The Use of Precision-cut Lung Slices to Assess Inflammation, Parenchymal Damage, and Collagen Deposition: Three Markers of Tobacco Exposure-induced Pulmonary Toxicity

Holger P. Behrsing[1]

Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA[1]

ABSTRACT

The Family Smoking Prevention and Tobacco Control Act (TCA) gives the Food and Drug Administration (FDA) broad authority to regulate tobacco products. The FDA-Center for Tobacco Products (CTP) calls for several research priority areas, including *in vitro* models and assays that will assess tobacco constituent safety and comparative product toxicity. Precision-cut lung slices (PCLS) retain normal lung architecture and endogenous cell types and have been effective in assessing acute pulmonary toxicity. Having demonstrated release of traditional leakage markers, inflammation, and tissue damage, recent advances in PCLS culture and choice of biomarkers suggest suitability for detecting specific events that contribute to the etiology of tobacco-induced chronic obstructive pulmonary disease (COPD). PCLS were exposed to various compounds known to cause acute and chronic pulmonary lesions. Acute toxicity was assessed via cytokine level, protein content, and the overt destruction of parenchymal tissue. Chronic exposures also included quantifying activated macrophages and assessing a marker of fibrosis (in addition to general histopathological evaluation). Acute toxicity elicited a rapid increase in cytokine levels, reduction in tissue leakage markers, and overt parenchymal damage. Chronic exposures lasting up to several weeks also resulted in decreased tissue viability, increased numbers (and location of) activated macrophages (ED-1 positive cells) and deposition of collagen. In summary, PCLS data from various chemical assessment studies are compiled to highlight the changes in different markers of acute and chronic toxicity, as related to the known events of tobacco-induced COPD etiology. It is expected that PCLS will prove to be a valuable tool in elucidating both acute and chronic effects of compounds found in tobacco or modified risk tobacco products such as e-cigarettes.

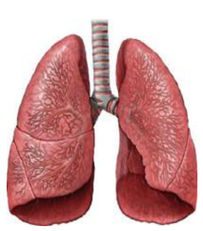
INTRODUCTION

With the expanded regulation of tobacco products and the need to assess inhaled toxicants, 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. Inflate lung tissue and create tissue cores

- Aseptic lung removal and storage in organ preservation solution. Inflation with 0.8% agarose, lobe dissociation, and tissue coring (8 mm).

2. Slice cores with Krumdieck slicer

- In thermostatically controlled cold UW, cores are sliced to 500 micron thickness

3. Slices are mounted onto HATF paper within titanium inserts and placed in vials and cultured in 1.7 mL serum-free, M199 medium

4. Vials are rotated at ~3-7 rpm in roller drum within humidified incubator set to 5% CO2/95% air at 37ºC

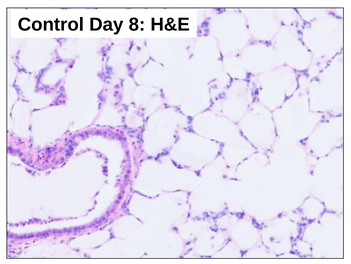
5. After acclimation period, inserts are transferred to vials containing treatment medium (replaced every 1-2 days). Medium is collected through a slice's lifespan until harvest.

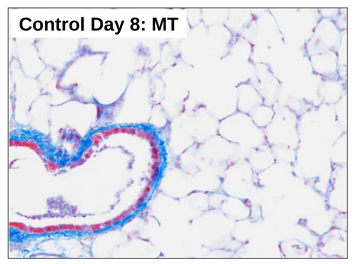
6a. At harvest, slices designated for biochemical evaluation are homogenized in 500 µL ice cold PBS+ 0.5% Triton X-100

6b. Slices designated for histological evaluation are placed into histology cassettes, submerged in 4% paraformaldehyde for ~24 hr. and then transferred to 70% EtOH solution until embedding, sectioning, and staining.

RESULTS

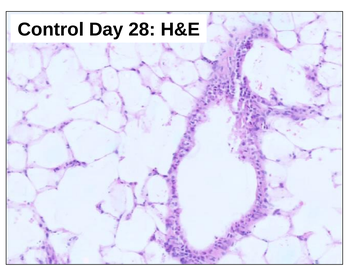
Culture Longevity: Histology

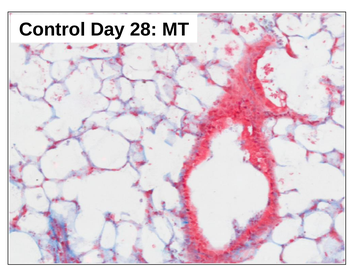




8 Days

- Alveoli are patent and bronchiolar epithelium intact.
- Only few strands of collagen fibers in alveolar walls; bronchiole shows thick layer of collagenous tissue



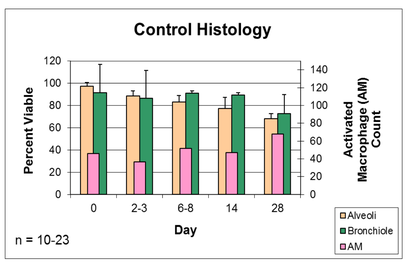


28 Days

- Bronchiole with intact epithelial lining cells.
- Histology is well-preserved
- Normal pattern of collagen deposition within interstitium

