Nrf2 Responses of Commercial Cigarette Whole Smoke and Aerosol from Six Electronic Nicotine Delivery Systems (ENDS) 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 whole smoke from a marketed combustible cigarette (CC) and whole aerosol from four different ENDS (Vuse Alto®) flavors varying in nicotine concentrations on cell viability and Nrf2 response in a 3D human airway model (EpiAirway™) transfected with a luciferase Nrf2 promoter.

EpiAirway™ tissues were exposed to whole smoke or aerosol generated under the Health Canada Intense or a modified ISO 20768:2018 regimen, respectively. Whole smoke/aerosol doses were controlled using dilution airflows of 0.5 to 6 L/min for CC, and undiluted to 3 L/min for Vuse Alto®. Eighteen hours post-exposure, luciferase activity and cell viability were measured. Relative luciferase fold activity was expressed as fold change over the air exposed control. Post-exposure, whole smoke/aerosol deposition was quantified using chemical analysis (e.g., glycerol, nicotine, carbonyls).

Differential Nrf2 activation was observed following exposure to whole smoke compared to the ENDS aerosol. A peak response for the CC was ~79 times higher and occurred at ~164 lower equivalent nicotine concentration than Vuse Alto®. Cell viability remained >80% at all airflows for all ENDS test articles and >60% for the CC. Moreover, the minimum exposure-correlated nicotine concentration required to induce a >2-fold increase (threshold response) in Nrf2 activation was >100x lower for CC than the four different Vuse Alto® flavors.

These data show that the 3D Nrf2 EpiAirway™ *in vitro* model can be used to assess and discriminate responses from a biomarker (oxidative stress) for disease pathways associated with tobacco product usage (e.g., respiratory and cardiovascular disease).

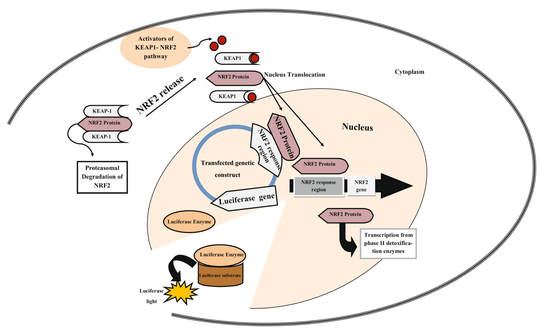

Figure 1: Schematic of the Nrf2 pathway with luciferase linked Nrf2 gene expression. Adapted from Mozaheb et al., 2019

References

- ISO 20768:2018. Vapour products – Routine analytical vaping machine – Definitions and standard conditions (1st edition)
- ISO 3204:1999. Tobacco and tobacco products – Atmosphere for conditioning and testing (4th edition)
- Health Canada Method T-115:1999, Determination of 'Tar', nicotine, and carbon monoxide in mainstream tobacco smoke
- Mozaheb et al., Scientific Reports, 9: 3248, 2019

