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Non-Trainee

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Basic Science

Abstract Title
Oxidized Phospholipids Inhibit Glucocorticoid Receptor Nuclear Translocation and Expression of Anti-inflammatory Genes in Airway Epithelial Cells:

Background
Inhaled glucocorticoids (GC) are primary controller therapies for asthma. Upon binding GC, glucocorticoid receptors (GR) translocate to the nucleus, bind GRE elements , and activate anti-inflammatory gene transcription. Oxidized phosphatidylcholines (OxPAPC) accumulate in the airways of asthmatics to promote cytokine release, airway narrowing and airway hyperresponsiveness. OxPAPC activate intracellular kinases, and transient receptor potential ankyrin 1 (TRPA1)-mediated Ca2+ influx in airway epithelial and smooth muscle cells.

Objective
We tested the hypothesis that OxPAPC inhibit GC-induced anti-inflammatory gene expression by modulating nuclear translocation of GR.

Methods
We measured fluticasone propionate (FP; 1μM, 5 hrs) induced transcription in the human bronchial epithelial luciferase reporter cells, 2XGRE-BEAS 2B. In some experiments, we pre-exposed cells to pathophysiological concentrations of OxPAPC (80μg/mL, 0-18 hours). Luciferase was measured by Firefly Luciferase assay. In some studies, we pre-incubated cells with TRPA1 inhibitor (HC030031, 10μM). We used qRT-PCR to measure GC-inducible mRNA (below), and tracked nuclear co-localization of GR using quantitative fluorescent immunocytochemistry. Expeeriments included at least five replicates, and data was analyzed using one-way ANOVA and Dunnett's post hoc testing.

Results
In 2xGRE BEAS-2B cells, OxPAPC pre-exposure time dependently reduced FP-induced luciferase transcription up to 62% (3,641±29.7 vs 9,622±705.7 units, 18hrs). TRPA1 inhibition did not alter FP-induced luciferase, however it markedly inhibited OxPAPC suppression of FP-induced genes: KLF9 (-93.7%, $p<0.01$), FKBP5 (-94.8%, $p<0.001$) and GILZ (-79.8%, $P<0.058$). TRPA1 inhibition partially (~25%), but not significantly, reversed the suppressive effects of OxPAPC. Nuclear co-localization of GR after FP exposure (1μM, 2 hrs) was also inhibited by OxPAPC (40 μg/mL) (ie. Nuclear Co-localization Coefficient 0.852 vs 0.621, $P<0.01$).

Conclusion
OxPAPC reduces GR-dependent transcription and GC-inducible gene expression, likely by inhibiting GR nuclear translocation in airway epithelial cells. This suggests a mechanism for OxPAPC to contribute to steroid insensitivity and promote persistent inflammation in asthma.

Authors

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