Cigarette smoke induces biomarkers of COPD in fresh 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 ITEM, 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, 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).

Methods

Human Precision-Cut Lung Slices (PCLS) were exposed to Csc in a submerse culture or the whole Cs in an Air-Liquid Interface (ALI) using the *in vitro* exposure device P.R.I.T.® ExpoCube®. Cs concentration was defined by deposited particle mass during the exposure time. Tissue viability (mitochondrial activity (WST-1), LIVE/DEAD®), release of cytokines and extracellular matrix (ECM) proteins were analysed. Inhibitors dexamethasone (Dxm) and roflumilast (Roflu) were applied to suppress inflammatory responses of tissue to Cs.

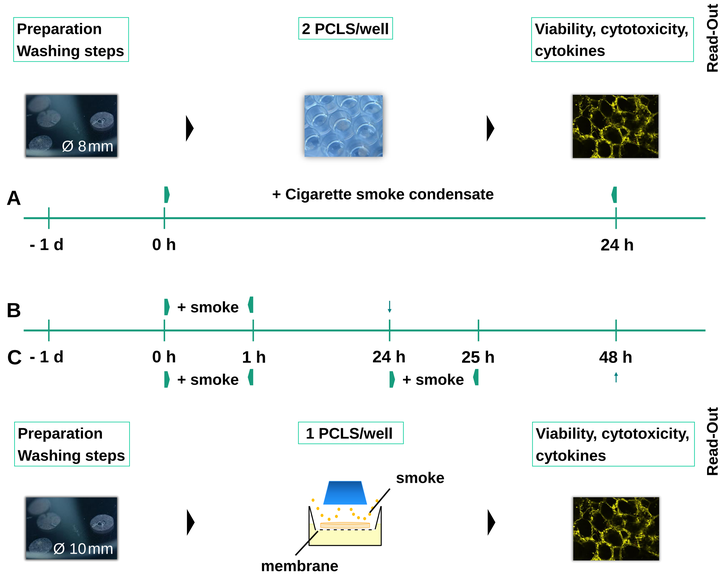

Fig. 1: Experimental setup for submerse exposure of PCLS to Csc for 24 h (A) and for exposure of lung tissue to cigarette smoke at ALI (B, C). Tissue was exposed for up to one hour and post-cultivated for 24 h (B) or daily exposed for one hour and post-cultivated for 48 h (C).

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).

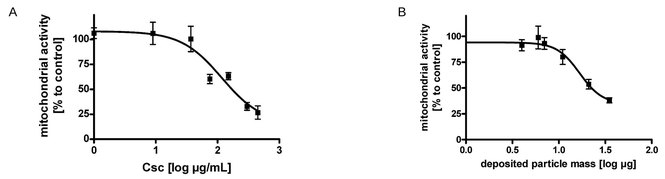

Fig. 2: Viability of human PCLS after 24 h exposure to increasing concentrations of Csc (A) or Cs (B) was assessed by WST-1 assay. N=4 for Csc, N=3 for Cs, technical duplicates.

Viable lung tissue stained with calcein was detected in medium control. Dead cells were stained using ethidium homodimer, shown in Triton control. Tissue treated with Csc present concentration dependent loss of viable cells in the tissue and increased dead cells.

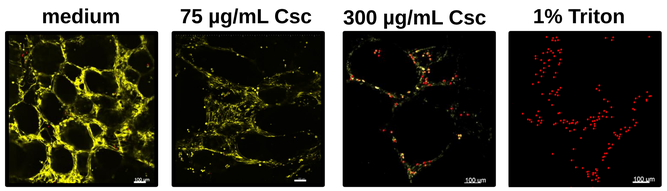

Fig. 3: Lung tissue exposed to increasing concentrations of Csc was used to assess tissue viability by LIVE/DEAD® staining. Viable lung tissue indicated in yellow and cell nuclei in red.

Human lung tissue exposed to clean air (0 µg deposited particles) shows no impaired viability. Tissue exposed to the smoke of two cigarettes (7 µg deposited particles) shows loss of viable cells and an increase in dead cells (Fig. 4).

