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Effect of flavoring chemicals on free radical formation in electronic cigarette aerosols

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ABSTRACT

Background: Flavoring chemicals, or flavorants, have been used in electronic cigarettes (e-cigarettes) since their inception; however, little is known about their toxicological effects. Free radicals present in e-cigarette aerosols have been shown to induce oxidative stress resulting in damage to proliferation, survival, and inflammation pathways in the cell. Aerosols generated from e-liquid solvents alone contain high levels of free radicals but few studies have looked at how these toxins are modulated by flavorants.

Objectives: We investigated the effects of different flavorants on free radical production in e-cigarette aerosols.

Methods: Free radicals generated from 49 commercially available e-liquid flavors were captured and analyzed using electron paramagnetic resonance (EPR). The flavorant composition of each e-liquid was analyzed by gas chromatography mass spectroscopy (GCMS). Radical production was correlated with flavorant abundance. Ten compounds were identified and analyzed for their impact on free radical generation.

Results: Nearly half of the flavors modulated free radical generation. Flavorants with strong correlations included β-damascone, δ-tetradecalactone, γ-decalactone, citral, dipentene, ethyl maltol, ethyl vanillin, ethyl vanillin PG acetal, linalool, and piperonal. Dipentene, ethyl maltol, citral, linalool, and piperonal promoted radical formation in a concentration-dependent manner. Ethyl vanillin inhibited the radical formation in a concentration dependent manner. Free radical production was closely linked with the capacity to oxidize biologically-relevant lipids.

Conclusions: Our results suggest that flavoring agents play an important role in either enhancing or inhibiting the production of free radicals in flavored e-cigarette aerosols. This information is important for developing regulatory strategies aimed at reducing potential harm from e-cigarettes.

1. Introduction

Since their inception, electronic cigarettes (e-cigarettes) have been sold and marketed with flavored e-liquids; however, little is known regarding the products formed by these flavoring additives when heated at the high temperatures found in e-cigarettes. Many of the flavoring chemicals, or flavorants, found in these liquids are “generally recognized as safe” (GRAS) when consumed orally (US FDA 21CFR 182.1320); however, the thermal breakdown of these compounds in e-cigarette aerosols has yet to be fully evaluated, particularly in a toxicological context. In fact, the organization responsible for certifying food-safe flavorings for the FDA, the Flavor Extracts Manufacturers Association (FEMA), has specifically stated that they do not evaluate flavor ingredients for use in e-cigarettes or any other exposures other than ingestion [1].

The development of many tobacco-related diseases, such as cardiovascular disease, chronic obstructive pulmonary disease (COPD), and cancer are all thought to be influenced or induced by oxidative stress and oxidative damage [2–5]. Oxidative stress can be induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS), of

Abbreviations: AA, arachidonic acid; COPD, chronic obstructive pulmonary disease; DHA, cis-4,7,10,13,16,19-docosahexaenoic acid; ELISA, enzyme-linked immunosorbent assay; EPA, cis-5,8,11,14,17-eicosapentaenoic acid; EPR, electron paramagnetic resonance; GLY, glycerol; PBN, phenyl-N-tert-butylnitrone; PG, propylene glycol; RNS, reactive nitrogen species; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxyl; TRIS-HCl, tris(hydroxymethyl)aminomethane hydrochloride

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which free radicals are a major constituent [6]. In 2010, the Surgeon General released a report in which it identified free radical induced oxidative stress from tobacco smoke as being a contributor to the development of smoking-related diseases [7]. Free radicals are found in high concentrations in cigarette smoke ($> 10^{16}$ molecules/puff) [8–10]. Similarly, previous studies done by our lab and others have shown relatively high levels of reactive free radicals in e-cigarette aerosols ($> 10^{13}$ molecules/puff) by electron paramagnetic resonance (EPR) [11–14]. We found that free radical generation was highly dependent on the propylene glycol content of the e-cigarette liquid [14]. In addition to free radicals, a number of other studies have also found an assortment of other toxic agents in e-cigarette aerosols including nitrosamines, heavy metals, diethylene glycol, and reactive organic compounds such as formaldehyde and acetaldehyde [15–21].

Flavoring additives represent an important component of tobacco products as they have been shown to directly influence tobacco product preference and use, and have historically been used to attract younger consumers [22–26]. Despite banning characterizing flavors (fruits, candy, *etc.)* in cigarettes in the 2009 Family Smoking Prevention and Tobacco Control Act, flavorings are still utilized in virtually all other tobacco products, including e-cigarettes [23,27]. Recent studies, including the Population Assessment of Tobacco and Health (PATH) Study, reported that flavor was the primary reason for using a particular tobacco product among youth and young adults [28,29]. Nationwide, a survey of young adults reported that their first and usual e-cigarette flavor was something other than tobacco flavored [29]. Preferences for non-tobacco flavored e-cigarettes have also been seen in adults as a recent study found that over 75% of adult users of e-cigarettes preferred flavors other than tobacco for their e-liquids [30]. While the popularity of flavored e-cigarette products continues to grow, the potential harms from these flavoring additives remains largely unknown.

