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Impact of e-liquid flavors on nicotine intake and pharmacology of e-cigarettes

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ABSTRACT

Objectives: To describe the effect of e-liquid flavors on nicotine intake and pharmacology of e-cigarettes.
Methods: 11 males and 3 females participated in a 3-day inpatient crossover study with strawberry, tobacco, and their usual flavor e-liquid. Nicotine levels were nominally 18 mg/mL in the strawberry (pH 8.29) and tobacco (pH 9.10) e-liquids and ranged between 3–18 mg/mL in the usual brands (mean pH 6.80). Each day consisted of a 15-puff session followed by 4 h of abstinence, then 90 min of *ad libitum* use. Subjects used a KangerTech mini ProTank 3.
Results: After 15 puffs, the amount of nicotine inhaled and systemically retained were not significantly different between the strawberry and tobacco e-liquids but plasma $AUC_{(0 \rightarrow 180)}$ was significantly higher with the strawberry e-liquid. While not significantly different, C_{max} was 22% higher and various early time point AUCs to measure rate of rise of nicotine in blood ranged between 17 and 23% higher with the strawberry e-liquid compared to the tobacco e-liquid. During *ad libitum* use, systemic exposure to nicotine ($AUC_{(0 \rightarrow 90)}$) was the same for the tobacco and usual brand e-liquids but were both significantly lower than after using the strawberry e-liquid. The usual flavors were more liked and satisfying than the strawberry and tobacco e-liquids.
Conclusion: Flavors influence nicotine exposure through flavor liking, may affect rate of nicotine absorption possibly through pH effects, and contribute to heart rate acceleration and subjective effects of e-cigarettes. E-cigarette users titrate their nicotine exposure but the extent of titration may vary across flavors.

1. Introduction

Flavored e-liquids are commonly used in electronic cigarettes (e-cigarettes). Several flavorants are toxic and could be harmful to e-cigarette users. Diacetyl and its related compound, 2,3-pentanedione, both of which give a buttery flavor, cause bronchiolitis obliterans in humans exposed in occupational settings (Kreiss et al., 2002; van Rooy et al., 2007), and have been used as constituents of flavorants in e-liquids (Allen et al., 2016; Farsalinos et al., 2015). Some e-liquids and specific flavorants such as cinnamaldehyde, 2-methoxycinnamaldehyde, vanillin, and 2,5-dimethypyrazine (chocolate flavoring) have cytotoxic effects in *in vitro* studies (Bahl et al., 2012; Behar et al., 2014; Sherwood and Boitano, 2016). Flavors are also significant sources of toxic aldehydes produced during thermal decomposition of e-liquid constituents (Khlystov and Samburova, 2016).

From a regulatory perspective, use of flavors in e-liquids is controversial. Over 7000 different flavors have been identified, including tobacco, sweet flavors, menthol, and combinations (Krishnan-Sarin et al., 2015; Zhu et al., 2014). Sweet flavors, in particular, appeal to youth and may contribute to e-cigarette uptake (Kong et al., 2015; Krishnan-Sarin et al., 2015). On the other hand, flavors may be an important consideration for the acceptability of e-cigarettes to smokers who are trying to quit smoking (Farsalinos et al., 2013; Shiffman et al., 2015), a balance the U.S. Food and Drug Administration has acknowledged in its deeming rule of e-cigarettes as tobacco products (Food and Drug Administration, 2016).

Flavors might also influence nicotine pharmacokinetics from e-cigarettes, which has implications for their abuse liability. Flavors enhance the rewarding and reinforcing effect of nicotine-containing e-cigarettes (Audrain-McGovern et al., 2016), and one flavor decreased

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the maximum plasma nicotine concentration, C_{max}, in a study of an industry e-cigarette prototype (Walele et al., 2016). To the best of our knowledge, no study has assessed differences in nicotine intake and pharmacology of flavored e-liquids when used in commercially available e-cigarettes.

The objectives of this pilot study were to describe differences in nicotine intake, pharmacokinetics, and physiologic and subjective effects during prescribed vaping, and determine the intake of nicotine and extent of titration of nicotine blood levels during *ad libitum* access comparing different nicotine-containing flavored e-liquids.

2. Methods

We performed a 3-arm crossover study over three consecutive inpatient days in healthy e-cigarette users to examine the effects of e-liquid flavors on e-cigarette use. This paper focuses on the effects of e-liquid flavors on nicotine intake and pharmacology during a standardized session of 15 puffs and during *ad libitum* access. Changes in puff topography and vaping behavior with e-liquid flavors will be reported separately.

2.1. Subjects

A convenience sample of 14 subjects (3 females, 11 males) completed the study. Participants were recruited *via* Craigslist.com, flyers, and college campus newspapers. They were screened for eligibility at a clinical research facility. Exclusive e-cigarette users or dual users of fewer than 5 tobacco cigarettes per day, who used second and/or third generation e-cigarettes at least 25 days per month for the past 3 months or more, and had saliva cotinine levels $\geq$30 ng/mL were eligible. Exclusion criteria were unstable chronic medical conditions, current or past severe mental illness, pregnancy, current substance abuse other than marijuana, and sole users of first generation e-cigarettes (cig-a-likes). The study was approved by the Committee on Human Research at the University of California San Francisco. Written, informed consent was obtained from each participant and all participants were financially compensated.

