Evaluation of In Vitro Models of the Rat and Human Airway Epithelium for Assessment of Acute Toxicity

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1 Introduction
Acute airway toxicity is an important consideration for the development of pharmaceuticals, chemicals, cosmetics and agrochemicals. Accurate toxicity information is required to ensure safe use of products, procedures for accidental exposure and dose range selection for in vivo studies. Currently validated methods require the use of animals yet it is widely acknowledged that these methods may not be appropriate for accurate prediction of human risk. To combat this, and as part of a 3Rs strategy (refine, reduce, replace), in vivo models are being developed as an alternative strategy for assessment of inhalation toxicity.

One such commercially available airway model is Epithelium™ (MatTek Corp., Ashland, MA, USA) constructed from primary human airway epithelial cells. At present there is no means to correlate the responses of these tissues to human outcomes as available in vivo data has been produced in rodents. To address this information gap, an in vitro model has been generated in a similar manner to the human Epithelium™ model using cells from the airway epithelium of Charles River rats. In this study the rat and human models were compared on three independent occasions by two laboratories (Charles River and MatTek) using 14 test chemicals.

2 Methods

Human Epithelium™: The model was prepared by seeding normal human bronchial epithelial cells on a Milipore collagen coated membrane insert. Until a confluent monolayer was formed, the inserts were cultured submerged. When a confluent monolayer had developed, the inserts were further cultured at the air-liquid interface for up to 21 days. A fully differentiated airway model was formed (Figure 2).

Rat Epithelium™: Airway epithelial cells isolated from the conducting airways of 8-week old male CD rats (Charles River Laboratories, MA, USA) and seeded onto Milipore membrane inserts. These cultures were culminated at the air-liquid interface for up to 27 days (Figure 2).

Histological Evaluation: Human and rat cultures were fixed, embedded, sectioned and stained in H&E and examined.

3 RESULTS
Pre-dose TEER measurements from air liquid interface (ALI) tissues were found to be high indicating the successful production of a robust barrier in both human and rat Epithelium™. Histological evaluation of the Epithelium™ models revealed a pseudostriatified epithelium and mucociliary functionally.

The MTT and TEER data from Charles River and MatTek are presented in Table 1. IC₅₀ values from the MTT data are presented in Figure 5 and Figure 6. In each species, a clear dose response was observed in the MTT data. There were instances in both laboratories where it was not possible to obtain stable TEER measurements or a clear dose response in the TEER data. At Charles River, this was mostly pronounced with the rat Epithelium™ where an IC₅₀ was calculated on 3 or more occasions for only 7 chemicals. At MatTek, instances of this were dispersed between human and rat Epithelium™.

In conclusion, the panel of 14 test items were successfully tested on human and rat Epithelium™ tissues. The TEER and MTT viability results for both the rat and human Epithelium™ tissues were highly reproducible. The responses of the rat and human Epithelium™ tissues were generally similar, with the notable exception of methyl methacrylate and ethyl alcohol, for which the rat tissue was considerably more sensitive.

However, the results produced across each test run within each species were very consistent. In addition, the testing was carried out by two independent laboratories and the two datasets were in general agreement. Correlation of these results with in vivo results will be important for showing the predictiveness of these models for toxicity screening and safety assessments.

This work was funded in part by National Institute of Environmental Health Sciences grant R04ES014312-05 and by the Charles River Innovations Fund.

4 CONCLUSION