Published OnlineFirst on July 20, 2010 as 10.1158/1055-9965.EPI-10-0288

Research Article

Cancer Epidemiology, Biomarkers & Prevention

A Clinical Laboratory Model for Evaluating the Acute Effects of Electronic "Cigarettes": Nicotine Delivery Profile and Cardiovascular and Subjective Effects

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Abstract

Background: Electronic "cigarettes" are marketed to tobacco users as potential reduced exposure products (PREP), albeit with little information regarding electronic cigarette user toxicant exposure and effects. This information may be obtained by adapting clinical laboratory methods used to evaluate other PREPs for smokers.

Methods: Thirty-two smokers participated in four independent Latin-square ordered conditions that differed by product: own brand cigarette, "NPRO" electronic cigarettes (NPRO EC; 18 mg cartridge), "Hydro" electronic cigarettes (Hydro EC; 16 mg cartridge), or sham (unlit cigarette). Participants took 10 puffs at two separate times during each session. Plasma nicotine and carbon monoxide (CO) concentration, heart rate, and subjective effects were assessed.

Results: Own brand significantly increased plasma nicotine and CO concentration and heart rate within the first five minutes of administration whereas NPRO EC, Hydro EC, and sham smoking did not. Own brand, NPRO EC, and Hydro EC (but not sham) significantly decreased tobacco abstinence symptom ratings and increased product acceptability ratings. The magnitude of symptom suppression and increased acceptability was greater for own brand than for NPRO EC and Hydro EC.

Conclusions: Under these acute testing conditions, neither of the electronic cigarettes exposed users to measurable levels of nicotine or CO, although both suppressed nicotine/tobacco abstinence symptom ratings.

Impact: This study illustrates how clinical laboratory methods can be used to understand the acute effects of these and other PREPs for tobacco users. The results and methods reported here will likely be relevant to the evaluation and empirically based regulation of electronic cigarettes and similar products. *Cancer Epidemiol Biomarkers Prev; 19(8); OF1–9. ©2010 AACR.*

Introduction

A variety of potential reduced exposure products (PREP) are/were marketed to cigarette smokers with explicit or implied claims that their use is associated with less exposure to lethal smoke constituents (1-3). These PREPs for smokers include products that involve burning specially cured tobacco that contains lower levels of some toxicants, that primarily heat rather than burn tobacco, or smokeless tobacco (4-6). As has been argued many times (1, 7, 8), objective empirical evaluation of these products is critical as a means of determining the extent to which PREP use is associated with reduced toxicant exposure, e.g., to nicotine or carbon monoxide (CO), and suppression of tobacco/nicotine abstinence symptoms. Clinical laboratory work has been very revealing, and sometimes shows reduced toxicant exposure and often shows a failure to suppress aversive abstinence symptoms fully (9, 10). In theory, toxicant exposure reduction may be associated with long-term decreased health risk, but this theoretical decreased risk is very unlikely with a product that is not used because it fails to suppress abstinence symptoms and/or is otherwise unacceptable.

So-called electronic "cigarettes" are one of the newest types of PREPs available, and anecdotal evidence suggests that, at least for some smokers, electronic cigarettes can completely replace tobacco cigarettes (11, 12). Many electronic cigarette brands are available in retail outlets and over the internet. Electronic cigarettes consist of a rechargeable battery, heater, and a cartridge that contains a liquid made of propylene glycol, nicotine, and other chemicals (13). When the battery-powered heater is activated, it heats the solution to produce a vapor that can then be inhaled by the user (14, 15). Electronic cigarettes are

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Note: The results of this study were presented in poster form at the annual Society for Research on Nicotine and Tobacco meeting held in Baltimore, MD February 24-27, 2010.

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doi: 10.1158/1055-9965.EPI-10-0288

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marketed as PREPs for smokers, with manufacturer claims such as "alternative to smoking that will satisfy your nicotine urges and cravings" (16), "helps smokers quit, cut down or smoke healthier" (17), and "gives smokers all the pleasure and satisfaction of traditional smoking without all the health, social and economic problems" (18). To date, however, there are few objective data to substantiate these claims. Indeed, there is little objective information describing electronic cigarette toxicant content and yield or user toxicant exposure and effect.

The little available data suggest that electronic cigarette cartridges and vapors may contain trace amounts of impurities and tobacco-specific nitrosamines (13, 19). Electronic cigarette cartridge nicotine content may be less than the product labeling indicates, and the vapor produced from the cartridge may yield very little nicotine (13, 19). The scant data from human laboratory studies suggest that electronic cigarette use is likely to involve little nicotine exposure and tobacco abstinence symptom suppression that is far less than that produced by a tobacco cigarette (20, 21). Existing clinical models that have been used to evaluate combustible and noncombustible PREPs (10, 22, 23) might be adapted to quickly and efficiently provide necessary information regarding electronic cigarette toxicant exposure and effect.

