.

REFERENCES

1. Van Seventer GA, Shimizu Y, Horgan KJ, Shaw S. The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. *J Immunol* 1990;144:4579-86.
2. Damle NK, Klussman K, Linsley PS, Aruffo A. Differential costimulatory effects of adhesion molecules B7, ICAM-1, LFA-3, and VCAM-1 on resting and antigen-primed CD4⁺ T lymphocytes. *J Immunol* 1992;148:1985-92.
3. Greve JM, Davis G, Meyer AM, et al. The major human rhinovirus receptor is ICAM-1. *Cell* 1989;56:839-47.
4. Uncapher CR, DeWitt CM, Colonna RJ. The major and minor group receptor families contain all but one human rhinovirus serotype. *Virology* 1991;180:814-7.
5. Papi A, Johnston SL. Rhinovirus infection induces expression of its own receptor intercellular adhesion molecule 1 (ICAM-1) via increased NF- κ B-mediated transcription. *J Biol Chem* 1999;274:9707-20.
6. Stanway G. Rhinoviruses. In: Webster R, Granoff A, editors. *Encyclopedia of virology*. London: Academic Press; 1994. p. 1253-9.
7. Gwaltney JM Jr, Phillips CD, Miller RD, Riker DK. Computed tomographic study of the common cold. *N Engl J Med* 1994;330:25-30.
8. Arola M, Ziegler T, Puhakka H, Lehtonen OP, Ruuskanen O. Rhinovirus in otitis media with effusion. *Ann Otol Rhinol Laryngol* 1990;99:451-3.
9. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ* 1995;310:1225-9.
10. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 1993;307:982-6.
11. Johnston SL, Pattemore PK, Sanderson G, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. *Am J Respir Crit Care Med* 1996;154:654-60.
12. Gern JE, Galagan DM, Jarjour NN, Dick EC, Busse WW. Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. *Am J Respir Crit Care Med* 1997;155:1159-61.
13. Papadopoulos NG, Bates PJ, Bardin PG, et al. Rhinoviruses infect the lower airways. *J Infect Dis* 2000;181:1875-84.
14. Papi A, Johnston SL. Respiratory epithelial cell expression of vascular cell adhesion molecule-1 and its up-regulation by rhinovirus infection via NF- κ B and GATA transcription factors. *J Biol Chem* 1999;274:30041-51.
15. Johnston SL, Papi A, Bates PJ, Mastrorade JG, Monick MM, Hunninghake GW. Low grade rhinovirus infection induces a prolonged release of IL-8 in pulmonary epithelium. *J Immunol* 1998;160:6172-81.
16. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. *Am J Respir Crit Care Med* 1995;151:879-86.
17. Calhoun WJ, Dick EC, Schwartz LB, Busse WW. A common cold virus, rhinovirus 16, potentiates airway inflammation after segmental antigen bronchoprovocation in allergic subjects. *J Clin Invest* 1994;94:2200-8.
18. Doull IJ, Lampe FC, Smith S, Schreiber J, Freezer NJ, Holgate ST. Effect of inhaled corticosteroids on episodes of wheezing associated with viral infection in school age children: randomised double blind placebo controlled trial. *BMJ* 1997;315:858-62.
19. Wilson NM, Silverman M. Treatment of acute, episodic asthma in preschool children using intermittent high dose inhaled steroids at home. *Arch Dis Child* 1990;65:407-10.
20. Vignola AM, Crampette L, Mondain M, et al. Inhibitory activity of loratadine and descarboethoxyloratadine on expression of ICAM-1 and HLA-DR by nasal epithelial cells. *Allergy* 1995;50:200-3.

