Pathophysiological changes in the extracellular matrix but not inflammation were induced by cigarette smoke as early events in fresh human lung tissue

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Introduction

Cigarette smoke inhalation is a main reason to develop chronic obstructive pulmonary disease (COPD), characterised by degradation of alveoli, mucus hypersecretion, emphysema development and inflammation (1). The pathophysiology of COPD is not well understood so the mechanisms that underlie various components of COPD need to be modelled in vitro, specifically using Cs. We assessed the pathophysiological effects of cigarette smoke (Cs) and Cs condensate (Csc) in fresh human lung tissue.

Materials and Methods

Human Precision Cut Lung Slices (PCLS) were exposed to Csc submersed or whole Cs in an Air-Liquid Interface using the in vitro exposure device P.R.I.T.® ExpoCube®. Cytotoxicity, release of cytokines and extracellular matrix (ECM) proteins were measured and gene expression analysis upon RNA isolation from PCLS was performed.

Results

PCLS exposed to Csc submersed or Cs in ALI culture showed a concentration-dependent increase in cytotoxicity after 24 h. EC50 values were determined using WST-1 assay. EC50 values of 196 µg/mL for Csc and 16 µg particles deposited on human lung tissue for Cs were calculated (Figure 2).

Conclusions

These results indicate that early stages of cigarette smoke induced lung tissue changes are primarily not provoked by immunological processes but by apoptosis, increased specific metabolic activity and damage of the epithelium.

References


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Figure 2: Viability of human PCLS after 24 h exposure to increasing concentrations of Csc (A) or Cs (B) was assessed by WST-1 assay. N=4 for Csc, N=3 for Cs, technical duplicates.

Figure 3: Fold regulation of selected mRNAs in human lung tissue slices treated with LPS or Csc for 24h or exposed to Cs and post-cultivated for 24h. The heat maps indicate that LPS and Csc stimulation for 24h induces expression of inflammatory and epithelial genes in human PCLS, respectively. Different concentrations of Cs indicate an increased expression of Cyp7a1, Cyp4a1, Cyp4b1, Lep, and Cyp4a11. N=4 for LPS and Csc, n=3 for Cs.

Figure 4: Relative regulation of selected mRNA levels in response to LPS or Cs compared to the respective control groups at 24h. Significant differences are indicated by *p<0.05, **p<0.01. N=3-4.

Figure 5: Relative regulation of selected mRNA levels in response to LPS or Csc compared to the respective control groups at 24h. Standard RT² Profiler PCR Array for Fibrosis (Qiagen). N=4.

Figure 6: Production of pro-inflammatory cytokines and ECM proteins in PCLS after Cs exposure were quantified by ELISA. Statistical significance is indicated by *p<0.05, **p<0.01. N=3-4.

Figure 1: Experimental setup for submersed exposure to Csc for 24 h (B) and for exposure of lung tissue to cigarette smoke at ALI (A).