Inhalational Exposures As Potential Risk Factors For COVID-19 In Adolescents

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E-cig Use — A Significant Risk Factor For COVID-19 in Adolescents?

In 2020, about 1.8 million fewer U.S. youths are current e-cigarette users compared to 2019.

However, 3.6 million U.S. youths still currently use e-cigs.

There is a notable uptick in use of disposable e-cigs by youth.

More than 8 out of 10 current youth e-cig users use flavored e-cigs.
Innate Defense Mechanisms of the Respiratory Tract

- We inhale 11,000 liters of air per day (volume of a concrete mixer truck)
- There are approximately 5,400,000 bacteria in 11,000 liters of air
- Approximately 5,170,000 virus particles

White Blood Cells
- Macrophages
- Neutrophils
- Natural Killer (NK) Cells

Epithelial Cells

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Do **flavored e-liquids** Impact the Respiratory Tract? Example - Cinnamaldehyde

- Macrophages
- Neutrophils
- Natural Killer Cells

\[ \text{Flavored E-liquids} + \quad ? \]
Cinnamaldehyde Inhibits Immune Cell Functions

- **Macrophages**: Ability to Ingest Bacteria
- **Neutrophils**: Ability to Ingest Bacteria
- **Natural Killer Cells**: Ability Kill Tumor/Virus-infected Cell

Clapp et al., 2017
Sini-cide Alters Macrophages

PG/VG

0.25% Sini-cide
E-cig Flavorings Significantly Affect Neutrophil Function

• E-liquid flavorings can impair neutrophil functions at concentrations that do not produce cytotoxicity.

• Not all flavoring chemicals are created equal. Chemical classes can influence effects.

• Vaping aromatic aldehyde-containing e-liquids could result in impaired innate immune system response to airway infection.
Do **flavored e-liquids** Impact the Respiratory Tract? Example - Cinnamaldehyde
1% Sinicide E-liquid on HBECs

Cilia Beat Frequency (Hz)

Percent Active Area
1% Sinicide E-liquid on HBECs
1% Sinicide E-liquid on HBECs
1% Sinicide E-liquid on HBECs
Acquisition and Analysis of Samples from the Nasal Mucosa

Nasal Scrape Biopsy

Obtain Nasal Biopsy from Healthy Volunteers

Analysis of Immune Gene Expression

Indication of Changes in Respiratory Immune Status of Smokers and E-cigarette Users

Comparison of Non-Smokers, Smokers, and E-cigarette Users

Subjects were classified based on self-recorded smoking/vaping status and 3-week smoking/vaping behavior diary.

Individuals identified as mixed users based on the smoking diaries were excluded from the analyses

Martin et al., 2016
E-cig Usage Induces Greater Changes Than Cigarettes

All changes in immune gene expression were down-regulation!

Martin et al., 2016
E-cigarette cause an overall suppression of key host defense functions and immune dysfunction in the respiratory tract!
E-Cigarettes and Respiratory Host Defense

• E-cigarette aerosols, e-liquids, and their components can alter the function of airway cells and respiratory immune cells in multiple model systems and with a wide variety of exposure paradigms.

• Impairs antiviral pulmonary immune defenses in a mouse model. (Sussan et al 2015, Madison et al 2019)

• E-cigarette exposure can change virulence, bacterial persistence, and development of biofilm (Gilpin et al 2019)

• E-cigarettes increase antibiotic resistance (Hwang et al 2016)
  • E-cigarettes increase Staphylococcal virulence, causes resistance to antimicrobial peptides, and increases biofilm formation.
Young people who ever used e-cigarettes are 5 times more likely to be diagnosed with COVID-19.

Young people who ever used e-cigarettes plus conventional cigarettes are 7 times more likely to be diagnosed with COVID-19.

Original article
Association Between Youth Smoking, Electronic Cigarette Use, and Coronavirus Disease 2019
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Human \textit{in vivo} studies of Influenza Infections

- FluMist™ is a cold-adapted Live Attenuated Influenza Virus (LAIV) vaccine
  - "cold-adapted", thus replication limited to nasal cavity (32°C)
  - It generates a replicative but self limited viral infection with innate and immune host defense responses
  - Provides a safe tool to study influenza virus infections \textit{in vivo}
Study Timeline

Subjects Recruited

3-4 weeks

Screen

Baseline sample collection

Day 0

LAIV (FluMist®) Infection

Day 1

Day 2

Day 8

Sample collection post-infection

Day 21

• ELF
• NLF
• Nasal biopsy

Noah et al (2011) EHP
Analysis Methods

Nasal Scrape Biopsy

RNA extraction
Analysis of Immune Gene Expression
Comparison of Non-Smokers, Smokers, and E-cigarette Users

ELF and NLF

ELF extraction and NLF processing

Comparison of Non-Smokers, Smokers, and E-cigarette Users

IgA ELISA and multiplexed ELISA
Evidence That E-cigs Affect Viral Infections

Live-attenuated influenza virus (LAIV)
- Live – it replicates like normal flu
- Attenuated – milder and limited to nose

Recruited 1) Non-smokers, 2) Smokers, and 3) E-cig Users

Decreased Expression of Immune Genes
- Cigarettes
  - 28
  - 76 ↓ 4 ↑
- E-cigarettes
  - 52
  - 160 ↓ 31 ↑

Decreased Levels of LAIV-specific Antibodies

Rebuli et al., 2020
Summary

• The gene expression changes induced in the nasal mucosa of smokers and e-cigarette users are consistent with an immunosuppressive phenotype.

• Gene expression changes induced in the nasal mucosa of e-cigarette users overlap with those induced in cigarette smoker, but were greater (in # and magnitude) in e-cigarette users

• Antiviral host defense responses are compromised in smokers and e-cig users; nasal mucosal antibody levels are reduced
What does this mean for vaping adolescents?

• Even though their COVID-19 morbidity is usually mild, does vaping modify the disease?

• How does vaping affect nasal mucosal/systemic anti-SARS-CoV-2 antibodies? Magnitude? Persistence?
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Questions?
Summary of Previous Findings in Cigarette Smokers

Experimental virus infections (using the live attenuated influenza virus vaccine) resulted in blunted immune responses and enhanced markers of viral replication in the nose. (Noah et al., 2011, 2012)

These effects included reduced recruitment and activation of immune cells as well as decreased levels of key soluble mediators known to orchestrate host defense responses (Horvath et al., 2011, 2012)

General Immunosuppressive phenotype in Cigarette Smokers