Early biomarkers indicate COPD induced by whole cigarette smoke in live human lung tissue

Helena Obernolte[1], Detlef Ritter[1], Jan Knebel[1], Peter Braubach[2], Danny Jonigk[2],Gregor Warnecke[2], Patrick Zardo[2], Hans-Gerd Fieguth[3], Olaf Pfennig[3], Armin Braun[1], Katherina Sewald[1]

[1] Fraunhofer Institute for Toxicology and Experimental Medicine; [2] Medical School Hannover; [1,2] Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease (BREATH) research network, Member of the Cluster of Excellence Regenerative Biology to Reconstructive Therapy (REBIRTH), Hannover, Germany; [3] KRH clinics, Hannover, Germany

Introduction

Cigarette smoke (Cs) inhalation is a main reason to develop chronic obstructive pulmonary disease (COPD). It is characterised by degradation of alveoli, inflammation and mucus hypersecretion. Mechanisms that underlie various components of COPD can be modelled *in vitro*, specifically using cigarette smoke with fresh human lung tissue. The aim of the study is to establish pathological changes of COPD in vital lung tissue by using Cs and cigarette smoke condensate (Csc).

Materials and Methods

Human Precision-Cut Lung Slices (PCLS) were exposed to Csc or whole Cs in an Air-Liquid Interface (ALI) using the *in vitro* exposure device P.R.I.T.® ExpoCube®. Cytotoxicity, release of cytokines and extracellular matrix (ECM) proteins were analysed. Pharmacological treatments were applied to inhibit inflammatory responses of tissue to Cs.

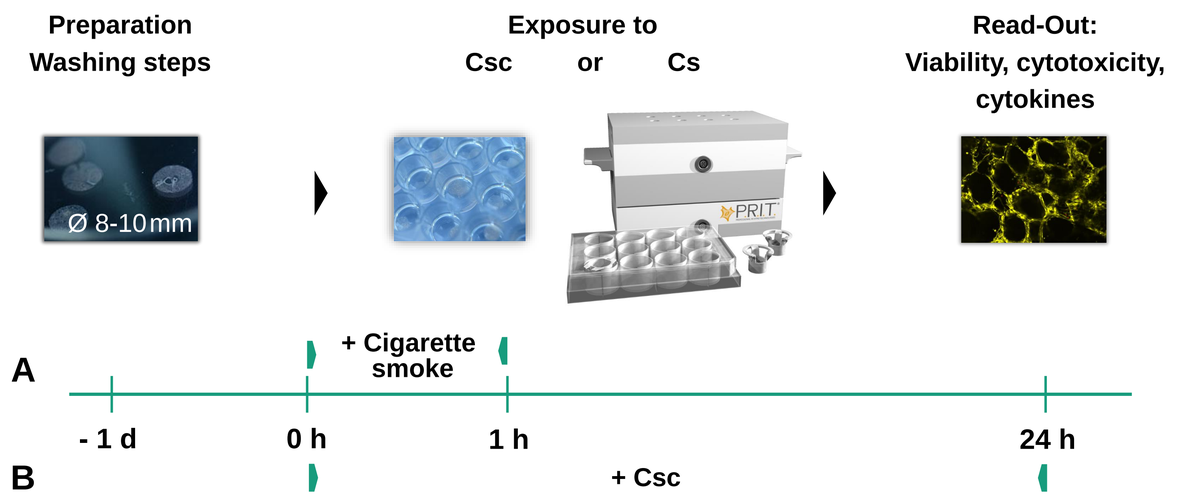

Fig. 1: Experimental setup for submerse exposure to Csc for 24 h (B) and for exposure of lung tissue to cigarette smoke at ALI (A).

Results

Concentration dependent cytotoxicity was observed in human PCLS after 24 h Csc submerse exposure and cigarette smoke exposure in ALI culture. 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 (Fig. 2).

