Inflammatory Response Elicited by Propylene Glycol/Vegetable Glycerin, Menthol, and Tobacco Flavored E-cigarette Aerosols in C57BL/6J and BALB/cJ Mice

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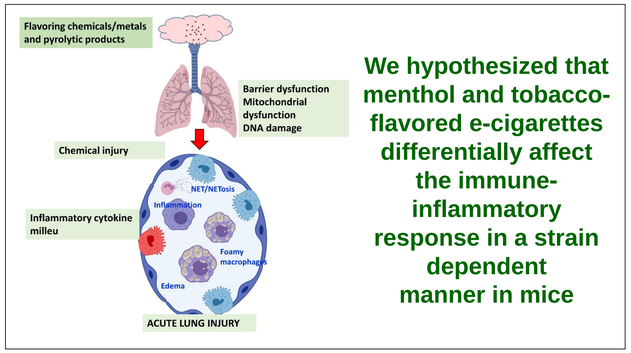
INTRODUCTION

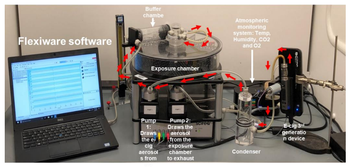
- Electronic Nicotine Delivery Systems (ENDS) are available in various flavors and nicotine strengths.
- These flavors contain propylene glycol, vegetable glycerin, and flavoring chemicals that impart flavors.
- Menthol and Tobacco flavored ENDS are still legal in many states and claimed to be a safer alternative to traditional combustible cigarettes
- In this study we investigated the acute inflammatory effects of exposure to menthol and tobacco flavors.

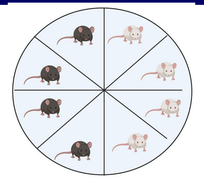
HYPOTHESIS



We hypothesized that menthol and tobacco-flavored e-cigarettes differentially affect the immune-inflammatory response in a strain dependent manner in mice

METHODS





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

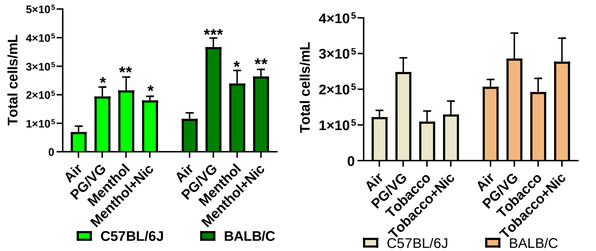
METHODS

Differential cell counts by FACS analysis:
Flow cytometric analysis of immune inflammatory cells was performed using cell-type specific monoclonal antibodies for macrophages (F4/80 PE), neutrophils (LY6B.2 Alexa fluor 488), T-lymphocytes (CD8a PE-cy5) and leukocytes (CD45 APC). Flow cytometry data acquisition was performed on a Guava easyCyte8i flow cytometer and analyzed using Guavasoft software.

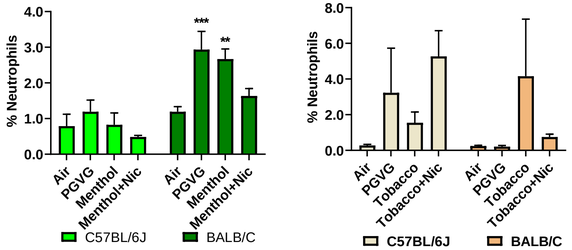
Inflammatory mediator analysis by Luminex :
Levels of Eotaxin ,G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10 ,IL-12 (p40), IL-12 (p70), IL-13, IL-17α, KC, MCP-1 (MCAF), MIP-1α, MIP-1β, RANTES, and TNF-α were determined using Bio-Plex Pro Mouse Cytokine 23-plex Assay #M60009RDPD using FLEXMAP3D instrument in bronchoalveolar lavage (BAL) fluid and lung homogenate.