21. Barnett A, Iorio LC, Kreutner W, Tozzi S, Ahn HS, Gulbenkian A. Evaluation of the CNS properties of SCH 29851, a potential non-sedating antihistamine. *Agents Actions* 1994;43:149-56.
22. Du Buske LM. Clinical comparison of histamine H1-receptor antagonist drugs. *J Allergy Clin Immunol* 1996;98:S307-18.
23. Haria M, Fitton A, Peters DH. Loratadine. A reappraisal of its pharmacological properties and therapeutic use in allergic disorders. *Drugs* 1994;48:617-37.
24. Eda R, Sugiyama H, Hopp RJ, Bewtra AK, Townley RG. Effect of loratadine on human eosinophil function in vitro. *Ann Allergy* 1993;7:373-8.
25. Kleine-Tebbe J, Josties C, Frank G, et al. Inhibition of IgE- and non-IgE-mediated histamine release from human basophil leukocytes in vitro by a histamine H1-antagonist, desethoxycarbonyl-loratadine. *J Allergy Clin Immunol* 1994;93:494-500.
26. Johnston SL, Tyrrell DAJ. Rhinoviruses. In: Lennette EH, Schmidt NJ, editors. *Diagnostic procedures of viral, rickettsial and chlamydial infections*. Washington (DC): American Public Health Association; 1997. p. 553-63.
27. Papi A, Papadopoulos NG, Degitz K, Holgate ST, Johnston SL. Corticosteroids inhibit rhinovirus-induced intercellular adhesion molecule-1 up-regulation and promoter activation on respiratory epithelial cells. *J Allergy Clin Immunol* 2000;105:318-26.
28. Muller S, Kammerbauer C, Simons U, et al. Transcriptional regulation of intercellular adhesion molecule-1: PMA-induction is mediated by NF kappa B. *J Invest Dermatol* 1995;104:970-5.
29. Lippert U, Kruger-Krasagakes S, Moller A, Kiessling U, Czarnetzki BM. Pharmacological modulation of IL-6 and IL-8 secretion by the H1-antagonist decarboethoxy-loratadine and dexamethasone by human mast and basophilic cell lines. *Exp Dermatol* 1995;4:272-6.
30. Molet S, Gosset P, Lassalle P, Czarlewski W, Tonnel AB. Inhibitory activity of loratadine and descarboxyethoxyloratadine on histamine-induced activation of endothelial cells. *Clin Exp Allergy* 1997;27:1167-74.
31. Genovese A, Patella V, De Crescenzo G, De Paulis A, Spadaro G, Marone G. Loratadine and desethoxycarbonyl-loratadine inhibit the immunological release of mediators from human Fc epsilon RI+ cells. *Clin Exp Allergy* 1997;27:559-67.
32. Abdelaziz MM, Devalia JL, Khair OA, Bayram H, Prior AJ, Davies RJ. Effect of fexofenadine on eosinophil-induced changes in epithelial permeability and cytokine release from nasal epithelial cells of patients with seasonal allergic rhinitis. *J Allergy Clin Immunol* 1998;101:410-20.
33. Simons FER. Histamine and antihistamines. In: Kay AB, editor. *Allergy and allergic diseases*. Oxford, UK: Blackwell Science Ltd; 1977. p. 421-39.
34. Hogan MB, Greenberg PA. Histamine. In: Barbes PJ, Grunstein MM, Leff AR, Woolcock AJ, editors. *Asthma*. Philadelphia: Lippincott-Raven; 1997. p. 537-47.
35. Yoneda K, Yamamoto T, Ueta E, Osaki T. Suppression by azelastine hydrochloride of NF-kappa B activation involved in generation of cytokines and nitric oxide. *Jpn J Pharmacol* 1997;73:145-53.
36. Arnold R, Rihoux J, Konig W. Cetirizine counter-regulates interleukin-8 release from human epithelial cells (A549). *Clin Exp Allergy* 1999;29:1681-91.
37. Ledebur HC, Parks TP. Transcriptional regulation of the intercellular adhesion molecule-1 gene by inflammatory cytokines in human endothelial cells. Essential roles of a variant NF-kappa B site and p65 homodimers. *J Biol Chem* 1995;270:933-43.
38. Look DC, Pelletier MR, Holtzman MJ. Selective interaction of a subset of interferon-gamma response element-binding proteins with the intercellular adhesion molecule-1 (ICAM-1) gene promoter controls the pattern of expression on epithelial cells. *J Biol Chem* 1994;269:8952-8.
39. Jahnke A, Johnson JP. Synergistic activation of intercellular adhesion molecule 1 (ICAM-1) by TNF-alpha and IFN-gamma is mediated by p65/p50 and p65/c-Rel and interferon-responsive factor Stat1 alpha (p91) that can be activated by both IFN-gamma and IFN-alpha. *FEBS Lett* 1994;354:220-6.

Bound volumes available to subscribers

Bound volumes of *The Journal of Allergy and Clinical Immunology* are available to subscribers (only) for the 2001 issues from the Publisher, at a cost of \$107.00 for domestic, and \$128.00 for international subscribers for Vol. 107 (January-June) and Vol. 108 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. Copies are shipped within 30 days after publication of the last issue in the volume. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby, Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32887; phone (800) 654-2452 or (407) 345-4000.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.