Glucocorticoid receptor α and β expression in airway epithelial cells infected with NTHi

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Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease with distinct inflammatory phenotypes. Patients with non-eosinophilic inflammation typically respond poorly to corticosteroid treatment[1] and have higher incidences of bacterial infection[2]. *Haemophilus influenzae* is the most frequent bacterium detected in patients with COPD[3]. Here we investigate the effect of non-typeable *Haemophilus influenzae* (NTHi) infection on the expression of the glucocorticoid receptor (GR), through which corticosteroids act, in airway epithelial cells.

Hypothesis

NTHi infection downregulates the active GR alpha-isoform (GRα) and upregulates its inhibitory beta-isoform (GRβ) in airway epithelial cells, thereby inhibiting their response to corticosteroids.

Methods

- Beas2B cells, A549 cells and primary human bronchial epithelial cells were grown to 90% confluency in 24-well plates.
- Cells were treated with fluticasone propionate (FP) at 1 or 100nM for 2 hours prior to infection with 1.5 x 10^4, 1 x 10^6 and 1.5 x 10^7 CFU/ml NTHi (low, medium and high load respectively).
- 6 hours post-infection supernatants were collected and RNA extracted from cells by the E.Z.N.A. Total RNA Kit I Protocol.
- RNA was reverse transcribed using the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit.
- Quantitative PCR was carried out according to the Fast SYBR Green Master Mix Protocol to determine the relative cDNA levels of GRα, GRβ and GAPDH in triplicate.
- Results were analysed by the Pfaffl method.
- Statistical analyses were performed with GraphPad Prism 7.03.

Results

FIGURE 1: Expression of GRα and GRβ in A549 and Beas2B cells treated with low, medium or high concentrations of NTHi and/or FP at 1nM or 100nM, relative to untreated (control) cells. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

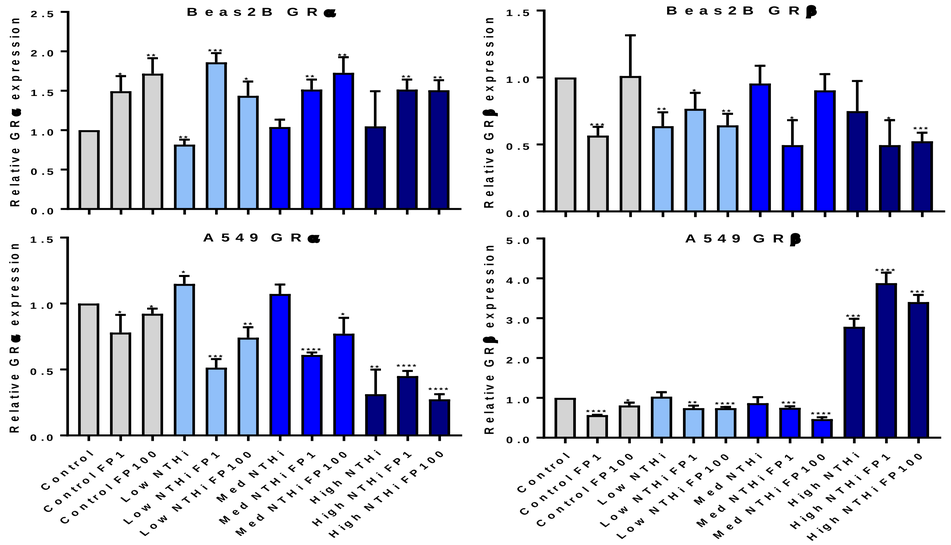

FIGURE 1: NTHi had no consistent effect on GRα and GRβ expression in the Beas2B cells, though there was a small stepwise increase in GRα expression with FP treatment to 1nM to 100nM (mean fold changes from control +0.495 and +0.717 respectively, p<0.05).

In the conditions with the high NTHi concentration in the A549 cells, GRα expression decreased (mean fold changes from control: -0.687, -0.549, and –0.725 for 0nM, 1nM and 100nM FP respectively, p<0.01) while GRβ expression increased (mean fold changes from control: +1.78, +2.88, and +2.41 for 0nM, 1nM and 100nM FP respectively, p<0.001).

FIGURE 2: Expression of GRα and GRβ in HBECs treated with low, medium or high concentrations of NTHi and/or FP at 1nM or 100nM, relative to untreated (control) cells. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

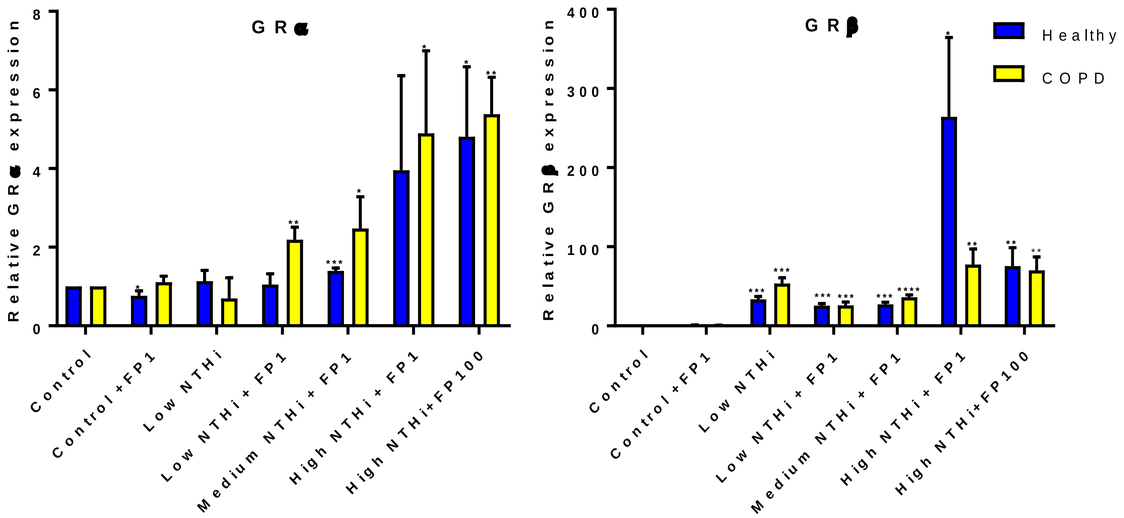

FIGURE 2: In both the HBECs, GRα expression increased in conditions with the high NTHi concentration and pre-treatment with FP. Moreover, GRβ expression was significantly increased in all conditions that included NTHi (e.g. for the high NTHi concentration and 100nM FP mean fold changes from control: +74.5 and +69.3 for healthy and COPD HBECs respectively, p<0.01).

Conclusion

- GRβ expression significantly increased with the high NTHi concentration in A549 cells and with all three NTHi concentrations in both the healthy and COPD HBECs.
- This trend was apparent with and without pre-treatment with FP, but was not observed in the Beas2B cells.
- GRα expression was not consistent between the different cell types, though both the healthy and COPD HBECs showed a similar response.

References

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Funded By

Oxford Biomedical Research Centre

NHS National Institute for Health Research

All authors have no commercial support or relevant financial interests to disclose.