

The Effect of H1 Antagonists Carebastine and Olopatadine on Histamine Induced Expression of CC Chemokines in Cultured Human Nasal Epithelial Cells

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ABSTRACT

Background: CC chemokines have been shown to play an important role in inducing selective recruitment of inflammatory cells into local allergic inflammatory sites. CC chemokines are also known as histamine releasing factors. We previously showed that histamine enhances transcription of CC chemokines from nasal mucosa which leads to further induction of histamine release. This cyclic cascade may cause prolonged allergic inflammation. The aim of this study is to clarify the relationship between histamine and CC chemokine production by using human nasal epithelial cells (HNECs) and to examine the potential of H1 receptor (H1R) antagonists in new therapeutic approaches for the treatment of nasal allergy.

Methods: HNECs were isolated from the nasal turbinates of patients diagnosed with nasal allergy. HNEC monolayers were cultured for 48 hours with or without histamine (10^{-3} to 10^{-5} mol/L). Furthermore, an H1R antagonist, either carebastine or olopatadine, was added to the supernatant (10^{-3} to 10^{-7} mol/L) 30 minutes before incubation with histamine. The expression of Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES) and monocyte chemotactic protein-1 (MCP-1) in the culture media were measured by ELISA.

Results: The release of RANTES and MCP-1 was significantly upregulated by histamine compared with the control group. Both carebastine and olopatadine inhibited the release of CC chemokine production to the control level in both groups.

Conclusions: This study suggests that the interaction between histamine and CC chemokines may prolong allergic inflammation in human nasal mucosa. We also demonstrate the potential use of H1R antagonists in new therapeutic approaches to the treatment of nasal allergy through inhibiting this histamine-CC chemokine interaction.

KEY WORDS

allergic rhinitis, carebastine, CC chemokine, histamine, human nasal epithelial cells, monocyte chemotactic protein-1, olopatadine, RANTES

INTRODUCTION

Allergic rhinitis, one of the typical type I immunological reactions, has been well studied and its pathophysiologic mechanisms are unraveling. The immediate phase response during allergic rhinitis is

associated with IgE-mediated mast cell activation and results in the release of primary inflammatory mediators, which cause sneezing and rhinorrhea. The early phase of an allergic reaction is followed by the late phase reaction characterized by selective recruitment of inflammatory cells, particularly eosinophils and ba-

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sophils. The pathogenesis of the late phase reaction is so complicated that the precise mechanism of inflammatory cell recruitment is not yet fully understood. Results from several studies have led investigators to conclude that CC chemokines, including monocyte chemotactic protein (MCP)-1, MCP-3, Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES), and eotaxin, may play an important role in inducing selective recruitment of these cells to the allergic inflammatory site.¹ On the other hand, histamine is generally recognized as an important mediator of allergic rhinitis.² Histamine is released from mast cells in the immediate phase and basophils in the late phase.^{3,4} It is also reported that histamine induces production and expression of various cytokines⁵ and adhesion molecules.⁶ Previously, we showed, using the reverse transcription-polymerase chain reaction (RT-PCR) technique that in the nasal mucosa upregulated the mRNA expression of CC chemokines, MCP-1, MCP-3, RANTES and eotaxin.⁷ We also demonstrated that the histamine H1 receptor antagonist carebastine inhibited this upregulation. These results led us to the hypothesis that histamine may induce CC chemokine production of histamine in nasal mucosa and may form a prolonged inflammatory cycle in the histamine-CC chemokine axis in allergic rhinitis. The aim of this study is to prove the relationship between histamine and CC chemokine more precisely by using human nasal epithelial cells. Moreover, we examined the anti-inflammatory effect of antihistamines and accumulated more findings, which support the clinical efficiency and potential of these agents for new therapeutic approaches in the treatment of nasal allergy.

METHODS

REAGENTS

Carebastine and olopatadine, H1R antagonists, were kindly provided by Dainippon Sumitomo Pharmaceutical Co., Ltd. (Osaka, Japan) and Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan), respectively.

