Validation of in vitro models of tobacco-related harm and their role in evaluating modified risk tobacco products


British American Tobacco, Group Research & Development, Regents Park Road, Southampton, SO15 8TL, United Kingdom

Correspondence: Gary_Phillips@bat.com

INTRODUCTION

Cigarette smoking is associated with an increased risk of developing diseases such as cardiovascular disease, chronic obstructive pulmonary disease (COPD) and cancer. We are developing a scientific framework to evaluate whether modified risk tobacco products (MRTPs), as referred to in the US Family Smoking Prevention and Tobacco Control Act, that yield substantially reduced levels of tobacco smoke toxics are also associated with reduced health risks compared with conventional cigarettes. One important part of such a framework is the evaluation of the biological effects of cigarette smoke in vitro models of smoking-related diseases.

Here we describe in-house validation studies conducted on two in vitro smoking-related disease models; cardiovascular disease (endothelial cell migration: wound repair) and COPD (bronchial epithelial cell mediator release: Inflammation and tissue remodelling) using reference, commercial control and a reduced toxicant prototype (RTP) cigarette [1].

METHODS

Particulate matter generation

Cigarettes were machine-smoked according to the ISO regime and the particulate matter (PM) from reference (3R4F; University of Kentucky (9.4mg ISO tar yield)), conventional (6mg ISO tar yield) and an equivalent ISO tar yield RTP (6mg) were collected on a Cambridge filter pad, eluted with DMSO to 24mg/ml and diluted in culture medium prior to use.

Endothelial cell migration

Experiments were carried out on human umbilical vein endothelial cells (HVECs; Lifetime Cell Systems). Confluent monolayers were wounded using a pipette tip, creating a scratch wound (~800µm in width). Cell migration across the wound was measured by image capture and computer analysis (InCytuse; Essen Instruments). PM dilutions were applied to the cells immediately after wounding and migration measured over a period of 20-24 hours. Data is expressed either as a percentage of initial wound width or rate of wound closure.

Bronchial epithelial cell mediator secretion

NCI-H292 (bronchial epithelial) cells were seeded in 6 well plates, incubated for 48 hours in RPMI 1640 medium and then in UltraCulture for a further 24 hours. Cells were exposed to PM at concentrations between 0 and 50µg/ml for 24 hours and the secreted inflammatory (IL-8, IL-6) and tissue remodelling (MMP-1) mediators measured. All mediators were measured by electrochemical luminescence detection (MesoScale Discovery).

VALIDATION

Reproducibility and repeatability

To examine the effect of three separate experimental factors on data variability, a Plackett-Burman factorial design (Table 1) was applied to both models using PM derived from 3R4F cigarettes. The effects of experimenter, cell passage number and PM batch were assessed on the endothelial migration assay and PM age, cell density and incubation time assessed on the bronchial epithelial cell mediator release assay. Positive controls

Cytochalasin D and endostatin (1µg/ml) were added to the culture medium of HVECs and bleomycin and LPS (10µg/ml) to the culture medium of NCI-H292 cells. Migration and mediator secretion were then measured as detailed above.

Response to a RTP

HUVEC migration rate following exposure to conventional and RTP PM at 0, 24 and 48µg/ml were determined. PM from the same products were also applied to the culture medium of NCI-H292 cells (0-50µg/ml) and mediator secretion measured following 24 hour exposure.

STATISTICAL ANALYSIS

All data were analysed using the General Linear Model ANOVA. A p value of < 0.05 was considered significant.

Table 1. Plackett-Burman factorial design to study the effects of three factors on the reproducibility and repeatability of the endothelial migration and bronchial epithelial mediator release assays

<table>
<thead>
<tr>
<th>Expt No.</th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For endothelial migration, Factor 1 represents experimenter, Factor 2 PM batch and Factor 3 passage number. This was repeated twice at 3 PM doses (0, 24 and 48µg/ml). For bronchial epithelial mediator release, Factor 1 represents cell density (1.9 or 2.1 x 10⁶ cells/ml), Factor 2 incubation time (22 or 26h) and Factor 3 PM age (1 or 8 days). Each group of 8 experiments (block) was repeated 4 times with each combination performed in triplicate.

RESULTS

Reproducibility and repeatability

No significant effect of PM batch, operator or cell passage number was observed on migration. Also no significant effect of PM age or incubation time on mediator secretion was observed. However, there was a significant increase in IL-8 and MMP-1 concentrations with increased cell density (Table 2)

Table 2. The effects of cell density, incubation time and PM age on NCI-H292 cell inflammatory and tissue remodelling responses

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Cell density</th>
<th>Incubation time</th>
<th>PM age</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>0.001</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IL-6</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.004</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data were derived from 4 independent experimental blocks performed in triplicate. ns = not significant.

Response to RTP

There was a significant effect of RTP exposure on IL-8 secretion. There was a significant effect of DPI exposure on IL-8 secretion, p < 0.05, ANOVA. Both models were responsive to positive controls, with significant decreases in migration (Figure 1) and increased mediator secretion (Table 5).

Exposure to PM from an RTP resulted in a significant increase in migration rate (Figure 2) and a decrease in mediator secretion (Figure 3), when compared to PM from a conventional product.

CONCLUSION

In-house validation studies on the endothelial cell migration and the bronchial epithelial cell mediator secretion assays indicate that these in vitro models are fit for purpose and can be used for future MRTP assessment.

REFERENCES


www.bat-science.com

Poster POS2-24 presented at 2012 SRNT 18th Annual Meeting, March 13-16, Houston, Texas, USA. This study was entirely funded by British American Tobacco