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

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 toxicants 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. 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 exposures of e-cigarette aerosol to 3D tissue resulted in no significant toxicity compared to matched air controls (Czekala et al, 2019). In this study we compared the in vitro toxicological responses of a 3D organotypic model of the human airway epithelium (MucIar™, Epithelix) followed repeated exposures to either myblu™ whole undiluted aerosol or Kentucky Reference Cigarette (3RF4) smoke (1:17 dilution) in a range of functional endpoints.

2. MATERIALS AND METHODS

2.0 Test Articles

- Kentucky 3RF4 Reference Cigarette
- myblu™ device (Figure 1) and pod (1.6% [w/w] nicotine; tobacco flavour), UK market

2.1 Tissues

MucIar™ tissues, a fully differentiated 3D airway epithelium, were purchased from Epithelix 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, tobacco 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 to In Vitro System (SAGEIVS) (Burghart Tabaktechnik, Wedel, Germany). Tobacco 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.

2.3 Dosimetry

The nicotine dose delivered to the 24 Multi-Well Plate (MWP) fitted with Phosphate-buffered saline (PBS) was analysed using an AB Sciex API 6500 (Q-Trap) with an Agilent (1290 Infinity) HPLC system to ensure smoke/aerosol delivery to the model. The Electrospray Ionisation (ESI) and multiple reaction monitoring in positive mode were performed using an AB Sciex API 6500 (Q-Trap) with an Agilent (1290 Infinity) HPLC system to ensure smoke/aerosol delivery to the model.

2.4 Tissue viability and barrier integrity

Tissue viability and barrier integrity was assessed using the Transepithelial Electrical Resistance (TER) using an EVM200 Epithelial Voltmoheter. Morphological changes were observed by microscope and documented.

2.5 Histology and immunofluorescence staining

Histological evaluation (H&E/Alcian Blue and FoxJ1) after 4 weeks of repeated exposure to 30, 60 and 90 puffs of undiluted 3RF4 cigarette smoke at 1/17 dilution of the smoke. The undiluted myblu™ aerosol at any dose tested did not alter cytokine secretion (IL-8, IL-6, MMP-1 – data shown, and IL-1β, TNF-α, MMP-3, MMP-9 – data not shown) in comparison to matched air control.

2.6 Data and statistical analysis

All data and statistical analysis were conducted using Microsoft Excel and GraphPad Prism. Statistically significant differences between samples were calculated using ANOVA with posthoc Dunnett’s test. All differences were considered statistically significant with a p-value < 0.05.

REFERENCES


3. RESULTS

3.1 Dosimetry of Smoke / Aerosol

The nicotine delivered to 24 MMP plates was quantified and results are presented in Table 1. The nicotine delivered to 3RF4 for the 3RF4 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 3RF4 smoke. This suggests a major loss of barrier integrity. No differences in TER were observed after exposure to the myblu™ aerosol in comparison to matched air control

3.2.2 Histology and immunofluorescence staining

3.2.3 Inflammatory markers assessment

3.3 Cilia Beat Frequency

The Cilia Beat Frequency (CBF) and Active Area (CAA) were not affected by any dose of undiluted myblu™ aerosol. The 3RF4 cigarette smoke caused a dose-dependent decrease of both CBF and CAA.

4. CONCLUSIONS

- Although myblu™ delivered significantly lower compared to the 3RF4 cigarette smoke, it did not trigger any significant toxicological response in any of the models compared with matched air controls. It demonstrates that a dose response to diluted (1/17) cigarette smoke was various endpoints assessed, including changes to tissue morphology at 30 (loss of cilia) and 90 puffs, with significant increases in selected cytokines (IL-8, IL-6, IL-1β, TNF-α, MMP-3, MMP-9 – data not shown) in comparison to matched air control.
- Where cigarette smoke significantly decreased CBF, CAA and number of ciliated cells at all doses tested, tissues exposed to myblu™ aerosol were indistinguishable from matched air control.
- In overall, these results add to a weight-of-evidence approach to substantiate the harm reduction potential of myblu™ for adult smokers.

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