To date, only a handful of studies have examined the toxicity of specific flavorants. Specifically, exposure to cinnamaldehyde, 2-methoxycinnamaldehyde, and diacetyl have been shown to cause cytotoxicity at concentrations typically found in e-cigarette liquids [31–34]. The flavorants acetoin and maltol also appear to be potent inducers of inflammation [35]. A wide variety of volatile organic compounds have been identified in both in flavored e-cigarette liquids and their aerosols [36]. More recently, benzene has been shown to form as a result of the thermal decomposition of benzaldehyde, a natural fruit flavorant common in many e-liquid flavors [37]. Benzaldehyde has also been shown to cause respiratory airways irritation in animal exposure studies [34]. Another study found that toxic aldehydes are produced primarily from the decomposition of flavor compounds during vaping. Altogether, these studies suggest that flavor compounds may play an important role in the potential toxicity of e-cigarettes [38].

While the effects that e-cigarette operating parameters and e-cigarette solvents have only recently been investigated with respect to the delivery of toxins, the effect that e-cigarette flavoring additives have on the generation of these toxic compounds remains largely unknown. Of the studies performed that specifically address this topic of flavorants, only a few compounds have been identified as being harmful [31,34,37]. While many of these studies demonstrated cytotoxic effects of various flavorants, there have been no studies that have looked at the effects of flavorants on the generation of free radicals in e-cigarettes. Thus, in this study, we systematically evaluated the free radical generation of forty-nine commercially available, nicotine-free e-liquid flavor concentrates in e-cigarette aerosols. We also identified the individual flavorants found in the e-liquids and evaluated the effects of ten specific flavorants on free radical generation.

2. Materials and methods

2.1. E-cigarette, coil, and atomizer tank

The e-cigarette used for this study was a Wismec Reuleaux RX200S Mod (MyVaporStore.com) in temperature control mode. Three high amperage Samsung INR18650-25R, 2500 mA h, 3.7 V batteries were used to power the device. The batteries were recharged after 250 puffs were performed using the device. The heating element used was a commercially available 0.5 Ω stainless steel coil (SS316) Uwell Crown Coil (MyVaporStore.com). The atomizer tank used had a capacity of 4 mL and was composed of stainless steel and glass (Uwell Crown Tank; MyVaporStore.com).

2.2. Reagents

Arachidonic acid (AA), cis-4,7,10,13,16,19-docosahexaenoic acid (DHA), cis-5,8,11,14,17-eicosapentaenoic acid (EPA), glycerol (GLY), hexane, phenyl-N-tert-butylnitrone (PBN), propylene glycol (PG), 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO), and tert-benzene, and tris(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl) were purchased from Sigma-Aldrich (St. Louis, MO) and used as received.

2.3. E-cigarette flavor concentrates and flavorants

A commercially available kit containing forty-nine popular nicotine free e-liquid flavor concentrates was purchased from NicVape.com (Spartanburg, SC). Food grade certified flavorants (β-damascone, δ-tetradecalactone, γ-decalactone, citral, dipentene, ethyl maltol, ethyl vanillin, linalool, and piperonal) were purchased from Sigma-Aldrich. Food grade certified ethyl vanillin propylene glycol acetal was obtained from Vigon (East Stroudsburg, PA).

2.4. Profiling of flavorants

E-liquid compositions were analyzed as described previously by gas chromatography-mass spectrometry (GCMS) [39]. Using a Gerstel MPS2 multipurpose autosampler (Gerstel GmbH & Co. KG. Mülheim an der Ruhr, Germany), samples (1 μL) were introduced and split 50:1 into an Agilent Technologies 7890 A gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA) with an Agilent 5975 C mass selective detector. The GC inlet was a silanized glass straight design inlet liner (78.5 mm long x 6.5 mm o.d. x 0.75 mm i.d.) (Supelco, Bellefonte, PA) and the column was an Agilent J&W VF-35ms capillary column (60 m x 0.25 mm x 0.25 μm) with helium (Airgas) as the carrier gas. The inlet and MS source were both maintained at 280 °C. The temperature profile consisted of: injection at 50 °C with a 2 min hold, a linear increase of 10 °C/min to 240 °C, and an isothermal hold at 240 °C for 10 min. The MS was set to a scan of 30–300 amu. Chromatogram peaks were analyzed using Mass Hunter Qualitative Analysis B.06.00, software and chemical identities were found by library searching against the NIST11 EI mass spectral database. Quantitation of specific chemicals was done using external standards dissolved in propylene glycol and run using the same method.

2.5. E-cigarette apparatus

The e-cigarette setup used here was similar to that used in our previous study [14]. In brief, the e-cigarette's fire button was activated by a 12 V relay timer switch (SainSmart; Amazon.com) and a second relay switch was connected to a 12 VDC solenoid valve (RioRand; Amazon.com). Upstream and downstream ends of the solenoid valve were connected to of the solenoid valve were connected an impinger and a flow meter respectively. The flow meter was connected to the house vacuum and adjusted to a flow rate of 500 mL/min. A diagram of this setup is shown in Supplemental Fig. 1.

2.6. Solvent components, temperature, and wattage factors

To investigate the thermal degradation of flavors, e-liquid flavor concentrates were diluted, per the manufacturer's instructions, to 20%