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Effect of desloratadine on epithelial cell granulocyte-macrophage colony-stimulating factor secretion and eosinophil survival

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Summary

Background Second-generation antihistamines are H₁ receptor antagonists and may have additional anti-inflammatory effects.**Objective** The aims of the study were to evaluate the effect of desloratadine (DL) on cytokine secretion by epithelial cells from both nasal mucosa (NM) and polyps (NP), and on eosinophil survival primed by epithelial cell secretions.**Methods** Epithelial cells were cultured and stimulated with fetal bovine serum (FBS), IL-1 β or TNF- α with and without DL for 24 h. Culture supernatant cytokines concentration were measured by ELISA. Peripheral blood eosinophils were incubated with human epithelial cell conditioned media (HECM) and DL. Eosinophil survival was assessed by Trypan blue dye exclusion. Results are expressed as mean $\pm$ SEM of cytokine concentration (pg/mL) or eosinophil survival index (%).**Results** FBS increased granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), IL-6, IL-8, and TGF- β_1 secretion in epithelial cell cultures from both NM and NP. Only GM-CSF secretion was significantly ($P < 0.05$) inhibited by a dose-response of DL compared with positive controls, in both NM (10^{-5} M: 125 ± 36 pg/mL, 10^{-6} M: 95 ± 22 pg/mL vs. control: 256 ± 91 pg/mL, $n = 6$) and NP (10^{-5} M: 80 ± 29 pg/mL, 10^{-6} M: 109 ± 45 pg/mL vs. control: 333 ± 212 pg/mL, $n = 6$). DL also showed an inhibitory effect on HECM-induced eosinophil survival from both NM and NP. At 72 h, DL significantly ($P < 0.01$) inhibited eosinophil survival induced by HECM from NM (10^{-5} M: $19.9 \pm 5.5\%$, $n = 9$; 10^{-6} M: $28.7 \pm 7.7\%$, $n = 9$) and NP (10^{-5} M: $6.2 \pm 2.8\%$, $n = 11$) compared with HECM alone (NM: $42.1 \pm 7.3\%$; NP: $45.3 \pm 8.1\%$).**Conclusion** The inhibitory effects of DL on epithelial cell GM-CSF secretion and on eosinophil survival induced by epithelial cell secretions, suggest that this H₁ antagonist may regulate eosinophil inflammation in upper airways.**Keywords** cytokines, desloratadine, eosinophil, epithelial cells, GM-CSF, IL-1 β , inflammation, nasal mucosa, nasal polyp, TNF- α

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Introduction

The inflammatory response in upper airway diseases such as rhinitis, rhinosinusitis, and nasal polyposis is associated to both eosinophil infiltration [1–4] and increased expression of pro-inflammatory cytokines. Concentrations of IL-1 β , IL-4, and IL-5 are increased in nasal secretions from patients with allergic rhinitis (AR) [1, 5, 6]. In chronic rhinosinusitis, mucosal levels of granulocyte-macrophage colony-stimulating factor (GM-CSF),

IL-1 β , IL-3, IL-4, IL-5, IL-6, IL-8, IL-13, and TNF- α are also up-regulated [7, 8]. Eotaxin, GM-CSF, IL-4, IL-5, IFN- γ , IL-1 β , IL-8, transforming growth factor β (TGF- β), and vascular endothelial growth factor (VEGF) are highly expressed in nasal polyps (NP) [9–11].

Inflammatory and structural cells appear to be the source of these cytokines. We have previously demonstrated that cultured epithelial cells from human nasal mucosa (NM) and NP express GM-CSF, IL-6, IL-8, and TNF- α mRNA [9]. Other studies have shown that eotaxin,

IL-1 β , IL-8, GM-CSF, and TNF- α , as well as IL-1 and TNF- α receptors are up-regulated in nasal epithelial cells from rhinitic patients [7, 12, 13]. Cultured epithelial cells from both human NM and NP release GM-CSF, IL-1 β , IL-6, IL-8, and TNF- α in response to fetal bovine serum (FBS) [9]. Similarly, IL-6, IL-8, and GM-CSF are released in response to TNF- α [14]. GM-CSF, along with TNF- α and IL-8 secreted from epithelial cells are involved in the promotion of eosinophil survival [15, 16] a finding that suggests that these cytokines may participate in the nasal inflammatory response.

