Superior effect of MP-AzeFlu than azelastine or fluticasone propionate alone on reducing inflammatory markers

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Abstract

Background: MP-AzeFlu, intranasal formulation of azelastine hydrochloride (AZE) and fluticasone propionate (FP), is superior to AZE or FP alone for treatment of allergic rhinitis (AR). However, the precise anti-inflammatory mechanism of action of MP-AzeFlu has not been characterized.

Objective: To investigate the anti-inflammatory effects of MP-AzeFlu compared with AZE or FP alone in an established in vitro model of eosinophilic inflammation.

Methods: Nasal mucosal epithelial cells and peripheral blood eosinophils were obtained from human volunteers. Epithelial cells were stimulated with 10% fetal bovine serum (FBS) in the presence of MP-AzeFlu, AZE, or FP (1:10² to 1:10⁵ dilution). Concentrations of interleukin (IL)-6, IL-8, and granulocyte–macrophage colony-stimulating factor (GM-CSF) were measured by ELISA. Eosinophils were incubated in human epithelial cell–conditioned medium (HECM) and survival assessed by trypan blue dye exclusion. Results are expressed as mean ± SEM percentage secretion/survival compared with FBS/HECM (respectively).

Results: FP and MP-AzeFlu (all dilutions) and AZE (1:10²) significantly reduced IL-6 secretion and eosinophil survival compared with positive controls. At 1:10² dilution, IL-6 secretion was significantly lower with MP-AzeFlu (38.3 ± 4.2%, compared with FBS = 100%) than with AZE (76.1 ± 4.9%) or FP (53.0 ± 4.9%). At 1:10² dilution, eosinophil survival was significantly lower with MP-AzeFlu at day 3 (17.5 ± 3.0%) and day 4 (2.4 ± 1.4%, compared with HECM = 100%) than with AZE (day 3: 75.2 ± 7.2%; day 4: 44.0 ± 9.7%) or FP (day 3: 38.5 ± 3.5%; day 4: 14.6 ± 4.0%).

Conclusion: Greater reductions in cytokine secretion and eosinophil survival observed with MP-AzeFlu in vitro may underlie MP-AzeFlu's superior clinical efficacy vs. AZE or FP alone observed in AR patients.

Keywords: Cytokines, Eosinophil survival, Nasal mucosa, Epithelial cells, MP-AzeFlu, Azelastine, Fluticasone propionate, Allergic rhinitis, In vitro model

Background

Allergic rhinitis (AR) is one of the most common chronic diseases, impairing quality of life and causing billions of dollars of lost productivity annually [1]. AR is characterized by upper airway inflammation, sneezing, and nasal congestion, drainage, and itching [1]. Inflammatory mediators and cells in AR include elevated levels of proinflammatory cytokines and eosinophil infiltration [2–5].

Current guidelines recommend intranasal corticosteroids for treatment of AR and, in some cases, the use of oral or intranasal antihistamines [1, 6, 7]. Unfortunately, many patients do not achieve full control of their symptoms and are not satisfied with their treatment [8]. Combination therapy may
be considered for patients with inadequate response to monotherapy [1] or when a prompt response to initial therapy is desired [6]. In particular, combined intranasal azelastine hydrochloride (AZE) and intranasal fluticasone propionate (FP) in a single intranasal formulation (MP-AzeFlu) is recommended as more effective than monotherapy [8] and as a first-line option for moderate-to-severe AR [7, 8].

In randomized studies, MP-AzeFlu was more effective among patients with seasonal AR and perennial AR than AZE or FP alone, [9–13] and was more effective among patients with non-AR than FP alone [14]. In addition, studies in real-world settings in Europe found significant improvements in AR symptoms with MP-AzeFlu therapy, [15, 16] and a 1-year study of MP-AzeFlu vs. FP alone provided support for the long-term efficacy and safety of MP-AzeFlu in persistent rhinitis [17]. A retrospective US claim database study of AR patients with comorbid asthma has shown, that the AR and asthma related therapy costs were lower when the patients have been treated with MP-AzeFlu than with a free combination of intranasal steroid and intranasal antihistamine [18].

