Cigarette Smoke Exposure Induces Cytotoxicity and Inflammation in Viable Lung Tissue Slices

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Introduction
Chronic obstructive pulmonary disease (COPD) is a common severe lung disease and a major cause for morbidity and mortality in the world. COPD is characterized by pulmonary emphysema, chronic bronchitis, and histological changes in the lung. A major reason for COPD is cigarette smoking. To understand the underlying biological mechanisms, the need emerged to develop relevant models. Aim of this study is to establish Precision-Cut Lung Slices (PCLS) as a chronic toxicity model by using cigarette smoke and cigarette-smoke condensates (Csc).

Methods
Human and rodent PCLS were prepared and exposed to Csc and LPS submersely, or to cigarette smoke at Air Liquid Interface (ALI). After exposure tissue was cultured up to 5 days. Induced toxicity was assessed by measurement of metabolic activity using WST-1 assay, or by detection of lactate dehydrogenase and LIVE/DEAD® viability staining. Induction of pro-inflammatory immune responses were evaluated by ELISA. Therapeutical intervention was assessed by addition of dexamethasone. Histological structure of lung parenchyma was visualized using hematoxylin and eosin staining.

Results
Csc induced concentration dependent cytotoxicity in murine and human PCLS after 24 h submersion exposure. EC₅₀ values for Csc were 85 µg/mL for murine and 144 µg/mL for human PCLS (Fig. 2).

Cigarette smoke induced concentration dependent toxicity in murine PCLS. The EC₅₀ could be determined at 255 ng/cm² (Fig. 5).

Fig. 2: Vitality of murine (A) and human (B) PCLS after 24 h of exposure to increasing concentrations of Csc was assessed by WST-1 assay. EC₅₀ values were calculated for murine and human PCLS. N=3 for murine PCLS, N=4 for human PCLS in duplicates, *p<0.05, **p<0.001.

Fig. 5: Vitality of murine PCLS after 1 h of exposure to increasing concentrations of cigarette smoke and 2 h post-incubation using WST-1 assay. EC₅₀ value was calculated. N=3.

Treatment of PCLS with Csc did not show histopathological changes of lung tissue after hematoxylin and eosin staining.

Exposure of mouse and human lung tissue to Csc significantly increased levels of pro-inflammatory cytokines (Fig 6). Dexamethasone inhibited Csc induced IL-1α production in human PCLS.

Conclusions
Csc induced tissue injury and inflammation in mouse and human PCLS. PCLS represent a promising model to reflect the toxic aspects of cigarette smoke. Further pharmacological intervention can be studied in PCLS.

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