In Vitro Systems Toxicology Assessment of Exposure to Aerosol from a Carbon Heated Tobacco Product as Compared with Exposure to Cigarette Smoke: The Impact on Nasal and Small Airway Epithelial Cultures

Arita R., Iskandar, Yannick Martinez, Carole Mathis, Patrice Leroy, Florian Martin, Alan Sewer, Laura Ortgea Torres, Shaobai Majedy, Keyur Trivedi, Stefan Fentzelt, Emmanuel Guerdj, Celine Merg, Ashraf Elamin, Nikolai V. Ivanov, Manuel C. Peitsch, Julia Hoeng
Philip Morris International R&D, Philip Morris International S.A., Neuchâtel (part of Philip Morris International group of companies)

Introduction
Toxicological assessment of tobacco products should provide relevant indication for public health. Advances in tissue engineering have allowed the development of in vitro organotypic cultures with an air-liquid interface, thus permitting the study of the long-term effects of aerosols on human small airway epithelial cultures. This study assessed the impact of an aerosol from a carbon heated tobacco product (CHTP1.2)—compared with cigarette smoke (3R4F)—on organotypic nasal cultures (reconstituted from the epithelial cells of a 41-year-old female nonsmoker donor) and organotypic small airway cultures (reconstituted from the epithelial cells of a 55-year-old female nonsmoker donor). A paired design was implemented so that in parallel to the exposure to cigarette smoke or to CHTP1.2 aerosol, the cultures were also exposed to the culture medium. The collection of transcriptome profiles used in the study was the network-based analysis of transcriptome profiles (NPA), a system biology approach to translate complex biological networks into output models. A series of experimental repetitions was conducted to increase the assessment robustness (N = 3 exposure runs/repetition).

Materials & Methods

Culture History - In vitro aerosol exposure system

<table>
<thead>
<tr>
<th>Culture Type</th>
<th>Exposure Duration</th>
<th>Concentration (mg nicotine/L)</th>
<th>CHTP1.2</th>
<th>3R4F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal</td>
<td>24 h</td>
<td>0.27</td>
<td>3R4F</td>
<td>0.27</td>
</tr>
<tr>
<td>Small Airway</td>
<td>48 h</td>
<td>0.27</td>
<td>CHTP1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>0.27</td>
<td>CHTP1.2</td>
<td></td>
</tr>
</tbody>
</table>

Characterization of the 3R4F smoke and CHTP1.2 aerosol

The impact of 24 h of exposure to C5 from 3R4F reference cigarette (University of Kentucky) was compared with CHTP1.2 aerosol in human organotypic nasal cultures (reconstituted from the nasal epithelial cells of a 41 year-old female nonsmoker donor) and organotypic small airway cultures (reconstituted from the small airway epithelial cells of a 55 year-old female, nonsmoker donor). A paired design was implemented so that in parallel to the exposure to the 3R4F smoke or to the CHTP1.2 aerosol, the cultures were also exposed to the culture medium. To increase the assessment robustness (N = 3 exposure runs/repetition). Characterization of the 3R4F smoke and CHTP1.2 aerosol in the exposure system (10% FSPTCA, 2009) includes the measurement of nicotine in the diluted 3R4F smoke and CHTP1.2 aerosol and the determination of nicotine concentration in the exposed cultures using Luminex® xMAP® technology.

Post-Exposure Measurements

Cytotoxicity was assessed by measuring the adenylate kinase activity in the basolateral medium of the exposed cultures using 100 ng of total RNA (per sample) that were isolated using human epithelial culture TRIzol. RNA was reverse transcribed using high temperature reverse transcriptase and the cDNA was used as template for quantitative real-time polymerase chain reaction (qPCR) with the mRNA levels of the following gene sets:

- Secreted mediators (MMP-1, MMP-9, TIMP-1, VEGFA, IL-1B, IL-6, IL-8, INFγ, TNFα, TGFβ)
- MicroRNA changes (hsa−miR−132−3p, hsa−miR−149−5p)
- Inflammatory response (NOS2, TNFα, IL-1B, IL-6, IL-8, INFγ, TGFβ)

The heatmap lists only the miRNAs that were differentially expressed following exposure at least in one contrast in both culture types. The results show that the 3R4F exposure was found to be sub-toxic at all tested concentrations (N = 9 per group in nasal; N = 6 per group in small airway studies).

Conclusions

The results show that the impact of CHTP1.2 aerosol was considerably less than 3R4F smoke at all tested concentrations, in regard to:

- Cytotoxicity
- Inflammatory responses (based on the secreted pro-inflammatory mediator levels) following exposure

A network-based systems biology approach was used to conduct qPCR to assess the expression of genes involved in respiratory tissues. CHTP1.2 exposure led to less upregulated inflammatory mediator levels than 3R4F smoke exposure at all tested concentrations.

References