Mechanistic studies have examined the effect of FP (alone and in combination with the antihistamine loratadine) on the expression of inflammatory mediators, including proinflammatory cytokines and eosinophils [19–24]. Typically, FP downregulated cytokine expression and reduced eosinophil survival in these studies, although findings are mixed. In addition, AZE—alone or in combination with other agents—was found to suppress inflammatory markers in several in vitro studies [25–27]. However, the mechanism of action of combined AZE and FP, specifically MP-AzeFlu’s effects on inflammatory mediators, has not been characterized. In particular, it is unknown whether there may be an enhanced anti-inflammatory effect of the two drugs in combination, compared with the individual components, which might underlie the superior clinical efficacy [9–14] of the combination compared with monotherapy.

To study the role of inflammatory mediators in upper airway diseases and the mechanism of action of anti-inflammatory drugs in these diseases, an in vitro model was developed utilizing cultured primary isolated nasal mucosal epithelial cell cultures and peripheral blood eosinophils [28–31]. This in vitro model has been used previously to compare the anti-inflammatory effects of a number of drugs including corticosteroids, chromones, anti-leukotrienes, and second generation antihistamines, [24, 28–34] demonstrating that it is a good model to study the mechanisms of action of these classes of drugs. In a previous study using this in vitro model, the combination of the corticosteroid mometasone furoate and the antihistamine desloratadine reduced interleukin (IL)-6 and (sICAM)-1 secretion and inhibited eosinophil survival induced by epithelial secretions compared with either agent alone [29].

The objective of the current study was to investigate the anti-inflammatory effects of MP-AzeFlu compared with AZE or FP alone in an established in vitro model of eosinophilic inflammation.

Materials and methods

Materials

AZE and FP were provided by MEDA Pharma (Bad Homburg, Germany). Other materials were purchased from commercial sources (see Additional file 1).

Study population

Nasal mucosa specimens were obtained from 12 patients (nine men, three women), ranging in age from 34 to 73 years (mean ± standard deviation, 58.2 ± 3.5 years), who underwent nasal corrective surgery for septal dysmophy, turbinate hypertrophy, or both. The diagnosis of septal dysmophy and turbinate hypertrophy was based on the clinical history and nasal endoscopic exploration. Skin-prick test was positive (allergen sensitization) in three patients (25.0%). None of the patients in this study had clinical AR, chronic rhinosinusitis (CRS), nasal polyps (NP), and/or asthma. Patients were excluded from this study if they were receiving topical or systemic glucocorticoids or antihistamine treatment 4 weeks prior to the surgery or had an upper or lower airway infection 2 weeks prior to the surgery. All patients gave informed consent to participate in the study at the time of surgery. Tissues used in this study were obtained from the Biobank BTIRCE—R100311-016 at Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Scientific and Ethics Committee of Hospital Clinic de Barcelona gave the ethical clearance for this process.

Normodense eosinophils were obtained from nine volunteers (seven women, two men), ranging in age from 39 to 74 years (mean ± standard deviation, 59.1 ± 13.6 years) with >3% peripheral blood eosinophils (mean ± standard deviation, 7.7 ± 1.4%). Patients were excluded if they received topical or systemic glucocorticoid or antihistamine treatment 4 weeks prior to blood extraction or if they had an upper or lower airway infection two weeks prior to blood extraction. Skin-prick test was positive (allergen sensitization) in four patients (44%). Two of the patients had AR (22%), four patients had CRS with NP (44%), and three patients had CRS with NP and asthma (33%). All patients gave informed consent to participate in the study prior to the venipuncture. Scientific and Ethics Committee of Hospital Clinic de Barcelona gave the ethical clearance for this process.
Epithelial cell isolation, characterization, and culture

Nasal mucosa specimens were placed in Ham's F-12 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 μg/mL amphotericin B (Ham's PS) and immediately transported to the laboratory. Epithelial cells from nasal mucosa were isolated by protease digestion using a technique reported previously [23, 24, 28–35] and described briefly in Additional file 1. Culture of epithelial cells is also described in Additional file 1.

Dilution of MP-AzeFlu

Both azelastine hydrochloride and fluticasone propionate were diluted with dimethyl sulfoxide (DMSO) up to 2.39 × 10⁻² M and 7.29 × 10⁻³ M, respectively. These dilutions from each drug (tenfold concentrated compared with MP-AzeFlu) were diluted with culture medium to 2.39 × 10⁻³ M (azelastine) and 7.29 × 10⁻⁴ M (fluticasone), i.e., the concentration of azelastine hydrochloride and fluticasone propionate present in MP-AzeFlu. Further dilutions (from dilution 1:10² to 1:10⁵) were prepared with culture medium.

