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A novel glucocorticoid-sensitising agent for the treatment of respiratory diseases

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Introduction: Glucocorticoid resistance limits the effective treatment of chronic inflammatory diseases of the respiratory tract, including severe asthma and chronic obstructive pulmonary disease (COPD). Our previous work has shown that transforming growth factor-beta (TGF-β) induces glucocorticoid insensitivity in bronchial epithelial cells through a signalling pathway distinct from Smad signalling, or MAP kinase pathways (1). Viral infection of the lower respiratory tract with respiratory syncytial virus (RSV) also results in a state of glucocorticoid insensitivity which we have shown to be mediated by TGF-β activity. Global inhibition of TGF-β signalling increases the risk of cancer and autoimmune disease. In the current study, we demonstrate that TGF-β-induced and RSV-induced glucocorticoid insensitivity is prevented by the casein kinase 1 δ/ε (CK1δ/ε) inhibitor, PF670462.

Methods: *In vitro*glucocorticoid responsiveness of bronchial epithelial cells (BEAS-2B, air-liquid interface (ALI)-differentiated primary human bronchial epithelial cells (HBEC)) was assessed by RT-qPCR analysis. Cells were pre-treated with PF670462 (0.1 - 10 μM) for 30min prior to incubation with TGF-β (40pM) for 24 h then dexamethasone (Dex; 10-100 nM) for 4 h. *In vivo* glucocorticoid responsiveness was assessed in Balb/C mice infected with RSV through intranasal insufflation. Mice were treated 2 days later with either Dex (1mg/kg/day, ip), PF670462 (30mg/kg/day, ip), or both for 3 days. Bronchoalveolar lavage (BAL) was performed from which protein content and cell number was determined. RT-qPCR was performed on mRNA from whole lung extracts.

Results: In BEAS-2B cells, 3 μM PF670462 restored Dex-induced ENaCα mRNA from 38±2% to 95±5% in the presence of TGF-β ($p<0.05$, n=5). Similarly, FKBP5 mRNA was enhanced from 59±4% to 139±11%, PLZF from 71±3% to 129±3%, and GILZ from 77±4% to 102±5% ($p<0.05$, n=5). In ALI-HBEC from 3 individual donors, TGF-β inhibition of Dex-induced expression of the genes ENaC and PLZF was prevented. RSV-induced increases in cell number in BAL fluid (from $0.74\pm0.04 \times 10^6$ to $1.58\pm0.19 \times 10^6$) was partially sensitive to Dex treatment ($1.16\pm0.10 \times 10^6$) but was further reduced by the Dex/PF670462 combination ($0.92\pm0.11 \times 10^6$; $p<0.05$, n=6). RSV-induced increases in BAL protein content and lung expression of IL-6 mRNA were reduced only by the Dex/PF670462 combination, not by either agent alone. No effect on viral load was detected following Dex/PF670462 treatment.

Conclusions: We have identified PF670462 as a novel glucocorticoid sensitising agent with therapeutic potential to restore anti-inflammatory efficacy in glucocorticoid-resistant disease.

References: 1. Keenan CR et al. (2014). *Respir Res* **15**:55