The purpose of this study was to describe clinical laboratory methods that could be used to characterize electronic cigarette users' nicotine and CO exposure, cardiovascular response, and ratings of tobacco/nicotine abstinence symptom suppression and product acceptability. Accordingly, this within-subject study used these outcomes to compare, in 32 tobacco cigarette smokers, the effect of two marketed electronic cigarette brands with own brand cigarettes and sham smoking (i.e., puffing on an unlit cigarette). Based on preliminary data presented elsewhere (20, 21) we hypothesized that, compared with own brand, electronic cigarettes would deliver less nicotine and no CO and would be less effective at reducing symptoms of tobacco abstinence while producing lower acceptability ratings.

Materials and Methods

Participants

This study was approved by the Institutional Review Board of Virginia Commonwealth University and was conducted in accordance with the Declaration of Helsinki. Sixty-six men and women recruited from the Richmond, Virginia area provided written, informed consent. Prior to participation in any experimental sessions, 18 individuals were disqualified based on health concerns (e.g., high blood pressure) or failure to meet other inclusion/exclusion criteria (see below). In addition, 2 participants withdrew from the study and another 14 were withdrawn due to failure to comply with study procedures ($n = 9$), poor venous access ($n = 3$), or because study enrollment was completed ($n = 2$). The remaining 32 participants (13 women; 18 white) completed the study proper and were included in the analyses. Participants smoked at least 15 cigarettes per day (mean, 22 cigarettes per day; SD, 8.8), were between the ages of 18 to 55 years (mean, 33.6; SD, 12), provided an afternoon screening CO of at least 15 ppm (mean, 23.5; SD, 8.8), and had a urine cotinine result of at least 4 on a 7-point scale (0-6; NicAlert, Nymox Corp.; mean, 5.9; SD, 0.2). Exclusion criteria included self-reported history of any chronic mental or physical health condition, pregnancy or breastfeeding, active menopause, self-reported use of electronic cigarettes, current smoking cessation attempt, current drug use (other than marijuana), and >20 days self-reported marijuana or alcohol use in the past 30 days.

Study design and procedures

This study was conducted on an outpatient basis at the Clinical Behavioral Pharmacology Laboratory at Virginia Commonwealth University. Participants completed four laboratory sessions; each approximately 150 minutes in duration. One of four Latin-square ordered conditions was presented each session (separated by at least 48 hours) that differed by product administered: own brand (i.e., a lit cigarette of the participant's preferred brand), sham (i.e., an unlit cigarette of the participant's preferred brand), "NPRO" electronic cigarettes (NPRO EC; 18-mg cartridge; NJOY), and "Hydro" electronic cigarettes (Hydro EC; 16-mg cartridge; Crown Seven).

Participants were asked to refrain from cigarette smoking for at least 12 hours prior to their scheduled session. Smoking abstinence was verified upon arrival at the laboratory by an expired air CO level ≤10 ppm. At the start of a session, a heparinized catheter was inserted into a forearm vein, physiologic monitoring equipment was attached, and continuous physiologic recording commenced. Thirty minutes after session onset, participants responded to the subjective effect questionnaires, blood (7 mL) was sampled, and product was administered. Product administration consisted of 10 puffs with a 30-second interpuff interval (IPI; puff number and IPI were monitored by study staff): package instructions state that electronic cigarettes should be used similarly to a tobacco cigarette, and 10 puffs with a 30-second IPI approximates *ad libitum* cigarette smoking (24). Five, 15, 30, and 45 minutes after the first of 10 puffs of the initial product administration, participants responded to the subjective effect questionnaires and blood (7 mL) was sampled. Expired air CO was recorded at 15, 30, and 45 minutes. At 60 minutes, participants responded to the subjective effect questionnaires, blood (7 mL) was sampled, and product was administered a second time (again, 10 puffs, 30-second IPI). Five, 15, 30, and 45 minutes after the second product administration, participants responded to the subjective effects questionnaires and blood (7 mL) was sampled. Expired air CO was recorded at 15, 30, and 45 minutes. At session's end, the venous catheter was removed, participants were compensated for their time, and, if necessary, additional sessions were scheduled.

Materials

NPRO EC was purchased from NJOY (25). The NPRO EC resembles a tobacco cigarette in length and diameter and consists of a disposable mouthpiece that houses a cartridge, a small heating element (vaporizer) that is activated when the user draws air through the device, and a rechargeable lithium battery. Cartridges come in a variety of flavors with varying nicotine content (0-18 mg). According to the NJOY website, cartridge ingredients include nicotine, propylene glycol, water, ethanol, glycerol, acetylpyrazine, guaiacol, mysomine, cotinine, and vanillin.

