The Effect of Corticosteroids on Internalisation of Non-Typeable *Haemophilus Influenzae* Into Epithelial Cells

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Introduction

Non-typeable haemophilus influenzae (NTHi) is the most commonly detected persistent bacteria in the airways of COPD patients[1], in particular those with non-eosinophilic inflammation[2]. Increased inhaled corticosteroid dose has been linked to increased airway bacterial load and higher frequency of developing pneumonia[1]. Internalisation of NTHi into epithelial cells of the bronchi poses a potential mechanism by which the bacteria evade clearance by the host response and antibiotic treatment. Corticosteroid treatment may enhance this mechanism in this group of patients.
Here we compare the effect of two inhaled corticosteroids on the amount of NTHi internalised into bronchial epithelial cells.

Hypothesis

Corticosteroid treatment enhances internalisation of NTHi into bronchial epithelial cells.

Methods

Human bronchial epithelial cells from three healthy non-smoking donors were grown to 90% confluence at the third passage, up to three times. The cells were treated with high, medium or low concentration corticosteroid (16nM, 1.6nM and 0.16 Budesonide, or 10nM, 1nM and 0.1nM Fluticasone propionate) for two hours prior to addition of $1x10^6$ CFU of NTHi (Diagram 1). Cultures were incubated for a further two hours before the epithelial cells were treated with gentamycin to remove external bacteria. DNA was extracted and NTHi presence tested by qPCR targeting the OMP P6 gene.

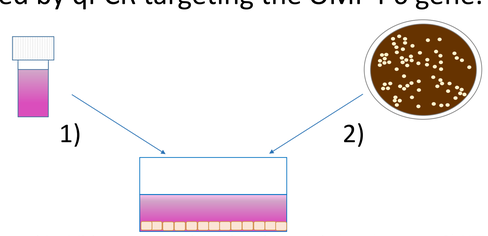

Diagram 1: 1) Corticosteroid addition to bronchial epithelial cells in basal culture. 2) NTHi growth on agar plate before addition to the epithelial cells.

Results

Results are shown as a percentage of the total bacteria initially added to the cells. Untreated epithelial cells showed 4.62% mean internalisation of NTHi (SD: 4.22) (Figure 1). Treatment of epithelial cells with corticosteroids showed a stepwise trend to decreased internalisation with the highest concentration added showing a 40.7% decrease in internalised NTHi detection (to mean: 2.74, SD: 1.89). No significant changes in internalisation were observed.

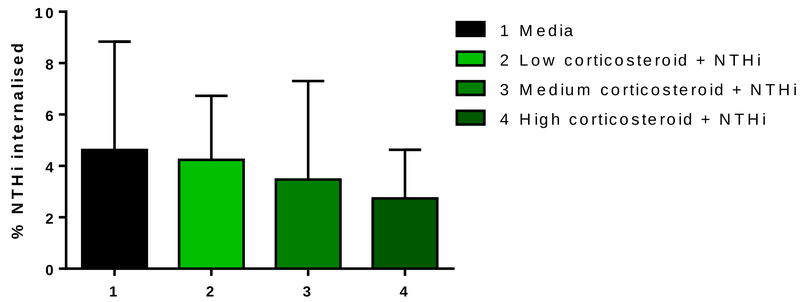

Figure 1: Percentage of total NTHi added which has internalised into bronchial epithelial cells. Cells were either untreated (media only), or treated with increasing concentrations of corticosteroids Fluticasone propionate or Budesonide individually. Low, medium and high concentrations relate to those shown in figure two.

Individually, Budesonide and Fluticasone propionate treatment do not result in differences in NTHi internalisation (Figure 2).

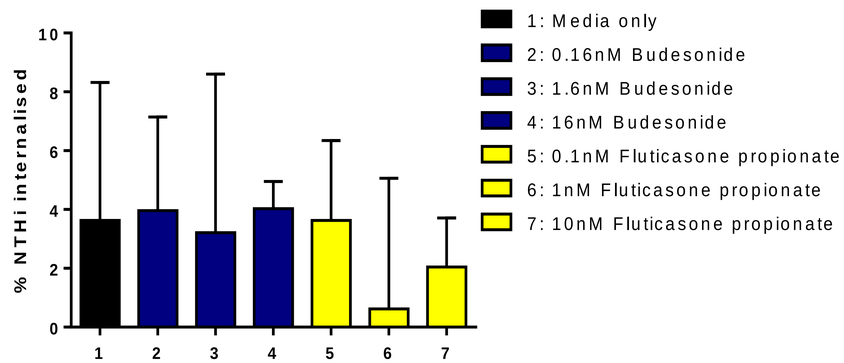

Figure 2: Percentage of total NTHi added which has internalised into bronchial epithelial cells. Cells were either untreated (media only), or treated with increasing concentrations of corticosteroids Fluticasone propionate or Budesonide.

Discussion

We have shown that corticosteroid treatment does not affect the amount of internalisation of NTHi into bronchial epithelial cells. The two inhaled corticosteroids Fluticasone propionate and Budesonide show this result on an individual basis. Levels of internalisation varied between cells from three donors. This study uses bronchial epithelial cells from healthy donors and therefore does not address differences in health and disease.

NTHi internalisation is thought to occur through attachment and penetration rather than lipid-raft mediated endocytosis[3,4]. This internalisation is likely to be enhanced when integrity of the bronchial epithelial cell barrier is low. Corticosteroid treatment improves epithelial barrier function *in vitro* to a greater extent in health compared to COPD, with less effect seen in more severe COPD[5]. Heijink et al relate this finding to oxidative stress, which is greater in more severe disease. Further studies are required to assess whether oxidative stress induced epithelial cell damage allows NTHi internalisation by impairing barrier integrity.

Conclusion

Internalisation of NTHi into human bronchial epithelial cells is not affected by corticosteroid treatment prior to their infection. This suggests that the epithelial barrier is not compromised by the inhaled corticosteroids tested to allow bacterial persistence by this route. No differences between NTHi internalisation with Budesonide or Fluticasone propionate treatment were seen.

References

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