Oxidative Stress Driven Inflammatory Responses in Lung Epithelial Cells

COPD, Inflammation, Epithelial cell

F. Tarhini1, L. Dunning1, A. Crilly1, J. Brzeszczynska1, L. McGarvey2, K. Thornbury3, C. S.Goodyear4, J. C.Lockhart1, G. J.Litherland1

1University of the West of Scotland - Paisley, Scotland (United Kingdom), 2Queens University Belfast - Belfast, Northern Ireland (United Kingdom), 3Dundalk Institute of Technology - Dundalk, Ireland (Ireland), 4University of Glasgow - Institute of Infection, Immunity & Inflammation (United Kingdom). This study was funded by the EU under the Interreg VA Programme, managed by the Special EU programmes body (SEUPB).

Cigarette smoke stimulates an inflammatory response and produces oxidants that cause oxidative stress in the lung, promoting pathophysiological changes related to chronic obstructive pulmonary disease (COPD). Hydrogen peroxide (H2O2) is an important oxidant detected in breath condensate of COPD patients2. We aim to understand how chronic exposure to H2O2 alone or in combination with other inflammatory mediators influences epithelial cell responses relevant to COPD lung pathology.

BEAS-2B cells were exposed chronically to H2O2 for 2 h/day for 3 days at different concentrations, alone or in combination with TGF-β (10 ng/ml) or LPS (100 or 500 ng/ml). Cell viability was assessed by MTT assay. Cytokines were measured by ELISA. Intracellular ROS production was detected by CM-H2DCFDA assay. Data were analysed using one-way ANOVA, followed by Multiple Comparison Test.

Cells tolerated a repeated exposure of H2O2 (up to 15 μM) ± TGF-β or LPS without significant loss of viability. Intracellular ROS was significantly elevated in the presence of LPS (mean ± SEM; 217±17 %; p<0.0001) or H2O2 (331±13 %; p<0.0001), with an additive effect of combined treatment (H2O2, 444±12 vs. LPS + H2O2, 604±35 %; p<0.0001). H2O2 stimulated modest release of IL-8 (38±2 pg/ml) and IL-6 (84±13 pg/ml). However, repeated 15 μM H2O2 exposure synergistically enhanced TGF-β induced IL-8 (TGF-β, 194±13 vs. TGF-β+ H2O2, 279±10 pg/ml; p<0.0001) but not IL-6 (TGF-β, 431±22 vs. TGF-β+ H2O2, 449±2 pg/ml). H2O2 synergistically enhanced LPS secretion of both IL-8 (LPS, 2487±21 vs. LPS+ H2O2, 3288±109 pg/ml; p<0.0001), and IL-6 (LPS, 2469±72 vs. LPS+ H2O2, 3277±62 pg/ml; p<0.0001).

Oxidative stress appears to be generated in BEAS-2B cells by LPS or H2O2 alone, and increased in combination. Repeated exposure to H2O2 induced minimal inflammatory response, but synergistically enhanced the effect of TGF-β and LPS on cytokine production. These data suggest combined exposure models may be useful to study the effects of epithelial cell challenges relevant to COPD pathology.
