Establishment of an *in vitro* human nasal epithelium model to study histamine effect & fexofenadine benefit as inverse agonist in prophylactic condition

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INTRODUCTION

- Histamine (HIS) is a major chemical mediator involved in allergic reactions which are mainly mediated by the histamine H1 receptor (H1R) (1)
- H1R activation results in the symptoms of allergic rhinitis (AR). The level of H1R gene expression is strongly correlated with the severity of allergy symptoms in patients with pollinosis (2).
- Fexofenadine (FEX) is a well-known non-sedating antihistamine molecule that binds H1R and prevents histamine binding and subsequent H1R activation. FEX is widely used to relieve symptoms of AR and urticaria.
- Interestingly, FEX, when acting as an inverse agonist, binds the inactive form of H1R and down-regulates constitutive receptor activity. Inverse agonist activity of FEX could prevent HIS induced H1R activation in a more effective manner by shifting the H1R equilibrium toward the inactive state (3).

OBJECTIVES

- Develop a new *in vitro* model based on fully reconstituted human nasal epithelium tissue.
- Assess the effect of HIS and select relevant biomarkers.
- Evaluate effect of FEX, as antihistamine, on inflammatory cytokine Interleukin 8 & 6 (IL-8 & IL-6) and H1R gene expression.
- Explore the benefit of inverse agonist activity of FEX in prophylactic condition versus that of concomitant with HIS.

MATERIALS AND METHODS

- Human nasal epithelial cells were obtained from donors and seeded on Transwell® inserts in MucilAir™ culture medium. Human differentiated nasal epithelium tissue was obtained from the air-liquid interface (ALI) culture **(Figure 1).**
- Effects of basal exposure to HIS or in combination with FEX on nasal epithelium tissue were studied by measurements of multiple endpoints: tissue integrity (transepithelial electrical resistance (TEER)) , cytotoxicity (lactate dehydrogenase (LDH) release), functional activities such, as cilia beating frequency (CBF) and mucociliary clearance (MCC), inflammatory cytokine secretion (IL-6 & IL-8) and H1R gene expression (real-time Taqman RT-PCR) **(Figure 2).**

➢ Different scenarios were studied to assess the impact of prophylactic vs. concomitant exposure of FEX:

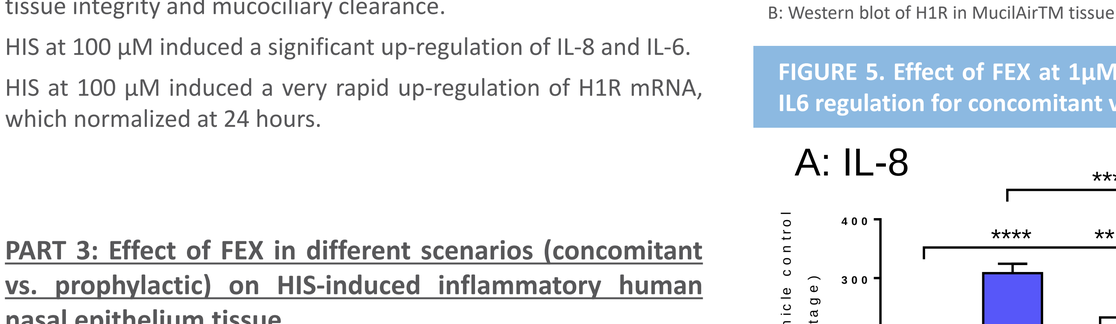
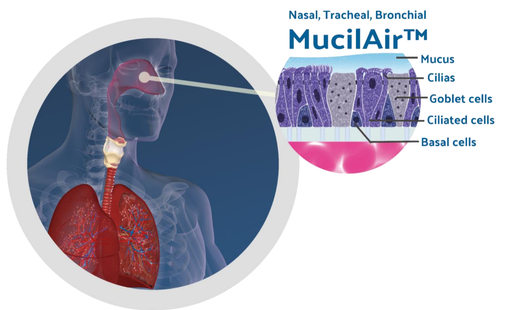
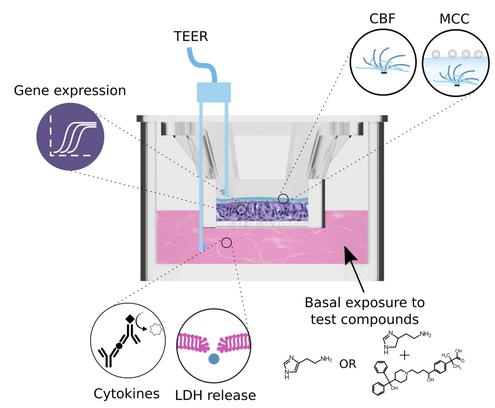


FIGURE 1. Human nasal epithelial cells



Cells were isolated from primary tissue and expanded once. Pooled nasal epithelial cells from 14 individual donors were seeded on 6.5-mm Transwell® inserts in MucilAir™ culture medium (EP04MM, Epithelix, Geneva, Switzerland). Once confluent, cultures were switched to air-liquid interface (ALI). At least 28 days are needed to obtain fully differentiated epithelia. Average culture time post ALI was 43 days.