Hydro EC was purchased from Crown Seven (26). Hydro EC is similar in composition to NPRO EC and, according to the manufacturer's website, cartridges also come in a variety of flavors and contain nicotine (0-16 mg), propylene glycol, water, and tobacco flavoring.

For electronic cigarette conditions, a new 16-mg (Hydro EC) or 18-mg (NPRO EC) nicotine cartridge was used and batteries were fully charged before each session. The flavor of each electronic cigarette was matched to the participants' usual brand of cigarettes (regular or menthol). Consistent with product instructions, participants were instructed to puff from the electronic cigarette devices as they would a normal cigarette.

Participants' usual brand of cigarette was used in the own brand and sham conditions. According to the Federal Trade Commission (FTC, 2001), on average, usual brand yield was 1.06 mg nicotine (27), 14.7 mg tar, and 14.6 mg CO (based on available data for 26 participants).

Outcome measures

Physiologic measures. Heart rate was measured every 20 seconds (Model 506, Criticare Systems, fitted with a reusable pulse oximeter sensor). Blood samples were centrifuged and plasma was stored at −70°C. Plasma samples were sent to the Bioanalytical Analysis Core Laboratories of Virginia Commonwealth University's Department of Pharmaceutics and were analyzed for nicotine content using liquid chromatography/tandem mass spectrometry [limit of quantitation (LOQ) = 2.0 ng/mL; see ref. 24 for details]. Expired air CO was assessed using a BreathCO monitor (Vitalograph).

Subjective effect questionnaires. Participants responded to a computerized version of the Tiffany Drobes Questionnaire of Smoking Urges Brief (QSU Brief; ref. 28). The questionnaire consists of 10 items rated from 0 (strongly disagree) to 6 (strongly agree). Items from this scale are loaded onto two previously validated factors; factor 1 (intention to smoke) and factor 2 (anticipation of relief from withdrawal).

Participants responded to three computerized questionnaires containing visual analog scale (VAS) items. Each word or phrase was centered above a horizontal line that represented a scale from 0 to 100 points; the left anchor was "Not at all" and the right anchor was "Extremely". A mouse-controlled cursor produced a vertical mark on the line that could be adjusted by the participant. Scores on visual analog scale items were calculated as the distance between the vertical mark and the left anchor and were expressed as a percentage of line length.

Eleven VAS items were used to assess nicotine/tobacco abstinence symptom suppression (29). The direct effects of nicotine were assessed using 10 VAS items sensitive to nicotine effects (30, 31). Participants also responded to 14 visual analog scale items that assessed the direct effects of tobacco.

Data analyses

Heart rate values were averaged for 5-minute periods prior to each product administration or blood sampling. One heart rate measurement was missing for one participant in one session, and the missing value was replaced with an average of values before and after it. For plasma nicotine, values below the LOQ were replaced by the LOQ as in previous work (10, 32, 33).

We had previously reported the interim findings of this study, in a letter that examined nicotine exposure, heart rate, and craving results (21). The first 16 participants were included in that analysis. Prior to analyzing the data from all 32 participants described here, we first compared results of the first group of 16 participants with those of the second group of 16 participants using a repeated measures ANOVA with two within-subjects factors: condition (4 levels) and time [10 levels for nicotine, subjective, and heart rate (HR) data; 7 for CO data], and one between-subjects factor: study group (first 16 or second 16). No significant main effects or interactions involving the study group factor were observed on any measure. Given this failure to observe any differences across the two groups, all data were combined. Thus, the data from all 32 participants for plasma nicotine, CO, heart rate, and subjective measures were analyzed using repeated measures ANOVA with two factors: condition (4 levels) and time (10 levels for nicotine, HR data, and subjective data; 7 levels for CO). Huynh-Feldt corrections were used to account for violations of sphericity. Tukey's Honestly Significant Difference (HSD) test was used to examine differences between means ($P < 0.05$).

Results

Table 1 presents the main effects and interactions for all measures. The primary results of interest are those for which a significant condition by time interaction were observed, indicating that changes over time observed on that measure depended upon product used in that condition.