The first line treatment of upper airway inflammation includes corticosteroids and antihistamines [17]. Inhibition of pro-inflammatory cytokine secretion from epithelial cells [9, 16, 18, 19] and the reduction of eosinophilic viability induced by pro-inflammatory cytokines [4] contribute to the anti-inflammatory effects of corticosteroids. Recent studies have suggested that antihistamines may also have anti-inflammatory functions. In enzymatically dispersed cells obtained from human NP, azelastine reduced TNF- α release [20], while terfenadine and mizolastine inhibited both TNF- α and GM-CSF release [21, 22]. In purified eosinophils from allergic patients, loratadine attenuated platelet-activating factor-induced eosinophil chemotaxis and O₂⁻ generation, but it did not exert any effect on platelet-activating factor-induced eosinophil cationic protein (ECP) release [23]. In allergic rhinitic subjects under natural allergen exposure, both loratadine and cetirizine reduced eosinophil infiltration, as well as ECP and eosinophil peroxidase levels in NM [24]. Desloratadine (DL), an active metabolite of the H₁-receptor antagonist loratadine [25], improves with efficacy nasal and non-nasal symptoms in AR patients [26]. A recent study reported that DL-induced apoptosis of eosinophils stimulated with IL-5 [27].

The objectives of this study were: (1) to examine the effect of DL in cytokine release from NM and NP cultured epithelial cells, and (2) to assess the effect of DL on eosinophil survival primed by cultured epithelial cell secretions from both healthy NM and NP.

Materials and methods

Materials

Ham's F-12 and RPMI 1640 medium was purchased from Bio Whittaker Europe (Verviers, Belgium); 24-well culture plates from Costar (Cultek SL, Madrid, Spain); DL from Schering Plough (Kenilworth, NJ, USA); recombinant human IL-1 β and TNF- α from R&D Systems (Minneapolis, MN, USA); penicillin-streptomycin, FBS from Invitrogen Corporation (Paisley, Scotland, UK); and amphotericin B from Squibb (Esplugues de Llobregat, Catalonia, Spain). Hydrocortisone, *N*-formyl-methionyl-leucyl-phenylalanine, human transferrin, bovine insulin, 3,3',5-triiodo-L-

tyrosine sodium salt, protease type XIV, light mineral oil, glutamine, trypan blue, and dimethylsulphoxide (DMSO) were obtained from Sigma-Aldrich Co. (Madrid, Spain); endothelial cell growth supplement and epidermal growth factor were supplied by Collaborative Research Inc. (Bedford, MA, USA); Percoll[®], cytokine ELISA kits from Amersham Biosciences Europe (Cerdanyola, Spain); and rat tail collagen type I from Upstate (Lake Placid, NY, USA).

Methods

Tissue handling. Nasal mucosa specimens were obtained from 12 patients (10 men, two women), ranging in age from 18 to 73 years (38.4 ± 4.2 years), undergoing nasal corrective surgery for septal dismorphism, turbinate hypertrophy, or both. Skin prick test (SPT) was positive in one patient (8.3%). None of patients were receiving topical or systemic glucocorticoid treatment at the time of surgery.

Nasal polyp specimens were obtained from 12 patients (eight men, four women), ranging from 24 to 81 years (49.4 ± 4.7 years), undergoing endoscopic nasal polypectomy. SPT was positive in four patients (33.3%). Five patients (41.7%) also had concomitant asthma and 11 patients (91.7%) were on regular treatment with intranasal steroids.

None of the patients had had an upper airway infection the 2 weeks before surgery. All patients gave informed consent to participate in the study, which was approved by the Scientific and Ethic's Committee of our Institution.

Epithelial cell isolation. Tissue specimens were placed in Ham's F-12 medium supplemented with penicillin (100 UI/mL), streptomycin (100 μ g/mL), amphotericin B (2 μ g/mL), and immediately transported to the laboratory. Epithelial cells from NM or NP were isolated by protease digestion using a technique previously reported [9, 15, 16]. Viability of cells was assessed by trypan blue dye exclusion using a haemocytometer (Assistent Sondheim, Germany). Cell population was characterized using smears obtained by cytocentrifugation (30g, 10 min) and stained with May-Grünwald-Giemsa or with mouse monoclonal anti-cytokeratin antibody using the immune-alkaline phosphatase method [9, 15]. After tissue protease digestion, cell viability was $96.8 \pm 0.7\%$ for NM and $91.4 \pm 1.4\%$ for NP, and the percentage of epithelial cell purity was $99.9 \pm 0.08\%$ for NM and $91.3 \pm 1.4\%$ for NP specimens. After cell culture, the percentage of epithelial cell purity was 100% for both NM and NP specimens.

