Assessment Of An In Vitro Model Of Lung Epithelial Cell Stress Responses Exposed To Aqueous Extracts Generated From A Heated Tobacco Device.

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INTRODUCTION

Cigarette smoking is an identified cause of a number of human disorders, including heart disease, lung disease and cancer. Although the mechanisms underlying these disorders are diverse, they are each underpinned by smoke-induced cellular oxidant, inflammatory and apoptotic responses. These cellular responses are mediated by many cell signalling pathways, including those controlled by the transcription factors Nrf2 (antioxidant responses) and NF-κB (inflammatory responses).

We are developing a range of products including e-cigarettes and heated tobacco devices which yield lower levels of toxins than cigarette smoke. For an in vitro testing strategy to compare different nicotine delivery products, we have developed an in vitro model of lung epithelial cell stress responses that include oxidative, pro-inflammatory, apoptotic and necrotic endpoints.

EXPERIMENTAL PROCEDURES

Cell culture

Human bronchial epithelial cells (NCI-H292; American Type Culture Collection, Middlesex, U.K.) were grown initially in 75cm² (T75) tissue culture flasks. H292 cells were maintained in RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS), 2mM glutamine, 50U/mL penicillin and 50µg/mL streptomycin, at 37°C in a humidified 5% CO₂ incubator.

Aerosol aqueous extract (AEaq) production

Conventional 3R4F reference cigarettes or a heated tobacco device were smoked or heated on a Borgwaldt-KC RM20H smoking machine under HCI puffing conditions (55/2/30, vents blocked on 3R4F). AEaq was generated by bubbling the smoke from a single cigarette (smoked) or stick (heated to 180°C) through 20mL of DMEM/F12 medium. The AEaq from the heated tobacco device contained all the aerosol generated by heating the tobacco.

Exposure

H292 cells were exposed to AEaq at the following concentrations: 0.0%, 12.5%, 25%, 50% & 100%.

GSH:GSSG-Glo™ assay (antioxidant response)

GSH:GSSG ratios were determined post 4h exposure via the Promega GSH/GSSG-Glo assay. Luminescence signals were read using a SpectraMax multimode plate reader.

DCF assay (intracellular reactive oxygen species generation)

Increases in intracellular ROS generation were measured, post 1h exposure via the ROS indicator probe DCF. Fluorescence signals were read using a SpectraMax multimode plate reader.

Glo Response H292-ARE-Luc2P assay (antioxidant response)

ARE activation was determined, post 6 hour exposure via the stably transfected Glo Response H292-ARE-Luc2P cells. Luminescence signals were measured using a SpectraMax multimode plate reader.

Multiplex MSD platform assay (inflammatory response)

The concentrations of secreted pro-inflammatory cytokines IL-1β, IL-6, IL-8 & TNF-α were assessed using the electrochemiluminescence MSD platform in a 96-well format. H292 cells were exposed to AEaq for 4h followed by a 20h recovery at 37°C.

Apolive-Glo™ duplex assay apoptosis and cytotoxicity

Caspase-3/7 activity and cell viability were assessed post 4h exposure using the Promega Apolive-Glo™ multiplex assay in a 96-well format. After each exposure fluorescence signals for cell viability were measured using a multimode plate reader.

RESULTS

- All data are means ± standard deviations from 4-5 experiments with 12 intra-plate replicates per dilution.
- Cellular responses indicative of oxidative stress were lower in cells exposed to heated tobacco AEaq compared to those responses in cells exposed to 3R4F AEaq.
- Inflammatory responses in cells exposed to heated tobacco AEaq were lower when compared to those seen in cells exposed to 3R4F AEaq.
- 3R4F AEaq reduced cell viability to a greater extent than heated tobacco AEaq. Similarly, caspase activity was lower in cells exposed to the heated tobacco compared to 3R4F AEaq.

CONCLUSIONS

- A panel of in vitro assays utilising lung epithelial cells to measure oxidative stress, inflammation and cytotoxicity was suitably sensitive to discriminate between AEaq derived from a combustible reference (3R4F) cigarette and a heated tobacco device.
- Reductions in all biological endpoints measured were observed in response to exposure with the heated tobacco AEaq compared to those responses in cells exposed to 3R4F AEaq.