Generation of human epithelial cell–conditioned media

When epithelial cell cultures reached 80% confluence, the hormonally defined serum-free media were switched to RPMI-1640 media supplemented with antibiotics (penicillin 100 U/mL and streptomycin 100 μg/mL), amphotericin B (2 μg/mL), glutamine (150 μg/mL), and HEPES buffer (25 mM). Because previous studies have shown that non-stimulated epithelial cells produce low levels of cytokines, [23, 24, 28–35] human epithelial cell–conditioned media (HECM) was generated by incubating cells with fetal bovine serum (FBS) at 10% for 24 h. The culture supernatant (HECM) was harvested from wells, centrifuged at 400 g (10 min, 25 °C), sterilized through 0.22 μm filters, and stored at −80 °C. In order to avoid variability in cytokine concentration caused by intrinsic effect on the ELISA final values. In order to avoid variability in cytokine concentration caused by differences in the number of cells present in each culture well, cytokine production was normalized by optical density value obtained by the cell proliferation assay.

Enzyme-linked immunoassays of cytokines and sICAM-1

Concentrations of granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-6, IL-8, and sICAM-1 were measured in HECM using commercial enzyme-linked immunosorbent assay (ELISA) kits. Cytokines selected for analysis were those found at detectable levels in previous studies in which this model was used. The assay detection ranges were 15.6–1000 pg/mL for GM-CSF, 9.38–600 pg/mL for IL-6, and 31.2–2000 pg/mL for both IL-8 and sICAM-1. To verify that the substances used in the different experiments (AZE, FBS, FP) 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 substances showed any intrinsic effect on the ELISA final values. In order to avoid variability in cytokine concentration caused by differences in the number of cells present in each culture well, cytokine production was normalized by optical density value obtained by the cell proliferation assay.

Isolation of peripheral blood eosinophils

Isolation of eosinophils from peripheral blood samples is described in Additional file 1.

Assessment of eosinophil survival

Eosinophils (2.5 × 10⁵ cells/well) were incubated on 24-well tissue culture plates with RPMI (2 mL) in the presence or absence of MP-AzeFlu (dilution 1:10² to 1:10⁵) or equivalent dilutions of AZE (from 2.39 × 10⁻⁵ M to 10⁻⁸ M) or FP (from 7.29 × 10⁻⁶ M to 10⁻⁵ M) for 1 h before the addition of epithelial cell secretions at 10%. 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. Because dead eosinophils become lysed and, consequently, the number of cells present in the culture wells decreases, the results were calculated using the eosinophil survival index instead of the percentage of surviving cells. The eosinophil survival index was calculated as follows: number of eosinophils recovered multiplied by percentage of eosinophil viability divided by number of eosinophils delivered on day 0.
To reduce the variability caused by the incubation of eosinophils with HECM obtained from different nasal mucosa, a mixture of HECM was created with the cell supernatants from all nasal mucosal epithelial cell cultures, and this HECM was used in all eosinophil experimental protocols. Because FP was diluted in DMSO and the HECM added to the eosinophil cultures contained 10% FBS, we investigated the effect of DMSO and FBS on eosinophil survival. Neither DMSO nor FBS at the higher final concentration had a significant effect on eosinophil survival (data not shown).

**Statistical analysis**

Statistical procedures were performed using SPSS 16.0 software (IBM, Armonk, NY, USA). Results are expressed as mean ± standard error of the mean normalized by the optical density value obtained by the cell proliferation assay. A non-parametric test, the Wilcoxon signed rank test, was used in cytokine secretion experiments, and analysis of variance (ANOVA) with the Dunnett multiple comparisons test was used for statistical comparisons in eosinophil survival experiments. P < 0.05 was considered statistically significant.

**Results**

**Effect of FBS on cytokine and sICAM-1 secretion**

In nasal mucosal epithelial cell cultures, FBS increased the secretion of IL-6, IL-8, GM-CSF, and sICAM-1 compared with control medium (Table 1).