Results

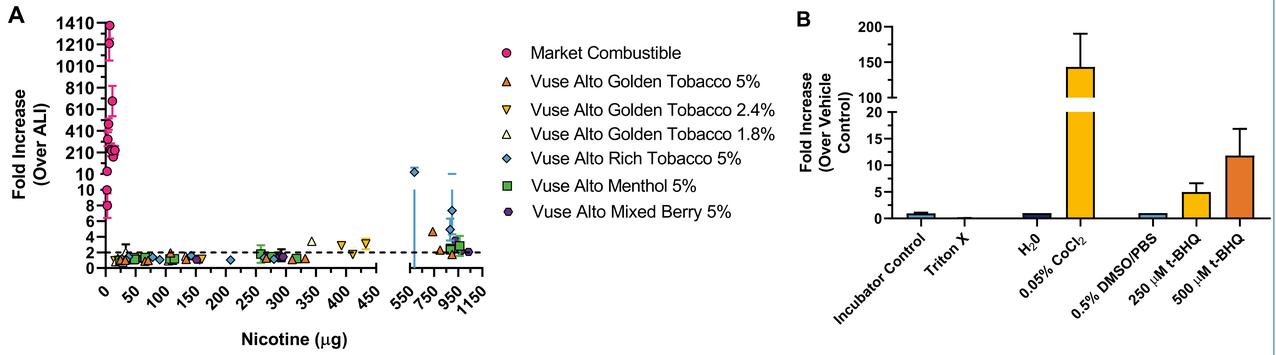

Figure 2. (A) EpiAirway™ Nrf2 tissues were exposed to whole smoke or aerosol for 24 or 220 minutes, respectively; then allowed to recover for 18 hours. (B) EpiAirway™ Nrf2 tissues were exposed to 1% triton X, 0.05% $CoCl_2$, *tert*-butylhydroquinone (t-BHQ), water ($CoCl_2$ vehicle), or 0.5% DMSO/PBS (t-BHQ vehicle) for 18 hours. Following (A) recovery or (B) exposure, cells were lysed for the determination of Nrf2 linked luciferase activity. Data are represented as mean ± SD, triplicate tissues, n=3 (A) or n=10 (B). ALI; air liquid interface

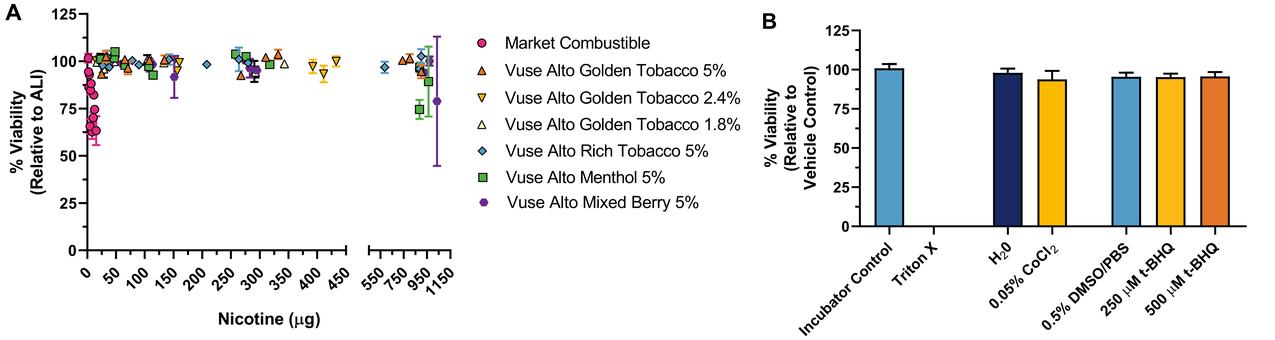

Figure 3. (A) EpiAirway™ Nrf2 tissues were exposed to whole smoke or aerosol for 24 or 220 minutes, respectively; then allowed to recover for 18 hours. (B) EpiAirway™ Nrf2 tissues were exposed to 1% triton X, 0.05% $CoCl_2$, *tert*-butylhydroquinone (t-BHQ), water ($CoCl_2$ vehicle), or 0.5% DMSO/PBS (t-BHQ vehicle) for 18 hours. Following (A) recovery or (B) exposure, LDH release into the basolateral media was measured. Data is represented as mean ± SD, triplicate tissues, n=3 (A) or n=10 (B). ALI; air liquid interface

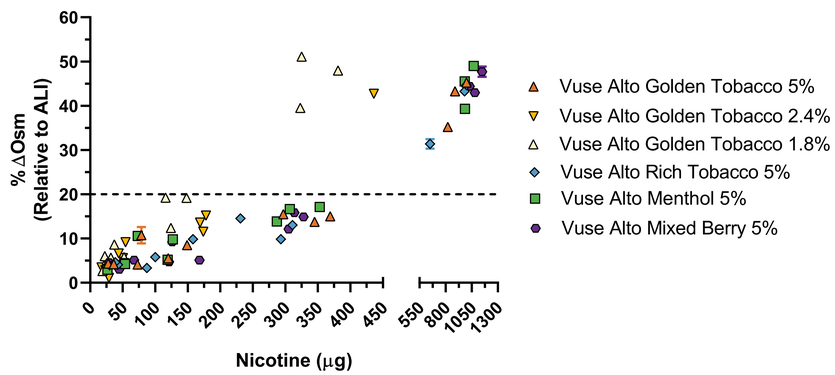

Figure 4. Osmolality was measured from basolateral media in the exposure well following 220-minute whole aerosol exposure. Percent change in osmolality vs. nicotine exposure concentration measured in dosimetry well was calculated. Data are represented as mean ± SD, n=3 independent experiments. ALI; air liquid interface

