Effects of E-Cigarette Flavoring Chemicals on Human Macrophages and Bronchial Epithelial Cells

Introductory Information

E-cigarettes utilize a wide range of flavoring chemicals whose respiratory health effects are not well understood. In this study, we used pulmonary-associated cell lines to assess the *in vitro* cytotoxic effects of thirty flavoring chemicals. Human bronchial epithelial cells (BEAS-2B) and both naïve and activated macrophages (THP-1) were treated with 10, 100, and 1000 µM of flavoring chemicals and analyzed for changes in viability, cell membrane damage, reactive oxygen species (ROS) production, and inflammatory cytokine release. Viability was most greatly affected by decanal, hexanal, nonanal, cinnamaldehyde, eugenol, vanillin, alpha-pinene, eugenol, and limonene. High amounts of ROS were elicited by vanillin, ethyl maltol, and the diketones (2,3-pentanedione, 2,3-heptanedione, and 2,3-hexanedione) from both cell lines. Naïve THP-1 cells produced significant levels of IL-1β, IL-8, and TNF-α when exposed to ethyl maltol and hexanal. Activated THP-1 cells released increased IL-1β and TNF-α when exposed to ethyl maltol, but many flavoring chemicals had an apparent suppressive effect on inflammatory cytokines released by activated macrophages, with varying degrees of accompanying cytotoxicity. The diketones, L-carvone, and linalool, suppressed cytokine release in the absence of cytotoxicity. These findings provide insight into patterns of cytotoxicity and inflammatory cytokine release potentially relevant to the development of pathological changes in the lungs of e-cigarette users.

Methods Collection

Cell Culture

- All cells were cultured at 37 °C in a 5% CO_2 atmosphere in a Thermo Forma incubator in DMEM:F12 media (ATCC) supplemented with 10% fetal bovine serum and 50 mg/mL penicillin/streptomycin (ATCC).
- The THP-1 monocytes were differentiated into naïve macrophages. To differentiate the naïve macrophages (M0) into classically activated macrophages (M1).

- Cell line authentication was performed by the Genomics Core Facility at West Virginia University, Morgantown, WV to confirm their identity.

Preparation of E-liquid solutions

- Due to low water solubility of several flavoring compounds, a vehicle solution of propylene glycol (PG) and vegetable glycerin (VG) was used.
- A 1% solution of a 50 PG/50 VG mixture was found to be sub-toxic,
- Concentrations of both the liquid and solid flavoring chemicals were added to the PG/VG vehicle and vortexed until the solution was homogenized at room temperature.
- The 100 mM flavoring chemical solutions were stored at 4 °C for the duration of the study.
- Cells were treated with flavoring chemicals using final concentrations of 10, 100, and 1000 µM diluted in PBS.
- All flavoring chemicals were classified as "Food Grade" and obtained from Sigma-Aldrich.

Endotoxin

- A Pierce™ Chromogenic Endotoxin Quantification assay was performed.

Cellular Viability

- Assessed with the alamarBlue assay.
- Cells were treated with 10, 100, and 1000 µM of flavoring chemicals and incubated for 4 and 24 hours.
- AlamarBlue (10 µL) was added to the medium in each well for a final volume of 100 µL and incubated for 4 hours before reading.
- Fluorescence was measured at 560ex/590em with a Synergy H1 microplate reader.

Lactate Dehydrogenase

- Membrane damage (LDH) was assessed with the Homogeneous Membrane Integrity Assay.
- The assay utilizes a coupled enzymatic reaction which converts resazurin into resorufin.

Intracellular ROS

- Intracellular ROS were measured using the cell permeable dye, 2′,7′-dichlorofluorescin diacetate (DCFH-DA).
- DCFH-DA is de-esterified intracellularly and turns to highly fluorescent 2′,7′-dichlorofluorescein (DCF) upon oxidation.
- Cells were incubated with the dye for 45 minutes, after which the cells were rinsed with PBS and treated with 10, 100, and 1000 µM of the flavoring chemicals.

Cytokines

- Cells were grown in 96-well plates as previously indicated and treated with 1000 µM of flavoring chemical solution for 4 and 24 hours.
- The cytokine analysis was conducted according to manufacturer's instructions using the V-PLEX proinflammatory panel II which quantifies IL-1β, IL-6, IL-8, and TNF-α.

Citations – Publications based on the dataset

Morris A, Leonard S, Fowles JR, Boots T, Mnatsakanova A, Olgun N, Attfield KR. Effects of E-Cigarette Flavoring Chemicals on Human Macrophages and Bronchial Epithelial Cells. Toxicol Vitr. [In clearance]

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