**Dose response of MP-AzeFlu, AZE, and FP on cytokine and sICAM-1 secretion induced by FBS in nasal mucosal epithelial cells**

AZE at 1:102 dilution significantly inhibited FBS-induced IL-6 release and increased FBS-induced GM-CSF secretion from nasal mucosal epithelial cells compared with FBS alone (Table 2). FP showed a dose-dependent inhibitory effect on FBS-induced secretion of IL-6, IL-8, and GM-CSF at 1:10² to 1:10⁵ dilutions. MP-AzeFlu, at dilutions 1:10² to 1:10⁵, showed a dose-dependent inhibitory effect on FBS-induced secretion of IL-6 and IL-8. GM-CSF secretion was inhibited by MP-AzeFlu from 1:10³ to 1:10⁵ dilutions, with no effect at 1:10² dilution. AZE, FP, and MP-AzeFlu showed no effect on FBS-induced sICAM-1 secretion.

**Comparison of MP-AzeFlu, AZE, and FP effects on FBS-induced cytokine secretion at the same drug dilutions**

When comparing the effect of FP with MP-AzeFlu (from dilutions 1:10² to 1:10⁵), there were no significant differences on the inhibition of GM-CSF or IL-8 secretion. However, with each drug at dilution 1:10⁵, the inhibitory effect of MP-AzeFlu on IL-6 secretion was significantly greater than that of AZE or FP, as shown by the lower levels of IL-6 secretion with MP-AzeFlu (Fig. 1; see Table 2 for underlying findings at dilution 1:10⁵). In addition, the effect of FP was significantly greater than the effect of AZE. At higher dilutions (1:10³ to 1:10⁵) of FP and MP-AzeFlu, there were no significant differences between drugs in the inhibition of IL-6 secretion.

**Time course of MP-AzeFlu (dilution 1:10²) effects on HECM-induced eosinophil survival from days 1 to 4**

HECM at 10% from nasal mucosal epithelial cells significantly increased eosinophil survival when compared with control medium from days 1 to 4 (Fig. 2). MP-AzeFlu at 1:10² dilution showed a time-dependent inhibitory effect on HECM-induced eosinophil survival from days 2 to 4.

**Dose response and time course of MP-AzeFlu, AZE, and FP on HECM-induced eosinophil survival at days 3 and 4**

At days 3 and 4, MP-AzeFlu and FP (dilution 1:10² to 1:10⁵) significantly inhibited HECM-induced eosinophil survival (Table 3). However, AZE showed an inhibitory effect only at dilution 1:10². At days 3 and 4, the inhibitory effect of MP-AzeFlu at dilution 1:10² was significantly greater than either AZE or FP at the same dilution, as shown by the lower levels of eosinophil survival with MP-AzeFlu (Fig. 3, see Table 3 for underlying findings at dilution 1:10²). In addition, the effect of FP at dilution 1:10² was significantly greater than the effect of AZE at the same dilution. No differences were found when comparing the inhibitory effect of FP with that of MP-AzeFlu from dilutions 1:10³ to 1:10⁵.

**Discussion**

This report shows clear mechanistic effects that are consistent with and may underlie the superior clinical efficacy of MP-AzeFlu compared with corticosteroid or antihistamine alone.

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**Table 1 Effect of FBS on cytokine secretion from epithelial cells**

| Cytokine     | pg/mL normalized by tetrazolium XTT | P     | N   |
|--------------|-------------------------------------|-------|-----|
|              | Control                              | 10% FBS |
| IL-6         | 6799 ± 1892                         | 24480 ± 539.7   | <0.001 | 9  |
| IL-8         | 41190 ± 9873                        | 12,685 ± 16240  | <0.001 | 9  |
| GM-CSF       | 1631 ± 40.7                         | 8202 ± 257.8    | <0.001 | 9  |
| sICAM-1      | 2877 ± 63.4                         | 4396 ± 101.6    | <0.05  | 9  |

