Cigarette Smoke induced Inflammation and Cytotoxicity in viable lung tissue

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Introduction

Chronic obstructive pulmonary disease (COPD) is a common leading cause of death worldwide, often developed due to cigarette smoke (Cs) inhalation. 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

Precision-Cut Lung Slices (PCLS) prepared from rodent, rhesus and human lungs were exposed to Csc in a submerse culture or to Cs in an Air-Liquid Interface (ALI) for up to 96 hours. Tissue viability (WST-1, Live/Dead staining) and cytotoxicity (LDH) were assessed. Pro-inflammatory cytokines and matrix metalloproteinases (MMPs) were determined by ELISA. Dexamethasone was used to inhibit inflammatory responses of tissue to Cs.

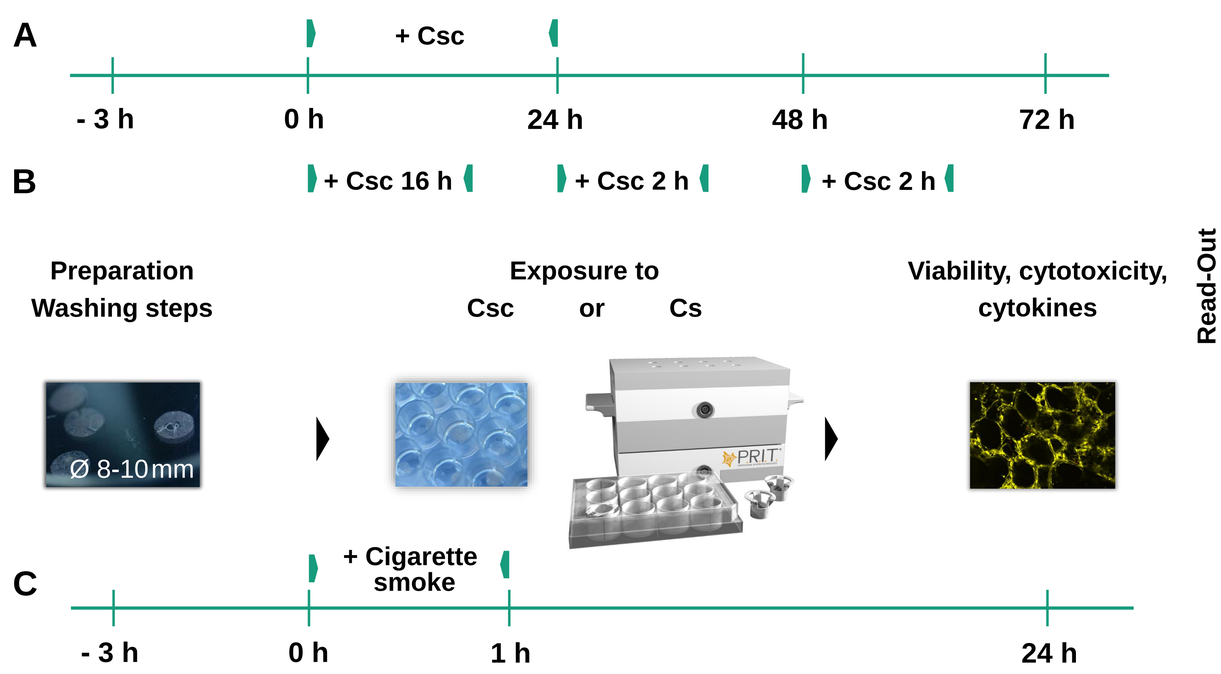

Fig. 1: Experimental setup for submerse exposure to Csc for 24 h (A), for repeated exposure for 72 h (B), and for exposure of lung tissue to cigarette smoke at ALI (C).

Results

Csc induced concentration dependent cytotoxicity in rat, rhesus and human PCLS after 24 h submerse exposure. EC_{50} values were determined by WST-1 assay (Fig. 2) (curves not shown). Pro-inflammatory cytokines interleukin 1α and -β (IL-1α/-β) were significantly increased in all species after 24 h Csc exposure of lung tissue. Dexamethasone inhibited Csc induced production of pro-inflammatory cytokines (Fig. 2C).