Physiologic measures

Plasma nicotine. A significant condition by time interaction was observed for plasma nicotine (Fig. 1A). Mean (SD) plasma nicotine increased from a preadministration level of 2.1 (0.32) ng/mL to a peak of 18.8 (11.8) ng/mL

Table 1. Results of statistical analyses for all outcome measures

Measure	Condition F (*P*)	Time F (*P*)	Condition × time F (*P*)
Heart rate*	13.5 (>0.001)	62.0 (>0.001)	16.1 (>0.001)
Plasma nicotine*	112.2 (>0.001)	53.0 (>0.001)	45.2 (>0.001)
Carbon monoxide†	105.5 (>0.001)	107.0 (>0.001)	205.5 (>0.001)
Hughes and Hatsukami*			
Anxious	4.8 (0.01)	5.7 (>0.001)	1.8 (0.05)
Craving a cigarette	37.7 (>0.001)	25.7 (>0.001)	10.6 (>0.001)
Depression/feeling blue	0.5 (ns)	1.5 (ns)	1.1 (ns)
Difficulty concentrating	0.5 (ns)	3.4 (0.02)	0.8 (ns)
Drowsy	0.6 (ns)	3.9 (0.007)	1.6 (ns)
Hunger	2.6 (ns)	7.8 (>0.001)	1.8 (ns)
Impatient	4.3 (0.009)	3.7 (0.006)	2.5 (0.005)
Irritability/frustration/anger	5.8 (0.004)	2.3 (ns)	3.3 (0.001)
Restless	1.5 (ns)	2.5 (0.05)	1.9 (0.025)
Desire for sweets	1.7 (ns)	1.7 (ns)	1.7 (ns)
Urge to smoke	40.8 (>0.001)	28.2 (>0.001)	9.7 (>0.001)
QSU*			
Factor 1	40.6 (>0.001)	35.7 (>0.001)	16.9 (>0.001)
Factor 2	19.5 (>0.001)	16.6 (>0.001)	7.6 (>0.001)
Direct effects of nicotine*			
Confused	1.0 (ns)	1.4 (ns)	1.4 (ns)
Dizzy	1.5 (ns)	5.2 (0.002)	4.1 (0.002)
Headache	0.7 (ns)	0.9 (ns)	1.6 (ns)
Heart pounding	0.4 (ns)	2.9 (0.05)	1.9 (ns)
Lightheaded	1.2 (ns)	7.6 (>0.001)	5.8 (>0.001)
Nausea	0.1 (ns)	0.8 (ns)	1.1 (ns)
Nervous	1.0 (ns)	1.0 (ns)	1.2 (ns)
Salivation	0.5 (ns)	0.2 (ns)	1.0 (ns)
Sweaty	0.8 (ns)	0.9 (ns)	1.3 (ns)
Weak	0.1 (ns)	0.8 (ns)	1.1 (ns)
Direct effects of tobacco*			
Satisfying	76.2 (>0.001)	61.6 (>0.001)	17.2 (>0.001)
Pleasant	66.5 (>0.001)	68.5 (>0.001)	17.6 (>0.001)
Taste good	47.7 (>0.001)	66.8 (>0.001)	14.4 (>0.001)
Dizzy	19.2 (>0.001)	17.6 (>0.001)	7.2 (>0.001)
Calm	42.0 (>0.001)	31.1 (>0.001)	10.3 (>0.001)
Concentrate	17.9 (>0.001)	15.8 (>0.001)	5.5 (>0.001)
Awake	27.0 (>0.001)	22.0 (>0.001)	6.6 (>0.001)
Reduce hunger	24.2 (>0.001)	19.4 (>0.001)	5.6 (>0.001)
Sick	3.6 (0.02)	3.6 (0.01)	0.9 (ns)
Taste like own brand	115.5 (>0.001)	60.0 (>0.001)	26.9 (>0.001)
Feel like own brand	65.9 (>0.001)	67.0 (>0.001)	21.6 (>0.001)
Harsh as own brand	64.9 (>0.001)	49.2 (>0.001)	21.3 (>0.001)
Mild as own brand	62.4 (>0.001)	54.0 (>0.001)	20.5 (>0.001)
Smoke another cigarette RIGHT NOW	6.5 (>0.001)	38.3 (>0.001)	4.8 (>0.001)

Abbreviation: ns, nonsignificant.

*$df_{Condition}$, 3,93; df_{Time}, 9,279; $df_{Condxtime}$, 27,837.

†$df_{Condition}$, 3,93; df_{Time}, 6,186; $df_{Condxtime}$, 18,558.

five minutes after the first administration under the own-brand condition. No significant changes in plasma nicotine were observed for the Hydro EC, NPRO EC, or sham conditions.

Heart rate. A significant condition by time interaction was also observed for heart rate (Fig. 1B). Heart rate increased from an average (SD) of 65.7 (10.4) bpm at baseline to a peak of 80.3 (10.9) bpm five minutes after the

first administration under the own-brand condition. No significant changes in heart rate were observed for the Hydro EC, NPRO EC, or sham conditions.

Expired air CO. A significant condition by time interaction was observed for expired air CO levels (Fig. 2). Mean (SD) CO increased from a preadministration level of 5.3 (2.1) ppm to a peak of 16.2 (4.5) ppm 15 minutes after the second administration under the own-brand condition. No significant changes in CO level were observed for the Hydro EC, NPRO EC, or sham conditions.

