Metabolic Dysregulation-Mediated Pulmonary Innate Immune Response in Acute Exposure to Menthol and Tobacco-Flavored E-cigarettes

Thivanka Muthumalage and Irfan Rahman

Department of Environmental Medicine, University of Rochester Medical Center, Rochester, NY, USA.

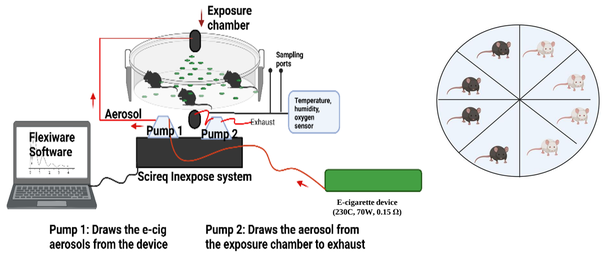
INTRODUCTION

- Electronic Nicotine Delivery Systems (ENDS) flavors legal in most states include tobacco and menthol.
- These flavors contain propylene glycol, vegetable glycerin, and flavoring chemicals that impart flavors.
- Our studies and others have shown suppressed immunity (inflammatory mediators) upon exposure to e-cigarettes, but the mechanisms are unknown.
- We investigated the metabolic and inflammatory effects of acute exposure to PG/VG, tobacco, and menthol flavors.

HYPOTHESIS

Menthol and tobacco-flavored e-cigarettes cause metabolic dysregulations in lung cells altering the immune-inflammatory response.

METHODS



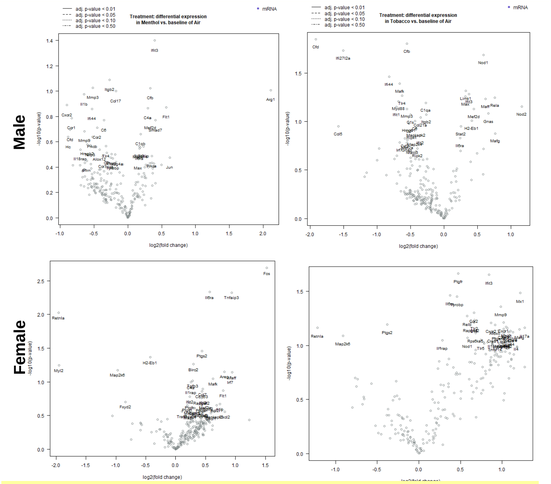
Aerosol exposure: Male and female C57BL/6J and BALB/cJ mice (2-months old) were exposed to propylene glycol/vegetable glycerin (PG/VG), menthol and tobacco (0 mg nicotine) 2 hrs a day for 3 days using Scireq Inexpose whole-body exposue setup (70 mL/puff, 2 puffs/min). Mice were sacrificed 24 hrs post exposure and bronchoalveolar lavage fluid and lung tissues were collected.

Inflammatory and metabolic gene expression analyses:

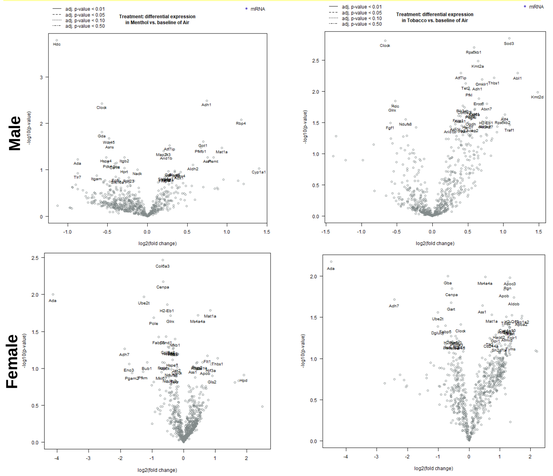
RNA was isolated from lung tissues of air, PG/VG, menthol, and tobacco flavor exposed mouse lung tissue using Zymogem DirectZol RNA kits. 50 ng of isolated RNA was hybridized with metabolic and inflammatory panels and processed through nCounter Sprint Profiler system. Normalized nanoString mRNA counts were used to perform advanced analyses using nSolver software. Differential expression of genes for male and female mice exposed to PG/VG, menthol, and tobacco was determined and preseted as volcano plots. By performing gene set analysis scores most affected direct pathways were assessed and presented as heatmaps and barplots of most affected cell types were presented.