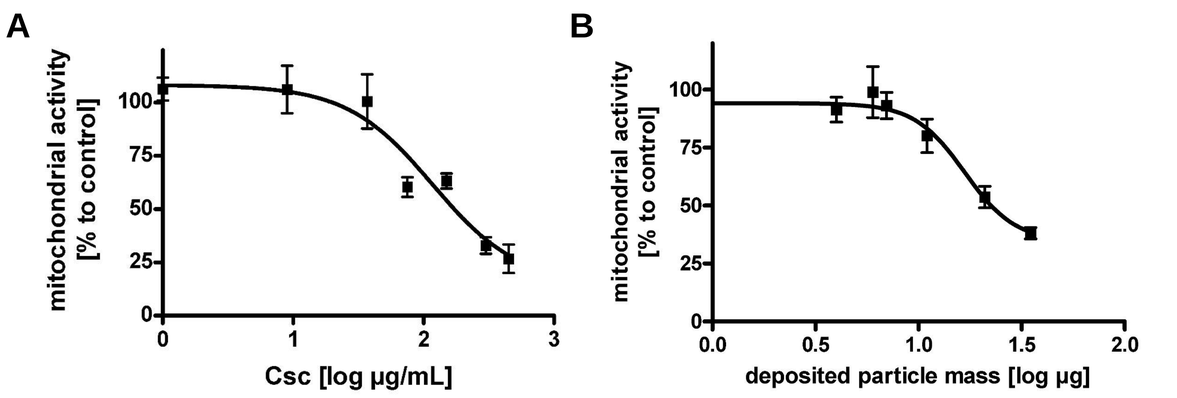

Fig. 2: Viability of human PCLS after 24 h exposure to increasing concentrations of Csc (A) or Cs (B) was assessed by WST-1 assay. N=4 for Csc, N=3 for Cs, technical duplicates.

Human lung tissue exposed to clean air (0 µg) shows no loss of viability, as did the respective non-exposed lung tissue control. PCLS exposed to the smoke of two cigarettes (7 µg) shows loss of viable tissue and an increase in dead cells (Fig. 4).

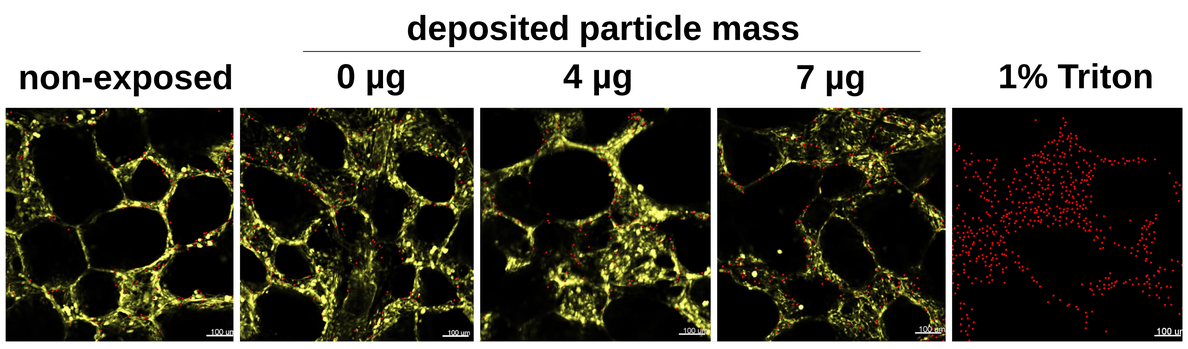

Fig. 3: Viability of lung tissue exposed to increasing concentrations of Cs was assessed by LIVE/DEAD® staining. Viable lung tissue stained with calcein was detected in non-exposed control (yellow). Dead cells were stained using ethidium homodimer-1 (red), shown in Triton control.

Pro-inflammatory cytokines interleukin (IL-) 1α and 1β and matrix metalloproteinases (MMP-9) were analysed by ELISA (Fig. 4). Significant increase of pro-inflammatory cytokines can be observed after 24 h Csc exposure of lung tissue. Dexamethasone inhibited the Csc-induced production of IL-1α.

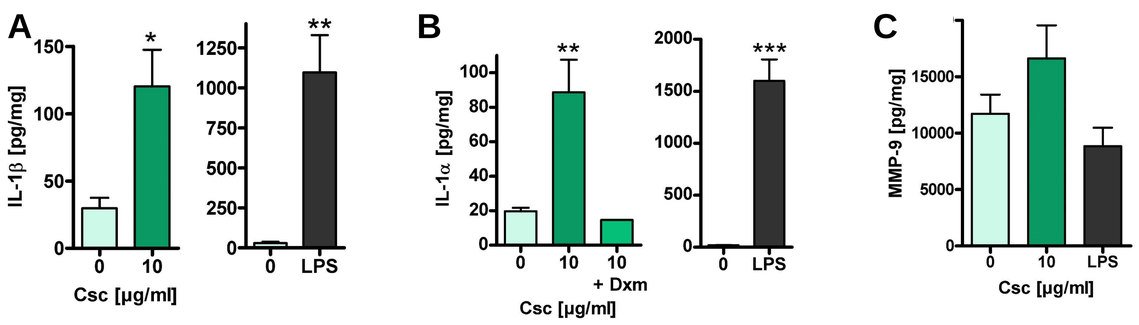

Fig. 4: Production of cytokines in PCLS after Csc exposure or Csc + Dxm was quantified by ELISA. Statistical significance is indicated by *p<0.05, **p<0.01, ***p<0.001. N=3.