Materials and Methods

3D Cell Model: EpiAirway™ tissues comprised of normal, human-derived tracheal/bronchial cells that have been cultured to form a highly differentiated model and transfected with a Nrf2 lentiviral luciferase reporter were obtained from MatTek, Inc. Tissues were maintained at the air-liquid interface according to the manufacturer's guidelines.
Test Articles: Market combustible and Vuse Alto® products were obtained by RAI Services Company. Test articles were conditioned in accordance with ISO 3402:1999 prior to each experiment.
Whole Smoke/Aerosol Generation: Whole aerosol was generated using a Vitrocell® VC10® Smoke Exposure System (serial #210311 (HCI), serial #200814 and 091215 (mISO)). The tissues were exposed to whole smoke from a market combustible 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 24 minutes (48 total puffs). Tissues exposed to Vuse Alto® whole aerosol generated under a modified ISO 20768:2018 (mISO) regime (55 mL volume, 3 sec duration, 30 sec puff interval, 60 second pause every 10 puffs) for a total of 220 minutes (240 total puffs). Three exposure wells in the module contained tissues and the fourth contained 0.9 mL PBS as a dosimetry trap.
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 18 hours post-exposure using the ONE Glo™ Luciferase Report Assay System according to the manufacturer's instructions (Promega, UK).
LDH Release: The lactate dehydrogenase (LDH) assay was performed according to the manufacturer's instructions (Takara Bio). LDH activity was determined by measuring the optical density of the sample at 490 nm.
Osmolality Determination: Duplicate osmolality measurements were obtained by sampling from the PBS dosimetry trap following each exposure using a Fiske 2020 osmometer which was calibrated prior to each use.
Nicotine Determination: Samples from the PBS dosimetry trap were analyzed using a LC-MS/MS. The linear range of the method was 0.08 to 50 µg/mL. The limit of detection and limit of quantification were 0.026 and 0.08 µg/mL, respectively.
Statistical Analysis: Linear interpolation was used to determine the lowest nicotine concentration to induce a 2-fold increase (SAS).

Summary and Conclusions

- **Apical application of chemicals known to induce Nrf2 luciferase-linked expression in the lung ($CoCl_2$, t-BHQ) elicited a 5 – 500-fold increase, with a dose dependent increase seen for t-BHQ (Figure 2B). No impact on cell viability was observed following any of the chemical exposures (Figure 3B).**
- **Market combustible whole smoke caused an increase in Nrf2 luciferase-linked expression with a peak response of 995 ± 585-fold at 4.82 ± 1.10 µg nicotine (Figure 2A).**
- **The largest increase in Nrf2 luciferase linked expression with ENDS test articles occurred with Rich Tobacco 5% whole aerosol, with a peak response of 12.51 ± 11.51-fold at 790.2 ± 176.3 µg nicotine (Figure 2A).**
- **Cell viability of EpiAirway™ Nrf2 tissues remained >60% and >80% at all airflows for the market combustible and ENDS test articles, respectively (Figure 3A).**
- **A minimum of a 1,400x difference in nicotine concentration was required to induce a 2-fold increase in Nrf2 luciferase-linked expression was observed between the market combustible (0.23 ± 0.17 µg) and ENDS test articles (329.4 ± 94.1 µg) (Figure 3A).**
- **Increases in >20% (>60 mOsm) osmolality over air control were measured in the basolateral media following exposure in all ENDS test articles at the undiluted (0 L/min) airflow.**
- **Overall, these results indicate these ENDS test articles elicit minimal oxidative stress compared to the market combustible at all nicotine concentrations.**