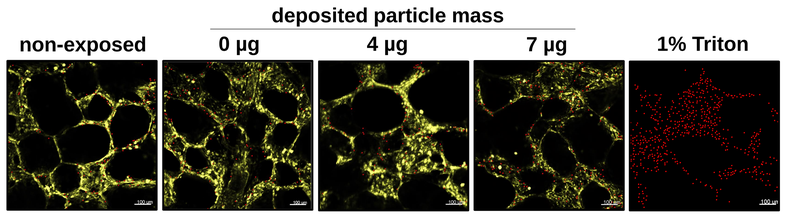

Fig. 4: Viability of lung tissue exposed to increasing concentrations of Cs was assessed by LIVE/DEAD® staining. Viable lung tissue indicated in yellow and cell nuclei in red.

Pro-inflammatory cytokines interleukin (IL-) 1α and 1β and matrix metalloproteinases (MMP-9) were analysed by ELISA (Fig. 5). 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.

Conclusions

Exposure of vital *ex vivo* human lung tissue to Csc and Cs induces tissue damage, early biomarkers of inflammation and changes in ECM proteins. Pharmacological intervention of inflammation induced by Cs can be studied in PCLS. The direct exposure of lung tissue to the complex mixture of cigarette smoke closely reflects the *in vivo* situation in the translational *ex vivo* model Precision-Cut Lung Slices.

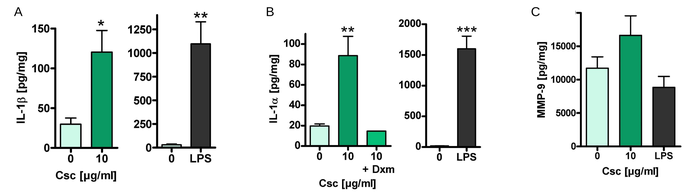

Fig. 5: Production of pro-inflammatory cytokines in PCLS after Csc exposure or Csc + Dxm was quantified by ELISA. Statistical significance is indicated by *p<0.05, **p<0.01, ***p<0.001. N=3.

Exposure of human lung tissue to Cs increased the release of pro-inflammatory cytokines significantly (Fig. 6A, B). MMP-9 and Pro-Col1α1 present significant changes in the ECM after Cs exposure (Fig. 6C, D). Repeated exposure revealed changes in MMP-9 and Pro-Col1α1 with a deposited particle mass of 7 µg (Fig. 6F, G). Pharmacological intervention reduced the Cs-induced inflammatory cytokines. Dexamethasone significantly reduced IL-1α production (Fig. 6E).

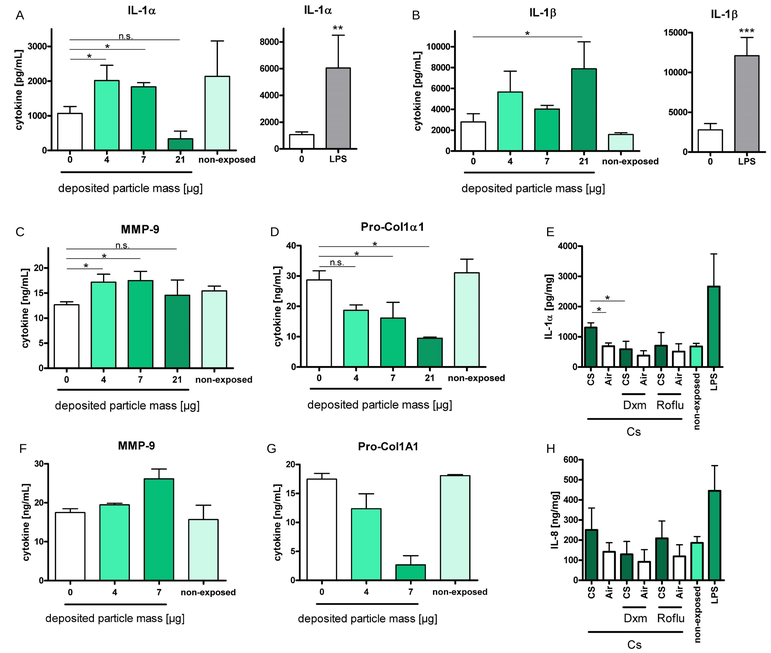

Fig. 6: Production of pro-inflammatory cytokines and ECM proteins in PCLS after one (A, B, C, D) and two exposures (F, G) to CS and pharmacological intervention after single exposure (E, H) were quantified by ELISA. Statistical significance is indicated by *p<0.05, **p<0.01, ***p<0.001. N=3 for single exposure and treatment, N=2 for repeated exposure.

Contact

Helena Obernolte, Fraunhofer ITEM, Hannover, Germany
helena.obernolte@item.fraunhofer.de