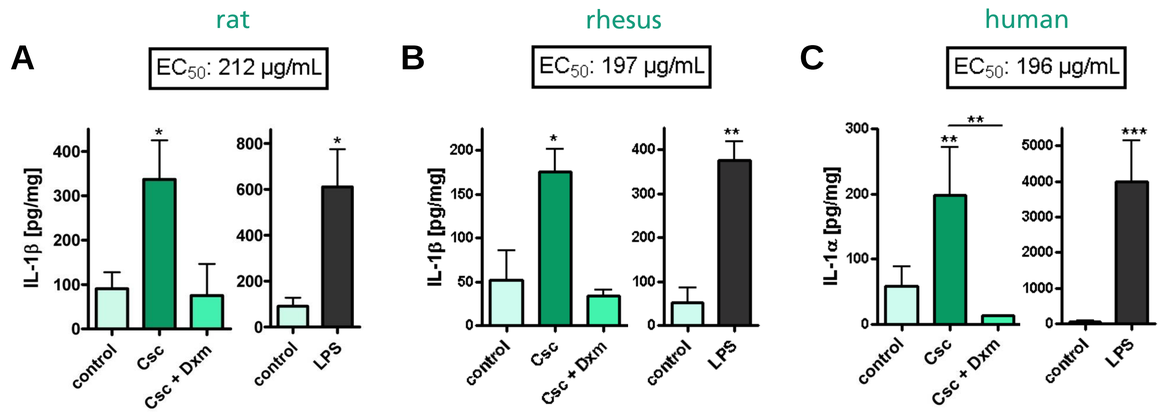

Fig. 2: Vitality and cytokine secretion after 24h Csc exposure. Viability of rat, rhesus and human PCLS after 24h of exposure to increasing concentrations of Csc was assessed by WST-1 assay. Production of pro-inflammatory cytokine IL-1β in rat (A) and rhesus (B) and IL-1α in human PCLS (C) after exposure to Csc, LPS or Csc + Dxm was quantified by ELISA. Statistical significance are indicated by *p<0.05, **p<0.01 and ***p<0.001. N=3 in duplicates, N=4 in duplicates for EC_{50} of rat and human PCLS.

Repeated exposure of human PCLS to Csc induced cytotoxicity (Fig. 4A). ALI cultivation of human PCLS did not induce loss of viability after 96 h (B).

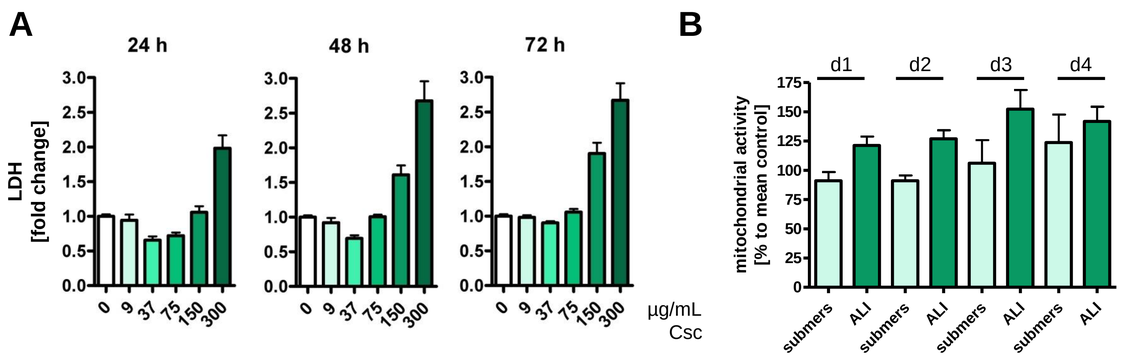

Fig. 3: Detection of lactate dehydrogenase (LDH) after repeated exposure of human PCLS to Csc (A). Viability of human PCLS after 4d ALI cultivation was determined using WST-1 assay. N=3 in duplicates.

Cs induced toxicity and increased secretion of pro-inflammatory cytokines. Increasing concentrations of Cs reduced mitochondrial activity and increased LDH release in rat and human PCLS (Fig. 4, 5).

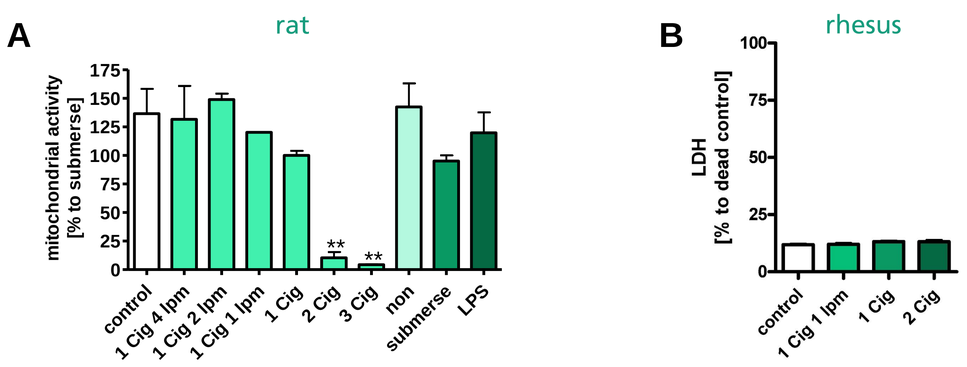

Fig. 5: Detection of mitochondrial activity and LDH of rat and rhesus PCLS after Cs exposure and 24 h post-incubation. N=1-3 for rat PCLS, N=4 for rhesus PCLS.

