Effect of corticosteroids on epithelial cell response to non-typeable *Haemophilus influenzae*

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Introduction

- Non-typeable *Haemophilus influenzae* (NTHi) is the most common bacteria present in the airway of patients with neutrophilic COPD (1). The bacteria is found persistently in 30% of patients and in 50% during exacerbation (2,3).
- Bacterial infection induces an inflammatory response of epithelial cells with increased release of cytokines including IL-8, which as a chemoattractant, recruits neutrophils.
- Neutrophilic patients show no clear response to inhaled corticosteroid (ICS) therapy (4) and there is evidence showing that ICS dose correlates with bacterial load (5), suggesting that ICS treatment might be causally linked to bacterial presence and persistence.
- We examine the effect of corticosteroids on the inflammatory response of human bronchial epithelial cells when infected with NTHi.

Hypothesis

Inflammatory response of bronchial epithelial cells upon infection with NTHi is reduced with corticosteroid treatment.

Methods

Summarised in Diagram 1 below.

1) Human bronchial epithelial cells (Lonza) from three non-smoking donors were grown to 90% confluence and treated with 0.16nM, 1.6nM and 16nM Budesonide (equivalent 0.0689µg/L, 0.689µg/L and 6.89µg/L respectively), or 0.1nM, 1nM and 10nM Fluticasone propionate (equivalent 0.05µg/L, 0.5µg/L and 5µg/L respectively), referred to as low, mid and high concentrations of corticosteroids, for two hours.

2) Epithelial cells were then infected with $1x10^6$ CFU of NTHi which had grown on chocolate agar for 24 hours.

2) Cultures were incubated for a further two hours before supernatants were collected and tested for IL-8 concentration by ELISA (R&D Systems). IL-8 release was taken as a measure of inflammatory response.

- Paired T tests were used to compare groups.

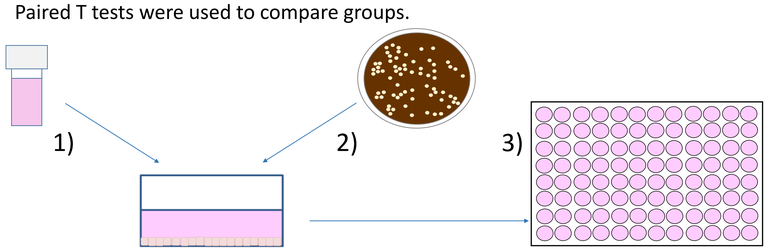

Diagram 1: 1) Corticosteroid addition to bronchial epithelial cells in basal culture. 2) NTHi growth on agar plate before addition to the epithelial cells. 3) Cell supernatants measured for IL-8 by ELISA.

Results

- IL-8 release from bronchial epithelial cells is increased two-fold upon infection with NTHi compared to basal release (mean: 175.7pg/ml (95% CI: 125.8-225.7), to 370.2pg/ml (95% CI: 257.6-482.6, p=0.0063).
- Results from both corticosteroids tested at comparable doses were pooled for corticosteroid class results (Figure 1).
- NTHi treated bronchial epithelial cells showed higher IL-8 release than with any dose of corticosteroid (mean: 370.2pg/ml (95% CI: 257.6-482.7) to 221.4pg/ml (95% CI: 158.8-283.9, p=0.0048), 225.1pg/ml (95% CI: 165.7-284.5, p=0.0054 and 189.2pg/ml (95% CI: 137.6-240.7, p=0.0021) respectively from lower to higher concentration).
- Corticosteroid treatment alone does not alter IL-8 release (p=0.3225).

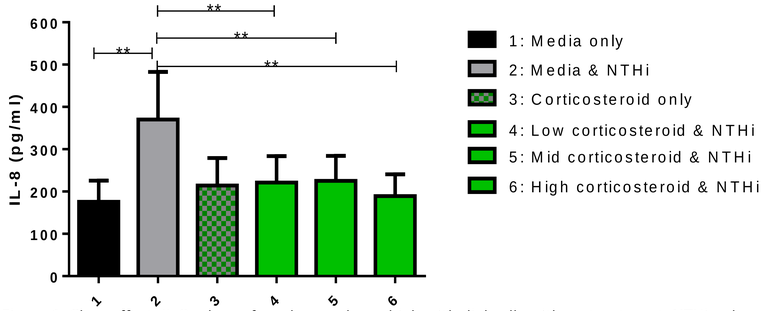

Figure 1: Class effect. IL-8 release from human bronchial epithelial cells with no treatment, NTHi only, steroid only or simultaneous NTHi and steroid treatment. (**=p<0.01).

- Addition of the corticosteroids was then analysed individually (Figure 2). Upon addition of Budesonide or Fluticasone propionate at any concentration, a trend to reduced IL-8 release from bronchial epithelial cells was observed compared to NTHi treatment alone, ranging from 33.4% to 49% reduction.
- This reduction reached statistical significance for the high concentrations of Budesonide (mean: 370.2pg/ml (95% CI: 195.2-544.9) to 189.1pg/ml (95% CI: 104.8-273.4, p=0.0451) and Fluticasone propionate (to 189.2 (95% CI: 115.9-262.5, p=0.0460).
- Budesonide and Fluticasone propionate addition to bronchial epithelial cells shows no change in IL-8 release compared to media only (p=0.5696 and p=0.4365 respectively).

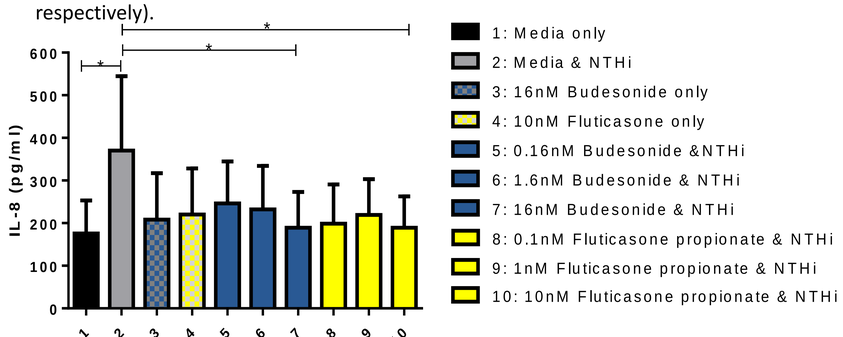

Figure 2: Drug effect. IL-8 release from human bronchial epithelial cells with no treatment, NTHi only, steroid only or simultaneous NTHi and steroid treatment. (*=p<0.05).

Discussion

- ICS are the mainstay treatment for patients with COPD exacerbation.
- IL-8 is a chemoattractant for neutrophils. NTHi infection causes an increase of IL-8 release to draw more neutrophils to the area to tackle the infection.
- This increase of IL-8 release is dampened upon corticosteroid treatment of bronchial epithelial cells, a major receptive area of ICS.
- Ineffective draw of neutrophils to the site of infection reduces the hosts immune defence to remove the infection, allowing bacterial persistence; and may be a causal link to the increased risk of pneumonia with ICS (5, 6). Further studies are warranted.

Conclusion

- We conclude that NTHi stimulates release of IL-8 from bronchial epithelial cells.
- As a class, corticosteroids dampen this stimulated release.
- 16nM Budesonide and 10nM Fluticasone propionate prevent stimulation of greater IL-8 release from human bronchial epithelial cells *in vitro*.
- This may in part be responsible for the higher neutrophil count associated with those COPD patients with persistent NTHi infection.
- Further work is required to determine how neutrophils are affected by the reduction of detectable IL-8 in this model.

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