PATIENTS

Twenty-two patients with allergic rhinitis who underwent turbinatectomy for the treatment of nasal obstruction (13 men and 9 women, range 12 to 58 years; 26.59 ± 11.19 years [mean $\pm$ SEM]) were included in this study. All patients had severe symptoms of nasal obstruction due to irreversible changes in the inferior turbinate or deflected nasal septum. All patients had positive allergy responses for mite nasal allergy, determined by a clinical history, a nasal smear positive for eosinophils, positive intradermal testing, or serum specific mite IgE antibodies (CAP system, Pharmacia Diagnosis, Sweden), as well as positive nasal provocation test responses and negative test responses for other common aeroallergens. All patients refrained

from taking topical corticosteroids for four weeks and from taking anti-histamines for two weeks prior to the study. The patients who were treated with immunotherapy were not included in this study. This study was approved by the Nippon Medical School's Ethics Committee and informed consent was obtained from all patients who participated in this research.

PREPARATION OF EXPLANT CULTURE WITH HUMAN NASAL EPITHELIAL CELLS

Epithelial cells of the nasal inferior turbinate were obtained from surgically resected nasal turbinate and dispersed as previously described by Otsuka *et al.*⁸ Briefly, after incubation in McCoy's 5A (GIBCO BRL; Grand Island, NY, USA) with 0.1% protease E (Quantikine Co., Minneapolis, MN, USA) at 4°C for 24 hours, epithelial cells were detached from the turbinate tissue by gentle agitation. Human nasal epithelial cells (HNECs) were cultured until they reached confluence (5–7 days) in type I collagen coated 24 well tissue culture plates under a humidified atmosphere at 37°C and 5% CO₂. Confluent cells, with a viability greater than 90% measured by trypan blue exclusion, were then incubated for 48 hours in serum-free McCoy's 5A medium with and without various concentrations (10^{-5} to 10^{-3} mol/L) of histamine dihydrochloride (ICN Biomedicals, Inc, Aurora, OH, USA). To investigate the effect of the histamine H1 receptor antagonists on CC chemokine expression, cells were pre-incubated in the medium containing 10^{-7} to 10^{-5} mol/L carebastine or olopatadine for 30 minutes before histamine was added. In the control group, cells were incubated with carebastine or olopatadine only.

ANALYSIS OF MCP-1 AND RANTES PRODUCTION

MCP-1 and RANTES production in prepared supernatant was examined using an immunoassay kit (R&D Systems, Inc., Minneapolis, MN, USA). The detection limit of MCP-1 was 5.0 pg/ml and that of RANTES was 2.0 pg/ml. The percent changes of these values by histamine or H1R antagonists were calculated. Samples were prepared in duplicate in accordance with the manufacturer's directions.

STATISTICAL ANALYSIS

The data are expressed as means $\pm$ SEM. The statistical significance of differences among groups were analyzed by one way ANOVA and subsequently by the Tukey-Kramer's analysis. StatView J-5.0 software (SAS Institute Inc., Cary, NC, USA) was used in all statistical analyses. The differences were considered significant only when the *p* value was less than 0.05.