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

FBS fetal bovine serum, GM-CSF granulocyte–macrophage colony-stimulating factor, IL interleukin, SEM standard error of the mean, sICAM-1 soluble intercellular adhesion molecule-1
Table 2 Effect of AZE, FP, and MP-AzeFlu on cytokine and sICAM-1 secretion from nasal mucosal epithelial cells

| Agent/dilution | Secretion, compared with FBS (%) | N |
|----------------|----------------------------------|---|
|                | IL-6                | IL-8      | GM-CSF   | sICAM-1  |
| Control media  | 15.3 ± 2.1*          | 18.0 ± 2.7* | 44.3 ± 4.7* | 65.9 ± 5.1* | 9 |
| 10% FBS        | 100                  | 100       | 100      | 100      | 9 |
| AZE            | 1:10^2               | 76.1 ± 4.9* | 90.1 ± 5.8 | 223.5 ± 34.9* | 95.2 ± 2.3 | 9 |
|                | 1:10^3               | 88.7 ± 4.3 | 87.0 ± 5.9 | 93.8 ± 6.0 | 92.8 ± 4.6 | 9 |
|                | 1:10^4               | 84.6 ± 8.3 | 93.3 ± 3.3 | 89.7 ± 5.8 | 96.9 ± 1.8 | 9 |
|                | 1:10^5               | 90.1 ± 3.8 | 91.0 ± 5.3 | 87.6 ± 3.8 | 98.9 ± 1.1 | 9 |
| FP             | 1:10^2               | 53.0 ± 4.9 | 58.5 ± 2.3 | 58.2 ± 5.0 | 852.3 ± 3.5 | 9 |
|                | 1:10^3               | 59.1 ± 5.4 | 62.0 ± 5.7 | 60.2 ± 5.8 | 818.5 ± 5.4 | 9 |
|                | 1:10^4               | 57.7 ± 6.6* | 60.4 ± 3.6 | 56.2 ± 6.5 | 870.3 ± 3.4 | 9 |
|                | 1:10^5               | 75.3 ± 6.9 | 65.4 ± 5.9 | 65.0 ± 7.2 | 849.7 ± 3.3 | 9 |
| MP-AzeFlu      | 1:10^2               | 38.3 ± 4.2 | 55.3 ± 3.4 | 126.9 ± 9.8 | 840.8 ± 4.8 | 9 |
|                | 1:10^3               | 55.0 ± 6.5 | 60.8 ± 4.6 | 61.7 ± 5.7 | 863.6 ± 6.0 | 9 |
|                | 1:10^4               | 52.9 ± 3.1 | 60.8 ± 3.2 | 57.9 ± 5.3 | 927.3 ± 3.2 | 9 |
|                | 1:10^5               | 72.4 ± 7.5 | 65.1 ± 5.2 | 69.7 ± 7.5 | 889.7 ± 2.3 | 9 |

Results are expressed as mean ± SEM. The Wilcoxon signed-rank test was used for analysis. *P < 0.05 compared with 10% FBS-induced secretion.

AZE azelastine hydrochloride, FBS fetal bovine serum, FP fluticasone propionate, GM-CSF granulocyte-macrophage colony-stimulating factor, IL interleukin, MP-AzeFlu intranasal AZE and intranasal FP in a single device, SEM standard error of the mean, sICAM-1 soluble intercellular adhesion molecule-1

Key findings of this study include: (1) FP and MP-AzeFlu at all tested dilutions, and AZE at 1:10^2 dilution, significantly reduced secretion of IL-6 compared with FBS-induced secretion; (2) at 1:10^2 dilution of each agent, the reduction of IL-6 secretion by MP-AzeFlu was significantly greater than with AZE or FP alone; (3) FP and MP-AzeFlu, at all tested dilutions, and AZE at dilution 1:10^2, significantly reduced eosinophil survival at days 3 and 4 compared with HECM alone; and (4) at 1:10^2 dilution of each agent, the decrease of eosinophil survival induced by MP-AzeFlu at days 3 and 4 was significantly greater than with AZE or FP alone.

Findings of the current study are largely consistent with previous research with this in vitro model, further validating the model. As in earlier studies, we found that secretion of IL-6, IL-8, GM-CSF, and sICAM-1 from nasal mucosa epithelial cells was increased in response to FBS, [23, 24, 28–31, 35] and HECM increased eosinophil survival [24, 28–31].

In the present study, the corticosteroid FP and the formulation MP-AzeFlu reduced IL-6 and GM-CSF secretions from nasal mucosal epithelial cells relative to FBS. This is consistent with previous findings for the intranasal corticosteroids budesonide, beclomethasone dipropionate, mometasone furoate, FP, and fluticasone furoate [23, 24, 29, 30, 35]. In this study, MP-AzeFlu findings for IL-8, GM-CSF, and sICAM-1 differed little from FP alone. We also found that FP and MP-AzeFlu reduced eosinophil survival relative to HECM, consistent with previous findings for intranasal corticosteroids [24, 29, 30].

