1. INTRODUCTION

OBJECTIVES

A range of tobacco-based and tobacco-free next generation products (NGPs) are commercially available and there is a growing scientific consensus that NGPs may present a less harmful alternative to cigarettes for adult smokers. With the increased popularity of NGPs amongst adult smokers it is important that their potential biological effects can be assessed quickly and accurately. Although not recommended for use by pregnant women, in order to address regulatory concerns, further research is required to assess whether NGP aerosols present a developmental risk to the unborn fetus. The objective of this study was to compare the potential developmental toxicity of NGP aerosols to cigarette smoke using Stemsta’s devTOXqPredict human pluripotent stem cell-based assay.

The devTOXq assay is a high-throughput in vitro developmental toxicity assay used to signal whether a compound has the potential to cause developmental toxicity in humans. Omnthe, and cysteine are both involved in metabolic pathways important for normal cell proliferation and differentiation during development. Changes in the ratio of ornithine/cystine levels have been experimentally associated with common mechanisms of developmental toxicity (Palmer et al, 2013). The devTOXq Predict assay uses the metabolic perturbation of the biomarkers ornithine/cystine ratio (o/c ratio) to predict whether a test compound exhibits developmental toxicity potential.

2. MATERIALS AND METHODS

2.1 Smoke / Aerosol Extract Generation Method

Aerosol from test products was generated with a Vitrocel VC10s (Vitrocell, Munich, Germany) smoking machine. The 3R4F smoke and the THP aerosol were generated using the ISO intense smoking regimen, with the HYB and myblu™ according to CORESTA Recommended Method N° 81. Smoke or aerosol extracts were prepared by bubbling the sample into 3 in-line impingers, each containing 10 mL Phosphate Buffered Saline (See Figure 1). A total stock solution of 30 ml per test article was used: 1.8 µg per ml for 3R4F and 4 µg per ml for NGP. Nicotine and carbonyls trapped in fresh PBS samples were quantified using an LC-MS/MS and HPLC-DAD method respectively.

2.2 devTOXq PredictAssay Method

- Human induced pluripotent stem (iPSC) cells (Cell Line: HYR0103; ATCC, Manassas, VA, USA) were maintained in the undifferentiated state in mTeSR (StemCell Technologies, Vancouver, BC, Canada) on Matrigel (Corning, Bedford, MA, USA).
- iPSC cells were exposed to eight concentrations (0.003-10%) of each test sample for 48 hours, with media changes after three test sample replacement every 24 hours. Each experimental plate contained a reference control (0.1% DMSO), positive control (1 µM Methotrexate, 3 wells, red circles), and negative control (0.005 µM Methotrexate, 3 wells, green circles) control treatments, as well as eight concentrations of two test articles (0.003-10%, 3 wells/concentration, blue circles). Media controls (lacking cells, a test article) were also included for each treatment to assess the impact of the test article on the sample matrix (filled squares).
- Ornithine and cystine were measured using liquid chromatography-high resolution mass spectrometry (LC-HRMS).
- Relative fold changes for each metabolite were calculated by normalizing the response of each treatment to the reference sample. The o/c ratio was determined by dividing the reference-normalized value for ornithine by the reference-normalized value for cystine.
- GraphPad Prism v8.0 (GraphPad Software, San Diego, CA, USA) was used to fit non-linear dose response curves to determine the developmental toxicity potential (dTP, o/c ratio) and toxicity potential (TP, cell viability) concentrations (Figure 3).
- An extra sum-of-squares F test was used to determine if the dose-response curve for the 3R4F sample was significantly different from the other three test samples (THP, HYB, myblu™).

The devTOXq assay has excellent accuracy, specificity and sensitivity with a wide range of chemotypes. The tested chemicals included pharmaceuticals, agrichemicals, cosmetic ingredients, industrial solvents, and environmental chemicals (Palmer, 2019).

3. RESULTS / DISCUSSION

3.1 DevTOXq Results Interpretation

The point where the o/c ratio dose-response curve (teal line) crosses the developmental toxicity threshold (DTT; red line) indicates the exposure level where a test sample has developmental toxicity potential (red point).

The cell viability potential (blue point) is the exposure level where the cell viability dose-response curve (black line) exceeds the DTT.

Described in Palmer et al., 2013.

3.2 DevTOXq Predict Assay Method

A two-way ANOVA followed by Fisher’s Least Significant Difference post hoc test was used to determine if the response for 3R4F was significantly different from the other three test samples. P-values were adjusted for multiple comparisons using Benjamin and Hochberg’s method to control the false discovery rate.

HYB and myblu™ extracts did not elicit a metabolic response, indicating that these test samples are predicted to have no potential for developmental toxicity in vivo at the concentrations tested.

4. CONCLUSIONS

- The dose-response curves for THP, HYB, and myblu™ were all statistically significantly different from 3R4F (extra-sum-of-square F test, p<0.0001) for both cell viability and o/c ratio.
- THP induced significantly less cytotoxicity and exhibited a significantly weaker metabolic perturbation in the o/c ratio compared to 3R4F (p<0.001).
- Both myblu™ and HYB induced no cytotoxicity under the conditions of test and showed no response in the o/c ratio predicting no potential for developmental toxicity in vivo.
- Although the devTOXq assay shows promise at predicting developmental toxicity, as with other in vitro models, it cannot fully reproduce all events contributing to the disruption of normal human development by exogenous chemicals. However, the devTOXq assay has utility as part of a weight of evidence approach for the assessment of NGPs.

REFERENCES