Oxidative Stress Driven Inflammatory Responses in Lung Epithelial Cells

COPD, Inflammation, Epithelial cell

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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 (H_2O_2) is an important oxidant detected in breath condensate of COPD patients[2]. We aim to understand how chronic exposure to H_2O_2 alone or in combination with other inflammatory mediators influences epithelial cell responses relevant to COPD lung pathology.

BEAS-2B cells were exposed chronically to H_2O_2 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-H_2DCFDA assay. Data were analysed using one-way ANOVA, followed by Multiple Comparison Test.

Cells tolerated a repeated exposure of H_2O_2 (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 H_2O_2 (331±13 %; $p<0.0001$), with an additive effect of combined treatment (H_2O_2, 444±12 vs. LPS + H_2O_2, 604±35 %; $p<0.0001$). H_2O_2 stimulated modest release of IL-8 (38±2 pg/ml) and IL-6 (84±13 pg/ml). However, repeated 15 μM H_2O_2 exposure synergistically enhanced TGF-β induced IL-8 (TGF-β, 194±13 vs. TGF-β+ H_2O_2, 279±10 pg/ml; $p<0.0001$) but not IL-6 (TGF-β, 431±22 vs. TGF-β+ H_2O_2, 449±2 pg/ml). H_2O_2 synergistically enhanced LPS secretion of both IL-8 (LPS, 2487±21 vs. LPS+ H_2O_2, 2898±109 pg/ml; $p<0.0001$), and IL-6 (LPS, 2469±72 vs. LPS+ H_2O_2, 3277±62 pg/ml; $p<0.0001$).

Oxidative stress appears to be generated in BEAS-2B cells by LPS or H_2O_2 alone, and increased in combination. Repeated exposure to H_2O_2 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.

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