Influence of cetirizine and loratadine on granulocyte-macrophage colony-stimulating factor and interleukin-8 release in A549 human airway epithelial cells stimulated with interleukin-1beta

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Background and Purpose: In addition to being antagonists of histamine receptors, some antihistamines modulate the pathogenesis of allergic inflammation by reducing mediator release, adhesion molecule expression and, consequently, recruitment of inflammatory cells. The aim of this study was to explore the effects of 2 second-generation antihistamines, cetirizine and loratadine, on granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-8 (IL-8) secretions in human airway epithelial cells.
Methods: A549 cells were pre-incubated with cetirizine (1, 5, 10 μM) or loratadine (1 μM) individually for 16 h followed by stimulation with IL-1beta for 8 h. The levels of GM-CSF and IL-8 were measured by an enzyme-linked immunosorbant assay.
Results: Cetirizine (10 μM) and loratadine significantly reduced the release of GM-CSF, by 37% and 40%, respectively ($p<0.05$). Cetirizine (5, 10 μM) inhibited the production of IL-8 by 19% ($p<0.05$). However, cetirizine (1 μM) and loratadine (1 μM) did not appreciably inhibit IL-8 release.
Conclusions: These observations indicate that these 2 second-generation antihistamines inhibit the release of GM-CSF and IL-8 beyond their antagonistic histamine H1 receptor activity and may thus exert clinically relevant anti-inflammatory effects in inflammatory airway disorders.

Key words: Cell line, cetirizine, granulocyte-monocyte colony-stimulating factor, interleukin-8, loratadine

Introduction

The histamine H1 antihistamines are among the most widely used medications in the world. Oral anti-histamines are first-line medications, used mainly for symptomatic relief, in the treatment of allergic hypersensitivity reactions including allergic rhinitis, rhinoconjunctivitis, urticaria, and pruritus. In addition to their antihistaminic effects, antihistamines can modulate immunological mechanisms involved in the pathogenesis of allergic inflammation by reducing mediator release, adhesion molecule expressions and, consequently, recruitment of inflammatory cells [1-3]. These effects may confer additional clinical benefits in the management of airway inflammation in allergic airway diseases.

Anti-inflammatory properties are not uniformly distributed among drugs of the antihistamine class. Recent investigations indicate that H1 antihistamines may modulate airway inflammation by down-regulating the activity of airway epithelial cells [4]. Airway epithelial cells act not only as the first barrier against the entry of injurious substances in the environment, but also play an important role in regulating the inflammatory process through their ability to express a wide range of proinflammatory cytokines and chemokines, including granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-6, IL-8 and eotaxin [5].

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Among these mediators, GM-CSF possesses significant functions in recruitment, activation, survival enhancement and inhibition of eosinophil apoptosis in the airways of symptomatic asthmatics [6-8]. Elevated levels of GM-CSF have been well demonstrated in bronchoalveolar lavage fluid, bronchial biopsy specimens, and sputum samples from asthmatics [9-11]. This exaggerated secretion may contribute to eosinophilia, a hallmark of allergic disease [12].

IL-8, a member of the C-X-C chemokine family, is a potent chemotactic factor and neutrophil activator. Prominent neutrophilic inflammation was observed during the acute exacerbation of asthma together with high levels of the neutrophil chemoattractant IL-8 [13]. Repeated intranasal administration of IL-8 (twice a week for 3 weeks) induced bronchial hyper-responsiveness and neutrophil accumulation in the lower airways of guinea pigs [14]. This administration also stimulated neutrophil degranulation, respiratory burst, and adherence to endothelial cells by CD11b/CD18. IL-8 also functions as an angiogenic factor and may contribute to angiogenesis, a prominent feature of the histopathology of several inflammatory diseases, such as asthma, rheumatoid disease, and inflammatory bowel disease [15].

Second-generation antihistamines, which are relatively non-sedating compared with their first-generation counterparts, are a heterogeneous group of compounds that display marked diversities in chemical structure, adverse effects, half-life, tissue distribution and metabolism, spectrum of antihistaminic properties, and anti-inflammatory effects. With regard to the latter, there is a growing understanding that some of these compounds might be beneficial as adjunct medications in asthma therapy [16].