Cell cultures. Epithelial cell suspensions (10^5 cells/well) were plated on 24-well plates coated with rat tail collagen type I in a hormonally defined serum-free media: F-12 culture medium (2 mL), antibiotics (penicillin, 100 UI/mL; streptomycin, 100 μ g/mL), amphotericin B (2 μ g/mL),

glutamine (150 µg/mL), transferrin (5 µg/mL), insulin (5 µg/mL), epidermal growth factor (25 µg/mL), endothelial cell growth factor supplement (15 µg/mL), triiodothyronine (200 pM) and hydrocortisone (100 nM). Epithelial cells were cultured in 5% CO₂ humidified atmosphere at 37 °C, the culture media being changed every 2 days.

Cytokine secretion from human nasal epithelial cell cultures. When epithelial cell cultures reached 80% subconfluence, the hormonally defined serum-free media was switched to RPMI-1640 media supplemented with antibiotics (penicillin, 100 UI/mL; streptomycin, 100 µg/mL), amphotericin B (2 µg/mL), glutamine (150 µg/mL) and HEPES buffer (25 mM). As previous studies have shown that non-stimulated epithelial cells produce low levels of cytokines [19], cultured epithelial cells were incubated with FBS (0.1–1–10%), IL-1β or TNF-α (both at 0.1–1–10 ng/mL) in the presence or absence of different concentrations of DL (10⁻⁸–10⁻⁵ M) for 1, 4 and 24 h. After incubation, cell supernatants (human epithelial cell conditioned media (HECM)) were harvested from cultures, centrifuged at 400 g (10 min, 25 °C), sterilized through 0.22 µm filters, and stored at -80 °C until used. In order to avoid different effects of FBS from different batches and sources, the same manufacturing batch was used in all experiments.

Cell viability and drug cytotoxicity. To test the potential cell toxicity of DL, we used a colorimetric assay (Roche Diagnostics, Indianapolis, IN, USA) based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into the supernatant. To assess the percentage of cytotoxicity, a low control (spontaneous LDH release), high control (maximum LDH release by cell lyses with 2% Triton X-100) and an experimental LDH activity were determined in six experiments. The drug cytotoxicity was calculated as follows [28]: (experimental LDH act - low control)/(high control - low control) × 100.

Cytokine enzyme-linked immunoassays. The concentrations of eotaxin, GM-CSF, IL-5, IL-6, IL-8, RANTES, TGF-β₁ and VEGF were measured in supernatants from NM and NP cultured epithelial cells using commercial ELISA kits. The assay ranges were: 31–2000 pg/mL for eotaxin, 15.4–600 pg/mL for GM-CSF, 15.7–1000 pg/mL for IL-5, 10–400 pg/mL for IL-6, 25–1000 pg/mL for IL-8, 51.2–2000 pg/mL for RANTES, 15.6–1000 pg/mL for TGF-β₁, and 31.3–2000 pg/mL for VEGF. To verify that the molecules used in the different experiments (DL, IL-1β, TNF-α, FBS) did not affect the ELISA results, wells containing either culture media alone or media with the highest drug concentration used in the different protocols were compared (*n* = 3). None of the selected drugs showed any intrinsic effect on the ELISA final values.

Eosinophil isolation. Normodense eosinophils were obtained from atopic and non-atopic subjects with > 3% in peripheral blood eosinophils. None of the patients had an upper airway infection in the 2 weeks before surgery and all patients gave informed consent to participate in the study, which was approved by the Scientific and Ethic's Committee of our Institution. Eosinophils were obtained by a previously described method [15, 16] using discontinuous Percoll[®] (Amersham Biosciences, Uppsala, Sweden) gradients. Eosinophil viability and purity (> 90%) were quantified by trypan blue dye exclusion and May-Grünwald-Giemsa staining, respectively.

