Development of the combined oxidative stress and cytotoxicity model in MucilAir tissues for the exposure of cigarette smoke and Heated Tobacco Product aerosols

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Introduction

Oxidative stress plays an important role in the progression of some diseases, such as cardiovascular disease, neurodegenerative diseases and various inflammatory conditions such as chronic obstructive pulmonary disease (COPD). Several clinical markers of oxidative stress exist including Nrf2 transcription factor and 8-isoprostane, a stable downstream marker following Nrf2 activation.

Cigarette smoking induces oxidative stress in the lungs of smokers due to the abundance of chemical toxicants in cigarette smoke. Heated Tobacco Products (HTP) are postulated as a potentially reduced risk product compared to cigarettes, and HTP aerosols show lower biological activity in *in vitro* and clinical studies as compared to cigarette smoke [1,2].

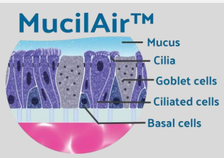
The objective of this study was to expose the 3D lung model, MucilAir, to cigarette smoke and a HTP aerosol to measure 8-isoprostane as a biomarker for oxidative stress and combine this with a measure of cytotoxicity using MTT reduction.

Methodology

MucilAir tissues

MucilAir tissues were obtained from Epithelix Sarl. They are an *in vitro* tissue model system that represents the morphological conditions of the human upper airway epithelium, and benefit from being cultured at the air-liquid interface (ALI).

Figure 1. Schematic representation of a MucilAir tissue, showing its three-dimensional structure and various cell types relevant to the respiratory tract.



Test Articles

Cigarettes

1R6F cigarettes were smoked under ISO 20778 (55mL puff volume, taken over 2 seconds, every 30 seconds, with vents 100% blocked) on a Vitrocell® VC 10®, and MucilAir tissues were exposed to smoke via the Vitrocell 48 mammalian exposure module (Figure 2) for approximately 30 minutes. Seven diluting airflows ranging from 10L/min to 0.5 L/min (low to high aerosol concentration, respectively) were used. Nicotine was used as a dosimetry marker.

HTP

A HTP aerosol was generated using the Vitrocell® VC 1/7® under ISO 5501-1 (55mL puff volume, taken over 2 seconds, every 30 seconds, with vents unblocked). 24 sticks were puffed over 120 minutes, using 7 diluting airflows ranging from 5L/min to 0 L/min (undiluted). Nicotine was used as a dosimetry marker.

Positive controls

Positive controls for oxidative stress inducers were also assayed, which consisted of Phorbol 12-myristate 13-acetate (PMA):Ionomycin and tert-butyl hydroperoxide (tBHP) that were dosed basolaterally and apically, respectively.

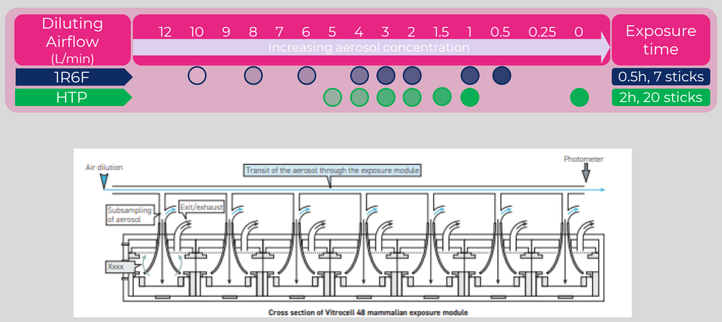


Figure 2. Schematic of the aerosol dilutions used for 1R6F and the HTP (top), and a cross section of the Vitrocell 48 mammalian exposure module (bottom).

Results

Positive controls

The positive controls, tBHP and PMA:Ionomycin induced high and low levels of oxidative stress, at a dose range that was not cytotoxic (Figure 3).