Subjective effect questionnaires

QSU brief. A significant condition by time interaction was observed for both factors of the QSU brief. Figure 3A shows data for factor 1 (intention to smoke; the QSU factor with the greater condition by time F value). Hydro EC and NPRO EC significantly decreased scores on both factors (relative to sham and/or baseline) at several time points. In contrast, own brand significantly decreased scores (relative to baseline and sham) on both factors at nearly every time point. In addition, the scores observed for own brand after product administration were significantly lower than Hydro EC and NPRO EC at almost every time point. A nearly identical pattern of results was observed for factor 2, although ratings were generally lower than those observed on factor 1.

Nicotine/tobacco abstinence symptom suppression. Significant condition by time interactions were observed on ratings of "anxious," "craving a cigarette/nicotine," "impatient," "irritability/frustration/anger," "restless,"

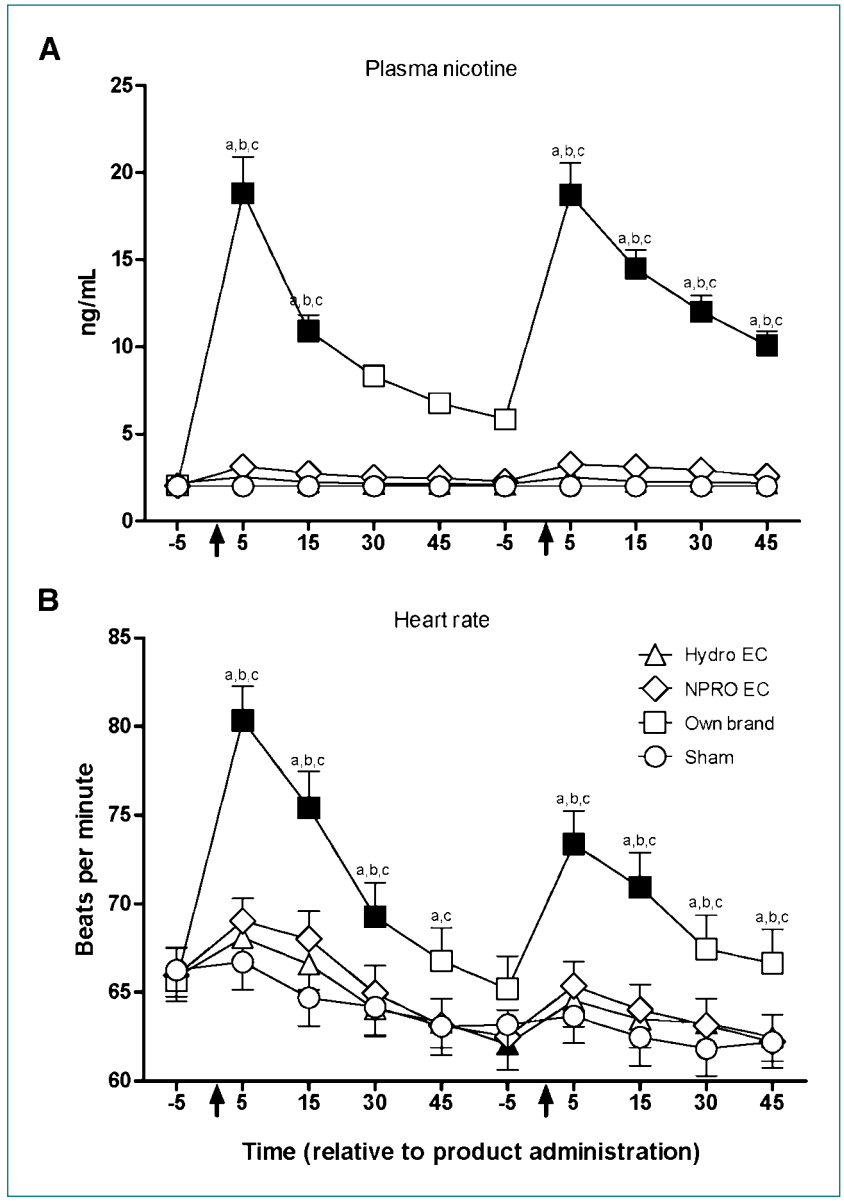

Figure 1. Mean data for nicotine blood plasma (A) and heart rate (B) as a function of condition and time. *X*-axes, time in minutes relative to product administration; arrows, first and second product administrations. *Y*-axes, A, nicotine blood plasma concentration (ng/mL); B, heart rate (beats per minute); filled symbols, significant difference from baseline. An "a," "b," or "c" indicates that own brand was significantly different from sham, Hydro EC, or NPRO EC at that time point. A "d" indicates that Hydro EC was significantly different from sham at that time point. An "e" indicates that NPRO EC was significantly different from sham at that time point (Tukey's HSD, $P < 0.05$). Unidirectional error bars, 1 SE.

