Toxic Insult to Rat Precision Cut Lung Slices Increases Tissue Cytokine Levels and Activation of Macrophages, and Causes Acute Damage, While Prolonged Insult May Lead to Increased Deposition of Collagen - A Marker of Fibrosis

Holger P. Behring1
Advanced In Vitro, LLC, Frederick, MD 21704, USA1

ABSTRACT

The use of in vitro or ex vivo models is intended to provide meaningful data that will identify or predict the adverse effects of tissue exposure. Precision-cut lung slices (PCLS) are used as a model that retains the heterogenous population of cells in the native architecture of the organ. The retention of native cells allows the study of the initial, dynamic events (such as inflammation) that occur following a toxic insult prior to overt tissue damage. The purpose of the reported studies was to identify initial inflammatory signals, acute toxicities, as well as markers associated with chronic toxicities of PCLS exposed to a toxic insult as a way to qualify the model for identifying such endpoints. Rat PCLS were exposed to several chemotherapeutics known to cause acute and/or chronic pulmonary damage. Time points for respective endpoints were chosen based on known response times of when relevant endpoints may change. Cytokines and acute toxicity were evaluated during initial days of exposure while activation of macrophages and collagen deposition were evaluated through 4 weeks of culture in other studies. Exposure of PCLS for 24 hours resulted in increased cytokine levels and 72 hour exposure caused overt toxicity, as assessed using tissue protein content and histologically using H&E and ED1 staining. Long term exposure of PCLS to two agents known to cause fibrosis (bleomycin and camptothecin) resulted in elevated numbers of macrophages and also increased collagen deposition. PCLS generate inflammatory cytokine signals and if levels persist out insult removal, these signals may predict subsequent tissue damage. The expression of adverse markers of chronic exposures (collagen deposition) in PCLS may signify risk of fibrosis. Cytokine responses, macrophage activation, and fibrosis are hallmarks of tobacco related exposures. PCLS may elucidate acute and chronic adverse pulmonary responses when exposed to tobacco products.

INTRODUCTION

With the expanded regulation of tobacco products and the need to assess inhalated toxians, researchers require models that allow accurate translation of results. In vivo models are not always suitable for mechanistic studies and with the 3Rs initiative to expand the use of in vitro models, researchers are often attracted to more complex 3-dimensional (3D) models that offer a heterogeneity of cell types for more diverse cell-cell signaling. Exposure of lungs to tobacco smoke, or modified risk tobacco products, results in a series of events that can include inflammation leading to acute damage, or with repeated exposure, may result in a chronic inflammatory state that may ultimately lead to fibrosis and/or chronic obstructive pulmonary disease. The complex sequence of events involves many cell types and diverse signaling between parenchyma and mediators of inflammation. While several 3D in vitro ex vivo airway epithelium models can offer multiple cell types, only ex vivo precision-cut lung slices (PCLS) are known to retain macrophages – a cell known to have a central role in pulmonary inflammation.

This study reviews PCLS as a model that demonstrates longevity in culture, responds to challenge by regulating cytokines, demonstrates acute damage, and also expresses biomarkers associated with chronic toxicity.

MATERIALS & METHODS

1. Inflated lung tissue and create tissue cores
   - Aspirate lung removal and storage in organ preservation solution. Inflation with 0.8% agarose, lobe dissection, and tissue (8 mm).

2. Slice cores with Krumdieck slicer
   - In thermostatically controlled cold UW, cores are sliced to 500 micron thickness

3. Culture maintenance
   - Insertioneal access in cold UW, cores are sliced to 500 micron thickness

4. Culture maintenance
   - After acclimation period, insertions are transferred to vials containing treatment medium (replaced every 48 hr) with 5% CO2. 5% CO2 is maintained until harvest.

5. Culture maintenance
   - After acclimation period, insertions are transferred to vials containing treatment medium (replaced every 48 hr) with 5% CO2. 5% CO2 is maintained until harvest.

6. PCLS Harvest:
   - a) PCLS for biochemical evaluation are homogenized in 500 µL ice cold PBS-0.5% Triton X-100
   - b) PCLS for histological evaluation are fixed with 4% paraformaldehyde for 24 hr, immersed in 70% ETCH, embedded, sectioned and stained

1. PCLS exhibit longevity and retention of viability for 1 month or more. This makes them suitable for long term culture and repeated exposure paradigms in a manner that can reflect consumer product use (e.g. tobacco product exposures over time).

2. The 3D, native lung parenchymal architecture, and inclusion of native cell types allows for a complex response (e.g. activation of macrophages, increased cytokine expression, and collagen deposition) to challenge.

3. The involvement of multiple cell types and biomarkers may be required for a long term disease such as COPD.

PCLS exhibit longevity and retention of viability for 1 month or more. This makes them suitable for long term culture and repeated exposure paradigms in a manner that can reflect consumer product use (e.g. tobacco product exposures over time).

The 3D, native lung parenchymal architecture, and inclusion of native cell types allows for a complex response (e.g. activation of macrophages, increased cytokine expression, and collagen deposition) to challenge.

A marker of fibrosis (COPD).

REFERENCES


ACKNOWLEDGMENTS

The author wishes to acknowledge Dr. Khalid Arif, Carman Tp, and Michael Furlan, all of whom provided expertise to the development of the PCLS protocol and utilization of the PCLS model for the assessment of adverse effects.

DISCLOSURES: The data presented was previously published and generated at SRI (via the leading author, Michael Furlan) and was presented at SRI (SRI contract no. HSHQDC-01-D-00006). None of the conclusions, interpretations, or opinions made represent the opinions or views of SRI, SRI Freddie, or the NCI.