Eosinophil survival assessment. Eosinophils (2.5 × 10⁵ cells/well) were incubated in 24-well tissue culture plates with RPMI (2 mL) in the presence or absence of DL (10⁻⁸–10⁻⁵ M) at 37 °C for 1 h before the addition of 10% HECM from NM or NP. Eosinophil survival index was assessed at 24 h (day 1), 48 h (day 2), 72 h (day 3), and 96 h (day 4) of incubation by trypan blue dye exclusion. The eosinophil survival index was calculated as follows: number of eosinophils recovered × percentage of eosinophil viability/number of eosinophils delivered on day 0.

Because DL was diluted in DMSO and the HECM added to the eosinophil cultures contained 10% FBS, we investigated the effect of both DMSO and FBS on eosinophil survival. Neither DMSO nor FBS at the higher final concentration present in the culture media (0.1% DMSO when DL was at 10⁻⁵ M, and 1% FBS when HECM was at 10%) had a significant effect on eosinophil survival (data not shown).

Statistics. Statistical evaluations were performed using the statistical software Microsoft SPSS 10.0. Results are expressed as mean ± standard error of the mean (SEM). A non-parametric (Wilcoxon's signed rank test) was used for statistical comparisons. *P* < 0.05 was considered statistically significant.

Results

Effect of desloratadine on cytokine secretion

Nasal mucosa and NP cultured epithelial cells were incubated with 10% FBS or media alone for 1, 4, and 24 h. After 1 and 4 h of incubation, FBS at 10% caused no effect on cytokine secretion from both NM and NP epithelial cell cultures (data not shown). At 24 h, FBS at 10% induced a significant release of GM-CSF, VEGF, IL-6, TGF-β₁, and IL-8 (Table 1). No effects on eotaxin, IL-5, and RANTES secretions were detected because the concentrations of these three cytokines in epithelial cell supernatants were always under the ELISA limit of detection.

Incubation with IL-1β or TNF-α for 1, 4, and 24 h had no significant effect on cytokine secretion from both NM and NP cultured epithelial cells (data not shown).

Table 1. Effect of fetal bovine serum on cytokine secretion from epithelial cells

Cytokine	Nasal mucosa epithelial cells (pg/mL)			Nasal polyp epithelial cells (pg/mL)		
	Control	10% FBS	<i>n</i>	Control	10% FBS	<i>n</i>
VEGF	< 31.3	140.0 ± 28.1*	6	< 31.3	178.1 ± 40.7*	6
IL-6	< 10	199.2 ± 53.1**	9	22.0 ± 17.0	209.7 ± 78.0*	6
TGF-β ₁	111.6 ± 27.7	812.2 ± 239.9*	6	133.7 ± 25.3	893.7 ± 252.1*	6
IL-8	188.5 ± 39.9	2262.7 ± 1265.7*	5	239.6 ± 67.6	2457.0 ± 1269.1*	5

Results are expressed as mean ± SEM. The Wilcoxon signed-rank test was used for analysis.

* $P < 0.05$ and ** $P < 0.01$ compared with control.

FBS, fetal bovine serum; VEGF, vascular endothelial growth factor.