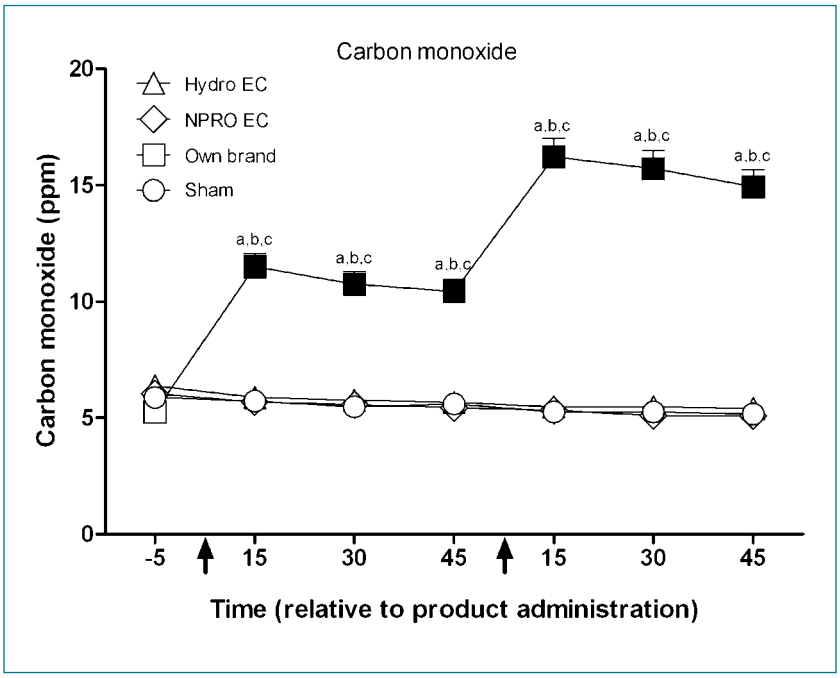

Figure 2. Mean data for carbon monoxide (CO) as a function of condition and time. *X*-axis, time in minutes relative to product administration; arrows, first and second product administrations. *Y*-axis, CO in parts per million (ppm); filled symbols, significant difference from baseline. An "a," "b," or "c" indicates that own brand was significantly different from sham, Hydro EC, or NPRO EC at that time point. A "d" indicates that Hydro EC was significantly different from sham at that time point. An "e" indicates that NPRO EC was significantly different from sham at that time point (Tukey's HSD, $P < 0.05$). Unidirectional error bars, 1 SE.

and "urge to smoke a cigarette." Own brand generally decreased ratings on all of these measures. NPRO EC and Hydro EC produced some abstinence symptom suppression on two measures: "craving a cigarette" and "urge to smoke." Figure 3B depicts data for ratings of "craving a cigarette" (the measure with the largest F-value). Ratings of "urge to smoke" decreased significantly 5 minutes following the first Hydro EC administration, and 5, 15, and 30 minutes following the first and second NPRO EC administrations relative to baseline. Ratings of "urge to smoke" decreased significantly at all time points following the first and second own-brand administrations relative to baseline, and own brand was significantly different from Hydro EC and NPRO EC at several time points. Similar effects of own brand, Hydro EC, and NPRO EC were observed on ratings of "craving a cigarette" (Fig. 3B).

Direct effects of nicotine. Significant condition by time interactions were observed on VAS items assessing "dizzy" and "lightheaded." Own brand significantly increased ratings of "lightheaded" and "dizzy" within the first five minutes following the first administration. NPRO EC, Hydro EC, and sham did not alter ratings significantly on these measures.

Direct effects of tobacco. Significant condition by time interactions were observed for ratings of "satisfying," "pleasant," "taste good," "dizzy," "calm," "concentrate," "awake," "reduce hunger," "taste like own brand," "feel like own brand," "harsh as own brand," "mild as own brand," and "smoke another cigarette right now." Ratings of "calm" and "satisfying" are shown in Fig. 3C and D. Ratings of "satisfying" and "pleasant" increased significantly at all time points under the Hydro EC, NPRO EC, and own-brand conditions (relative to baseline). Own brand increased ratings of "satisfying" and "pleasant" to a significantly greater degree than Hydro EC or NPRO EC. Ratings of "taste good," "calm," "concentrate," "awake," and "reduce hunger" increased significantly at all time points after own brand and at several time points after Hydro EC and NPRO EC (relative to baseline). Ratings of "taste like own brand," "feel like own brand," "harsh as own brand," and "dizzy" increased significantly at all time points under the own-brand condition (relative to baseline and sham). Ratings of "mild as own brand" increased significantly at all time points under the own-brand condition (relative to baseline and sham) and at nearly all time points under the Hydro EC condition (relative to baseline). Ratings of "smoke another cigarette right now" increased significantly relative to baseline at all time points under the Hydro EC and NPRO EC conditions, and 30 and 45 minutes postadministration 1 and 45 minutes postadministration 2 under the own-brand condition.

