E-cigarettes induce lower biological responses than conventional cigarettes: A comparison of in vitro toxicity following repeated whole aerosol exposure to human bronchial tissue for 4 weeks

Luukas Czekala,1 Roman Wiedencreutz,2 Edgar Treille Stiecklin,2 Lisa Maria Bode1 Liam Simms,1 Matthew Stevenson1

1. INTRODUCTION

Smoking is a cause of serious diseases in smokers, including lung cancer, heart disease and emphysema. There is scientific agreement that the harmful toxins formed during tobacco combustion are the cause of smoking-related diseases, not nicotine. For this reason numerous public health bodies and governments worldwide have indicated that e-cigarettes have a central role to play in tobacco harm reduction. Previously, we have shown that acute exposure of cigarette aerosol to 3D tissue did result in no significant toxicity compared to matched air controls (Czekala et al., 2019). In this study we compared the in vitro acute toxicity of a 3D organotypic model of the human airway epithelia (MucAl™, Epithelix) following exposure to aerosols of conventional cigarettes (3R4F) and myblu™ (1:17 dilution) in a range of functional endpoints.

2. MATERIALS AND METHODS

2.1 Tissues

MucAl™ tissues, a fully differentiated 3D airway epithelium, were purchased from Epithelix Sarl and cultured for more than 7 days before use. The tissues were exposed to test smoke/aerosol under the conditions listed in 2.2.

2.2 Smoke and Aerosol Generation

Test product aerosol/smoke was generated according to Table 1. Tissues were repeatedly exposed (3 times per week) at the Air Liquid Interface (ALI) for 4 weeks to either 30, 60 or 90 puffs of aerosol/smoke/filtered humidified air control using Imperial Brands’ Smoke Aerosol Exposure In Vitro System (EVI System) at Ghent University (Belgium). Cigarette smoke was diluted with filtered humidified air 1:17 times whilst myblu™ aerosol was applied undiluted. Following each individual exposure, tissues were incubated for 24 hours at standard culture conditions before further repeated exposures.

Table 1: Smoke and Aerosol Generation regimes

<table>
<thead>
<tr>
<th>Dose</th>
<th>3R4F Cigarettes</th>
<th>3R4F Cigarettes</th>
<th>3R4F Cigarettes</th>
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<tbody>
<tr>
<td>Exposure</td>
<td>30 puffs</td>
<td>60 puffs</td>
<td>90 puffs</td>
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2.3 Dosimetry

The nicotine dose delivered to the 24 Multi Well Plate (MWP) filled with Phosphate-buffered saline (PBS) was calculated using an AB Sciex API 6500 (Q-Trap) with an Agilent (1200 Infinity) HPLC system to smoke/use/vapour deliveries to the model. The Electrospray ionization (ESI) and multiple reaction monitoring in positive ion mode was used. The quantification was performed with an external calibration of nicotine with a range from 2.5-100 ng/ml.

3. RESULTS

3.1 Dosimetry of Smoke / Aerosol

The nicotine delivered to 24 MWP plates was quantified and results are presented in Figure 2. Nocotine delivery for the 3R4F is lower than corresponding myblu™ (undiluted) due to the 1:17 dilution of the smoke. A good dose correlation is observed.

3.2 In Vitro Toxicology

3.2.1 Tissue Viability and Barrier Integrity

The TEER value decreased significantly after exposure to the 3R4F smoke. This suggested a major loss of model barrier integrity. No differences in TEER were observed after exposure to the myblu™ aerosol in comparison to matched air control.

3.2.2 Histology and immunofluorescence staining

4. CONCLUSIONS

Although myblu™ delivered significantly more nicotine compared to the 3R4F cigarette smoke, it did not trigger any significant toxicological responses in any of the models compared with matched air controls.

Data demonstrated a dose response and also showed an increase in MMP expression with greater nicotine exposure, as would be expected when using repetitive nicotine exposure.

In conclusion, the results of this study appear to support the hypothesis that the aerosol from myblu™, using a 1:17 dilution, induced significantly lower biological responses than conventional cigarettes. This research was conducted in adherence with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

REFERENCES


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