The inflammatory response of the H292 lung epithelial model to cigarette smoke particulate generated using different smoking regimes and reduced toxicant prototypes

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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 smoke is a complex aerosol, made up of approximately 5700 identified constituents distributed in the particulate and vapour phases (2). The role of each component of cigarette smoke in the development of smoking related diseases is largely unknown, however, the retention of smoke particulate has been implicated to contribute to smoking related injury and disease (1,3). To address the role of the particulate fraction of cigarette smoke in disease development particulate matter (PM) extracts are used. We are developing 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 inflammatory/tissue remodelling mediator release). The model was utilised to assess the impact of particulate generated from cigarette smoke on inflammatory mediator release in response to reference (3R4F), reduced toxicant prototype (RTP) and commercial control (CC) cigarettes.

METHODS

• PM extracts were generated from 3R4F (University of Kentucky, USA: 9.4mg ISO tar), CC (6mg and 1mg ISO tar) and RTP (6mg and 2x1mg ISO tar) cigarettes. The two 1mg RTP products (RTPa and RTPb) incorporated different toxicant reduction technologies.
• Extracts were produced by trapping the cigarette smoke on a Cambridge filter pad (Fig 1) and eluting to a nominal 24mg/ml using a calculated volume of DMSO (4).

Two smoking regimes were tested: ISO smoking regime (35 ml puff volume drawn over 2 seconds once every minute: ISO 3308:2000) and an internal intense regime (IR) (55ml puff over 2 seconds every thirty seconds). PM extracts were generated for the 6mg products and the 3R4F using both the ISO and IR regimes but due to filter pad degradation, the 1mg product extracts were generated using the IR regime only.
• Lung epithelial cells (NCI-H292; ECACC, Salisbury, UK) at passage 89 and a seeding density of 2 x 10^5 cells/ml were grown in culture plates until confluent. Serum free media was then used 24 hours prior to exposure.
• Cells were exposed to a range of sub-toxic doses of PM extract (0 – 50μg/ml) for 24 hours, then media was removed and stored at -80°C.

Expression of inflammatory (IL-6, IL-8 and VEGF) and tissue remodelling (MMP-1,3,9) mediators was measured using Meso Scale Discovery’s electrochemiluminescence plate based assays.

Statistical Analysis

The effect of PM on inflammatory mediator secretion was analysed by first applying a ln transformation to the data, then 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 PM extracts produced using different smoking regimes on inflammatory mediator secretion from H292 cells

There was a significant difference (p<0.001) in secretion of all mediators in response to PM extract produced using different smoking regimes (Fig 2). The extracts produced using ISO smoking consistently induced the secretion of greater quantities of all inflammatory and remodelling mediators.

The effect of PM extracts produced using different product types on inflammatory mediator secretion from H292 cells

• All mediator levels increased proportionally with increasing concentration of PM extract, slopes generated for all products revealed the same pattern and were not significantly different to one another (Fig 3).

• When IR data alone were considered, significant differences were found between 1mg RTP products and their control (IL-6, p<0.001, Fig 4). Differences were also identified between 6mg RTP and the 3R4F control in both MMP-9, p=0.013 (data not shown) and VEGF, p=0.007 (Fig 5).

• ISO regime data (data not shown) identified differences between the 3R4F Reference cigarette and the 6mg RTP and the 3R4F control (p<0.005) was identified to cigarette smoke particulate generated using different smoking regimes.

• Expression of inflammatory (IL-6, IL-8 and VEGF) and tissue remodelling (MMP-1,3,9) mediators was measured using Meso Scale Discovery’s electrochemiluminescence plate based assays.

SUMMARY

• The model is sensitive to cigarette smoke PM and an inflammatory mediator response is induced at sub-cytotoxic doses.
• The model is able to discriminate between smoking regimes.
• Data is somewhat variable, not all mediators are able to differentiate RTPs from their CCs. This may be due to the normalisation of particulate levels across products and RTP modifications may not have reduced particulate phase toxicants sufficiently to have a measurable effect in this system.

CONCLUSION

This model should be used as part of a suite of tests which examine all fractions of smoke. It will find its greatest utility where product modifications have an impact on particulate profiles, or when examining differences in toxicity between combustible and novel alternative nicotine products.

REFERENCES