Acute Cigarette Smoke Exposure Induces Cytotoxicity and Inflammation in Living Tissue of Precision-Cut Lung Slices

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Introduction
COPD is a severe lung disease with high mortality and increasing prevalence. It is characterized by profound damage of lung parenchyma due to cigarette smoke-induced stress. Lipopolysaccharide (LPS) is a widely used model compound which does not directly induce oxidative stress due to lacking radicals. It therefore cannot reflect cigarette smoke-induced toxicity in the lung. The aim of this study is to establish precision-cut lung slices (PCLS) as a relevant toxicity model by using cigarette smoke and cigarette smoke condensate in comparison to LPS as model compounds.

Methods
Murine PCLS were prepared and exposed submerged to LPS and cigarette smoke condensate. And to cigarette mainstream smoke using the PRIT™ air liquid interface (ALI) culturing and exposure system. Induced toxicity was assessed by LIVE/DEAD® vitality staining and determination of metabolic activity using the WST-1 assay. Pro-inflammatory immune responses connected to toxic and subtoxic doses were quantified using ELISA. Additionally, therapeutic intervention with dexamethasone and roflumilast was assessed.

Results

Cigarette condensate induces toxicity after 24 h
Concentration dependent toxicity could be shown for cigarette smoke condensate. EC50 was calculated at 158 µg/mL (Fig. 2).

Cigarette smoke induces toxicity after 1 h of ALI-exposure
Cigarette smoke induces toxicity could be detected by LIVE/DEAD staining (Fig. 3). Concentration dependent toxicity could be shown for cigarette smoke with EC50 of 0.255 µg/cm² (Fig. 4).

Fig. 2 Viability of PCLS after 24 h of exposure to increasing concentrations of cigarette smoke condensate using WST-1 (A). EC50 was calculated to be 158 µg/mL (B).

Fig. 3 Viability of PCLS after 1 h of exposure to toxic concentration (1.26 µg/cm²) of cigarette smoke determined by LIVE/DEAD staining (B) compared to untreated control (A).

Cigarette condensate induces pro-inflammatory cytokines TNF-α and IL-1α after 24 h
Treatment with cigarette condensate with subtoxic doses increases the production of TNF-α and IL-1α significantly after 24 h (Fig. 5).

Fig. 4 Viability of lung slices after 1 h ALI-exposure (+23 h post incubation in DMEM) to increasing doses of cigarette smoke (A). EC50 values were calculated (B).

Conclusion
PCLS represent a promising model to reflect the toxic aspects of cigarette smoke-induced tissue damage occurring in COPD. It furthermore can be used to study pharmacological intervention.

Acknowledgements
funded by Fraunhofer ITEM

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