Discussion

The purpose of this acute study was to describe clinical laboratory methods that could be used to understand electronic cigarettes better by examining users' toxicant exposure, cardiovascular response, and subjective reports. The results of this study show the usefulness of the clinical model and suggest that, unlike puffing from a tobacco cigarette, two 10-puff bouts with the two electronic cigarettes described here expose users to no measurable nicotine or CO and do not increase heart rate. Despite the failure to deliver nicotine, acute use of

the two products tested in this study produced some tobacco abstinence symptom suppression and increased subjective ratings of acceptability. Relative to the effects produced by an own-brand tobacco cigarette, these subjective effects of electronic cigarettes were modest.

With regard to nicotine exposure, the observation that 20 puffs from Hydro EC (16-mg cartridge) and NPRO EC (18-mg cartridges) did not increase plasma nicotine concentration significantly is entirely consistent with our preliminary report regarding these products (21). The plasma nicotine results are also consistent with the heart rate data reported here. That is, heart rate increases are observed when nicotine is administered via pharmaceutical products (34, 35) or tobacco products (32, 33, 36), and we observed increased heart rate when participants in this study smoked their own brand of cigarette (that also delivered nicotine). However, no heart rate increases were observed in either electronic cigarette condition (where no significant increases in plasma nicotine were observed). Finally, the mean peak changes from baseline in plasma nicotine concentration observed for NPRO EC (1.4 ng/mL) and Hydro EC (0.5 ng/mL) during the first product administration were similar in magnitude to the maximum nicotine concentration reported for a third electronic cigarette brand with a 16-mg cartridge (1.3 ng/mL; ref. 20). The consistency of results across participants, measures, and laboratories highlights the reliability of the clinical methods reported here (see also ref. 24), while also calling into question the ability of the three different electronic cigarettes that have been tested to date to approximate the nicotine delivery of a tobacco cigarette under acute conditions.

In spite of delivering no measurable nicotine, both electronic cigarettes tested in this study reduced ratings of "craving a cigarette" and "urge to smoke" and increased subjective ratings of product acceptability (e.g., "satisfying," "taste good," "pleasant"). These results are consistent with anecdotal reports from long-term electronic cigarette users and support the notion that electronic cigarettes may provide an alternative, perhaps a substitute, to cigarette smoking in some cases (11, 12). Interestingly, denicotinized cigarettes have also been shown to suppress tobacco abstinence symptoms for as

