The in vitro assessment of an electronic cigarette (Vypen ePen) using a suite of pre-clinical tools

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INTRODUCTION

There has been significant growth in the number of smokers currently using next generation products including electronic cigarettes (e-cigarettes). E-cigarette products do not contain tobacco and the toxicant delivery of these aerosols are greatly reduced in comparison to conventional tobacco products, suggesting that they could hold promise as reduced risk products. In this study we describe the in vitro assessment of a commercially available e-cigarette, Vypen ePen, and compare the results relative to a reference 3R4F cigarette.

METHODS

The products used for these studies were the 3R4F reference cigarette (University of Kentucky, USA) and an e-cigarette (Vypen ePen). All test articles were generated under Health Canada Intense (HCI) smoking regime (55/3/30, vents blocked on 3R4F).

Smoke aerosol production

Conventional 3R4F reference cigarettes and Vypen ePen devices were smoked or activated on a Borgwaldt-KC RM20 smoking machine. Particulate matter (TPM) extracts were prepared as previously described. Aerosol aqueous extract (AEE) was generated by bubbling the smoke from a single cigarette, or 10x 3 second activations (Vypen ePen) in 20mL of cell culture medium.

Ames bacterial reverse mutation assay

Product TPM exposures were conducted according to the principles of OECD 471, however utilising only S. typhimurium strain TA98+9, for product whole aerosol (WA) exposures, the Ames assay was modified as previously described. Briefly, TA98 was exposed to WA using a Vitrocell VR AES 4 stainless steel module smoke engine, in a scaled-down 35mm plate format for a period of 64 minutes.

Cytotoxicity assay

Human bronchial epithelial cells (NCH-H22) were exposed to WA at the air liquid interface (ALI) for a period of 1 hour, using a Borgwaldt-KC RM20 smoking machine (Borgwaldt KC, Hamburg, Germany). Following exposure, cytotoxicity was assessed using Neutral Red Uptake as previously described.

Gamma H2AX assay

Human bronchial epithelial cells (BEAS-2B) were exposed to WA at the ALI for a maximum of 5 hours. Immunostaining and a high content screening (HCS) approach using the Cellomics ArrayScan® VTI platform was used to determine the frequency of DNA double strand breaks.

Bhas2 cell transformation assay

Bhas 42 cells (v-Ha-ras-transfected BALB/c 3T3) were treated with TPM from test products using the promotor protocol. Cells were treated for 7 days to 30 days with media change every 3 days, followed by a 7 day recovery. Plates were scored and results evaluated as previously described.

Antioxidant depletion

NCH-H22 cells were exposed to AEE for a period of 4 hours. The ratio of reduced to oxidized glutathione (GSH:GSSG) was assayed using the GSH:GSSG Glo assay kit (Promega, UK).

Endothelial cell migration assay

Human umbilical vein endothelial cells were grown to confluency, "wounded" using a pipette tip and exposed to various AEE for 22 hours in 24 well ImageLock plates (Eisen Instruments, Ann Arbor, MI, USA). Cell migration was quantified using the closure of the "wound" using an Incyto time lapse video camera and software (Eisen Instruments, Ann Arbor, MI, USA).

Pro-inflammatory cytokine secretion

Muhi™ primary airway epithelial cultures were exposed to whole aerosols (1:20 dilution ratio) for 4x 5 minute intervals with 30 minutes rest between each exposure, using a Borgwaldt RM20 smoking machine. Cytokines were quantified with the Meso Scale Discovery Kit, 3 cytokine and 3-Flex MMP Kits, according to manufacturer’s instructions (Gaithersburg, USA).

RESULTS

Aerosol dilution (aerolar, 1x)

% Cell viability

DNA Double Strand Breaks

Cell Transformation

Mutagenicity

Normalized DNA content

Pro-Inflammatory Cytokine Secretion

Comparison of cigarette smoke, Vypen ePen demonstrated significantly lower cytotoxicity.

VyPen ePen did not induce mutations, whereas cigarette smoke exposure uses a positive dose response.

VyPen ePen did not induce DNA double strand breaks across dose, whereas cigarette smoke demonstrated a positive dose response.

Cytokine expression was up-regulated (IC50=1.5 μg/ml of 0.95) following 3R4F smoke exposure with no significant increase in mediator release in response to VyPen ePen exposure.

Antioxidant Depletion

Endothelial Migration

VyPen ePen did not cause antioxidant depletion, whereas cigarette smoke demonstrated a positive dose response.

VyPen ePen did not impair endothelial cell migration, whereas cigarette smoke demonstrated a positive dose response.

CONCLUSIONS

- Under these test conditions using a number of in vitro assays, e-cigarette VyPen demonstrated reduced reduction in responses compared to a reference 3R4F cigarette, indicating a potential to be reduced risk versus cigarettes.
- Additional research including clinical and population studies are needed to substantiate disease relevant risk reduction in populations.

REFERENCES