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. Studies 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 (MucilAir™; EpitheliC) follow 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

MucilAir™ tissues, a fully differentiated 3D airway epithelium, were purchased from EpitheliC 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, tobacoo 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 (SAEIVS) (Burghart Tabaktechnik, Wedel, Germany). 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.

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 6000 (Q-Trip) with an Agilent (1200 Infinity) HPLC system to ensure smoke/aerosol delivery to the model. The Electrospray ionisation (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.

2.4 Tissue viability and barrier integrity

Cellular cytotoxicity was assessed via the LDH quantification in growth culture media at each exposure time (data not shown). Barrier integrity of each tissue was assessed weekly by measuring the Transepithelial Electrical Resistance (TEER) using an EVOM2 Epithelial Voltohmmeter. Morphological changes were observed by microscopy and documented.

2.5 History and immunofluorescence staining

Tissue architecture was assessed using H&E/AcanBlue staining. Fox-J1 and MUC-5 AC (not shown) immunostaining were used to stain for ciliated cells and mucin, respectively. IL-13 served as a positive control for goblet cell hyperplasia.

2.6 Inflammatory markers

Inflammatory markers (IL-1β, IL-6, IL-8; TNF-α; MMP-1, MMP-3, & MMP-9) secreted into the culture media were collected from each well prior to the exposure on the exposure day and were frozen at -80°C for further analysis. Assessment of cytokines, chemokines and matrix metalloproteinases were performed with Meso Scale Dx QuickPlex™ on custom made plates more according to the manufacturers recommendations (Meso Scale).

2.7 Cilia Beat Frequency and Active Area

Cilia activity were measured every week on Tuesday and Friday, 4 hours after exposure to each agent/control. Cilia Beat Frequency (CBF) and Active Area (CAA) were evaluated under 4x magnification using a software.

2.8 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.

3. RESULTS

3.1 Dosimetry of Smoke / Aerosol

The nicotine delivered to 24 MWP plates was quantified and results are presented in Figure 1 and Table 1. For the 3RF4 the nicotine dose delivered 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 suggest a major loss of model barrier integrity. No differences in TEER were observed after exposure to the myblu™ aerosol in comparison with matched air control. LDH levels (data not shown) did not distinguish between any groups during the study, except 90 puffs of 3RF4.

3.2.2 Histology and immunofluorescence staining

Cilia Beat Frequency (CBF) and Active Area (CAA) were not affected by exposure to the 3RF4 aerosol in comparison with matched air control. Despite smoke and aerosol, the 3RF4 cigarette significantly altered the secretion of all cytokines mentioned above at 60 and 90 puff dose (representative cytokines presented).

3.2.3 Inflammatory markers assessment

The unfiltered myblu™ aerosol at any dose tested did not alter cytokine secretion (IL-6, IL-8, MMP-1 – data shown, and IL-1β, TNF-α, MMP-3, MMP-9 – data not shown) in comparison to match air control.

3.4 Cilia Beat Frequency

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

4. CONCLUSIONS

- Although myblu™ delivered significantly less nicotine compared to the 3RF4 cigarette smoke, it did not trigger any significant toxicological response in any of the models compared with matched air controls.
- Nicotine delivered to the 3RF4 tissue resulted in a dose dependent decrease in CBF and 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.

REFERENCES

[3] Coresta Recommended Method Nº 81
[4] Imperial Brands PLC, 121 Winterstoke Road, Bristol, BS3 2LL, UK
[5] A dosage of 30, 60 or 90 puffs of aerosol/smoke/filtered humidified air control using Imperial Brands’ Smoke Aerosol Exposure to In Vitro System (SAEIVS) (Burghart Tabaktechnik, Wedel, Germany). 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.

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