The proinflammatory cytokine IL-1 is an important mediator of inflammation and immunity. Both proinflammatory forms of IL-1 (IL-1alpha [IL-1α] and IL-1beta [IL-1β]) are multifunctional cytokines that generally produce similar biological effects, including activation of T lymphocytes, augmentation of B-cell proliferation and immunoglobulin synthesis, stimulation of the synthesis of acute phase response peptides and adherence of leukocytes to endothelial cells, and augmentation of septic shock-mediated hypotension [15]. The airway epithelial cells have the potential to be a major source of IL-6, IL-8 and GM-CSF and cytokine production can be amplified substantially by IL-1β and tumor necrosis factor (TNF)-α [17].

This study investigated the influence of 2 second-generation antihistamines, cetirizine and loratadine, on the production of GM-CSF and IL-8 in IL-1β-stimulated A549 human airway epithelial cells.

Methods

Reagents
Human recombinant IL-1β was purchased from BD BioSciences (San Diego, CA, USA) and was stored as 1 µg/mL stock solution in phosphate-buffered saline supplemented with 0.1% bovine serum albumin at –20°C. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was purchased from Serva Electrophoresis GmbH (Heidelberg, Germany). Cetirizine and loratadine were purchased from UCB Pharma (Brussels, Belgium) and Schering-Plough (Levallois-Perret, France), respectively.

Cell culture and study design
The human airway epithelial cell line A549 (American Type Culture Collection [ATCC], Rockville, MD, USA), derived from carcinoma cells of type II pneumocytes [18] was seeded into a 75-cm^2 tissue culture flask (BD Biosciences) and grown to confluence in Roswell Park Memorial Institute-1640 (Cambrex Corp., East Rutherford, NJ, USA) medium plus 10% fetal bovine serum (ATCC), 100 U/mL penicillin (Invitrogen, Carlsbad, CA, USA), 100 U/mL streptomycin (Invitrogen). For subsequent experiments, the cell suspension was diluted to a final concentration of 1×10^5 cells/mL and 1mL of this suspension was transferred to each well in 6-well tissue culture plates (BD Biosciences) to allow for cell adherence after an overnight incubation.

Resulting confluent monolayers were pre-incubated for 16 h with various concentrations of cetirizine (1, 5, 10 µM) or loratadine (1 µM). The selected loratadine concentration was physiologically appropriate [19], while the cetirizine doses ranged from the physiological peak plasma concentration to 10-fold higher [20].

The cells were stimulated with IL-1β (1 ng/mL) for 8 h. Cell-free supernatants were collected by centrifugation and stored at –70°C until subjected to enzyme-linked immunosorbent assay (ELISA). At all stages of culture, cells were maintained at 37°C in a 5% carbon dioxide atmosphere. The experiments were performed independently at least 4 times.

Cell survival was measured based on the ability of mitochondrial enzymes in living cells to chemically

reduce a tetrazolium salt (MTT) into a colored formazan dye [21]. A549 cells (1×10^5) were seeded into 6-well plates (BD Biosciences) and incubated with cetirizine (1, 5 or 10 µM) or loratadine (1 µM) individually for 16 h. After the appropriate exposure time, the medium was removed and 100 µL of medium containing 0.2 mg MTT/mL was added to each well. The cells were then incubated at 37°C for at least 4 h. The supernatants were collected and the absorbance was recorded immediately using an ELISA reader (Thermo Labsystems, Vantaa, Finland).

Measurement of GM-CSF and IL-8

GM-CSF and IL-8 levels were detected and quantified in cell supernatants using a commercially available ELISA kit (BD Biosciences) according to the manufacturer's specifications. Absorbance was read at 450 nm with an ELISA reader (Thermo Labsystems).

Statistical analysis

The mean cytokine production per 10^5 cells was measured and expressed as a percentage by dividing the amount of cytokine in co-culture with antihistamine and IL-1β to that with IL-1β only. Data were collected from at least 4 independent experiments. Comparisons between groups were tested for significance using analysis of variance repeated measures. Differences with a probability value <0.05 were considered significant. The Statistical Package for the Social Sciences (SPSS) for Windows (Version 10.1; SPSS, Chicago, IL, USA) was used for statistical analysis.

