Characterisation and application of an in vitro model of cigarette smoke-induced lung injury for the assessment of a potentially modified risk tobacco product

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INTRODUCTION

Exposure to cigarette smoke and retention of smoke particulates in the lung has been implicated in the development of many smoking-related diseases, including cardiovascular disease, chronic obstructive pulmonary disease (COPD) and cancer. To assist in the evaluation of potentially modified risk tobacco products and the next generation of tobacco and nicotine related products we are developing a suite of in vitro cell culture systems that model many of the processes involved in disease development. Here we describe in-house studies to characterise and apply an in vitro model of COPD (bronchial epithelial cell cytotoxicity, inflammatory and tissue remodelling mediator secretion) using reference (3RF4), commercial control (CC) and a prototype cigarette with reduced toxicant levels in smoke (RTP2).

METHODS

A Borwaldt RM202 Smoking Machine (Borgwaldt, Germany) was used to generate and dilute smoke from 3RF4 (University of Kentucky, USA: 9mg ISO pack tar), CC (7mg ISO pack tar) and RTP2 (7mg ISO pack tar) cigarettes under the ISO smoking regime (35 ml puff volume drawn over 2 seconds once every minute: ISO 3308: 2000). Air, whole smoke (WS) or vapour phase (VP: particulate matter (PM) removed using an in-line Cambridge filter pad) was then delivered to purpose-built Perspex™ exposure chambers (Figure 1) for exposure of lung cells at the air-liquid interface (ALI). Following a 24 hour recovery period cell viability was measured using the Neutral Red uptake assay and secretion of inflammatory (IL-6 and IL-8) and tissue remodelling mediators (MMP-1) measured using electrochemiluminescence (MesoScale, USA).

The effect of WS exposure on cell viability, inflammatory and tissue remodelling mediator secretion from H292 cells

Lung epithelial cells (NCI-H292: ECACC, Salisbury, UK) at passage 89 and at a seeding density of 3 x 10^4 cells/ml were grown submerged on Transwell™ inserts until confluent. Cells were then transferred to exposure chambers, the surface culture medium removed and cells exposed for 30 minutes to a range of WS and VP dilutions (1:5 to 1:1000 (smoke:air, v/v)) at the ALI.

Figure 1: WS exposure chamber (A) and schematic cross section (B)

The effect of passage number and cell density on cell viability

Lung epithelial cells at passages 89 and 99 were initially seeded on to Transwell™ inserts at two seeding densities (2.85 x 10^5 cells/ml) and grown under culture medium until confluent. Exposure to whole smoke at toxic (1:5-1:100, smoke:air) and minimally toxic (equivalent to 95% cell viability: 1:200, smoke:air) doses were then undertaken.

STATISTICAL ANALYSIS

A sigmoidal dose response model was applied to the cell viability data and the EC50 (concentration of WS and VP (equivalent WS dose) that kills 50% of the cells) measured. Differences between groups were determined using the sum of squares F test. The effect of passage number, seeding density and WS on cell viability and inflammatory mediator secretion (Figure 3) were analysed using the General Linear Model ANOVA.

MP-1 concentrations following exposure to 3RF4, CC and RTP2 were log transformed and a linear regression analysis performed to determine differences in slope and Y intercept. A p value of <0.05 was considered significant.

RESULTS

The effect of WS and VP exposure on cell viability and inflammatory mediator secretion

Cell viability was reduced in a dose dependent manner following exposure to WS and VP (Figure 2). VP constitutes 82% of the toxicity derived from WS. Exposure to a minimally toxic concentration of WS resulted in a significant (p<0.05) increase in all inflammatory mediators measured in the culture medium (Table 1) when compared to air controls.

Figure 2: The effect of WS and VP exposure on cell viability (n=5). The EC50 for VP (5.8±1μg/cm²) was significantly (p<0.001) higher than the EC50 following WS exposure (4.8±0.9μg/cm²)

No significant difference in the slope or Y intercept of MMP-1 secretion was observed between CC and RTP2 (Figure 5).

SUMMARY

VP constitutes the majority of the cytotoxic effect of WS exposure and at minimally toxic doses of WS induces the secretion of inflammatory mediators. Lower passage numbered cultures are more sensitive to WS exposure and secrete more inflammatory and tissue remodelling mediators than higher passage numbered cultures. WS derived from RTP2 is significantly less cytotoxic but induces a similar tissue remodelling response to that of WS derived from CC cigarettes.

CONCLUSION

The current model is robust, and can be used to compare the cytotoxic effects of cigarette smoke exposure from a variety of combustible products. Further studies will be required to fully elucidate the role of cigarette smoke-induced inflammation in tissue injury and remodelling.