

Lecture: 7th cosmetics amendment – can all goals be achieved in time?

Respiratory toxicology and immunotoxicology in precision cut lung slices (PCLS)

Katherina Sewald⁰, Simone Switalla⁰, Jan Knebel⁰, Detlef Rittler⁰, Maja Henjakovic⁰, Norbert Krug⁰, Hermann-Josef Thierse⁰, Armin Braun¹

⁰ Fraunhofer ITEM (Hannover) (DE); ¹ Clinical Center Mannheim (Mannheim) (DE)
e-mail: katherina.sewald@item.fraunhofer.de

Introduction: There is a clear evidence that a variety of chemicals cause allergic sensitization of the respiratory tract. PCLS offer the opportunity to gain insight into lung morphology and physiology after *in vitro* exposure to chemicals. Aim of this study is the evaluation of PCLS as an *ex vivo* model to test for chemical-induced sensitization. This work is part of the EU project Sens-it-iv. Furthermore, air/lifted exposure of PCLS is under development to establish a method for the future application of insoluble respirable chemicals in a reproducible and *in vivo* relevant way.

Method: Lung tissue (mouse, human) was cut with a microtome. Firstly, PCLS were cultivated submers and exposed to lipopolysaccharides (LPS) and allergens. Secondly, tissue was air/lifted exposed to synthetic air using a method under development at Fraunhofer ITEM. Vitality was controlled by measurement of LDH activity and live/dead fluorescence staining and expressed as EC₅₀. Cytokines and chemokines were detected with Luminex technology and ELISA. Proteomic DIGE was performed.

Results: LPS induced profound pro-inflammatory effects on cytokines such as IL-1 alpha, TNF alpha, and Rantes in PCLS. This shows that PCLS provide *per se* a suitable *in vitro* model to predict immune modulating potencies of substances. Chemical-induced local respiratory irritation and inflammation were characterized in PCLS. *Ex vivo* EC₅₀ values were determined and induced cytokines were quantified. There is a clear evidence that chemical-induced loss of cell viability is accompanied by the production of pro-inflammatory cytokines TNF alpha, IL-1 alpha, and MIP-1 alpha. But, for some allergens we also found an induction of cytokines at subtoxic concentrations. For example, respiratory allergen AHCP increased TNF alpha, IL-1 alpha and IL-8 while the contact allergen cinnamaldehyde was not able to induce the same cytokines. It, therefore, still needs to be elucidated to which extent a concurrent irritation influences the inflammation processes induced by allergens. Gel-based proteome analysis revealed an up-regulation of enzymes involved in the production of ROS, sensing of reactive xenobiotics and energetic metabolism. Preliminary experiments with PCLS exposed air/lifted to synthetic air using varying flows of up to 30 ml/min/PCLS did not induce irritation.

Conclusion: PCLS provide a suitable *in vitro* model to predict immune modulating potencies of substances and to characterize local respiratory irritation and inflammation induced by chemicals. Future exposure of PCLS to gaseous compounds will offer the possibility to investigate *ex vivo* parameters for a wide repertory of chemicals in a complex biological test system.

Keywords: allergens, sensitizers, lipopolysaccharides, pro-inflammatory cytokines, air/lifted culture