

Metabolic Dysregulation-Mediated Pulmonary Innate Immune Response in Acute Exposure to Menthol and Tobacco-Flavored E-cigarettes **Thivanka Muthumalage and Irfan Rahman**

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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.



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.







METHODS

RESULTS





SUMMARY

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	TryptophanKynurenine Metabolism
	Pentose Phosphate Pathway
	Vitamin & Cofactor Metabolism
	NF-kB
	Amino Acid Synthesis
	IDH12 Activity
	TLR Signaling
	MAPK
	mTOR
	Arginine Metabolism
	Amino Acid Transporters
	Мус
	Nucleatide Salvage
	PI3K
	Antigen Presentation
	Endocytosis
	DNA Damage Repair
	Glycolysis
	TCR & Costimulatory Signaling
	Cytokine & Chemokine Signaling
	Nucleatide Synthesis
	Cell Cycle
	Glucose Transport
	Glutamine Metabolism
	Transcriptional Regulation
	KEAP1NRF2 Pathway
	Hypoxia
	Lysosomal Degradation
	Epigenetic Regulation
	Fatty Acid Oxidation
	AMPK
	p53 Pathway
	Reactive Oxygen Response
	Autophagy
	Fatty Acid Synthesis
	Mitochondrial Respiration
G/VG	

kocytes	
oacco . Air	Color Key
	Regulation of TLR by endogenous ligan
	Interferon Signaling
	Interleukin-12 family signaling
	Chemakine receptors bind chemakines
	Interleukin-2 family signaling
	Interleukin-1 family signaling
	Complement cascade
	Apoptasis
	Interleukin-6 family signaling
	Ecosanoid ligand-binding receptors
	NF-kB pathway
	Extracellular matrix organization
	Immunoregulatory interactions between
	Costimulation by the CD28 family
	Signaling by VEGF
	Interleukin-17 signaling
	Platelet homeostasis
	Metabolism
	C-type lectin receptors
	Cellular responses to stress
	Interleukin-20 family signaling
	Fc epsilon receptor (FCERI) signaling
	Signaling by FGFR3 Signaling by FGFR4
	Signaling by FGFR1
	Signaling by FGFR2

- Significant alterations in genes associated with inflammation including, Arg1, Mafg, Maff, Ifit3, Nod1, Cfd, Ifi2712a, Ifi44, Ifit1, Fos, Tnfaip3, Irf7, Retnla, II6ra, Ptgs2, H2-Eb1, Ager, Mknk1, TIr4, MyI2, II3, II10, Ifnb1, II23a, Ifng, II21, 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 ecig 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.



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

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