Cigarette smoke induces biomarkers of COPD in fresh human lung tissue

Helena Obernolte1, Detlef Ritter1, Jan knebel1, Peter Braubach2, Danny Jonigk2, Gregor Warnecke2, Patrick Zardo2, Hans-Gerd Fieguth3, Olaf Pfennig3, Armin Braun4, Katherina Sewald1

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 (BREATHE) 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 subculture 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 (Rolflu) were applied to suppress inflammatory responses of tissue to Cs.

Results

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

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.

Exposure of human lung tissue to Cs increased the release of pro-inflammatory cytokines significantly (Fig. 6A, B). MMP-9 and Pro-CoNh1 present significant changes in the ECM after Cs exposure (Fig. 6C, D). Repeated exposure revealed changes in MMP-9 and Pro-CoNh1 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).

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.

Contact

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