Species comparison of cigarette smoke condensate-induced cytotoxicity and inflammation in **Precision-Cut Lung Slices**

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### Introduction

Chronic obstructive pulmonary disease (COPD) is a severe lung disease and a reason for morbidity and mortality worldwide. Chronic bronchitis, inflammation and pulmonary emphysema are characteristics for COPD and prevalently developed by cigarette smoke-induced stress. Although lipopolysaccharide (LPS) is a widely used model compound to induce inflammation, it cannot entirely reflect cigarette smoke induced effects in the lung. The goal of this study was to establish features of COPD in murine, rat, and human *Precision-Cut Lung Slices* (PCLS) by exposure of lung tissue to cigarette smoke condensate (Csc).

### Results

Cigarette smoke condensate induced toxicity could be detected by LIVE/DEAD® staining after 24 h exposure to Csc (Fig. 2).

Concentration dependent toxicity could be detected by LIVE/DEAD® staining (Fig. 3) and metabolic activity in murine, rat, and human PCLS after 24 h exposure with Csc. EC50 values for Csc were calculated to be at 85 µg/mL in murine PCLS, 228 µg/mL in rat PCLS, and 127 µg/mL in human PCLS (Fig. 4). Hence, murine lung tissue showed highest sensitivity to Csc.

![Fig 2: Detection of lactate dehydrogenase after repeated exposure of rat PCLS to cigarette smoke condensate for five days. N=1 in duplicates.](image)

![Fig 3: Viability of mouse (A-C) and human (D-F) PCLS after 24 h of exposure to 300 µg/mL cigarette smoke condensate determined by LIVE/DEAD® staining compared to untreated control and negative control (1% Triton X-100). Red: ethidium homodimer, yellow: calcein, scale bar: 100 µm.](/image)

![Fig 4: Viability of murine (A, B), rat (C, D), and human (E, F) PCLS after 24 h of exposure to increasing concentrations of Csc using WST-1 assay. EC50 values were calculated for murine, rat, and human PCLS. N=3 for murine PCLS, n=4 for rat and human PCLS in duplicates, *p*<0.05, **p**<0.005, ***p***<0.001.](image)

![Fig 5: Production of pro-inflammatory cytokine TNF-α significantly three fold on protein level (Fig 4). In rat and human PCLS an increased release of TNF-α after Csc exposure was not observed. Cytokine release depends on amount of cells in PCLS which shows to be highest in mouse of tested species.](image)

### Conclusions

Cigarette smoke condensate induced tissue injury in murine, rat, and human PCLS after 24 h. Hence, PCLS from different species show the toxic effect of cigarette smoke condensate. Furthermore, we observed cytokines that mediate inflammation in murine PCLS on protein level after 24 h. To compensate differences in total number of cells in PCLS of tested species, regulation of pro-inflammatory cytokines has to be checked on RNA level. Repeated exposure of Csc on rat PCLS also indicate tissue damage.

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