FIGURE 2. Culture condition and endpoint measurements



Basal exposure to HIS (10 mM-10 nM) or in combination with FEX (100 µM and 10 mM-1 nM) has applied up to 4 days in different FEX scenarios: prophylactic (from 1 hour to 1 week) vs. concomitant exposure.

RESULTS

❑ **PART 1: Reconstituted tissue characterization (Figure 3)**

- Tissue differentiation observed by histological staining (A) highlighting 3 cell types:
  - ✓ Basal, goblet (in blue, containing the mucus) and ciliated (visible cilia on the top).
- Reconstituted tissue expressed H1R (B).

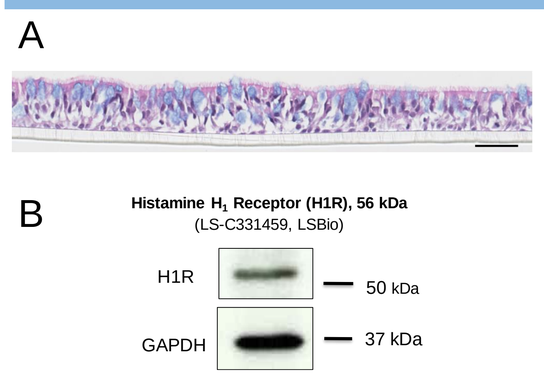
❑ **PART 2: Effect of HIS on reconstituted human nasal epithelium tissue (healthy MucilAir™-Pool) (Figure 4)**

- Maximum tolerated dose of HIS exposure was 100 µM, with no cytotoxicity (LDH measurements, data not shown) and unchanged tissue integrity and mucociliary clearance.
- HIS at 100 µM induced a significant up-regulation of IL-8 and IL-6.
- HIS at 100 µM induced a very rapid up-regulation of H1R mRNA, which normalized at 24 hours.

❑ **PART 3: Effect of FEX in different scenarios (concomitant vs. prophylactic) on HIS-induced inflammatory human nasal epithelium tissue**

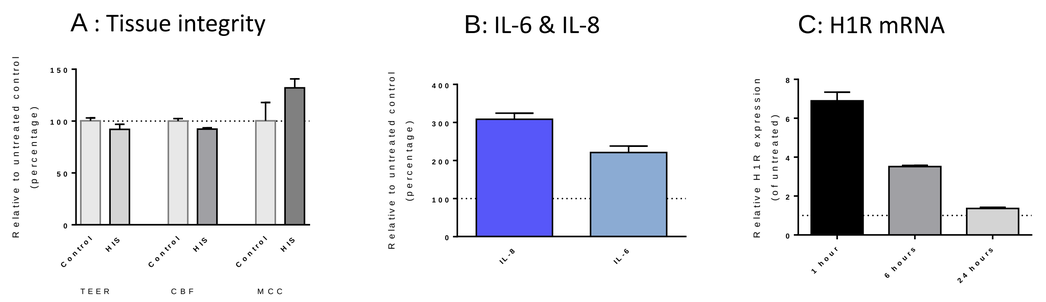
- Effect of FEX (1 µM) in prophylactic condition (1h before HIS exposure) induced significant:
  - ✓ Higher down-regulation of IL-8 & IL-6 vs. concomitant condition **(Figure 5).** Similar results observed at 24h or 1 week prior to HIS exposure (data not shown).
  - ➢ It seems that Higher effect on IL8 vs. IL6 in concomitant condition is observed.
- ✓ Higher down-regulation of H1R gene expression vs. concomitant condition in dose dependent manner **(Figure 6).**

FIGURE 3. Nasal Mucilair™-Pool reconstituted from 14 Healthy individual donors



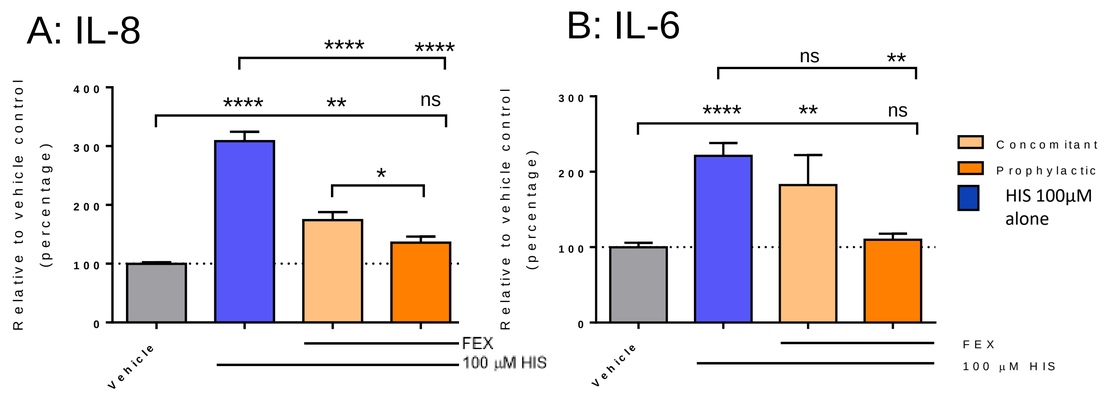
A: Haematoxylin-eosin and Alcian blue staining on transversal section of nasal MucilAirTM-Pool. Scale bar is 50 mm.
B: Western blot of H1R in MucilAirTM tissue