RESULTS

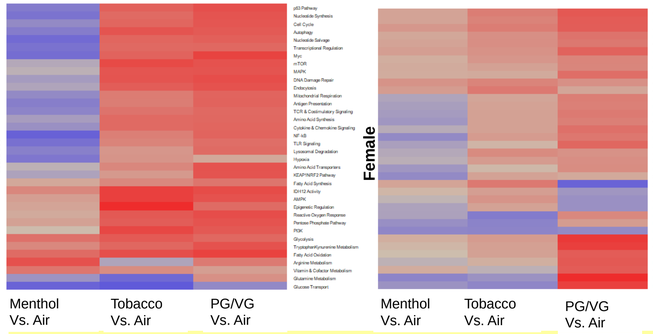
1. Menthol and tobacco exposure altered inflammatory genes



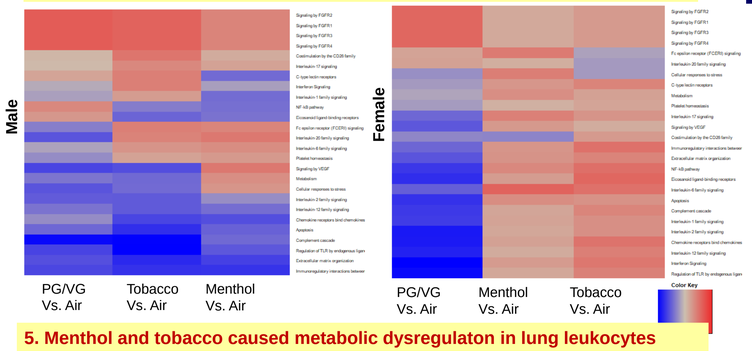
2. Menthol and tobacco exposure altered metabolic genes



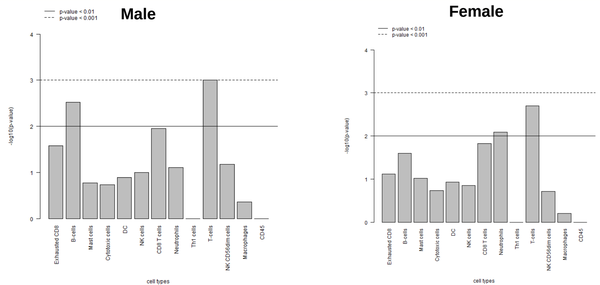
3. Acute menthol and tobacco exposures altered metabolic pathways.



4. Acute menthol and tobacco exposures altered inflammatory pathways.



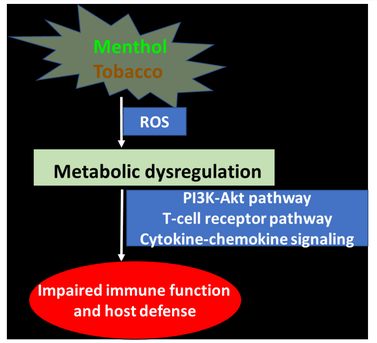
5. Menthol and tobacco caused metabolic dysregulaton in lung leukocytes



SUMMARY

- Significant alterations in genes associated with inflammation including, Arg1, Mafg, Maff, Ifit3, Nod1, Cfd, Ifi2712a, Ifi44, Ifit1, Fos, Tnfaip3, Irf7, Retnla, Il6ra, Ptgs2, H2-Eb1, Ager, Mknk1, Tlr4, Myl2, Il3, Il10, Ifnb1, Il23a, Ifng, Il21, Ptgfr, Ccr2, Mx1, were altered by e-cig menthol and tobacco flavor exposures.
- Metabolism-associated genes including, Rbp5, Mat1a, Pemt, Ass1, Adh1, Go1, Pfkb1, Atf7ip, Ada, Hdc, Clock, Gda, Abl1, Sod3, Atf4, Hadh, Hspa2, Uckl1, Mtor, Pcl1, Hdc, Sod3, Glrx, Col6a3, Nfkb1, Scf1, Traf1, Cox4i1, Cyp4a10, and Cyp1a2, were significantly altered by e-cig exposures.
- Most gene modulations were identified in neutrophils, mast cells, dendritic cells, and exhausted T-cells.
- mTOR, NFκB, PI3K, Fatty acid oxidation, Glucose transport, Arginine metabolism, mitochondrial respiration, FGFR signaling, TLR signaling, and key Interleukin signaling pathways were affected by flavor exposures.

CONCLUSION



Exposure to PG/VG, menthol, and tobacco flavored e-cigs caused adverse metabolic changes in immune cells affecting PI3K-Akt, T cell receptor, and chemokine-cytokine receptor signaling pathways potentially contributing to impaired innate immune function and host-defense.

ACKNOWLEDGEMENT

Supported by NIH U54CA228110 and NIEHS 1K99ES033835-1

Thivanka_Muthumalage@urmc.rochester.edu