RESULTS

1. Increased total inflammatory cells in BAL fluid by PG/VG, menthol, menthol 6 mg nicotine in both C57BL/6J and BALB/cJ mice

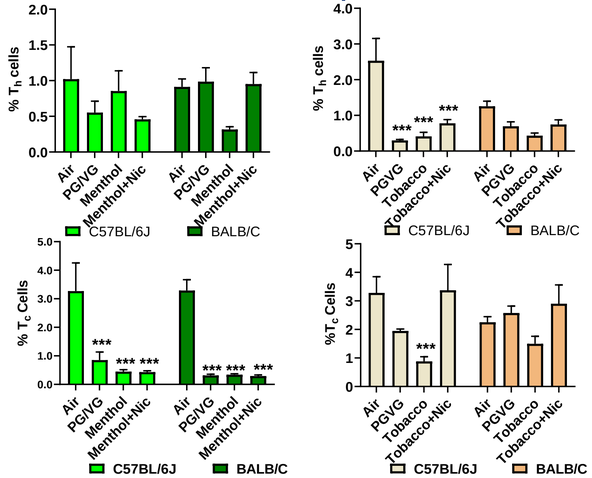


2. Increased neutrophil counts by PG/VG and menthol in BALB/cJ mice.

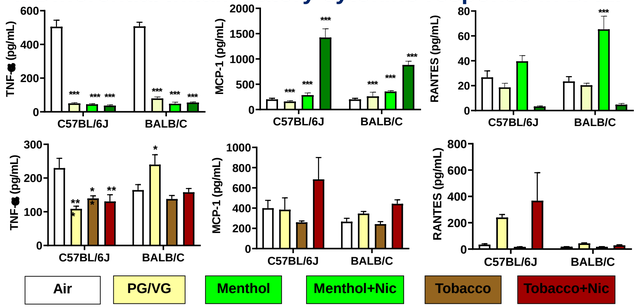


RESULTS

3. T lymphocytes were reduced in BAL fluid with PG/VG, Tobacco and Menthol flavor exposures in C57BL/6 mice

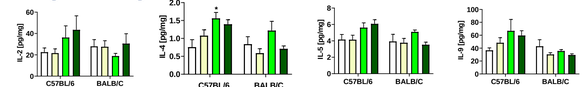


4. Differential Inflammatory cytokine response in BALF



Air | PG/VG | Menthol | Menthol+Nic | Tobacco | Tobacco+Nic

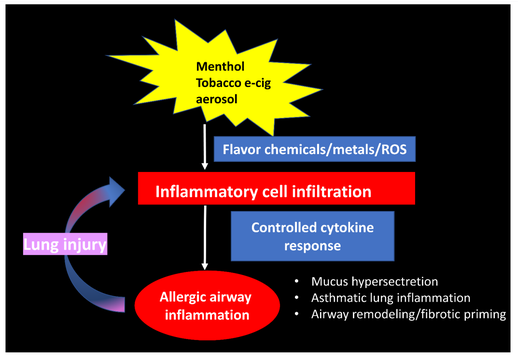
5. Anti-inflammatory/allergic tendency in response in lung homogenate



SUMMARY

- Acute exposure to PG/VG, menthol, and menthol with nicotine infiltrates inflammatory cells to BALF.
- Neutrophil influx seen in both C57BL/6J and BALB/cJ mice. However, in C57BL/6 mice a decrease in T-lymphocyte count was observed.
- BALF cytokine milieu suggests a controlled inflammatory response with allergic tendencies.
- Increased IL-2, IL-4, IL-5, and IL-9 in lung homogenates suggest limited/controlled T-cell differentiation and early recruitment, and allergic airway inflammation.

CONCLUSION



Acute exposure to PG/VG, menthol, and tobacco flavors induced an allergic airway inflammatory response which could result in lung injury and priming for lung remodeling. Elicited inflammatory response was strain-dependent and nicotine altered the immune response compared to the flavor exposure without nicotine.

ACKNOWLEDGEMENT

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