Results

A549 cell survival MTT test revealed that survival of A549 cells pre-incubated with various concentrations of cetirizine (1, 5, 10 µM) or loratadine (1 µM) for 16 h were all ≥98% compared with controls (Fig. 1). Drug concentrations used in the studies revealed no evidence of toxicity to A549 cells.

GM-CSF release

GM-CSF was undetectable without stimulation, with no basal secretion evident. Upon stimulation with IL-1β, secretion increased significantly. The mean GM-CSF release was expressed as a percentage by dividing the amount of cytokine in antihistamine co-cultured with IL-1β to that of IL-1β only. Pre-incubation with cetirizine (10 µM) and loratadine (1 µM) significantly inhibited IL-1β-induced GM-CSF release by 37% and
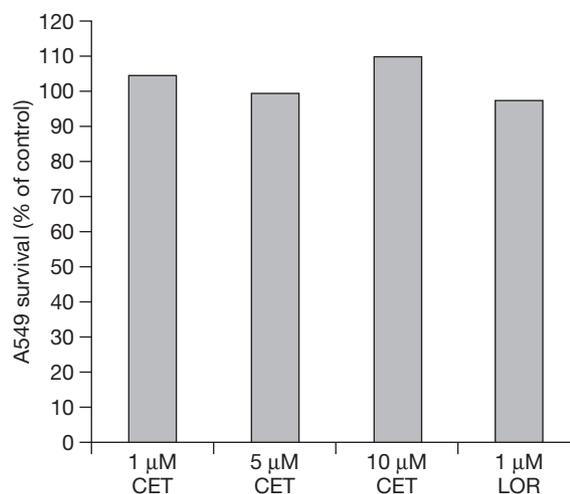
Fig. 1. A549 cell 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) survival test. MTT survival tests of A549 cells pre-incubated with various concentrations of cetirizine (CET; 1, 5, 10 µM) or loratadine (LOR; 1 µM) for 16 h were all ≥98% compared with the control group.

40%, respectively ($p<0.05$; Fig. 2). Pre-incubation of the cells with cetirizine (1 and 5 µM) reduced GM-CSF release by 2% and 20%, respectively. However, these decreases were not significant. GM-CSF release was significantly inhibited by the higher concentration of cetirizine (10 µM) compared to the lower concentrations (1 and 5 µM) [$p<0.05$].

IL-8 release

ELISA analysis of the supernatants from A549 cells detected the basal generation of IL-8. Upon IL-1β application, IL-8 secretion increased significantly. Pre-incubation with cetirizine concentrations of 5 or 10 µM both reduced the IL-8 production by 19% ($p<0.05$; Fig. 3). However, a lower concentration of cetirizine (1 µM) and loratadine had no significant effect on IL-8 secretion.