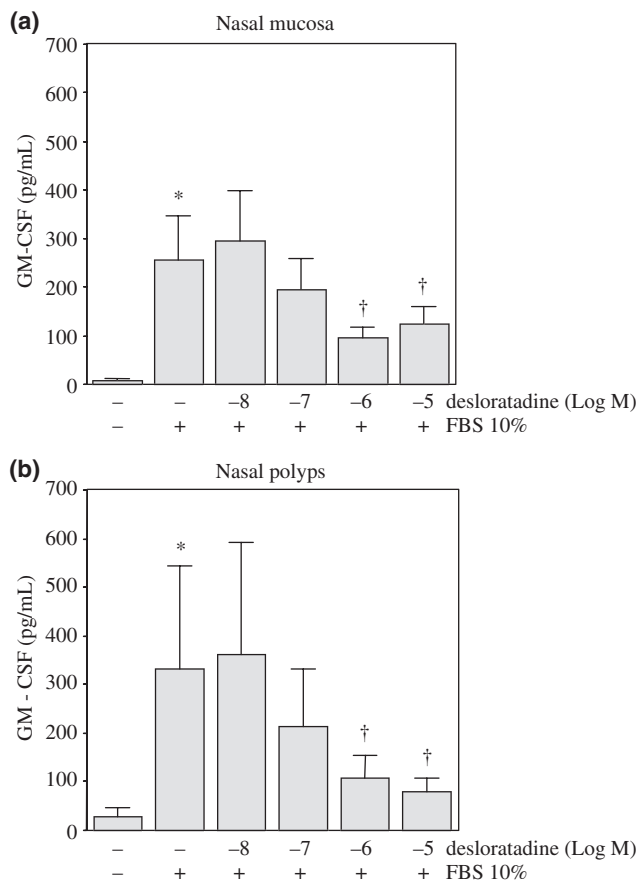


Fig. 1. Effect of desloratadine (DL) on granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion from epithelial cells. Fetal bovine serum (FBS) at 10% increased GM-CSF secretion from both nasal mucosa (a) and nasal polyp (b) cultured epithelial cells. DL from 10^{-6} to 10^{-5} M caused a dose-related inhibitory effect on FBS-induced GM-CSF secretion. Results are expressed as mean ± SEM. Wilcoxon's signed-rank test was used for analysis (* $P < 0.05$ compared with control † $P < 0.05$ compared with 10% FBS, $n = 6$).

To assess the effect on cytokine release, DL (from 10^{-8} to 10^{-5} M) was added to the cultures over 1 h prior to the addition of media alone or media with 10% FBS. GM-CSF, IL-6, IL-8, TGF-β₁, and VEGF concentrations in supernatants were assessed at 24 h.

In both NM and NP cell cultures, DL at 10^{-5} M showed a significant inhibitory effect on FBS-induced GM-CSF secretion in both NM (124.5 ± 35.5 pg/mL, $n = 6$, $P < 0.05$) and NP (79.9 ± 28.8 pg/mL, $n = 6$, $P < 0.05$), compared with FBS alone (mucosa: 255.6 ± 90.6 pg/mL; polyp: 332.9 ± 211.9 pg/mL). Desloratadine at 10^{-6} M also showed a significant inhibitory effect in both NM (95.4 ± 22.4 pg/mL, $n = 6$, $P < 0.05$) and NP (108.5 ± 44.6 pg/mL, $n = 6$, $P < 0.05$), compared with FBS alone. Desloratadine from 10^{-7} to 10^{-8} M had no significant inhibitory effect on GM-CSF secretion induced by FBS from both NM and NP cell cultures (Fig. 1). Desloratadine at 10^{-5} M had no significant effect on GM-CSF spontaneous secretion from either NM or NP cell cultures.

Desloratadine had no significant effect on spontaneous or FBS-induced secretion of IL-8, IL-6, TGF-β₁, or VEGF in either NM or NP cell cultures (data not shown).

Cell viability and drug cytotoxicity

DMSO, at the highest final concentration present in the culture media (0.1% DMSO) had no significant effect on either spontaneous or FBS-induced cytokine secretion in either NM or NP epithelial cell cultures (data not shown).

The LDH activity in the culture supernatants, expressed as cytotoxicity percentage, was not significantly different in the DL-treated cells for both NM ($10 \mu\text{M}$ DL: $-2.5 \pm 1.2\%$, $n = 6$; $10 \mu\text{M}$ DL + 10% FBS: $2.3 \pm 0.9\%$, $n = 6$) and NP ($10 \mu\text{M}$ DL: $1.0 \pm 0.8\%$, $n = 6$; $10 \mu\text{M}$ DL + 10% FBS: $3.8 \pm 1.0\%$, $n = 6$) with respect to media-treated cells.

Effect of desloratadine on eosinophil survival

Nasal mucosa HECM at 10% significantly ($P < 0.05$) increased the eosinophil survival, compared with control, from days 2 to 4, while NP HECM at 10% significantly ($P < 0.05$) increased the eosinophil survival index from days 1 to 4 (Table 2).

