Introduction

Cigarette smoking is an identified cause of a number of human diseases. We are developing a range of products, including tobacco heating products (THPs) which deliver lower yields of aerosol toxicants than cigarette smoke, potentially reducing harm to the user. As part of an integrated testing strategy to allow us to compare the relative biological effects of new nicotine delivery cytotoxicities with those of traditional cigarettes, we have developed a suite of in vitro assays to model smoking related disease processes.

The aim of this study was to compare the responses induced by aerosols from a novel hybrid tobacco heating product (h-THP), with those from a commercially available THP (c-THP) and a reference 3R4F cigarette in a suite of in vitro assays.

Test products

Table 1: Aerosol generation regimes

<table>
<thead>
<tr>
<th>Product</th>
<th>Puff Regimes</th>
<th>Puff Volume (mL)</th>
<th>Puff Frequency (puffs/min)</th>
<th>Puff Profile</th>
<th>Vent blocking</th>
<th>Coil pre-activation</th>
<th>Cell pre-activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R4F</td>
<td>CSM No. 8i</td>
<td>0.5</td>
<td>2</td>
<td>1</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>h-THP</td>
<td>CSM No. 8i</td>
<td>0.5</td>
<td>2</td>
<td>1</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>c-THP</td>
<td>HCM</td>
<td>0.5</td>
<td>2</td>
<td>1</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

TPM: Approximately 150 mg of TPM was collected on 44 mm Cambridge filter pads (Whatman, UK). DMSO (Sigma-Aldrich, UK) was used to elute the TPM from the pads to a stock concentration of 24 mg/mL.

AqE: AqE from test products were produced by bubbling 10 puffs from each product through 20 mL of non-supplemented DMEM/F12 medium (Gibco, USA) in a glass impinger.

Methods

Generation of test matrices

Three different test matrices were used for in vitro assessments: total particulate matter (TPM), whole aerosol (WA), and aqueous aerosol extract (AqE). These were produced according to the puffing regimes detailed in Table 1.

Cytotoxicity assay

Human bronchial epithelial cells (NCI-H292) were exposed to WA at the air-liquid interface (ALI) for 24 hours. cytosolic cell death was assessed using the Neutral Red Uptake (NRU) cell viability assay as previously described6.

Figure 1. In vitro biological effect of exposure to AqE from a 3R4F reference cigarette, a commercial THP and a novel hybrid tobacco product. Apoptotic response (a), generation of intracellular reactive oxygen species (b), GSH:GSSG ratio (c), and ARE activation (d) in lung epithelial H292 cells. Wound healing rates in HUVEC monolayers (e). Data shown are mean ± S.D. (n=5) (*Significantly different from untreated control (p<0.05).

Conclusions

• The novel hybrid tobacco product showed little or no activity in any of the in vitro assays in which it was tested.

References


Bhas cell transformation assay

The potential of TPM from the products to induce tumour development was evaluated using the Bhas 42 cell transformation assay, promoter protocol6. TPM was tested at various concentrations up to a maximum concentration of 48 µg/mL.

Oxidative stress and apoptosis

NCl-H292 cells were assessed for oxidative stress and apoptosis following exposure to AqE from both products as previously described3.

Endothelial cell migration assay

Artificial wounds were created in monolayers of human umbilical vein endothelial cells (HUVEC). Cells were treated with AqE, and wound repair was assessed over 22 hours using image analysis, as previously reported8.

Results

The hybrid tobacco product showed little to no biological activity in any of the in vitro assays in which it was tested, across each of the test matrices (Figures 1 and 2).

Figure 2. Cytotoxic, genotoxic, and proliferative effects of WA and TPM from a 3R4F reference cigarette, a commercial THP and a novel hybrid tobacco product. Mutagenic response of S. typhimurium TA98 exposed to TPM (a) and WA (b) in the Ames test. Cytotoxic responses of lung epithelial H292 cells exposed to WA in a NRU cell viability assay (c). DNA damage as measured by γH2AX assay (d), and in vitro tumour promotion in response to TPM in the Bhas cell transformation assay (e). Data shown are mean ± S.D. (n=3) (a, b, d, e) ± 6 (c). *Significantly different from untreated control (p<0.05).
The in vitro biological assessment of a novel hybrid tobacco product and comparison with cigarette smoke

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Cigarette smoking is a risk factor for many diseases including cardiovascular disease, lung disease, and cancer. Recently there has been an increase in the development and consumer acceptance of novel nicotine and tobacco products including tobacco-heating products (THPs) and vapour products such as e-cigarettes.

Using a number of in vitro test methods, recently outlined as part of a framework to substantiate the risk reduction potential of novel tobacco and nicotine products, we have assessed the toxicological and biological effects of a novel hybrid tobacco product, iFuse, designed to reduce toxicant exposures. Responses were compared to a commercially available THP (THS) and a 3R4F reference cigarette.

Exposure matrices assessed included total particulate matter, whole aerosol, and aqueous aerosol extracts obtained after machine-puffing using the Health Canada Intense smoking regime. The hybrid tobacco product had little or no activity across all the in vitro assays assessing endpoints including mutagenicity (Ames), genotoxicity (γH2AX), cytotoxicity (neutral red uptake), tumour promotion (Bhas cell transformation), oxidative stress (ROS formation, intracellular glutathione content and antioxidant response element activation) and endothelial cell migration (wound healing) when compared to a 3R4F reference product. The THS product also demonstrated significantly reduced responses. These in vitro assays have enabled the biological assessment of a novel hybrid tobacco product, and results suggest the product demonstrates reduced health risks. Further pre-clinical and clinical assessments are required to understand further the risk reduction of these novel products at individual and population levels.

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