Discussion

Cetirizine and loratadine are long-acting, second-generation H1 antihistamines that exhibit minimal sedative effects at recommended dosages. This study demonstrated that a physiologically relevant plasma concentration of loratadine (1 µM) and a physiologically elevated concentration of cetirizine (10 µM) significantly attenuated GM-CSF release from IL-1β-stimulated A549 cells. The latter was significantly different from the negligible effect observed using 1 or
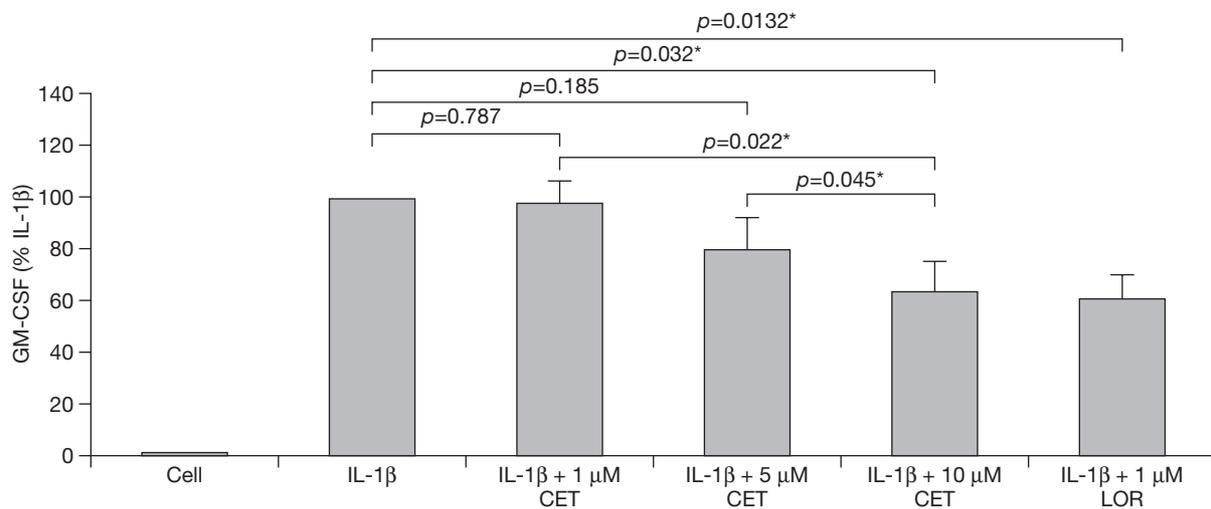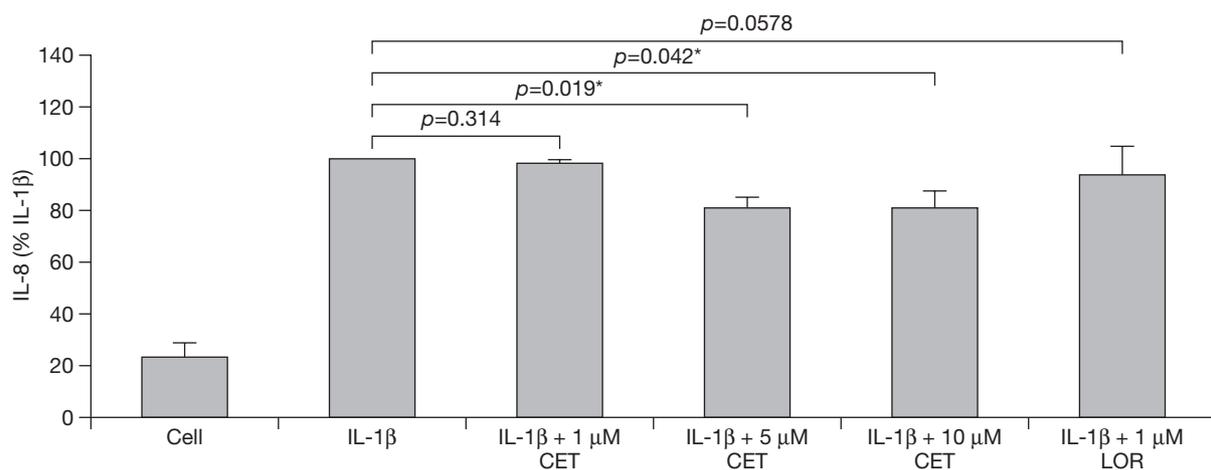
Fig. 2. Influence of cetirizine (CET) and loratadine (LOR) on the production of granulocyte-macrophage colony-stimulating factor (GM-CSF). Confluent A549 monolayers were pre-incubated with various concentrations of CET or LOR for 16 h and then stimulated with interleukin-1beta (IL-1β) for 8 h. Values are presented as mean ± standard error of the mean of the percentage of cytokine production on stimulation with IL-1β in 5 independent experiments. *$p<0.05$ for CET 10 μM + IL-1β and LOR 1 μM + IL-1β versus IL-1β.

5 μM cetirizine. Both higher levels of cetirizine (5, 10 μM) inhibited IL-8 production. However, loratadine did not reduce IL-8 release significantly.