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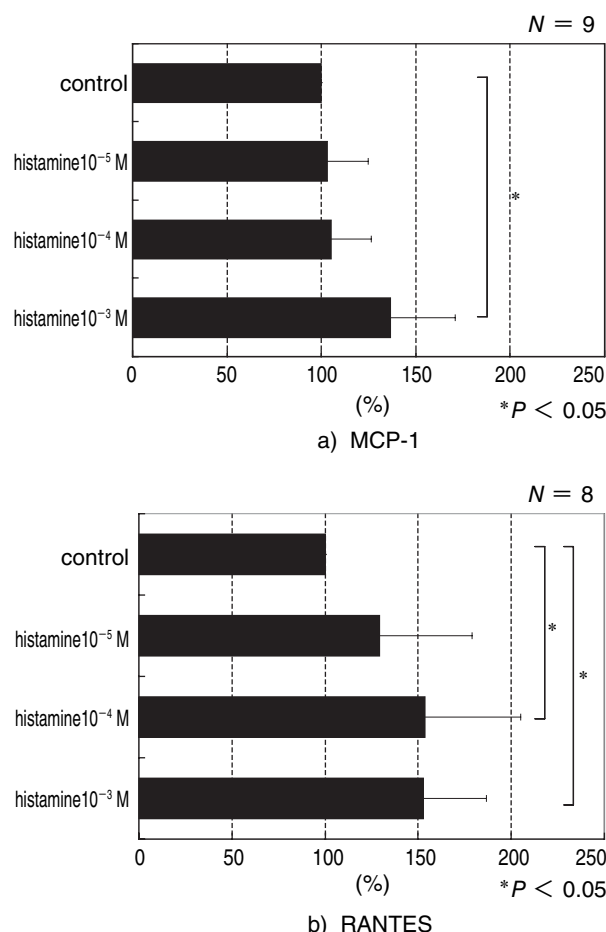


Fig. 1 The effect of histamine on CC chemokine production from HNECs. The expression of MCP-1 or RANTES in the culture media was measured by enzyme-linked immunosorbent assay (ELISA). **a)** Histamine tended to upregulate MCP-1 expression dose-dependently and the percent changes of MCP-1 production were increased significantly by 10^{-3} mol/L of histamine, compared with the non-histamine control ($p < 0.05$). **b)** The percent changes of expression of RANTES from HNECs were significantly up-regulated by 10^{-3} mol/L of histamine ($p < 0.05$). The production of RANTES also tended to be up-regulated by histamine in a dose-dependent manner.

RESULTS

BASAL CC CHEMOKINE EXPRESSION BY HISTAMINE STIMULATION

The baseline production of MCP-1 was 4140.8 ± 3925.4 pg/ml. MCP-1 expression in HNECs was significantly upregulated to $137.3 \pm 33.9\%$ by 10^{-3} mol/L of histamine ($p < 0.05$) (Fig. 1a).

The baseline production of RANTES was 16.5 ± 11.11 pg/ml. As showed in Figure 1b, the expression in HNECs of RANTES was significantly upregulated by histamine in a dose-dependent manner (10^{-3} mol/L

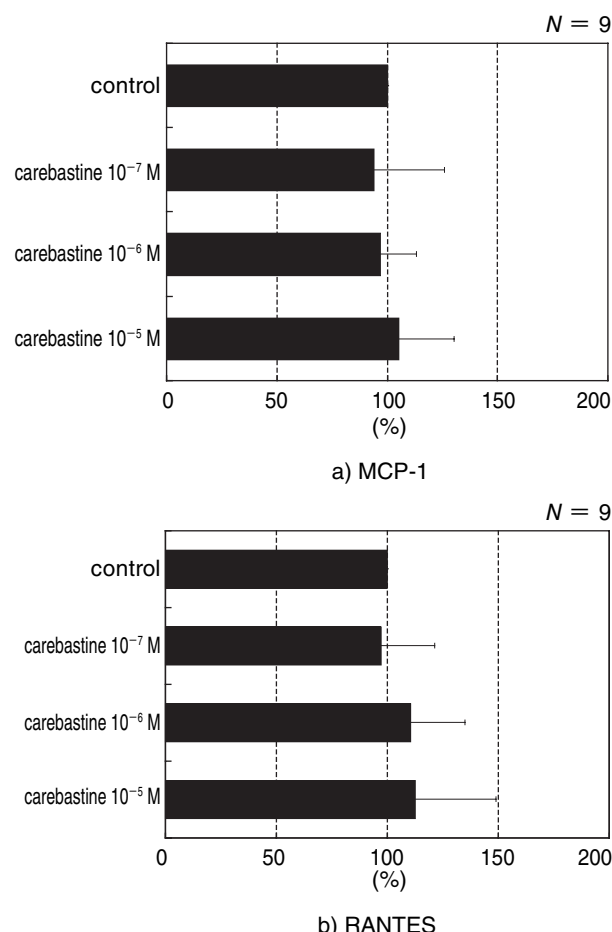


Fig. 2 The effect of carebastine alone on chemokines expression. **a)** The expression of MCP-1 from HNECs was not affected by carebastine. **b)** The expression of RANTES from HNECs was not affected by carebastine.

