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

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1. Introduction

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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, a 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-liquid aerosols on Epairway\(^\text{TM}\) 3D tissue and a variety of biological endpoints.

2. Materials and Methods

2.0 Test Articles

A bi-PLUS\(^\text{TM}\) 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 bi-PLUS\(^\text{TM}\) 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 VITOREC\(\text{TM}\) 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 both exposure exposures was 1 L/min.

2.2 Three-dimensional in vitro respiratory tissue exposures

Epairway\(^\text{TM}\) tissues (MatTek Corp., Ashland, MA, USA) are a 3-dimensional (3D) in vitro organotypic model of the human respiratory epithelium cultured at the Air-Liquid Interface (AI). 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 bi-PLUS\(^\text{TM}\) e-cigarettes with either the base e-liquid or Blueberry-flavoured e-liquid with equal nicotine concentrations. Triton X-100 (Sigma-Aldrich) was included as a control. Following exposure, tissues were cultured for a further 24 hours, according to the manufacturer’s instructions, before harvesting for analysis.

2.3 Tissue viability and barrier integrity

Epairway\(^\text{TM}\) 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 (TEER) using an EVCMM voltmetermeter (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 Q cm\(^2\), 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 cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8). Samples were analysed using the Quantikine ELSA 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). Absorbance was measured using a SpectraMax M2 spectrophotometer (Molecular Devices).

2.5 Histology and immunofluorescence

Tissue morphology was assessed by H&E staining. Immunofluorescent staining was conducted for specific markers of proliferation (Ki67), data not shown and DNA damage (γ-H2AX). Sections were permeabilized, blocked and incubated in the primary antibody (Abcam, Cambridge, MA, USA) for one hour at room temperature. Then sections were washed, incubated in the secondary antibody (Invitrogen, Carlsbad, CA, USA) for one hour at room temperature, incubated in DAPI (MatTek Corp.) to stain the nuclei and a coverslip applied. All slides were imaged using an Olympus VS120 Virtual Slide microscope (Lympho, Shinjuku, 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 85% and 73% following exposure to 27 and 45 puffs of cigarettes, respectively. Tissues remained 100% viable 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 ≤0.3 Ohm and 3.7 Ohm respectively (1% and 3% of the baseline value). 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 fold and 45 puffs: ~1 fold higher than matched air control). There was no statistical difference in IL-6 secretion between aerosol-exposed tissues and their matched air control. *p-value ≤ 0.05

3.4 The oxidative stress response

Cigarette smoke produced significantly increased amounts of 8-isoprostanate in a dose-dependent manner. B-isoprostane levels did not alter for samples exposed to cigarette e-aerosol, with or without blueberry-flavoured e-cigarette aerosol, as a positive control for cell death and demonstrated increased positive staining for γ-H2AX. A slight increase of 4% of nuclei positive cells was observed for the 27 puff dose of cigarette smoke; however the quantification of the 27 puff and 45 puff dose of cigarette smoke-exposed tissues may have been affected by the substantial destruction and loss of tissue. There were no significant differences between air-exposed tissues compared to smoke- or e-cigarette aerosol-exposed tissues at any of the doses tested.

4. Summary/Future work

• Under the experimental conditions, cigarette smoke impaired barrier function and reduced cell viability to approximately 50% after exposure to 45 puffs and induced secretion of inflammatory cytokines.

• E-cigarette aerosol up to 400 puffs did not alter barrier function, cellular viability or cytokine secretion compared to air matched controls. E-cigarettes up to the highest dose, did not induce DNA double strand breaks, as shown using γ-H2AX staining.

• The IL-6 and IL-8 levels remained largely unaffected by e-cigarette aerosols (except slight, non significant increase for the highest dose, 400 puffs of flavoured e-liquid).

• 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 suggests that the flattening did not impact the tissues’ oxidative stress response.

• We believe that the use of this 3D in vitro organotypic model 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 transcriptomic, proteomic and functional responses to repeated smoke/aerosol exposure from the next generation products.

References

