Nrf2 Response to Whole Smoke and Aerosol of Two Different Tobacco Product Types in a 3D Human Airway Model

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Abstract

The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, activated in human lung cells by cigarette smoke, regulates genes involved in the antioxidative stress response. Here, we evaluated the effect of cigarette whole smoke (3R4F reference cigarette) and whole aerosol from a commercially available tobacco heating product (THP) on cell viability and Nrf2 response in a 3D human airway model (EpiAirway™) transfected with a luciferase Nrf2 promoter.

EpiAirway™ tissues were exposed to smoke/aerosol generated under the Health Canada Intense regimen. Smoke/aerosol doses were controlled using dilution airflows of 0.5 to 6 L/min for 3R4F, and undiluted (0 L/min) to 3 L/min for the THP. Eighteen hours post-exposure, luciferase activity and cell viability were measured. Post-exposure, smoke/aerosol deposition was also quantified using chemical analysis (e.g., glycerol, nicotine) and fluorescence of captured particulate matter in a dosimetry well within the exposure module.

Differential Nrf2 activation was observed following exposure to 3R4F smoke compared to the THP aerosol. A peak response of 1,484 ± 184-fold Nrf2 activation at 1.5 L/min (7.09 ± 1.58 µg nicotine) was observed for 3R4F while no meaningful response (<2 fold) was generated by the THP under comparable conditions. Undiluted aerosol (37.67 ± 2.44 µg nicotine) was required for the THP to induce a response: 445 ± 103-fold change. Cell viability remained >80% at these airflows for the THP. Moreover, the minimum exposure-correlated nicotine concentration required to induce a >2-fold increase in Nrf2 activation was significantly ($p<0.001$) lower for 3R4F than THP.

Collectively, these data show that changes in the Nrf2 promoter response may be useful in discriminating response to smoke/aerosol exposures from different tobacco product types.

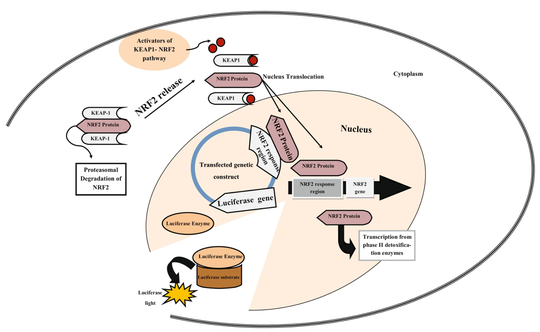

Figure 1: Schematic of the Nrf2 pathway with luciferase linked Nrf2 gene expression. Adapted from Mozaheb et al., Scientific Reports, 9:3248, 2019)

Results

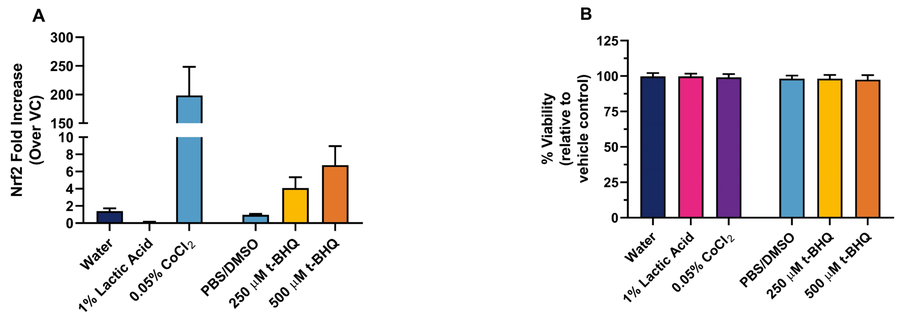

Figure 2. EpiAirway™ Nrf2 tissues were exposed to 1% lactic acid, 0.05% $CoCl_2$, *tert*-butylhydroquinone (t-BHQ), water (vehicle), or PBS/0.5% DMSO (t-BHQ vehicle) for 18 hours. Cells were lysed for determination of Nrf2 linked luciferase activity (A) and LDH release into the basolateral media (B).