L and 10^{-4} mol/L; $152.7 \pm 33.9\%$, and $153.6 \pm 51.3\%$ respectively) ($p < 0.05$).

INHIBITORY EFFECT OF CAREBASTINE OR OLOPATADINE ON HISTAMINE-STIMULATED PRODUCTION OF CC CHEMOKINES

The expression of MCP-1 or RANTES in HNECs was not affected by either carebastine or olopatadine alone (Figs. 2, 3).

Carebastine significantly inhibited histamine-stimulated production of MCP-1 and RANTES in HNECs (Fig. 4 respectively) ($p < 0.05$). The production of MCP-1 and RANTES, stimulated with histamine, was inhibited by carebastine in a dose-dependent manner. 10^{-5} mol/L of carebastine decreased MCP-1 secretion to $70.0 \pm 13.5\%$, while 10^{-6} mol/L decreased MCP-1 secretion to $73.4 \pm 19.9\%$. Also RANTES was reduced to $71.9 \pm 26.3\%$ by 10^{-5} mol/L of carebastine and to $73.2 \pm 24.9\%$ by 10^{-6} mol/L of carebastine. In a manner similar to carebastine, olopatadine significantly decreased MCP-1 or RAN-

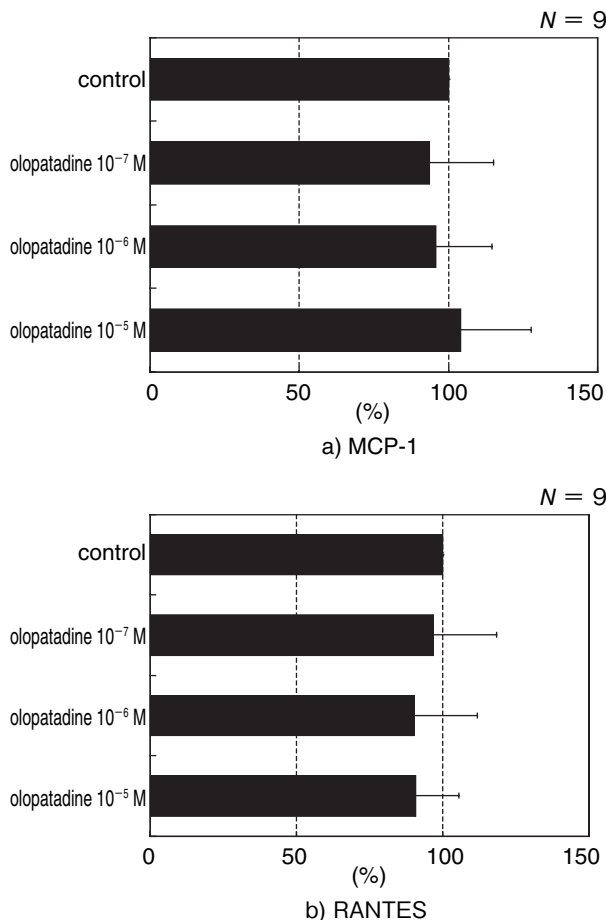


Fig. 3 Effect of olopatadine alone on chemokines expression. **a, b)** The expression of MCP-1 or RANTES in HNECs was not affected by olopatadine alone.

TES (Fig. 5) secretion in HNECs in a dose-dependent manner. 10^{-5} mol/L of olopatadine decreased MCP-1 secretion to $78.9 \pm 22.1\%$, while 10^{-6} mol/L decreased MCP-1 secretion to $79.0 \pm 16.7\%$. Also RANTES was reduced to $77.5 \pm 16.1\%$ by 10^{-5} mol/L of olopatadine to $77.0 \pm 12.4\%$ by 10^{-6} mol/L of olopatadine and to $77.9 \pm 13.1\%$ by 10^{-7} mol/L of olopatadine.