In the present study, the antihistamine AZE reduced IL-6 and GM-CSF secretion and eosinophil survival only at 1:10^2 dilution. These reductions are consistent with previous findings for the antihistamine desloratadine [29, 31]. Our findings for AZE are also consistent with those of previous in vitro studies that found AZE decreased inflammatory markers [25–27]. A possible mechanism for these effects has been suggested by research showing that AZE exhibits direct activity on transient receptor potential vanilloid 1 ion channels in mouse neuronal cells [36]. We found that AZE did not significantly reduce ICAM-1 expression, in contrast to an earlier study that found such a reduction [37].

The stronger effects of MP-AzeFlu on IL-6 secretion and eosinophil survival in the current study, compared with either AZE or FP alone, mirror and possibly underlie the stronger clinical efficacy of the MP-AzeFlu combination compared with its components [9–14]. The relative magnitude of in vitro effects for MP-AzeFlu vs. AZE or FP alone (Figs. 1, 3) appear similar to the relative magnitude of clinical effects of these agents in treatment of AR. For example, the reduction in total nasal symptom
Fig. 2 Time course of MP-AzeFlu effects on HECM-induced eosinophil survival. HECM at 10% from nasal mucosa (black circles) significantly increased eosinophil survival compared with control medium (white circles) from days 1 to 4. MP-AzeFlu (grey circles) at dilution 1:10^2 significantly decreased eosinophil survival induced by HECM from days 2 to 4. Results are expressed as mean ± SEM. ANOVA with Dunnett multiple comparison test was used for analysis (N= 7). *P < 0.05; **P < 0.01 compared with HECM. ANOVA analysis of variance, HECM human epithelial cell–conditioned media, MP‑AzeFlu intranasal azelastine hydrochloride and intranasal fluticasone propionate in a single device. SEM standard error of the mean.

Table 3 Effect of AZE, FP, and MP-AzeFlu on eosinophil survival induced by human epithelial cell–conditioned media

| Dilution | Eosinophil survival index, compared with HECM (%) | N  |
|----------|-------------------------------------------------|----|
|          | Day 3                                           | Day 4 |
| Control medium | 4.3 ± 0.8*                                     | 0.8 ± 0.5*         | 7 |
| 10% HECM | 100                                             | 100   | 7 |
| AZE      |                                                 |                   |
| 1:10^2   | 75.2 ± 7.2*                                     | 44.0 ± 9.7*       | 7 |
| 1:10^3   | 93.7 ± 3.5                                      | 78.8 ± 6.5        | 7 |
| 1:10^4   | 93.1 ± 3.7                                      | 74.2 ± 8.2        | 7 |
| 1:10^5   | 97.9 ± 1.4                                      | 76.1 ± 6.7        | 7 |
| FP       |                                                 |                   |
| 1:10^2   | 38.5 ± 3.5*                                     | 146 ± 4.0*       | 7 |
| 1:10^3   | 54.4 ± 7.3*                                     | 189 ± 4.1*       | 7 |
| 1:10^4   | 55.2 ± 8.3*                                     | 265 ± 5.6*       | 7 |
| 1:10^5   | 57.1 ± 9.0*                                     | 291 ± 6.1*       | 7 |
| MP‑AzeFlu |                                               |                   |
| 1:10^2   | 17.5 ± 3.0*                                     | 24 ± 1.4*        | 7 |
| 1:10^3   | 60.2 ± 5.5*                                     | 226 ± 5.1*       | 7 |
| 1:10^4   | 57.6 ± 7.0*                                     | 216 ± 4.6*       | 7 |
| 1:10^5   | 57.7 ± 6.4*                                     | 304 ± 7.6*       | 7 |

Results are expressed as mean ± SEM. ANOVA with Dunnett multiple comparison test was used for analysis. *P < 0.05 compared with 10% HECM-induced survival. ANOVA analysis of variance, AZE azelastine hydrochloride, HECM human epithelial cell–conditioned media, FP fluticasone propionate, MP‑AzeFlu intranasal AZE and intranasal FP in a single device. SEM standard error of the mean.
conditioned medium; IL: interleukin; MP‑AzeFlu: azelastine hydrochloride streptomycin, and 2 µg/mL amphotericin B; HECM: human epithelial cell–conditioned media, Ham's F‑12 medium supplemented with 100 UI/mL penicillin, 100 µg/mL GM‑CSF: granulocyte–macrophage colony‑stimulating factor; Ham's PS: immunosorbent assay; FBS: fetal bovine serum; FP: fluticasone propionate; CRS: chronic rhinosinusitis; DMSO: dimethyl sulfoxide; ELISA: enzyme‑linked immunosorbent assay; ANOVA: analysis of variance; AR: allergic rhinitis; AZE: azelastine hydrochloride; Abbreviations

Additional file

Supplementary materials and methods.