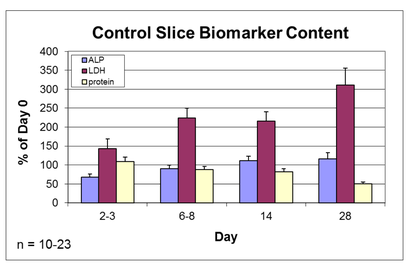
MT = Masson's Trichrome

Viability and Biochemical Stability



Viability and macrophages

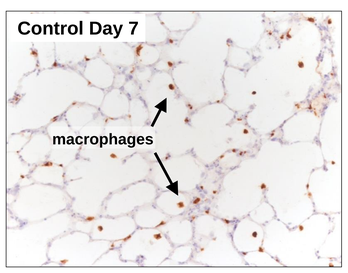
- High degree of alveolar and brochiolar viability retained over 28D
- Retention of viable, activated macrophages



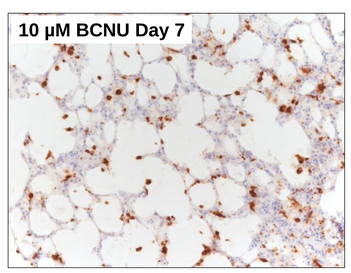
Biomarkers

- Retention of tissue markers over 28 days in serum-free M-199 medium
- Some loss of protein over time (coincides with minor loss of cellularity)

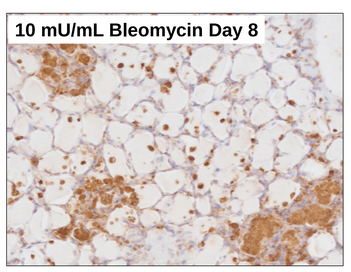
Hallmark of Inflammation: Activated Macrophages



- Untreated and vehicle control slices exhibit baseline levels of activated macrophages , as indicated by ED-1 immunostaining

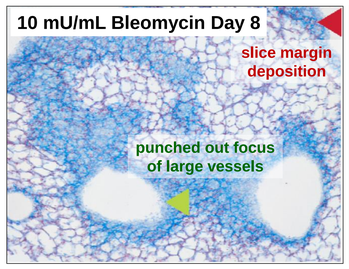


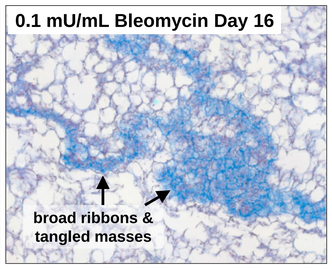
- BCNU (carmustine) exposure shows numerous macrophages, many of which have infiltrated alveolar walls mimicking interstitial pneumonitis



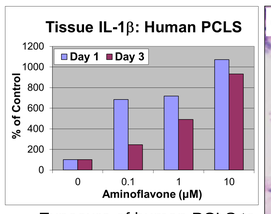
- Bleomycin treatment results in patches of activated macrophages filling alveolar spaces; many solitary macrophages also seen

Hallmark of Fibrosis: Collagen Deposition

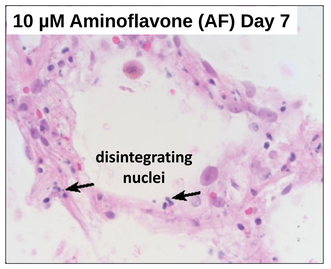




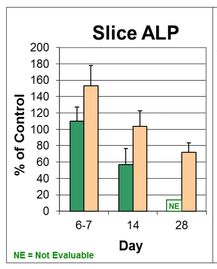
Mediators of Inflammation: Cytokines Precede Cell Death

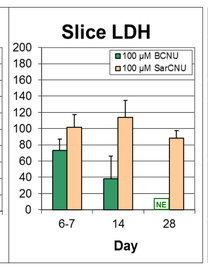


- Exposure of human PCLS to 10 µM AF causes cytokine increases in < 24 hr.



Comparison of Molecular Analogs (SarCNU and BCNU): Differential Toxicities



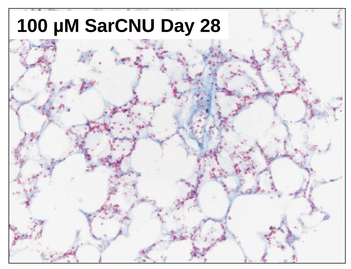


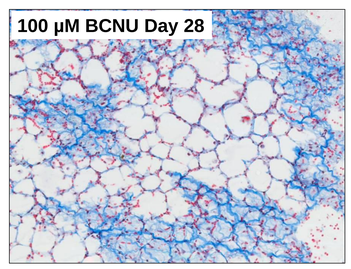
Activated Macrophages in Lung Slices

Compound	µM	Counts [a] Day 6-7	Day 14	Day 28
Vehicle	0	52	48	59
BCNU	1	60	94**	-
	10	67*	71*	-
	100	66	102**	81*
SarCNU	1	56	52	-
	10	61	57	-
	100	69*	76*	71

[a] Means of 3 measures on 3-4 replicates
* p<0.05 ** p<0.01

- Comparison of two analogs accurately demonstrates clinically noted differences in toxicity at the same dose range
- PCLS biomarker (LDH) content, numbers of activated macrophages, and observed levels of collagen deposition all reflect greater BCNU toxicity vs SarCNU





CONCLUSIONS

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

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