DISCUSSION

Nasal epithelial cells are major cell sources of pro-inflammatory cytokines and chemokines,^{1,9} which contribute to the pathogenesis of nasal allergy. Several studies have shown that histamine induces secretion of various inflammatory cytokines from airway epithelial cells.¹⁰⁻¹⁴ Moreover, histamine has also been reported to activate endothelial cells and bronchial epithelial cells to express adhesion molecules.¹⁵ Consequently, the logical question was raised as to whether H1-receptor antagonists could affect these up-regulatory effects of histamine on nasal epithelial cells. Some of the new generation H1-antihistamines, olopatadine, fexofenadine, levocabastine, and

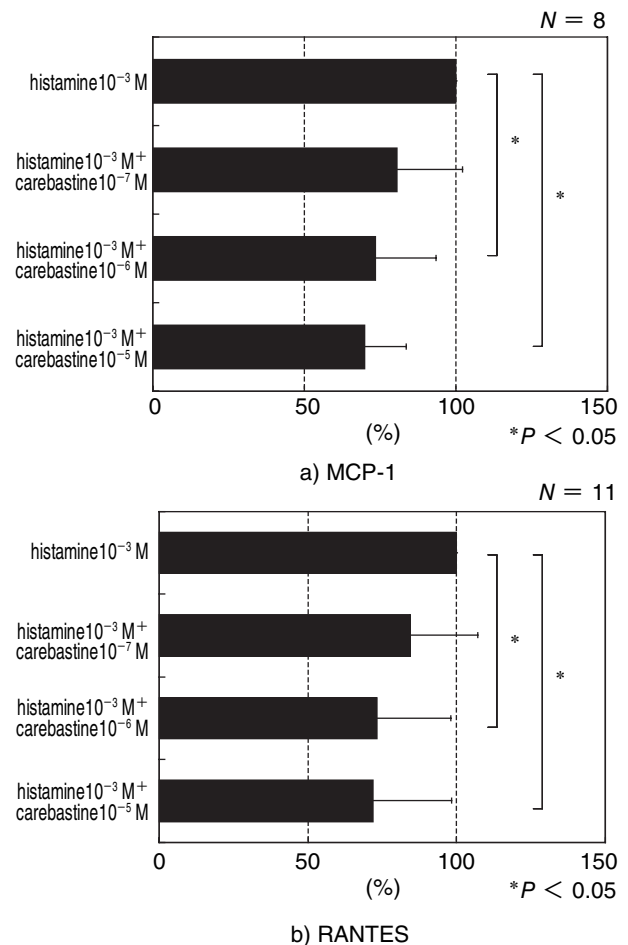


Fig. 4 The effect of carebastine on expression of chemokines with histamine. HNECs were preincubated in the medium with carebastine, previous to histamine addition. MCP-1 or RANTES expression was measured by ELISA. **a)** Carebastine dose-dependently inhibited the stimulatory effect of histamine on MCP-1 production from HNECs (histamine (10^{-3} M)/histamine with carebastine (10^{-5} M): $p < 0.05$, histamine (10^{-3} M)/histamine with carebastine (10^{-6} M): $p < 0.05$). **b)** Carebastine also dose-dependently inhibited the enhanced expression of RANTES with histamine stimulation (histamine (10^{-3} M)/histamine with carebastine (10^{-5} M): $p < 0.05$, histamine (10^{-3} M)/histamine with carebastine (10^{-6} M): $p < 0.05$).

emedastine, have been reported as having several anti-inflammatory effects including down-regulation of pro-inflammatory cytokines and chemokines production from conjunctival, nasal, and bronchial epithelial cells *in vitro*.^{11,14,16,17} However, the mechanism of the anti-inflammatory effects of these new generation H1-antihistamines have not been fully understood yet. Although our previous study suggests the presence of H1 receptors in nasal mucosal tissue, mucosal tissue might contain epithelium and fewer other cells such as fibroblasts and endothelial cells. In this

