Comparison of the impacts of an acute exposure to electronic cigarette aerosol and cigarette smoke on small airway epithelial cultures: In vitro systems toxicology assessment

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Introduction and Objectives

With increasing popularity of electronic cigarettes (EC), it is important to assess the potential toxicity of EC aerosol exposure. In the context of a harm reduction approach, we examined the effects of whole EC aerosol exposure relative to the effects of mainstream cigarette smoke (CS) exposure using human organotypic small airway cultures. Cultures were exposed at the air-liquid interface to 112 puffs of either undiluted aerosols generated from a MarkTen® cartridge containing various e-liquids (with aerosol formers alone [“Carrier”] with 4% nicotine [“Base”], with 4% nicotine and flavors [“Test Red”] or to diluted CS, in Vitrocell® exposure systems. We conducted a series of independent exposure repetitions to strengthen the accuracy of the observation. The concentrations of the deposited nicotine and carbonyls in the exposure chamber were measured as markers of exposure. Biological endpoints investigated included histology, cytotoxicity, inflammatory mediators, and gene microarray. Alterations in morphology or cytotoxicity were not observed in small airway cultures exposed to undiluted EC aerosols despite resulting in higher nicotine deposition in the chamber than that found following CS exposure. Increased loss of DNA was recorded in cultures exposed to CS but not in water exposed to any EC aerosol. Media of cultures exposed to EC had generally lower levels of inflammatory mediators in comparison to cultures exposed to EC aerosols. CS exposure elicited a greater number of differentially expressed genes. Based on a network-based enrichment analysis, the transcriptional data showed that the exposures impacted different cellular processes (e.g., cell fate, proliferation, stress, and inflammatory response) with greater impacts following CS exposure than following EC aerosol exposure. These collective endpoints demonstrated that EC aerosols had significantly lower biological impact in small airway cultures, in comparison to CS exposure.

Methods

ENDPOINTS

Time Point of Measurement

Pre-Exposure 24 h Post-Exposure

Determined using exposed PBS samples

- Deposited nicotine in the exposure chamber

Determined using the epithelial cultures

- Culture histology

- Ciliary beating frequency

- Transepithelial resistance

Determined using the basolateral medium samples

- Secreted inflammatory mediators

Culture Morphology Following Exposure

<table>
<thead>
<tr>
<th>Dose of Exposure</th>
<th>Culture Morphology Following Exposure</th>
<th>Ciliary Beating Frequency</th>
<th>Network Enrichment Based on the Transcriptome Changes</th>
<th>Secretion of Inflammatory Mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>7% 3R4F CS for 28 min</td>
<td>Apoptosis Score</td>
<td>Weighted Frequency</td>
<td>log2(fold change)</td>
<td>Cell Fat</td>
</tr>
<tr>
<td>100% Test Red for 28 min</td>
<td>100% Carrier for 28 min</td>
<td>100% Base for 28 min</td>
<td>100% Test Red for 28 min</td>
<td>100% Base for 28 min</td>
</tr>
<tr>
<td>100% Base for 28 min</td>
<td>100% Carrier for 28 min</td>
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<tr>
<td>100% Carrier for 28 min</td>
<td>100% Carrier for 28 min</td>
<td>100% Base for 28 min</td>
<td>100% Carrier for 28 min</td>
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</tr>
</tbody>
</table>

Ciliary beating frequency

- Reduced ciliary beating frequencies were detected following exposure to 7% 3R4F CS for 28 minutes equivalent to ~12 µg nicotine/mL PBS*
- Equivalent to ~0 µg nicotine/mL PBS for 100% Carrier EC aerosol for 28 min
- Equivalent to ~12 µg nicotine/mL PBS for 100% Test Red EC aerosol for 28 min
- Equivalent to ~154 µg nicotine/mL PBS for 100% Base EC aerosol for 28 min
- Equivalent to ~254 µg nicotine/mL PBS for 100% Carrier EC aerosol for 28 min

Network Enrichment Based on the Transcriptome Changes

<table>
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<th>Secretion of Inflammatory Mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>7% 3R4F CS for 28 min</td>
<td>MMP−1</td>
<td>Cell Proliferation</td>
</tr>
<tr>
<td>100% Test Red for 28 min</td>
<td>TIMP−1</td>
<td>Cell Stress</td>
</tr>
<tr>
<td>100% Base for 28 min</td>
<td>IL−1β</td>
<td>Inflammatory Processes</td>
</tr>
<tr>
<td>100% Carrier for 28 min</td>
<td>IL−13</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

In conclusion, when compared to air exposure, exposure to Test Red Base, or Carrier EC aerosols resulted in a lower impact than exposure to CS on in vitro human organotypic small airway epithelial cultures. Overall, marked differences in the measured endpoints (culture morphology, ciliary beating frequencies, secretion of inflammatory mediators, and global gene expression changes) were not observed following exposure to Test Red Base, or Carrier EC aerosols. The observation further suggested minimal specific effects of nicotine or flavor ingredients in the alternations of various biological endpoints measured in the study.

References


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*Former Altria Client Services employee.