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 heterogeneous 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 ED-1 staining. Long term exposure of PCLS to two agents known to cause fibrosis (bleomycin and camustine) resulted in elevated numbers of macrophages and also increased collagen deposition. PCLS generate inflammatory cytokine signals even if levels persist after insult removal, these signals may predict subsequent tissue damage. The expression of adverse markers of chronic toxicities (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.

RESULTS

CONCLUSIONS

With the expanded regulation of tobacco products and the need to assess inhalated toxins, 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 epithelial 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. Inflamed lung tissue and create tissue cores
   - Aspirate lung removal and storage in organ preservation solution. Inflation with 0.8% agarose, lobe dissecting, and tissue coring (8 mm).

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

3. Culture maintenance
   - After acclimation period, slices are transferred to vials containing treatment medium (replaced every 24 hours). Staining with Alcian Blue is collected through a slice’s lifetime until harvest.

4. 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 1% paraformaldehyde for 24 hr, transilluminated 70% EOB, embedded, sectioned and stained

5. Culture maintenance
   - After acclimation period, slices are transferred to vials containing treatment medium (replaced every 24 hours). Staining with Alcian Blue is collected through a slice’s lifetime until harvest.

6. PCLS Harvest:
   a) PCLS for biochemistry analysis are homogenized in 500 µL ice cold PBS 0.5% Triton X-100.
   b) PCLS for histopathological evaluation are fixed with 4% paraformaldehyde for 24 hr, transilluminated 70% EOB, 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 expression cytokine, and collagen deposition) to challenge. The involvement of multiple cell types and biomarkers may be required for a long term disease such as COPD.

3. PCLS have historically been employed to compare compound toxicities and the model has repeatedly demonstrated differential effects and severity of response across the compounds tested. By extension, PCLS are well suited to make specific tobacco product or product combination comparisons.

4. The identification of a no adverse effect level and a level at which increased inflammatory markers subside after treatment removal will help guide product dosing decisions.

REFERENCES

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DISCLAIMER

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