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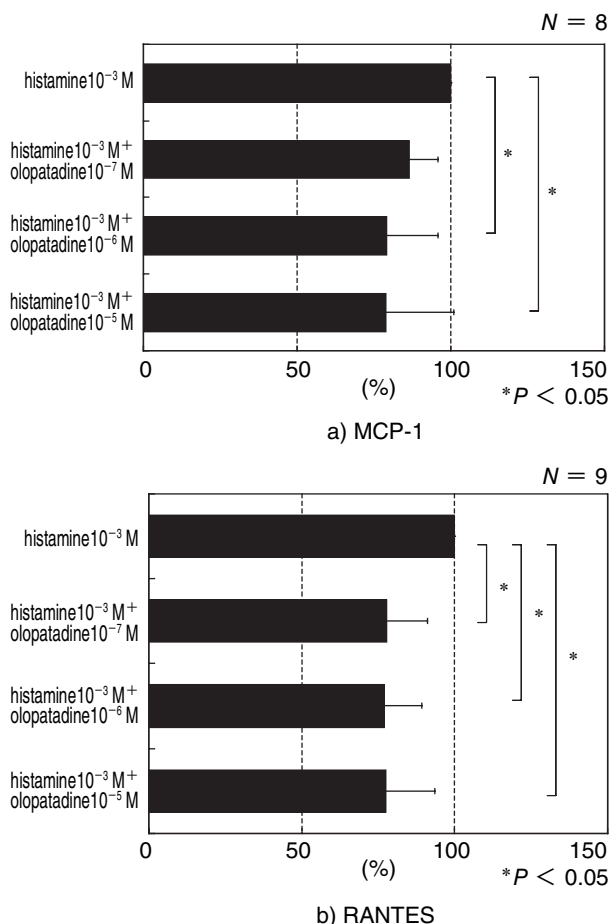


Fig. 5 The effect of olopatadine on expression of chemokines with histamine. As with experiments involving carebastine, olopatadine was added to the medium in advance of histamine stimulation of HNECs. Expression of chemokines was assessed by ELISA. **a)** Olopatadine significantly decreased MCP-1 production with histamine in a dose-dependent manner (histamine (10^{-3} M)/histamine with olopatadine (10^{-5} M): $p < 0.05$, histamine (10^{-3} M)/histamine with olopatadine (10^{-6} M): $p < 0.05$). **b)** Olopatadine dose-dependently decreased RANTES production from HNECs (histamine (10^{-3} M)/histamine with olopatadine (10^{-5} M): $p < 0.05$, histamine (10^{-3} M)/histamine with olopatadine (10^{-6} M): $p < 0.05$, histamine (10^{-3} M)/histamine with olopatadine (10^{-7} M): $p < 0.05$).

study, our findings, together with the evidence presented by other studies using the RT-PCR technique, suggest that the nasal epithelium expresses histamine H1 receptors.¹⁸⁻²⁰ The autoradiographic studies by Okayama *et al.* on human nasal turbinate mucosa failed to demonstrate the presence of H1 receptors in epithelium.²¹ Given this controversy, the localization of H1 receptors in human nasal mucosa needs to be more fully clarified in future investigations.

CC chemokines have been shown to play an important role in inducing selective recruitment of inflam-

matory cells, especially eosinophils and basophils, into local allergic inflammatory sites.^{1,22-26} Previously we demonstrated that CC chemokines act as a histamine releasing factor and are up-regulated by histamine.^{7,27} By forming this prolonged inflammatory cycle, the histamine-CC chemokine interaction may contribute to the protraction of nasal allergy. Additionally, this reaction may cause subsequent infiltration of inflammatory cells. In this study, we demonstrated that the newly generated antihistamines ebastine and olopatadine may inhibit the formation of this inflammatory cycle. Fexofenadine decreased constitutive and eosinophil-induced release of RANTES from nasal epithelial cells, but this decrease was not statistically significant.¹⁷ Loratadine decreased NO₂-induced release of RANTES from bronchial epithelial cells.²⁸ Several investigators reported down-regulation of IL-8,^{29,30} which is a CXC-chemokine. As described above, newly generated antihistamines are known to have anti-inflammatory effects. We selected olopatadine and ebastine for use in this study as a focus for future clinical investigation, due to the fact that they are commonly being used as a new generation of antihistamines in Japan.

