The use of human 3D reconstructed bronchial tissue to study the effects of cigarette smoke and e-cigarette aerosol on a wide range of cellular endpoints

Lukasz Czekala1, Matthew Stevenson1, Liam Simms1, Nicole Tschiertske1, Anna G. Maione2, Tanvir Walele3

1 Imperial Tobacco Ltd, 121 Wintertonke Road, Bristol, BS3 2LL UK; 2 MatTek, 200 Homer Ave., Ashland, MA, USA; 3 Fontem Ventures B.V., an Imperial Brands PLC Company, Radwarweg 60, 1043 NT Amsterdam

Visit our science website: www.fontenscience.com

1. Introduction

In 2015, Public Health England characterised e-cigarettes as being around 95% less harmful than smoking. In 2016, the UK Royal College of Physicians concluded that the long-term health risks associated with e-cigarettes are unlikely to exceed 5% of those associated with smoked tobacco products, substantially less. However, some recent data has reported that e-cigarette aerosol can potentially produce reactive oxygen species which may give rise to inflammation, DNA damage and reduced cell viability. To investigate these claims, we studied the effect of two different e-cigarette aerosols on EpAirway™ 3D tissue and a variety of biological endpoints.

2. Materials and Methods

A box PLUS® closed system e-cigarette device was used to generate aerosol using two different e-liquids (Blueberry 2.4% nicotine and a flavourless base liquid containing 2.4% nicotine). Conventional cigarettes and blu® PLUS devices were obtained from local vendors (Ashland, MA, USA).

2.1 Smoke and aerosol generation

Cigarette smoke and e-cigarette aerosol were generated using a VITRECELL™ VC 1 smoking machine following the Health Canada Intensive (HCI) cigarette smoke and the CORESTA Recommended Method No 81 (CRM N° 81) (e-cigarette aerosol). The exposure module contained six chambers; three for smoke exposures and three for air exposures in parallel. The dilution rate used for in vitro tissue exposures was 1 L/min.

2.2 Three-dimensional in vitro respiratory tissue exposures

EpAirway™ tissues (MatTek Corp., Ashland, MA, USA) are 3-dimensional (3D) in vitro organotypic model of the human respiratory epithelium cultured at the Air-Liquid Interface (ALI). Tissues were exposed in triplicate to 2, 27 or 45 puffs of whole smoke generated from cigarettes (1, 3 or 5 cigarettes, respectively) or to 80, 240 or 400 puffs of aerosol from blu® PLUS e-cigarettes with either the base liquid or blueberry flavoured e-liquid with equal nicotine concentrations. Triton X-100 (Sigma-Aldrich) was included as a positive control. Following exposure, tissues were cultured for an additional 24 hours, according to the manufacturer’s instructions, before harvesting for analysis.

2.3 Tissue viability and barrier integrity

EpAirway™ tissue viability was assessed 24 hours after exposure using the MTT assay (MatTek Corp.). Barrier integrity of each tissue was assessed by measuring Transepithelial Electrical Resistance Integrity (TER) using an EVOM2 voltmeter (World Precision Instruments, Sarasota, FL, USA). Measurements were made immediately prior to exposure and 24 hours after exposure. Barrier function was considered intact if the measurement was greater than or equal to 300 Ω·cm², according to the tissue manufacturer.

2.4 Assessment of cytokine secretion and oxidative stress

Media were collected from each tissue model 24 hours after exposure to determine tissue secretion of the pro-inflammatory cytokine interleukin 6 (IL-6) and interleukin 8 (IL-8). Samples were analysed using the Quantikine ELISA kits according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA). Presence of B-isoprostane is considered to be a relative indicator of oxidative stress and antioxidant deficiency. The concentration of B-isoprostane in conditioned media was assessed using a competitive ELISA kit according to the manufacturer’s instructions (Cayman Chemical, Ann Arbor, MI, USA). Absorption was measured using a SpectraMax M2 spectrophotometer (Molecular Devices).

1.1 Histology and immunofluorescence

Tissue morphology was assessed by H&E staining. Immunofluorescence staining was conducted for specific markers of proliferation (Ki67), data not shown and DNA damage (γ-H2AX). Sections were permounted, blocked and incubated in the primary antibody (Abcam, Cambridge, MA, USA). Samples were analysed using an Olympus VSI120 Virtual Slide Microscope (lymphim, Shijishu, Tokyo, Japan).

2.6 Data and statistical analysis

All data and statistical analysis was conducted using Microsoft Excel and GraphPad Prism Software. Statistically significant differences between samples were calculated using one-way ANOVA with appropriate post hoc tests. A difference was considered statistically significant with a p-value < 0.05.

3. Results

3.1 Tissue viability

Tissue viability declined to 45% and 27% following exposure to 27 puffs and 45 puffs of cigarettes, respectively. Tissues maintained 100% viability with exposure to either the base liquid aerosol or blueberry e-liquid aerosol up to 400 puffs. *p-value < 0.05

3.2 Transepithelial electrical resistance (TEER)

Exposure to cigarette smoke, 27 and 45 puffs, significantly reduced TEER to 88±3% and 80±2% respectively (7% and 9% of the base liquid control). The e-liquid aerosols did not impact barrier function up to the highest dose tested. *p-value < 0.05

3.3 Cytokine secretion: IL-6

IL-6 increased with increasing number of puffs for cigarette smoke aerosol (27 puffs = 3.8 fold and 45 puffs = 4.6 fold more IL-6 than matched air control). There was no statistical difference in IL-6 secretion between aerosolised flue-shorter and their matched air control. *p-value < 0.05

3.4 The oxidative stress response

Cigarette smoke aerosol produced significantly increased amounts of isoprostane in a dose-dependent manner. Isoprostane levels did not alter for samples exposed to e-cigarette aerosol, with or without blueberry flavouring. *p-value < 0.05

1.2 Under the experimental conditions, cigarette smoke impaired barrier function and reduced cell viability to approximately 30% after exposure to 45 puffs and induced secretion of inflammatory cytokines.

1.3 The e-liquid aerosol exposures did not significantly alter the B-isoprostane compared to the matched controls at any of the doses tested. The 27 puffs and 45 puffs of cigarette smoke, were statistically higher than exposure to all aerosol doses of both e-liquids. The results suggest that the flavouring did not impact the tissues’ oxidative stress response.

1.4 We believe that the use of 3D in vitro organotypic models of the human respiratory epithelium should be a part of a wider risk assessment framework.

Future work:

Future work will include studies with 3D, air-liquid interface lung models addressing transcripomic, proteomic and functional responses to repeated smoke/aerosol exposure from the next generation products.

References


Poster ID: 2803

Figure 1. Tissue viability

Figure 2. Transepithelial electrical resistance (TEER)

Figure 3a. Cytokine secretion: IL-6

Figure 3b. Cytokine secretion: IL-8

Figure 4. The oxidative stress response

Figure 5. Histological evaluation of tissues by H&E staining following smoke and aerosol exposure at AI

Figure 6. γ-H2AX staining and quantification as a marker of DNA double-strand breaks

Poster ID: 2803

Image 1155x1476 to 1658x1823

Image 1192x989 to 1726x837

Image 1183x489 to 1726x1462

Image 1155x2128; Figure 5.

Image 1192x2128; Figure 6.