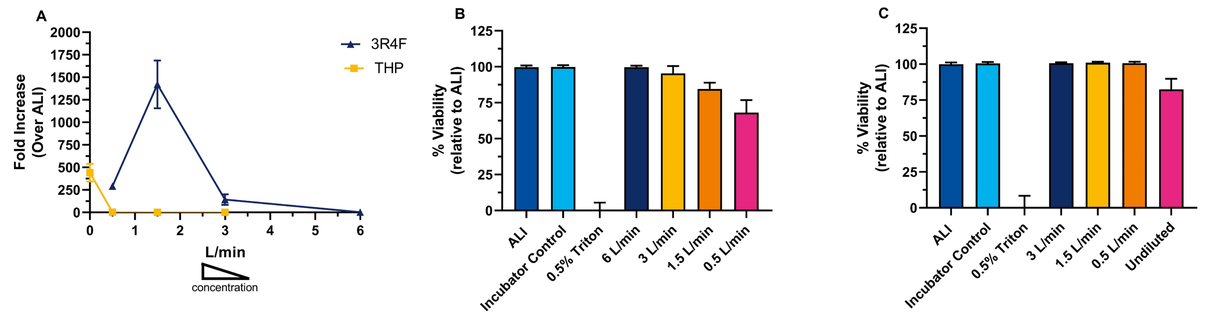

Figure 3. EpiAirway™ Nrf2 tissues were exposed 3R4F whole smoke or THP whole aerosol for 22 – 24 minutes. Following an 18-hour recovery, cells were lysed for determination of Nrf2 linked luciferase activity (A) and LDH release into the basolateral media for 3R4F (B) or THP (C) exposed cells.

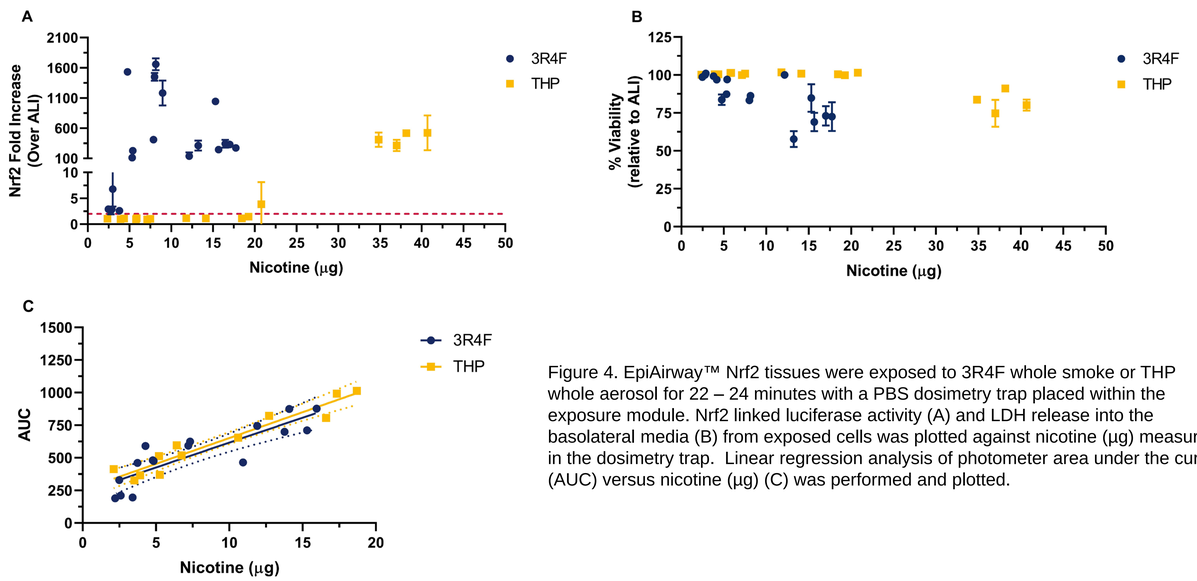

Figure 4. EpiAirway™ Nrf2 tissues were exposed to 3R4F whole smoke or THP whole aerosol for 22 – 24 minutes with a PBS dosimetry trap placed within the exposure module. Nrf2 linked luciferase activity (A) and LDH release into the basolateral media (B) from exposed cells was plotted against nicotine (µg) measured in the dosimetry trap. Linear regression analysis of photometer area under the curve (AUC) versus nicotine (µg) (C) was performed and plotted.