The pre-incubation of eosinophils with DL decreased their viability in a dose-related manner in experiments using HECM from both NM and NP. Desloratadine at 10^{-5} M showed a significant inhibitory effect on

Table 2. Effect of desloratadine on eosinophil survival induced by epithelial cell conditioned media

	Eosinophil survival index (%)				
	Day 1	Day 2	Day 3	Day 4	<i>n</i>
Nasal mucosa					
Control media	77.6 ± 4.4	30.9 ± 8.2	8.3 ± 3.4	4.7 ± 1.8	9
10% HECM	87.2 ± 4.1	65.1 ± 7.0**	42.1 ± 7.3*	22.4 ± 6.3*	9
+DL 10 μM	80.8 ± 4.6	50.6 ± 10.4 [‡]	19.9 ± 5.5 [‡]	12.8 ± 4.0 [‡]	9
+DL 1 μM	91.6 ± 2.9	64.9 ± 6.1	28.7 ± 7.7 [‡]	14.9 ± 4.8	9
+DL 0.1 μM	87.7 ± 1.8	59.1 ± 7.6	30.1 ± 8.6	16.4 ± 5.0	9
Nasal polyps					
Control media	80.9 ± 4.1	44.6 ± 8.0	19.6 ± 5.2	7.3 ± 1.8	11
10% HECM	94.1 ± 2.3**	73.5 ± 5.7**	45.3 ± 8.1*	36.6 ± 7.1*	11
+DL 10 μM	57.0 ± 13.7 [‡]	36.1 ± 11.6 [‡]	6.2 ± 2.8 [‡]	4.0 ± 1.8 [‡]	11
+DL 1 μM	87.6 ± 2.8 [‡]	76.5 ± 5.9	43.9 ± 8.4	25.0 ± 8.3 [‡]	11
+DL 0.1 μM	92.0 ± 1.4	74.0 ± 5.5	50.0 ± 8.5	29.4 ± 8.6	11

Results are expressed as mean ± SEM. The Wilcoxon signed-rank test was used for analysis.

* $P < 0.05$ compared with control, [†] $P < 0.05$ and [‡] $P < 0.01$ compared with HECM-induced survival.

DL, desloratadine; HECM, Human epithelial cell conditioned media.

HECM-induced survival from days 2 to 4 when the epithelial secretions were from NM, and from days 1 to 4 when the secretions were from NP. Desloratadine at 10^{-6} M also showed a significant inhibitory effect on eosinophil survival at day 3 when using NM secretions, and at days 1 and 4 when using NP secretions, compared with the effect of HECM alone (Table 2).

Finally, DL at 10^{-5} M showed an inhibitory effect on spontaneous eosinophil viability only at day 1 ($38.7 \pm 10.4\%$, $n = 10$, $P < 0.05$), compared with control media ($67.3 \pm 8.2\%$).

Discussion

The main findings of our study are: (1) FBS-induced the secretion of GM-CSF, IL-6, IL-8, TGF- β_1 , and VEGF by cultured epithelial cells from both NM and NP; (2) in both NM and NP epithelial cells, DL selectively blocked the induced secretion of GM-CSF; (3) DL caused an inhibitory effect on eosinophil survival induced by both NM and NP epithelial cell secretions.

We have shown that cultured human NM and NP epithelial cells increased the release of GM-CSF, IL-6, IL-8, TGF- β_1 , and VEGF in response to FBS. These findings are in keeping with previous studies which show that cultured NM and NP epithelial cells express and release GM-CSF, IL-1 β , IL-6, IL-8, and TNF- α [9] and that enzymatically dispersed NP cells release TNF- α and GM-CSF [21]. Our results demonstrate that FBS, but not IL-1 β and TNF- α , stimulate cytokine secretion from primary nasal epithelial cells. However, using other *in vitro* experimental models, it has been shown that IL-1 β and TNF- α can induce cytokine secretion from airway epithelial cells. For instance, TNF- α and IL-1 β increased the release of IL-8 from the human lung epithelial cell line A549 [29] and

the levels of immunoreactive GM-CSF, IL-6, and IL-8 in primary human bronchial epithelial cell cultures [30]. Similarly, TNF- α increases the release of IL-6, IL-8, and GM-CSF in cultured nasal epithelial cells [14].

