JUUL and Vape Pen Flavors Produce Reactive Oxygen Species, Potentially Eliciting Differential Cellular Oxidative Stress and Inflammatory Responses

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Disclosure

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Relevant financial relationships with a commercial interest

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JUUL AND EMERGING DEVICES AND FLAVORS
Background

• JUUL is made of an aluminum shell, lithium battery, circuit board and pressure sensor

• JUUL constituents include PG/VG, nicotine, benzoic acid, and flavoring chemicals

• Smoother hit even at high nicotine levels due to benzoic acid
Flavors and Flavorings (chemical groups) present in ENDS, cigarillos, and waterpipe flavorings for toxicity testing.

**Flavors**
- Tobacco
- Menthol
- Berry
- Chocolate
- Piña Colada

**Flavorings**
- Aldehyde
- Quinoline
- Pyridine
- Alcohol
- Ketone
- Ester
- Pyrrole
- Acetal
- Amino acid
- Ether
- Peptide
- Monoterpene
- Pyrazine

**Chemical Structures**
- $R \cdot CH_2 \cdot OH$
- $R \cdot CH_2 \cdot OH$
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- $R \cdot CH_2 \cdot OH$

**Examples**
- Tobacco
- Menthol
- Berry
- Chocolate
- Piña Colada
Hypothesis

JUUL flavors and flavoring chemicals produce reactive oxygen species (ROS) and the exposure to these flavors result in an inflammatory response.

- Oxidative stress
- Activate NF-κB-driven inflammatory mediators
- Inflammation
  - Bronchiectasis
  - Asthma
  - Bronchiolitis obliterans
  - Chronic Obstructive Pulmonary Disease
- Mitochondrial dysfunction
- DNA damage
Cell-free ROS assessment in JUUL flavors

Principle: 2’,7’dichlorofluorescein diacetate (H2 DCF-DA) fluorogenic probe.

DCFH-DA (non-fluorescent)  $\xrightarrow{\text{NaOH}}$  DCFH (non-fluorescent)  $\xrightarrow{\text{OX/ROS}}$  DCF (fluorescent)

1. Prepare H$_2$O$_2$ standards (0 through 50 uM)
2. Bubbling JUUL aerosol (using SciReq InExpose through the DCFH solution).
3. Read fluorescence (ex 485 nm/em 535 nm)
4. Report OX/ROS as H$_2$O$_2$ equivalents.

E-liquid puffing was an adaptation of Behar profile: Cell-free ROS produced was measured by bubbling JUUL vapors (5,10, and 15 puffs, each puff lasting 3 seconds)
JUUL aerosols generate acellular reactive oxygen species (ROS)
(Virginia Tobacco, Cool Mint, Fruit Medley, and Crème Brulee flavors with 5% nicotine strength)

- Dose-dependent increase in acellular ROS generation by JUUL.
- Cool mint, crème brulee, and cool cucumber showed highest.
- Significantly less ROS levels generated compared to cigarettes.
Vaping pens with different flavors and products

Vitamin vaping clip
Vaping pen ingredients

- **VitaminVape B-12**
  - Composed of vitamin B-12

- **Nutrovape Energy**
  - Vegetable Glycerin, Propylene Glycol, Caffeine, L-Theanine, Vitamin B-12, Natural and Artificial Flavorings

- **Nutrovape Sleep**
  - Vegetable Glycerin, Propylene Glycol, Melatonin, Chamomile Extract, Passion Flower Extract, L-Theanine, Natural and Artificial Flavorings

- **Nutrovape Performax**
  - Vegetable Glycerin, Propylene Glycol, Horny Goat Weed Extract, Passion Flower Extract, Kava Kava Root Extract, Longjack Extract (Tongkat Alie), Tribulus Terrestris Extract, Guarana Extract, Ginseng Extract, Maca Extract, L-Theanine, Natural and Artificial Flavorings
Dose-dependent increase in acellular ROS generation by vaping pens.

- B12 showed highest approaching ROS levels generated by conventional cigarettes
- Energy, sleep, and performax showed significantly less ROS levels generated compared to cigarettes.
A session of exposure was defined as **3 puffs per minute for 22 minutes**. Let the cells be exposed for 8 more minutes in the chamber (total 30 minutes of JUUL exposure per session).
Exposure and epithelial junction disruption

Acute exposure to Cool Cucumber JUUL flavor impairs barrier function

Crème Brulee flavored aerosol affects barrier function

16-HBE cells grown in 24-well transwell plates exposed to three-sessions (each 66 puffs) of JUUL with equal intervals of 12 hours between sessions. After the final exposure, the resistance was measured by EVOM2 device.

N=3-12 per group

**p<0.01, ***p<0.001 vs. Control
Acute exposure to JUUL aerosols elicits an inflammatory response in 16-HBE bronchial epithelial cells

16-HBE cells grown in 24-well transwell plates exposed to three-sessions of JUUL aerosol with equal intervals between sessions. After the last exposure, IL-8 was measured in conditioned media
Inflammatory response by a single Cool Mint flavored JUUL session in bronchial epithelial Beas2b cells

BEAS 2B cells were grown in 6 well plates and exposed to a single session (22 puffs) of JUUL aerosol with minimum media during exposure. 24-hours after incubation the conditioned media was used for IL-8 analysis and cell viability was measured by AO/PI staining.

*p<0.05 vs. Control, N=6 per group
JUUL flavors induce cytotoxicity and inflammation in monocytes

U937 cells were cultured in a 12-well plate in complete medium. Serum deprived for 24 hours and treated with 0.5% JUUL flavors by well-volume. 24 hours later the cell viability was assessed by AO/PI staining and the conditioned media was used for cytokine assessment.
JUUL flavors differentially induce **PGE2 secretion** in different cells

*U937 (liquid)*

*BEAS 2B (aerosol)*

*16 HBE (aerosol)*

*p<0.05, **p<0.01 vs. respective control. (N=2/per group)*

U937, BEAS-2B, and 16-HBE cells were exposed to JUUL flavors (liquid or aerosols) in serum deprived medium. Prostaglandin E2 was measured in conditioned media.
Summary

• JUUL flavors generate ROS (less than CS)

• JUUL Vitamin B12 e-vape pen generate more ROS vs energy and sleep pen

• JUUL flavors (crème brulee, cool cucumber) caused epithelial barrier dysfunction.

• JUUL flavors induced cell type dependent differential inflammatory response.
  • IL-8 levels were elevated by cool mint, cool cucumber, classic menthol, just mango (strawberry coconut), and café latte.
  • PGE2 levels were elevated by classic menthol and just mango (strawberry coconut)
  • IL-6 levels were elevated by classic menthol.
Conclusions

- JUUL flavors induce an inflammatory response mediated by reactive oxygen species.
- It is important to assess and quantify the chemicals imparting these flavors to provide insights into appropriate regulation of JUUL flavors and constituents.

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