Cytokine Production after Acute Exposure of Precision Cut Lung Slices to NO_2 and O_3

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Introduction

The lung plays a decisive role for toxic inhalable substances to enter the body. The toxic potency of chemicals has to be estimated for the assessment of human health risks.

The imperative to develop alternative methods in the field of acute inhalation toxicology in the context of REACH and 3Rs is currently the basis for the employment of live lung tissue. With the use of precision cut lung slices (PCLS) as an *ex vivo* model of "acute inhalation injury" chemicals can partly be tested for inhalation toxicology without experiments in live animals.

Methods

Lungs (mouse) were filled with an agarose/medium mixture, cooled on ice and cut into 250 µm thick slices. PCLS were washed and exposed one or three hours to synthetic air, O_3, and NO_2 in an air-liquid interface culture. Viability was controlled by Wst-1 and LIVE/DEAD® staining. Cytokines were quantified by ELISA or Luminex technology (Fig. 1).

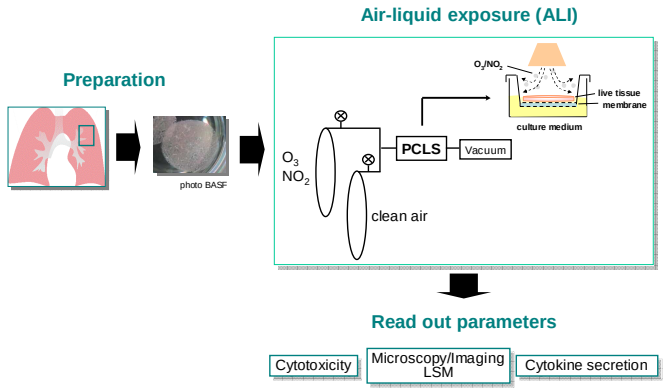

Fig. 1: Experimental set up for the exposure of PCLS using air-liquid interface conditions. A vacuum was generated to direct exposure of vital organotypic tissue to synthetic air, O_3, and NO_2. PCLS were supplied with medium from below the membrane.

Results and discussion

Air-liquid interphase culture of PCLS versus submerged culture

The conditions for air-liquid interphase cultivation of live lung slices on microporous membranes were defined firstly. The set-up allowed a humidified microclimate around the tissue and enabled a nutrification from the basal side of the membrane solely (Fig. 1).

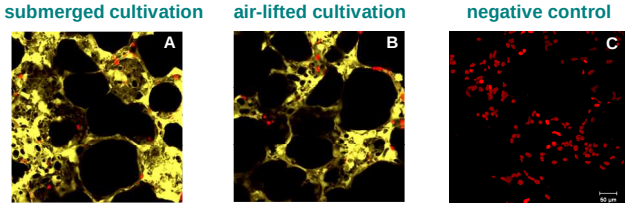

Fig. 2: Image analysis of PCLS after cultivation with different culturing systems. Tissue slices were stained with calcein AM and EthD-1 after 24 hours of submerged cultivation (**A**), 24 hours ALI cultivation (**B**), and after cell lysis with Triton X-100 (**C**). The images were examined by confocal microscopy and analyzed with IMARIS 4.5.2. Red colour shows cell nuclei (∅ 5 µm) of dead cells and yellow colour the cytoplasm of viable cells.

Quantitative image analysis of viability of air-liquid cultivated PCLS remained stable at about 100 % to standard submerged cultivation. This indicates that no progressive cellular damage occurs during the first 24 hours of air-liquid cultivation (Fig. 2).

Influence of flow rates on tissue viability

The viability of live tissue was nearly unaffected at flow rates of 10 mL/min maximum compared to the “air-liquid interphase” control.

Effect of *in vitro* exposure of PCLS to NO_2 and O_3

Live lung tissue was exposed to synthetic air (control), NO_2 and O_3 using ALI technique. Single acute exposure to O_3 did not induce immediate toxicity in alveolar tissue (Fig. 3A) but gave a rapid secretion of pro-inflammatory cytokines IL-1α and RANTES (Figs. 3B & C).

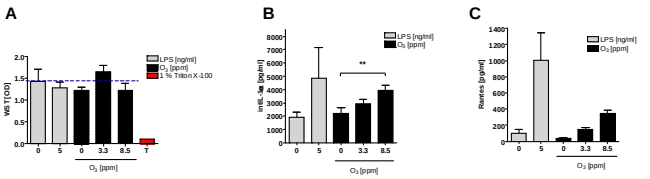

Fig. 2: Viability measured by WST-1 in PCLS after exposure to O_3. Triton X-100 (T) was measured as positive control for cytotoxicity (**A**). Intracellular increase of IL-1α (**B**) and RANTES (**C**) in PCLS exposed to O_3. Data are presented as mean ± S.E.M. (IL-1α : 0-10 ppm $O_{3\ n=4}$, Rantes: 0-10 ppm $O_{3\ n=2}$). ** p<0.01.

In contrast, a brief high exposure to NO_2 induced cell death (Fig. 3A & B). But contrary to our expectations, although there was a trend, we did not observe a significant increase in the pro-inflammatory cytokine IL-1α in live lung tissue (Fig. 3C).

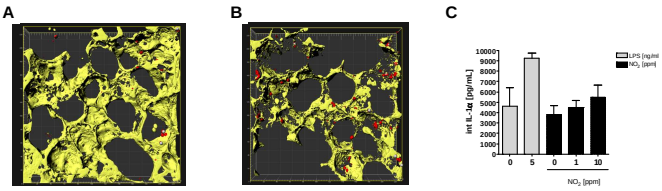

Fig. 3: Exposure of PCLS to synthetic air (**A**) or 80 ppm NO_2 (**B**) for 1 hour. Red colour of the LIVE/DEAD® staining shows cell nuclei of dead cells and yellow colour the cytoplasm of viable cells. The images were examined by confocal microscopy. The cytokine IL-1α tend to increasing intracellular concentrations (**C**).

In our study, the range of exposure doses were selected to cover high gaseous concentrations. Our result indicate that acute NO_2 exposure leads to cell damage. Thus, the sensitivity of this tissue based *in vitro* method may reflect closely the *in vivo* situation after exposure to brief high doses of NO_2 (Table 1).

Table 1: Comparison of our results for organotypic culture to other exposure cell systems.

	In vitro toxicity data				*In vivo* toxicity data			
Gas	**Cell type**	**LD_{50}**	**Exposure**	**Ref.**	**Species**	**LD_{50}**	**Exposure**	**Ref.**
NO_2	Human A549	11 ppm	1 h	Bakand *et al.*, 2007	Human	30-90 ppm	40 - 70 min	NIOSH, 2008
	Murine PCLS	80 ppm	1 h	this study	Rat	117 ppm	1 h	NIOSH, 2008
O_3	THP-1	0.5 ppm	30 min	Klestadt *et al.*, 2002	Human	50 ppm	30 min	NIOSH, 2008
	Murine PCLS	> 8.5 ppm	1 h	this study	Mouse	15 ppm	2 h	NIOSH, 2008

Toxicity data of several inhalation toxicity studies are available by the Registry of Toxic Effects of Chemical Substances (RTECS) (http://www.cdc.gov/niosh/).

Although no cytotoxicity was reached for O_3 in PCLS, we can assume that the lethal concentration is higher than 8.5 ppm what is already very close to the *in vivo* situation in laboratory animals.

Conclusion

Our study demonstrate that live lung tissue offers a suitable model to assess the effects of inhaled chemicals under direct air-liquid interphase exposure conditions.