In human NP epithelial cells, mizolastine and terfenadine inhibited the release of TNF- α and GM-CSF [21, 22], while azelastine reduced the allergically stimulated TNF- α production [20]. In human bronchial epithelial cell cultures, loratadine attenuated the NO₂-induced release of RANTES and soluble intercellular adhesion molecule-1 [31]. In allergic rhinitic patient's loratadine also reduced eosinophil infiltration, ECP, and eosinophil peroxidase in nasal secretions, as well as ICAM-1 expression on nasal epithelial cells [24]. Loratadine also caused a decrease of leukotriene B₄ production by neutrophils [32], inhibited GM-CSF secretion from human leukaemic mast cells (HMC-1), and TNF- α , IL-3, and IL-8 secretion from basophilic cells (KU812) [33]. In human basophils, DL showed an inhibitory effect on IL-4 and IL-13 secretion induced by anti-IgE antibody, calcium ionophore, IL-3, and phorbol ester [34].

In our model, DL showed a selective inhibitory effect on FBS-induced GM-CSF secretion in both NM and NP epithelial cell cultures. In addition, there was no evidence that the effect of DL in our model was caused by a decrease in epithelial cell viability. GM-CSF is a cytokine involved in the promotion of eosinophil chemotaxis, survival and activation, and topical steroids may decrease eosinophil survival by abrogating the promoting effect of epithelial cells by modulating GM-CSF release [9, 16, 18]. Our findings suggest that DL may decrease eosinophil number by abrogating GM-CSF release from epithelial cells. It is of interest to note that the inhibitory effect of DL on epithelial cells and eosinophil survival was reached at higher concentrations than those found after regular

clinical treatment. However, it is possible that cell culture environment does not exactly reproduce the *in vivo* conditions. It remains to be determined whether the FBS and the epithelial conditioned media exerts its effect on epithelial cells and eosinophils, respectively, by different pathways than *in vivo* or whether it represents a strong induction of cytokine secretion and eosinophil survival. As a consequence, the concentration of the inhibitory drug may be higher *in vitro* than *in vivo* conditions. In support of our findings, numerous studies investigating the anti-inflammatory effects of antihistamines have used higher concentrations than those reached *in vivo*. For instance, using different culture conditions, the concentration of oxatomide and cetirizine that decreased eosinophil survival was 20 and 100 μM , respectively [35, 36].

HECM from NM and NP epithelial cells increase eosinophil survival, compared with media-treated cells, until 4 days after culture. Previous studies from our laboratory have demonstrated that supernatants obtained from NM and NP cultured epithelial cells increase eosinophil survival *in vitro* [9, 16, 18]. The stimulatory effect of epithelial cell secretions on eosinophil viability has been previously used to compare the anti-inflammatory effect of drugs. This *in vitro* model has proved to be sensitive to detect differences in drug anti-inflammatory potencies between different topical and systemic anti-inflammatory drugs [4, 18]. Desloratadine showed an inhibitory effect on both spontaneous and epithelial cell-induced eosinophil viability, a finding that suggests that this antihistamine may exert effects by decreasing eosinophil survival.

These results are in keeping with studies demonstrating an anti-inflammatory effect of other antihistamine drugs on eosinophil survival/function. Ciprandi et al. [24] reported that in patients under natural allergen exposure, loratadine and cetirizine significantly reduced eosinophil and meta-chromatic cell nasal counts, the levels of ECP and eosinophil peroxidase in nasal secretions, and ICAM-1 expression on nasal epithelial cells. Similarly, loratadine reduced the eosinophil counts in nasal secretions of subjects with non-AR with eosinophilia [37]. Agrawal and Berro [27] have recently demonstrated that DL induces apoptosis of eosinophils stimulated with IL-5 at 24 and 48 h.

Nasal inflammatory diseases are also characterized by mucus hypersecretion. Cytokines such as TNF- α , IL-1 β , GM-CSF, as well as ECP, may stimulate the output of secretory cells in NM [28]. As DL had an inhibitory effect on both GM-CSF secretion from nasal epithelial cells and eosinophil survival, it may exert inhibitory indirect effect on nasal hypersecretion.

In summary, the present study suggests that DL may exert anti-inflammatory effects by decreasing the secretion of GM-CSF from NM and NP epithelial cells, and also by reducing eosinophil survival. These effects may contribute to the reduction of eosinophilic-related inflamma-

tion in diseases such as rhinitis, nasal polyposis and chronic rhinosinusitis.

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