2.2. Study e-liquid flavors and e-cigarette

For each of the three experimental arms, participants exclusively used one flavor of e-liquid: strawberry, tobacco or their usual brand of e-liquid. The strawberry and tobacco test e-liquids were purchased from Bulkejuice.com. We chose a strawberry flavor because it was one of the most highly rated single fruit flavors on Bulkejuice.com at the time. The tobacco flavor was chosen to represent tobacco-flavored e-liquids. Both flavors were labeled 50/50 VG/PG (vegetable glycerin/propylene glycol) and 18 mg/mL nicotine. We chose 18 mg/mL nicotine because users of smaller tanks commonly use e-liquids with high nicotine content (*e.g.*, 18 or 24 mg/mL) (Goniewicz et al., 2013; Wagener et al., 2016).

KangerTech Mini ProTank 3 clearomizer (1.5 ohm) connected to a KangerTech 3.7 V, 1000 mAh battery was the study e-cigarette, purchased directly from Kangertech.com. A new clearomizer was used for each assigned flavor. The nominal power of the e-cigarettes was 9.1 W.

2.3. General procedures

The study was conducted on the Clinical Research Center (CRC) at Zuckerberg San Francisco General Hospital. Each of the three study days ran from about 4p.m. to 4p.m. of the next day. Subjects and study personnel could not be blinded to the e-liquid administered because each e-liquid produced a strong distinct odor. On admission to the CRC, the participants' own e-cigarette(s) and e-liquid(s) were immediately removed. From 4 to 10p.m. (Acclimatization Session), subjects could vape *ad libitum* to become acclimatized to the assigned flavor for the next day's procedures. This time was considered sufficient for acclimatization to the e-liquid because all participants were experienced users of similar push-button tank e-cigarettes or third generation rebuildable atomizers. Participants were abstinent overnight until the morning standardized session of 15 puffs, which was followed by 4 h of abstinence, and then a 90-min *ad libitum* use session.

2.4. Standardized session experimental procedures

On each morning, participants were awakened at 7:00a.m. An intravenous (IV) line for blood sampling was placed in the forearm at 8:00a.m followed by a light breakfast. At about 8:30a.m, subjective questionnaires were administered, and three heart rate measurements were made within 10 min by pulse oximeter (average was used as the baseline heart rate for each day); baseline blood was sampled and urine collected at about 9:10a.m. At 9:28a.m, participants took 15 puffs, one puff every 30 s, from the e-cigarette. Puff duration was not controlled by the study. The amount of nicotine exhaled was collected as previously described (Havel et al., 2016; St.Helen et al., 2016a). E-cigarettes were weighed before and after vaping to determine amount of e-liquid consumed. Blood was sampled at 2, 5, 15, 30, 45, 60, 90, 120, and 180 min and heart rate was measured at 5, 10, 15, 20, and 30 min after the final puff. Subjective questionnaires were administered between the 5-min and 15-min blood samples.

2.5. Ad libitum session experimental procedures

After about 4 h of abstinence, subjective questionnaires were administered and a blood sample was taken. E-cigarettes were filled with the same e-liquid used during the standardized session and weighed before and after the session. Starting at 2:00p.m., participants vaped the study e-cigarette as desired for 90 min. During that time, subjects watched television, browsed the Internet through their personal computers or smartphones and/or read books. Participants were not allowed to sleep or doze off. Blood samples were taken every 15 min and subjective questionnaires were administered again at the end of the 90-min session.

2.6. Questionnaires

We measured nicotine withdrawal, craving, and positive and negative affective states before and after e-cigarette use with the Minnesota Nicotine Withdrawal Scale (total score) (MNWS) (Hughes and Hatsukami, 1986); the Questionnaire for Smoking Urges (QSU-Brief) modified for e-cigarettes using the total score, factor 1 subscale (a desire and intention to smoke with smoking perceived as rewarding), and factor 2 subscale (an anticipation of relief from negative affect with an urgent desire to smoke) (Cox et al., 2001); and the positive and negative affect subscales of the Positive and Negative Affect Schedule (PANAS) (Becoña et al., 1998), respectively. We used the five subscales of the modified Cigarette Evaluation Questionnaire (mCEQ) (Rose et al., 1999), further modified for e-cigarettes, to measure satisfaction, reward, aversive effects, enjoyment of sensation at the back of the throat and chest, and craving reduction. The mCEQ item "Did the e-cigarette taste good?" measured after each *ad libitum* session, was used as a proxy for 'liking' of the e-liquids.

2.7. Analytical chemistry

Nicotine was measured in the pooled 0.02 N HCl trap solution from the three gas dispersion tubes and mouthpiece and in e-liquids by LC–MS/MS using previously described methods (St.Helen et al., 2016a; Trehy et al., 2011). The LOQ was 0.5 ng/mL. Nicotine concentration in plasma was determined by GC–MS/MS (Jacob et al., 1991) modified for tandem mass spectrometry for improved sensitivity. The limit of quantitation (LOQ) was 0.2 ng/mL. The pH of all e-liquids was