FIGURE 4. Effects of HIS on reconstituted human nasal epithelia



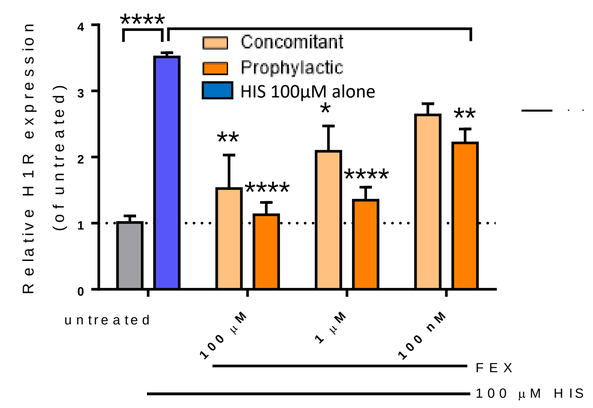
A : Tissue integrity: Effect of repeated (4 days) basal exposure to 100 µM HIS in healthy MucilAirTM-Pool .Representative data are shown for tissue integrity (TEER, day 2), cilia beating frequency (CBF, day 4) and mucociliary clearance (MCC, day 4) (n= 4 cultures, mean+SEM, Student's t tests, $p<0.05$, GraphPad Prism).
B: IL-8 and IL-6 secretion in basal culture medium of MucilAirTM-Pool after 100 µM HIS repeated (2 days) exposure. Cytokine secretions were measured by ELISA at day 2 (8 independent experiments, n=51 cultures).
C: H1R gene expression level at different times (from 6h to 24h) by RT-PCR after 100 µM HIS exposure in the tissue (n= 3 cultures, mean+SEM).

FIGURE 5. Effect of FEX at 1µM on HIS-induced inflammatory tissue model: comparison of IL-8 & IL6 regulation for concomitant vs. prophylactic scenarios.



Inhibitory effect of FEX on HIS-induced increase of IL-8 (A) and IL-6 (B) : 2 days after HIS application. Repeated (2 days) basal exposure to 100 µM HIS in MucilAirTM-Pool with basal co-exposure of 1 mM FEX concomitantly or with 1 hour before HIS exposure (prophylactic scenario). Co-exposure data are expressed in % of vehicle treated cultures (0.01 % DMSO) (vehicle n=32, HIS n=51, concomitant FEX n=22, and prophylactic scenario n=19 cultures, mean+SEM). One-way ANOVA with Dunnetts multiple comparison post-tests, *p<0.05, **p<0.001, ***p<0.001, ****p<0.0001 (GraphPad Prism).

FIGURE 6. Effect of FEX at different concentrations on HIS-induced inflammatory tissue model: comparison of H1R gene expression level for concomitant vs. prophylactic scenarios.



Dose-dependent inhibitory effect of FEX on HIS-induced increase of H1R expression 6 hours after HIS exposure. FEX was applied concomitantly or 1 hour before HIS exposure (prophylactic scenario) . Relative gene expression is calculated by normalization of GAPDH gene and relative to untreated cultures (fold change) (n=3, mean+SEM). Student's t test or one-way ANOVA with Dunnett's multiple comparison post-tests, *p<0.05, **p<0.001, ***p<0.001, ****p<0.0001 (GraphPad Prism).

CONCLUSION

- These data suggest that IL-8, IL-6 & H1R gene expression levels are promising biomarkers of HIS-induced inflammatory in human nasal epithelium model.
- In prophylactic scenario, higher anti-inflammatory activity of FEX (inhibition of both IL-8 & IL-6 inflammatory cytokines & down-regulation of H1R gene expression) has been demonstrated; these new findings correlate with inverse agonist FEX mode of action.
- These data suggest that starting FEX administration prior to allergen exposure -before the symptoms appear- or when the first symptoms are occurring could have a protective effect and better control of the intensity of nasal symptoms during allergic episodes.
- Histamine induced inflammation state on in vitro nasal epithelium could be a useful platform to study or screen anti-inflammatory compounds or new modalities like probiotics.

References

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Disclosures

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