INTRODUCTION
Prolonged cigarette smoke exposure is known to be a causative factor in the development of cardiovascular disease, chronic obstructive pulmonary disease (COPD) and cancer (1). Cigarette whole smoke (WS) is a complex aerosol, made up of approximately 5700 identified constituents distributed in the particulate and vapour phases (VP), with the latter constituting ~95% by weight (2). The role of each component of cigarette smoke in the development of smoking related diseases is largely unknown, however, exposure to both phases and the retention of smoke particulate has been implicated to contribute to smoking related injury and disease (1,3).

To address the role of cigarette smoke in disease development we are developing a suite of in vitro models to assist in the evaluation of potentially modified reduced toxicant prototypes and the next generation of tobacco and nicotine related products. Here we describe in-house studies conducted using an in vitro smoking-related disease model of COPD (bronchial epithelial cell cytotoxicity and inflammatory/tissue remodelling mediator release). The model was utilised firstly to assess the contribution of the particulate and vapour phases of cigarette smoke on cell cytotoxicity and inflammatory mediator release and then to apply the model to evaluate reduced toxicant prototype (RTP2), reference (3R4F) and commercial control (CC) cigarettes.

METHODS

- Lung epithelial cells (NCI-H292; ECACC, Salisbury, UK) at passage 89 and a seeding density of 3 x 10⁵ cells/ml were grown in submerged culture on Transwell® inserts until confluent.
- The surface culture medium was removed to transition cells to the air-liquid interface (ALI) and cells were then transferred to purpose-built Perspex™ exposure chambers (Figures 1a & 1b).

Figure 1a. BAT’s whole smoke exposure chamber. Figure 1b. Schematic cross section of exposure chamber.
- Air (AC), cigarette WS and VP (particulate matter removed using an in-line Cambridge filter pad) were then delivered to the lung cells within the exposure chambers maintained at 37° C at the ALI.
- Cells were exposed at the ALI for 30 minutes or a total of 4 cigarettes (~30 minutes) for 3R4F WS/VP and R2TP/CC/3R4F experiments respectively.
- A range of smoke dilutions were used (1:2.5 to 1:4000 smoke:air, v/v).
- A Borgwaldt RM205 Smoking Machine (Hamburg, Germany) was used to generate, dilute and deliver the cigarette smoke (Figure 2).

Figure 2. Borgwaldt RM205 Smoking Machine.
- 3R4F (University of Kentucky, USA; 9.4mg ISO tar), CC (7mg ISO tar) and R2TP (7mg ISO tar) cigarettes were used.
- Cigarettes were smoked to the ISO smoking regime (35 ml puff volume drawn over 2 seconds once every minute: ISO 3308:2000).
- Following a 24 hour recovery period under culture medium cell viability was measured using the neutral red uptake assay.
- Secreted inflammatory (IL-6 and IL-8) and tissue remodelling (MMP-1) mediators were measured using electrochemiluminescence.
- For product assessment experiments smoke dilutions were converted to deposited particulate mass using Quartz Crystal Microbalances fitted within the exposure chambers to quantify dose.

STATISTICAL ANALYSIS

A sigmoidal dose response model was applied to the cytotoxicity data and the effect of whole smoke and vapour from all products on the EC₅₀ (concentration of WS and VP that kills 50% of the cells) determined using the sum of squares F test.

The effect of whole smoke on inflammatory mediator secretion was analysed using the General Linear Model ANOVA. A pairwise comparison (Tukey test) was made between groups to assess effect of exposure and dose on inflammatory mediator release. A p value of <0.05 was considered significant.

RESULTS

The effect of WS and VP exposure on the cytotoxic and inflammatory mediator secretion from H292 cells

Cell viability was reduced in a dose dependent manner following exposure to WS and VP (Figure 3a). VP constitutes 82% of the toxicity derived from WS as determined by the difference in EC₅₀. Exposure to a minimally toxic concentration of WS (equivalent to 95% cell viability) resulted in a significant (~p<0.05) increase in all inflammatory mediators measured in the culture medium (Figure 3b).

The effect of WS exposure from 3R4F, CC and R2TP cigarettes on the cytotoxic and inflammatory response of H292 cells

Although there was no difference in the EC₅₀ between CC and 3R4F, exposure to RTP2 resulted in a significant increase compared to CC (Figure 4a). No significant difference in the rate or the total concentration of MMP-1 secretion was observed between CC and RTP2. Total MMP-1 secretion was significantly greater for the reference product than for CC or RTP2 (Figure 4b).

SUMMARY

- VP constitutes the majority of the cytotoxic effect of WS exposure and at minimally toxic doses of WS the secretion of inflammatory mediators is induced.
- WS from R2TP is significantly less cytotoxic but induces the equivalent tissue remodelling mediator secretion as WS derived from CC.

CONCLUSION

This in vitro smoking-related disease model of COPD can be used to compare the cytotoxic and inflammatory/tissue remodelling effects of cigarette smoke exposure from different fractions of smoke and a variety of combustible products.

REFERENCES