These new generation antihistamines are able to effectively antagonize H1 receptors expressed in the human nose. Some of them have additional properties, in particular anti-inflammatory activities that may be linked to their action on H1-receptors. Church has suggested that many of these anti-inflammatory properties require 10 to 1000 times higher doses than those used for symptom relief in allergic rhinitis or urticaria.³¹ In our study, both carebastine and olopatadine inhibited histamine-induced upregulation of CC chemokines at concentrations which were not far from those used clinically (about 2 to 5 times higher). This benefit may demonstrate one therapeutic option for treating allergic rhinitis. Recently, several studies have confirmed the anti-inflammatory properties of antihistamine *in vivo* but failed to demonstrate the down-regulation of pro-inflammatory cytokines or chemokines.³²⁻³⁴

In this study, we used 10^{-3} mol/L of histamine to activate nasal epithelial cells to evaluate the down-regulatory effects of anti-histamines. Jeannin *et al.*¹² reported that the concentration of histamine after mast cell degranulation in nasal tissue was 10^{-6} to 10^{-4} mol/L, however, the nasal epithelial cells were located very close to the mast cells in the nasal tissue. Consequently, we considered 10^{-3} mol/L of histamine as a reasonable concentration for activation of nasal epithelial cells. We concluded that it was very meaningful that both carebastine and olopatadine, in dosages close to clinical use, successfully inhibited chemokine production of nasal epithelial cells even when stimulated by such high concentrations of histamine.

Definitive evidence regarding whether the anti-

inflammatory effects of antihistamines are H1-receptor mediated or has not been shown yet. Although inhibition of calcium influx in cells and NF- κ B activation are thought to be involved,³⁵ precise mechanisms underlying these anti-inflammatory effects are not well understood. Furthermore, it has not been determined whether the observed anti-inflammatory effects of each antihistamine are drug specific or a property common to all of them. Our findings that blockade of the H1 receptor in nasal mucosa down-regulates histamine-induced expression of CC chemokines reflects a simple mechanism and seems to be a common property of antihistamines. To resolve these important issues, more careful investigations are needed in the future.

Uncontrolled allergic rhinitis leads to worsening of coexisting asthma and, conversely, treatment of allergic rhinitis improves coexisting asthma. Relief of inflammation is important for the management of both disorders. Orally administrated H1-antihistamine, in the doses ordinarily used for allergic rhinitis, improved coexisting mild seasonal asthma symptoms. It has been demonstrated that minimal persistent inflammation exists in patients with allergic rhinitis, even in the absence of clinically active symptoms.³⁶ This concept suggests the need for long-term anti-inflammatory treatment to adequately control allergic disorders.³⁷ The Consensus Group on New Generation Antihistamines (CONGA) recommended that the anti-allergic properties of antihistamines should be demonstrable *in vivo*, in humans, at therapeutic doses and under natural exposure to the offending allergens.³⁸ In our study, the anti-inflammatory properties of ebastine and olopatadine were demonstrated in humans, at doses which are not far above the therapeutic doses. Although an *in vivo* study in humans would not be easy to design and carry out, the results of our study suggest that it would be meaningful to carry out an *in vivo* study in the near future.

Antihistamines may produce anti-inflammatory effects through blocking histamine and CC chemokine interaction. These effects lead to reduction of the prolonged inflammatory allergic cycle. The results of this study suggest that continuous treatment with antihistamines will be useful in reducing basal levels of allergic inflammation. Further investigations regarding the size, term, and method of administration of an effective antihistamine dose are required.

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