Fig. 3 Comparison of AZE, FP, and MP‑AzeFlu effects on HECM‑induced eosinophil survival. Eosinophils were incubated for 3 and 4 days with culture medium (white column), 10% HECM (black), or 10% HECM plus AZE (diagonally striped), FP (horizontally striped), or MP‑AzeFlu (grey) at dilution 1:10². Results are expressed as mean ± SEM percentage of eosinophil survival compared with HECM. ANOVA with the Dunnett multiple comparison test was used for analysis (N = 7). *P < 0.05 compared with 10% HECM. ANOVA analysis of variance, AZE: azelastine hydrochloride, HECM: human epithelial cell–conditioned media, FP: fluticasone propionate, MP‑AzeFlu: intranasal AZE and intranasal FP in a single device. SEM standard error of the mean.

with AR were significantly higher when compared with placebo [56, 57]. In addition, it has been shown that GM-CSF and ICAM-1 are important in determining the function of eosinophils, since in the presence of GM-CSF ICAM-1 has been shown to cause significant release of eosinophil-derived neurotoxin and EOS superoxide anion (O2−) generation [58].

Conclusion

In conclusion, we found that both MP-AzeFlu and FP reduce expression of important cytokines and reduce eosinophil survival. MP-AzeFlu lowers both nasal epithelial cell cytokine secretion and eosinophil survival more potently than antihistamine (AZE) or corticosteroid (FP) administered alone. This translational study demonstrates a mechanism of action that may underlie the superior clinical effect of MP-AzeFlu on AR and non-AR when compared with the component drugs used as monotherapy.

Additional file

Additional file 1. Supplementary materials and methods.

Abbreviations

ANOVA: analysis of variance; AR: allergic rhinitis; AZE: azelastine hydrochloride; CRS: chronic rhinosinusitis; DMSO: dimethyl sulfoxide; ELISA: enzyme-linked immunosorbent assay; FBS: fetal bovine serum; FP: fluticasone propionate; GM-CSF: granulocyte–macrophage colony-stimulating factor; Ham's PS: Ham's F-12 medium supplemented with 100 UI/mL penicillin, 100 µg/mL streptomycin, and 2 µg/mL amphotericin B; HECM: human epithelial cell-conditioned medium; IL: interleukin; MP-AzeFlu: azelastine hydrochloride and fluticasone propionate; PBMC: peripheral blood mononuclear cells; SEM: standard error of the mean; sICAM: soluble intercellular adhesion molecule-1.

Authors' contributions

JRF, LP, MPG, IA, BC, SVA, MF, AV, and CP have no competing interests to declare. DC and DTN have competing interests, DC and DTN are employees of Meda Pharma GmbH & Co. KG (A Mylan Company). JM has conducted research/received research grant support or speaker/consultancy fees from Meda Pharma GmbH & Co. KG (A Mylan Company), Allakos, ALK-Abelló A/S, Genetech-Roche, GlaxoSmithKline, Menarini Group, MSD, Novartis, Sanofi-Genzyme-Regeneron, UCB Pharma, and URIACH Group. JRF, LP, MPG, IA, BC, SVA, MF, AV, and CP have no competing interests to declare. DC and DTN are employees of Meda Pharma GmbH & Co. KG (A Mylan Company).

Availability of data and materials

The datasets generated during the current study are not publicly available because the data reside in a proprietary database maintained by Meda.
Pharma GmbH & Co. KG (A Mylan Company); however, data are available from the corresponding author on reasonable request and with permission of Mylan Inc.

Ethics approval and consent to participate
This study was given ethical clearance by the Scientific and Ethics Committee of Hospital Clínic de Barcelona and conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice. All patients gave informed consent to participate in the study. Tissues used in this study were obtained from the biobank at Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS).

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