Exposure of human lung tissue to Cs significantly increased the release of pro-inflammatory cytokines (Fig. 5). MMP-9, Pro-Col1α1 and extracellular RAGE present significant changes in the ECM after Cs exposure (Fig. 6A-C). Increased ratio of MMP-9 to TIMP-1 are biomarkers for a emphysema development (Fig. 6D). Pharmacological intervention reduced the Cs-induced inflammatory cytokines. Dexamethasone significantly reduced IL-1α production (Fig. 6E, F).

Fig. 5: Production of pro-inflammatory cytokines in PCLS after Cs exposure were quantified by ELISA. Statistical significance is indicated by *p<0.05, **p<0.01, ***p<0.001. N=3.

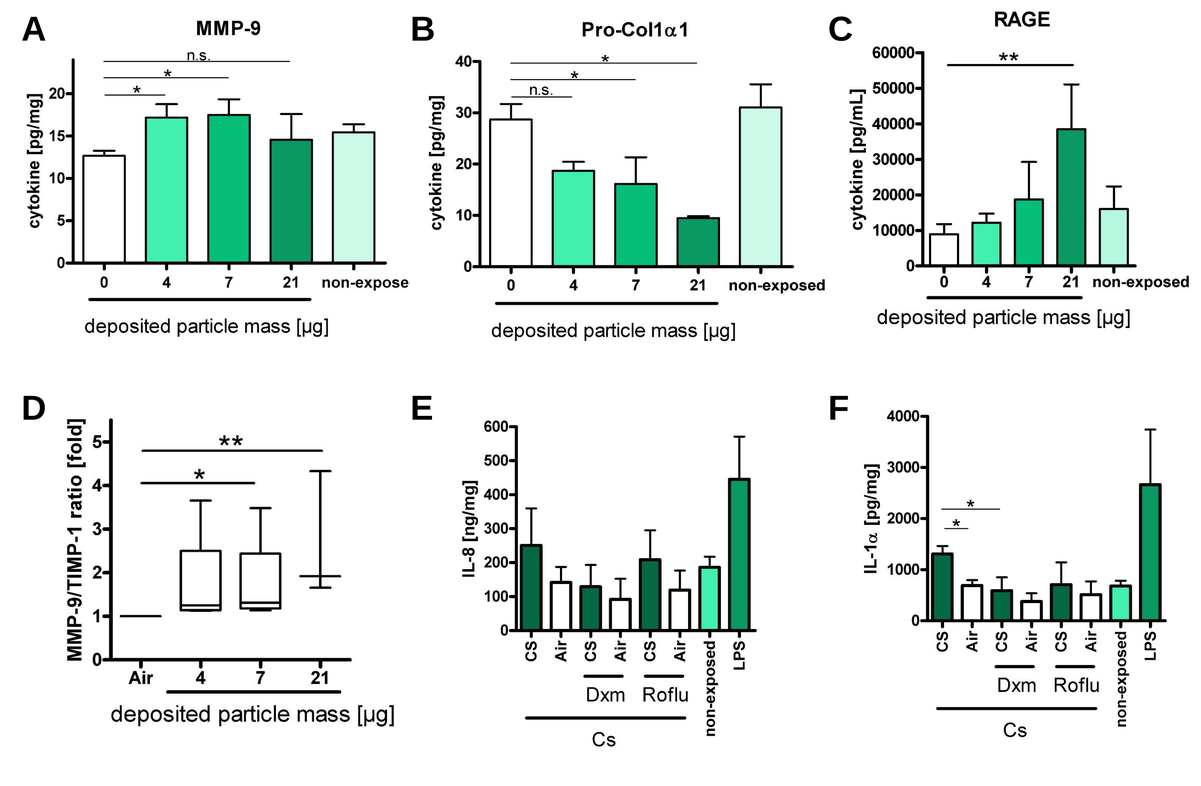

Fig. 6: Production of ECM proteins in PCLS and pharmacological intervention of the production of pro-inflammatory cytokines after Cs exposure were quantified by ELISA. Statistical significance is indicated by *p<0.05, **p<0.01. N=3 for A-B and E-F, N=4 for C, D.

Conclusions

Csc and Cs induced concentration dependent tissue injury, early biomarkers of inflammation and changes in ECM proteins in live *ex vivo* human lung tissue. The exposure of the complex mixture of whole cigarette smoke closely reflects the *in vivo* situation in human lung tissue.

Contact

Helena Obernolte, Fraunhofer ITEM, Hannover, Germany

helena.obernolte@item.fraunhofer.de, www.item.fraunhofer.de