Materials and Methods

3D Cell Model: EpiAirway™ tissues transfected with a Nrf2 lentiviral luciferase reporter were obtained from MatTek, Inc., which are comprised of normal, human-derived tracheal/bronchial cells that have been cultured to form a highly differentiated model. Tissues were maintained at the air-liquid interface according to the manufacturer's guidelines.
Test Articles: 3R4F reference cigarettes were obtained from the University of Kentucky. The Tobacco Heated Product (HTP) was commercially available in the UK and obtained by Labcorp.
Whole Smoke/Aerosol Generation: Whole aerosol was generated with a Vitrocell® VC10® Smoke Exposure System. The tissues were exposed to 3R4F whole smoke generated under Health Canada Intense (HCI) regime (55 mL volume, 2 sec duration, 30 sec puff interval, 100% vent blocking) with 20 mL/min vacuum for 22 minutes (4 cigarettes). Tissues exposed to THP whole aerosol generated under modified HCI (HCI parameters without vent blocking) for 24 minutes (4 consumables). Three places in the module contained tissues and the fourth contained 0.9 mL PBS as a dosimetry trap.
Photometers: Four photometers were harmonized by connecting to a Vitrocell® dilution bar and output signal was adjusted to be comparable for photometers following whole smoke generated by one 3R4F under HCI.
Chemical Exposures: 1% lactic acid, 0.05% $CoCl_2$, *tert*-butylhydroquinone (t-BHQ; 250 or 500 µM), water (vehicle), or PBS/0.5% DMSO (t-BHQ vehicle) were added apically to the EpiAirway™ tissues for 18 hours.
Luciferase Activity: The amount of luciferase activity was measured using the ONE Glo™ Luciferase Report Assay System according to the manufacturer's instructions (Promega, UK) 18 hours post-exposure.
LDH Release: The lactate dehydrogenase (LDH) assay was performed according to the manufacturer's instructions (Takara Bio USA, cat. #MK401). The LDH activity was determined by measuring the optical density of the sample at 490 nm.
Nicotine Determination: Samples were analyzed using a LC-MS/MS with Dionex Ultimate 3000 low pressure quaternary analytical HPLC system fitted with a Waters XBridge BEH Shield RP18 (2.5µm) 3.0 x 50 mm analytical column. The linear range of the method was 0.08 to 50 µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were 0.026 and 0.08 µg/mL, respectively.
Statistical Analysis: Maximum fold change across the airflows was calculated for each experiment and a t-test with unequal variance performed (SAS) to determine statistical significance ($p<0.05$) versus the 3R4F response.

Summary and Conclusions

- Chemicals known to induce Nrf2 luciferase-linked expression in the lung ($CoCl_2$, t-BHQ) elicited a robust response, whereas lactic acid did not. No impact from all chemicals exposures to cell viability was observed.
- 3R4F whole smoke caused an increase in Nrf2 luciferase-linked expression from 6 – 0.5 L/min with a peak response of 1,484 ± 184-fold at 1.5 L/min (7.09 ± 1.58 µg nicotine).
- THP whole aerosol caused a dose-dependent increase in Nrf2 luciferase linked expression from 3 – 0 L/min (undiluted) with a peak response of 445 ± 103-fold at the undiluted airflow (37.67 ± 2.44 µg nicotine).
- Cell viability of EpiAirway™ Nrf2 tissues remained >70% at all airflows for both 3R4F whole smoke and THP whole aerosol.
- Nicotine concentration required to induce a 2-fold increase in Nrf2 luciferase-linked expression was significantly ($p<0.001$) lower for the THP than 3R4F.
- Whole smoke/aerosol delivery into the exposure module measured by photometer area under the curve (AUC) was linearly correlated ($r^2>0.73$) with nicotine deposited into the dosimetry well within the module.
- The EpiAirway™ Nrf2 model can differentiate the Nrf2 response between a THP and a combustible cigarette.