Assessment of Lung Toxicity Induced by Chemical Allergens in Human Precision-Cut Lung Slices (PCLS)

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Introduction

A number of low-molecular weight (LMW) chemicals at workplaces are involved in the development of occupational asthma. There are no currently accepted and validated test methods to identify chemicals with the potential to cause respiratory sensitization. Risk assessment is normally performed in animal experiments; however, in the context of REACH and its principle of 3Rs there is an increasing public demand for alternative methods. Human precision-cut lung slices (PCLS) is an ex vivo approach in which nearly all relevant cell types of lung tissue are present in their natural position. The aim of the study was to analyse chemical-induced inflammation and irritation by assessing a variety of immunotoxic endpoints in human PCLS.

Methods

Human PCLS were prepared from peripheral tumour-free tissue from resected lung lobes of cancer patients. PCLS were incubated with 20 industrial chemicals in serum-free DMEM under standard cell culture conditions. PCLS without test substances were incubated as controls. After 24 hours incubation, induced toxicity was assessed by viability staining and determination of enzymatic activity using WST-1 assay. The concentrations of chemicals resulting in tissue viability of 75% (EC75) with respect to vehicle controls were calculated for all chemicals. Cytokine contents were detected by ELISA.

Results

Determination of 50% and 75% cell viability of human PCLS

Viability of human PCLS was determined by mitochondrial activity using WST-1 assay and LIVE/DEAD® staining after incubation of tissue sections with the selected set of chemicals in serum-free DMEM for 24 h (Fig. 2).

In order to determine the degree of correlation of the results obtained in human PCLS with in vitro and in vivo findings EC50 and EC75 values were assembled in linear regression analysis models with CV% and LD50 values achieved from in vitro and in vivo published data.

Individual EC50 values correlated significantly with data published for in vitro approaches with human cell lines THP-1 and NCTC (Pearson r= 0.86 and 0.78, respectively) (Fig. 3). Furthermore EC50 of human PCLS correlated with LD50 data published for in vivo rat inhalation toxicity with Spearman r of 0.53 (p value of 0.08) (Fig. 3).

Respiratory allergens TMA and HClPt induced a dose-dependent and significant production of TNFα up to 210% and 400% and IL-1α up to 1090% and 270%, respectively (Fig. 4A, 4B).

Conclusion

The toxicity of chemicals in human PCLS resembles the in vitro situation very closely and also correlates with LD50 values from in vivo studies. Pro-inflammatory effects could be shown in particular for respiratory allergens. However, some respiratory allergens like glutaraldehyde did not induce cytokine due to the chemical properties. Altogether, PCLS can be regarded as an ex vivo immunotoxicity model, displaying chemical-induced inflammation and irritation on all relevant cell types of lung tissue.

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