The observed differences in GM-CSF release and IL-8 production with higher concentrations of second-generation antihistamines in this study are consistent with established knowledge of their influence on the production of different cytokines. These findings also suggest the mechanisms responsible for the drugs' anti-inflammatory properties and effectiveness in the treatment of inflammatory airway diseases. Many studies have also demonstrated that second-generation H1 antihistamines exert anti-inflammatory effects, including inhibition of cell migration, mediator release from diverse cell types, adhesion and adhesion molecule expressions [22]. However, there are limited data on the effects of cetirizine and loratadine on GM-CSF and IL-8 secretions in human airway epithelial cells. Previous study showed that pre-incubation of the A549 cells with cetirizine for 30 min in the physiologic dose range of

Fig 3. Influence of cetirizine (CET) and loratadine (LOR) on the production of interleukin (IL)-8. Confluent A549 monolayers were pre-incubated with CET (1, 5, 10 μM) or LOR (1 μM) for 16 h and then stimulated with IL-1beta (IL-1β) for 8 h. Values are presented as mean ± standard error of the mean of the percentage of cytokine production on stimulation with IL-1β in 4 independent experiments. *$p<0.05$ for cetirizine 5 μM + IL-1β and cetirizine 10 μM + IL-1β versus IL-1β.

0.1-1.0 µg/mL (0.22-2.2 µM) significantly diminished TNF-α and PMA-induced IL-8 release by reducing IL-8 gene expression [23]. Bayram et al demonstrated that nitric oxide (NO_2) can induce the release of RANTES (regulated on activation, normal T expressed and secreted) and a soluble intercellular adhesion molecule-1 (sICAM-1), and that IL-8 production was significantly attenuated by incubation of human bronchial epithelial cells with 25 µM loratadine. Incubation with 2.5 µM loratadine significantly attenuated the NO_2-induced release of RANTES and sICAM-1, while IL-8 did not display similar behavior [24]. In contrast, production of GM-CSF or IL-8 by human airway epithelial cells with or without TNF-α stimulation was not inhibited by prolonged pre-incubation with up to 26 µM loratadine or 3.3 µM cetirizine [25].

In this study, the concentration of cetirizine that effectively inhibited GM-CSF release was 10-fold higher than the plasma level that is typically achieved clinically [20]. Indeed, it could be argued that the effect we observed was in fact due to drug toxicity. Negating this argument, MTT assay clearly demonstrated that the higher cetirizine concentration had no lethal effect on cells. Previous studies which reported a significant effect on inflammatory cell function used drug concentrations that were hundreds or thousands of times higher than those that can be achieved in vivo. Indeed, with some exceptions, the majority of the non-H1 receptor-mediated effects of antihistamines require higher concentrations [26]. The clinical implications of the present and previous findings related to concentration require further study.

The role of antihistamines seems to be of little relevance in the management of asthma in comparison with corticosteroids and β_2-agonists. However, the use of the second-generation H1 antihistamines significantly decreased the symptoms of both rhinitis and asthma as well as the use of β_2-agonist medications, and the H1 antihistamines may even improve pulmonary function [16]. These findings suggest a possible role for second-generation antihistamines in treating mild and moderate asthma, which might require administering doses greater than those commonly used to treat allergic rhinitis. The Early Treatment of the Atopic Child study, which examined the role of cetirizine in preventing or delaying bronchial asthma in children with atopic dermatitis, showed that asthmatic symptoms can be prevented in aeroallergen-sensitized children during cetirizine treatment and, furthermore, that cetirizine delays the onset of wheezing after treatment discontinuation [27].

Additional studies are required to further define the usefulness of H1 antihistamines in this role.

The exact mechanisms of anti-inflammatory effects are not clearly identified but may involve down-regulation of the H1 receptor-activated nuclear factor-kappaB (NF-κB), a ubiquitous transcription factor that binds to the promoter and enhancer regions of many genes that regulate the production of proinflammatory cytokines and adhesion proteins [28]. Several second-generation H1 antihistamines have been shown to down-regulate NF-κB expression in parallel with inhibiting the generation of the cytokines and adhesion molecules, IL-1β, IL-6, IL-8, TNF-α, GM-CSF and ICAM-1 [29-31]. Further investigation is needed to identify the cellular mechanisms by which these drugs exert their anti-inflammatory effects. This may also facilitate the development of more effective anti-inflammatory therapy.

In conclusion, this study has shown that 2 second-generation antihistamines, cetirizine and loratadine, can inhibit the release of GM-CSF and IL-8 beyond their antagonistic H1 receptor activity. These agents might exert anti-inflammatory effects in the course of inflammatory airway disorders.

Acknowledgments

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