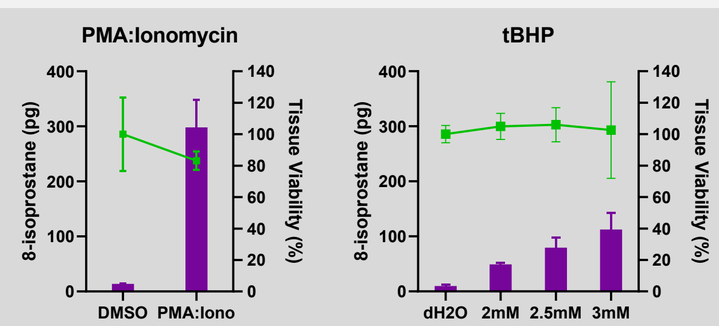


Figure 3. Oxidative stress (■) and cytotoxicity response (■) following exposure of MucilAir tissues to tBHP and PMA:Ionomycin for 3 hours exposure.

1R6F & HTP

As the model was an attempt to combine the cytotoxicity and oxidative stress responses, doses were carefully selected to ensure an adequate number of doses were in the non-cytotoxic range to measure 8-isoprostane release, along with doses that induced cytotoxicity.

Cytotoxicity

Both 1R6F and HTP induced cytotoxicity in MucilAir tissues (Figure 4), and IC_{50} values could be calculated for both products (Table 1).

Product	IC_{50}
1R6F	11.05
HTP	67.13

Table 1. IC_{50} values (µg deposited nicotine/mL) calculated for 1R6F and the HTP.

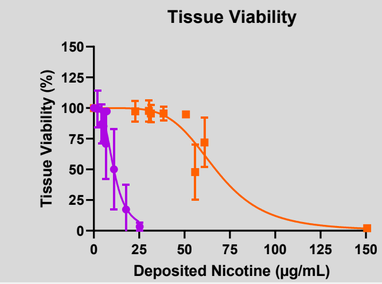


Figure 4. Tissue viability, determined via MTT reduction in MucilAir tissues following exposure to 1R6F smoke (■) and HTP aerosol (■) for 30 minutes and 2 hours, respectively.

Oxidative stress

At the non-cytotoxic doses tested (> 80% viability), 1R6F induced dose related increases in 8-isoprostane release (Figure 5) typically exceeding 4-fold induction. The HTP also induced some increases in 8-isoprostane, reaching 1.6-fold, and this was significantly less than 1R6F, despite a longer exposure (Figure 5).

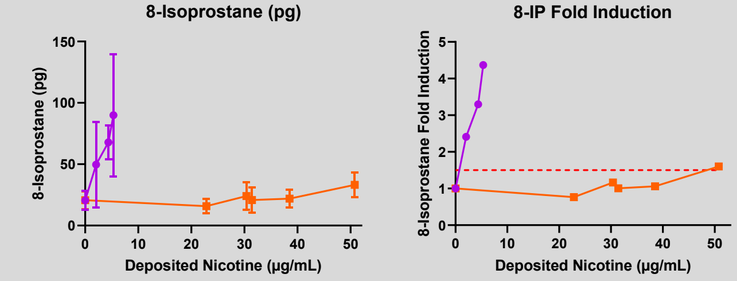


Figure 5. Induction of 8-isoprostane following exposure to 1R6F smoke (■) and HTP aerosol (■) in terms of quantity (left) and fold increases (right) relative to the vehicle control (air).

Conclusion

A combined model to determine 8-isoprostane release and cytotoxicity in the same 3D *in vitro* lung tissue system has been developed.
The positive controls induced significant increases in 8-isoprostane release at different levels of induction, demonstrating the potential of the assay to detect low and high responses to tested aerosols.
Exposure to whole aerosol from 1R6F and HTP showed dose related increases in 8-isoprostane release at non-cytotoxic doses, and complete cytotoxicity as measured by MTT reduction.
Clear differentiation between 1R6F and the HTP was observed, for 8-isoprostane release and cytotoxicity.

References

1. Thorne D, Breheny D, Proctor C, Gaca M. (2018). Assessment of novel tobacco heating product THP1.0. Part 7: Comparative in vitro toxicological evaluation. *Regul Toxicol Pharmacol.* Mar;93:71-83
2. Gale N, McEwan M, Hardie G, Proctor CJ, Murphy J. (2022). Changes in biomarkers of exposure and biomarkers of potential harm after 360 days in smokers who either continue to smoke, switch to a tobacco heating product or quit smoking. *Intern Emerg Med* Oct;17(7):2017-2030.

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