Fig. 5: Detection of mitochondrial activity and LDH of human PCLS after Cs exposure and 24 h post-incubation. N=4 in duplicates

Exposure of viable rat, rhesus and human lung tissue to Cs increased release of pro-inflammatory cytokines (Fig. 6). High concentrations of Cs induced an anti-inflammatory effect in IL-1α production in human PCLS (Fig. 6E). Matrix metalloproteinase-9 is increasingly released after Cs exposure in human PCLS as well as after Csc exposure in rhesus and human PCLS (Fig. 6C and data not shown).

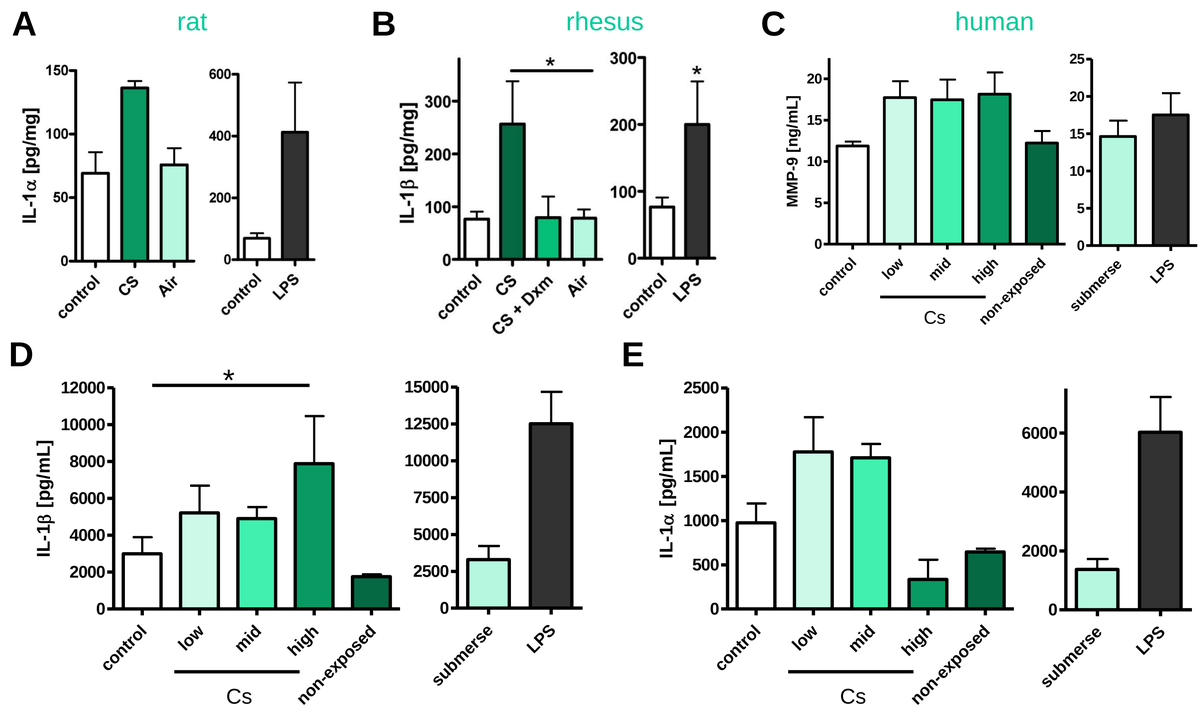

Fig. 6: Production of pro-inflammatory cytokines in rat (A), rhesus (B) and human PCLS (D, E) and MMP-9 in human PCLS (C) after exposure to Cs or Cs + Dxm was quantified by ELISA. Statistical significance is indicated by *p<0.05. N=2 for rat, N=4 human and N=3 for rhesus cytokines.

Conclusions

Csc and Cs induced tissue damage and early biomarkers of inflammation in rodent, rhesus and human PCLS. The exposure of lung tissue to the complex mixture of whole cigarette smoke closely reflects the *in vivo* situation in PCLS. Therapeutical intervention of inflammatory responses can be studied in PCLS.

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