Menthol and Tobacco Flavored Electronic Cigarettes Induced Dysregulated Repair in Lung Fibroblast and Epithelium

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INTRODUCTION

- Electronic cigarettes (e-cigs) have become increasingly popular and they are considered as a safer alternative to cigarette smoke.
- Initially, flavor additives were added to reduce the throat irritation, but unexpectedly also attracted teenagers.
- Previous publications have presented that e-cig exposure causes oxidative stress, lung inflammation, and dysregulated repair in lungs from both cells and mice models.
- The toxic effects of flavored e-cigs on respiratory health is lacking.

HYPOTHESIS

Menthol and tobacco flavored e-cigs induce inflammatory responses and dysregulated repair responses in lung fibroblast and epithelium.

APPROACH

Cell culture and exposure:

Lung fibroblast (HFL-1) and epithelium (BEAS-2B) was culture in DMEM/F12K medium with 10% FBS and 1% Pen-Strep. Around 300K cells were used for microtissue seeding. HFL-1 cells in culture plates or HFL-1 formed microtissues were serum deprived overnight before the e-cig exposure. E-cig aerosols generated from flavorless, menthol, and tobacco flavored e-cigs (EC-blend) were released into a Enzyscreen chamber (Enzyscreen, Netherlands) with 2 puffs/min, total 2 mins, and additional 8 mins post exposure were allowed for equilibrating and deposition of the e-cig aerosols. Cells were then cultured for 2 days for sample collecting.

Cytotoxicity measurement:

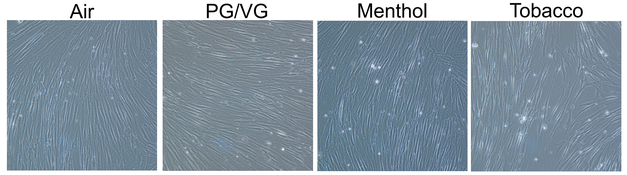
After e-cig exposure, cells were cultured for additional 2 days. Cellular morphology and confluence were monitored and conditioned media was collected for cytokine measurement (IL-8 and IL-6). Viable cell counts were conducted by AO/PI assay.

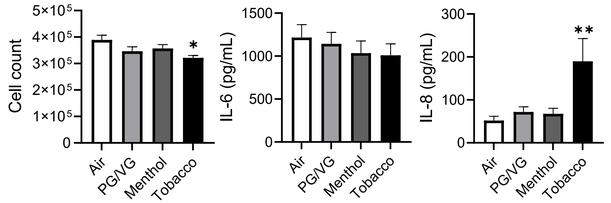
Quantification of dysregulated repair markers:

Cells were lysed in RIPA buffer for protein isolation and Trizol for RNA isolation. Respective protein and RNA markers were measured by western blot and qRT-PCR. GAPDH was used as endogenous control for normalization.

RESULTS

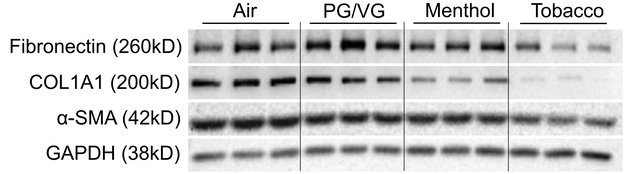
Tobacco Flavored E-cigs induced cytotoxicity and inflammatory responses in HFL-1

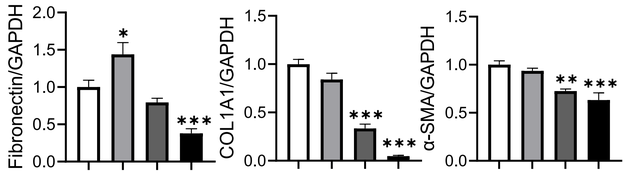




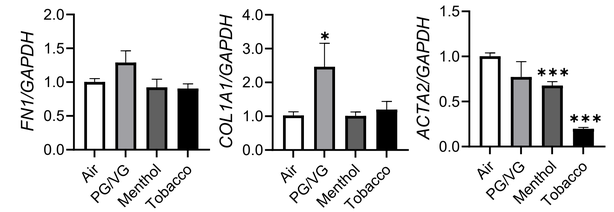
n=6 for cell count; n=9 for IL-6 and IL-8 measurement.
*P<0.05,**P<0.01 vs Air group

Tobacco and Menthol Flavored E-cig inhibit wound healing markers in HFL-1



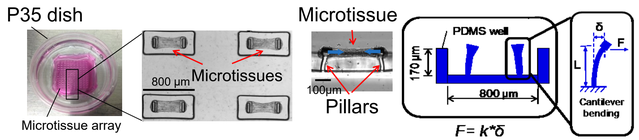


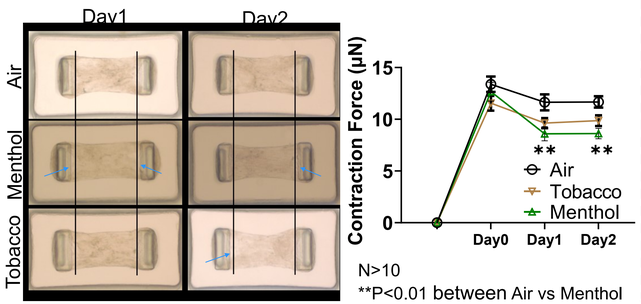
Gene expression:



n=6 *P<0.05,**P<0.01, and ***P<0.01 vs Air group

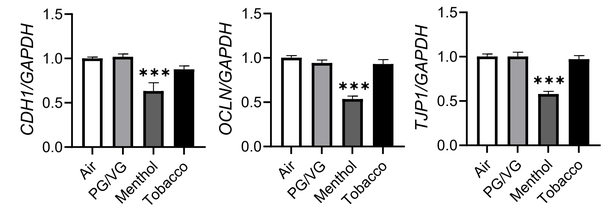
Menthol Flavored E-cig decrease tissue contractility in lung fibroblast formed microtissues





N>10
**P<0.01 between Air vs Menthol

Menthol Flavored E-cig dysregulated barrier function in lung epithelium (BEAS-2B)

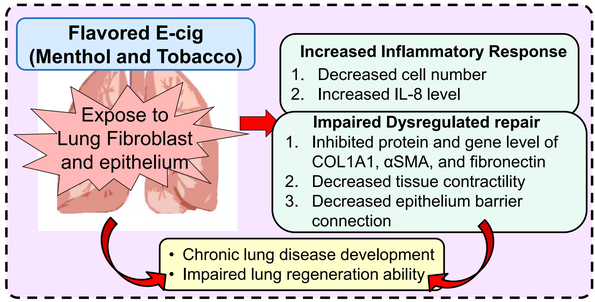


n=6, ***P<0.01 vs Air group

SUMMARY

1. HFL-1 cells exposed to tobacco flavored e-cig showed decreased cell number, and increased cellular inflammation (Increased IL-8)
2. Wound healing protein markers such as COL1A1, αSMA, and fibronectin were decreased after exposure to either menthol or tobacco flavored e-cigs.
3. Decreased tissue contraction force in HFL-1 formed microtissue when exposed to menthol and tobacco flavored e-cig was observed.
4. Epithelium barrier function interrupted by menthol e-cig exposure with decreased e-cadherin (CDH1), occludin (OCLN) and tight junction protein 1(TJP1)

CONCLUSION



CONCLUSION

1. Unregulated flavored e-cigs can lead to lung cellular dysfunction.
2. Inhibited wound healing ability by flavored e-cigs may predispose individuals to secondary challenges by respiratory toxicants.

ACKNOWLEDGEMENT

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