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 submerged 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.

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

Results

PCLS exposed to Csc submerse or Cs in ALI culture showed a concentration-dependent increase in cytotoxicity after 24 h. EC_{50} values were determined using WST-1 assay. EC_{50} values of 196 µg/mL for Csc and 16 µg particles deposited on human lung tissue for Cs were calulated (Figure 2).

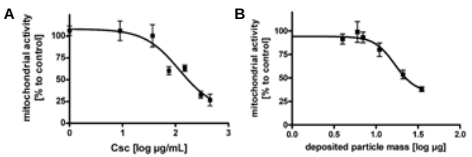

Figure 2: Viability of human PCLS after 24 h exposure to increasing concentrations of Csc (A) or Cs (B) was assessed by WST-1 assay. N=4 for Csc, N=3 for Cs, technical duplicates

Gene expression analysis upon RNA isolated from human PCLS exposed to a non-toxic dose of Csc of literature-based COPD-relevant genes indicate epithelial damage by upregulation of genes involved in tissue injury (MMPs) and metabolic activity (CYPs). Samples exposed to LPS as a control substance did not show an increase in emphysema-associated genes but in genes indicating inflammation (interleukins) (Fig. 3).

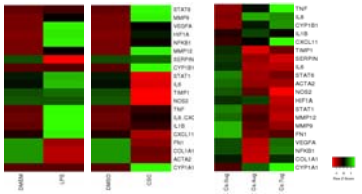

Figure 3: Fold regulation of selected mRNAs in human lung tissue slices treated with LPS or Csc for 24h or exposed to Cs and post-cultivated for 24h. The heat maps indicate that LPS and Csc stimulation for 24h induces expression of inflammatory and epithelial genes in human PCLS, respectively. Different concentrations of Cs indicate an increased expression of CYPs. RT² Profiler PCR Array (Qiagen). N=4 for LPS and Csc, n=3 for Cs.

In detail, fig. 4 presents expression of genes upregulated by Csc (A, B) and genes upregulated by LPS but not regulated by Csc (C-F).

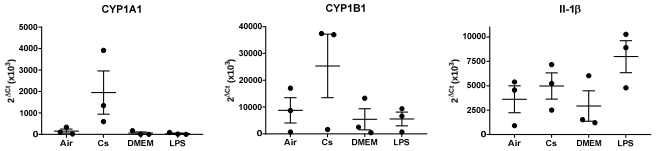

Figure 4: Relative regulation of selected mRNA levels in response to LPS or Cs compared to the respective control groups at 24h. Standard RT² Profiler PCR Array for fibrosis (Qiagen). N=3.

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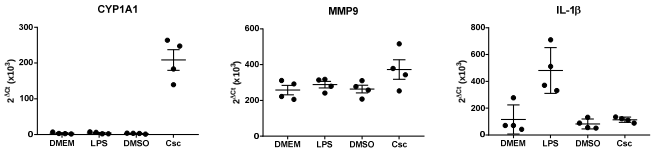

Figure 5: : Relative regulation of selected mRNA levels in response to LPS or Csc compared to the respective control groups at 24h. Standard RT² Profiler PCR Array for fibrosis (Qiagen). N=4.

These data can be supported on protein level. Increased production of proteins related to the extracellular matrix (e.g. MMP9, RAGE) were induced and expression in pro-inflammatory cytokines was reduced after Cs exposure in ALI. Supportive results were presented in BAL samples from COPD subjects showing markers of apoptosis (2).

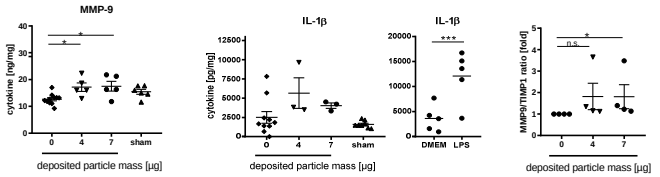

Figure 6: : Production of pro-inflammatory cytokines and ECM proteins in PCLS after Cs exposure were quantified by ELISA. Statistical significance is indicated by *p<0.05, **p<0.01. N=3-4.

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

(1) Shaykhiev, Renat; Crystal, Ronald G. (2014): Annals of the American Thoracic Society 11 Suppl 5, S252-8. DOI: 10.1513/AnnalsATS.201402-049AW.

(2) Hodge, S.; Hodge, G.; Holmes, M.; Reynolds, P. N. (2005): The European respiratory journal 25 (3), S. 447–454. DOI: 10.1183/09031936.05.00077604.