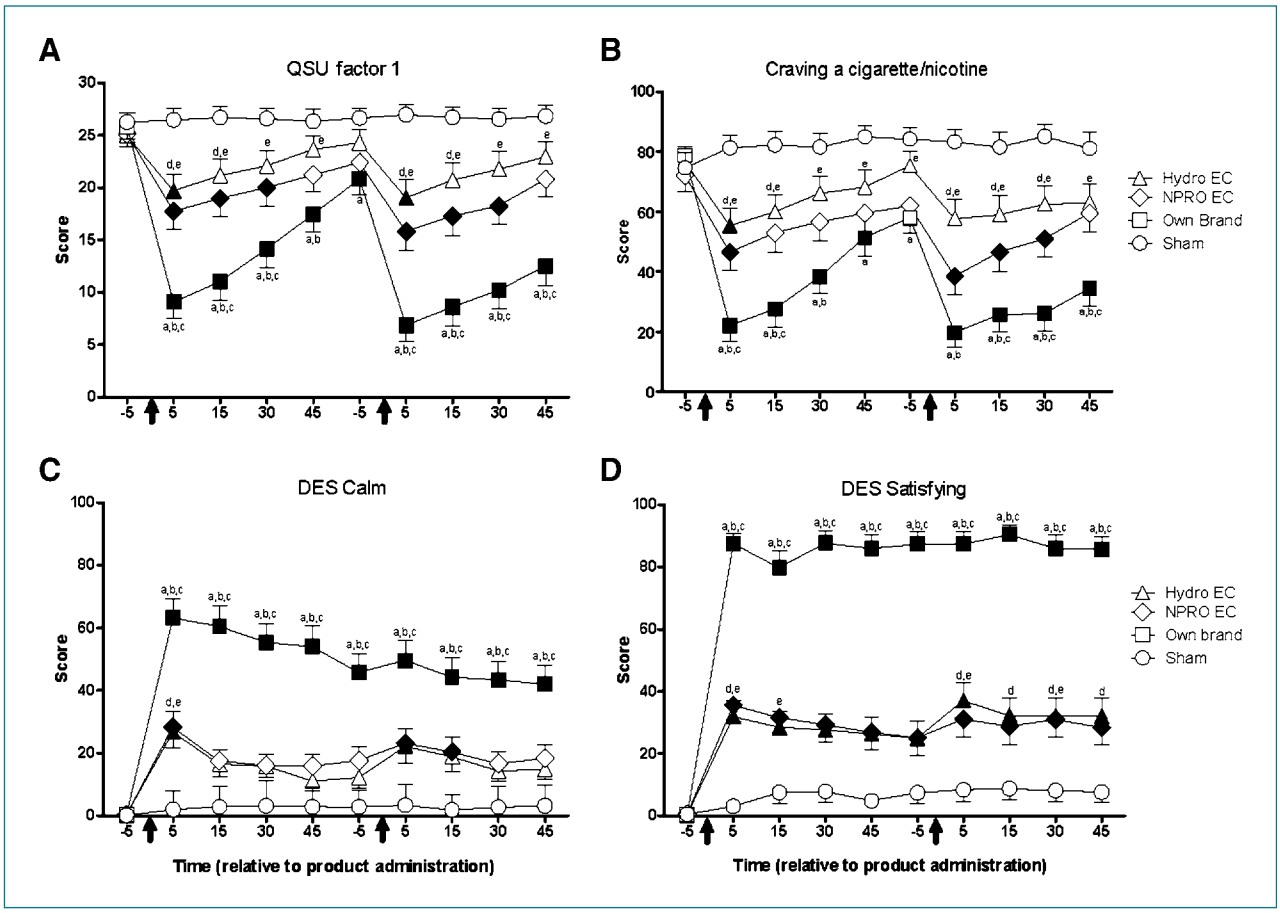

Figure 3. Mean data for QSU factor 1 ratings (A), ratings of "craving a cigarette" (B), ratings of "calm" (C), and ratings of "satisfying" (D) as a function of condition and time. *X*-axes, time in minutes relative to product administration; arrows, first and second product administrations. *Y*-axes, subjective ratings; filled symbols, significant difference from baseline. An "a," "b," or "c" indicates that own brand was significantly different from sham, Hydro EC, or NPRO EC at that time point. A "d" indicates that Hydro EC was significantly different from sham at that time point. An "e" indicates that NPRO EC was significantly different from sham at that time point (Tukey's HSD, $P < 0.05$). Unidirectional error bars, 1 SE. DES, direct effects of smoking.

long as 96 hours (37). However, under the acute conditions reported here, the two electronic cigarettes did not suppress nicotine/tobacco abstinence symptoms fully, relative to own-brand smoking. Other PREPs that failed to suppress abstinence symptoms fully have been shown to supplement rather than substitute for cigarette smoking (9, 24, 37, 38). Further controlled evaluation is needed to determine the extent to which the effects reported here are sufficient for electronic cigarettes to substitute for tobacco cigarettes, and in what proportion of smokers and under what conditions this substitution effect might occur.

Importantly, neither of the electronic cigarettes tested in this study was associated with any measurable CO exposure. Long-term CO exposure has been linked to cardiovascular disease caused by tobacco cigarette smoking (39). In part for this reason, substituting noncombustible tobacco or nicotine products for cigarettes has been suggested as a potentially effective strategy for reducing the harm of tobacco smoking (40). Following this same logic, and taking into account the trace levels of tobacco-specific nitrosamines found in some electronic cigarette products (13), electronic cigarettes may also warrant careful empirical examination by those interested in harm reduction for smokers. Clinical laboratory methods have an important role to play in this empirical examination, and can reveal carcinogen exposure and abstinence symptoms suppression over several days' PREP use (22, 24, 33, 41). These methods will likely be extremely important to any future regulation of electronic cigarettes either as tobacco products or drug delivery devices in the United States (i.e., by the Food and Drug Administration) and elsewhere.

Methodologic considerations of the current study include the brief electronic cigarette exposure period, rigorous control over some aspects of smoking behavior, use of two brands of electronic cigarette with similar cartridge nicotine content, and inclusion of electronic cigarette–naïve participants who may be representative of cigarette smokers sampling an electronic cigarette for the first time, but not of a more experienced electronic cigarette user population. The results of some outcome measures might be influenced by longer-term use, different puffing profiles, other electronic cigarette models/cartridge strengths, and/or the user's previous experience with electronic cigarettes. In future studies, control conditions other than own brand and sham smoking might be of interest. Indeed, a recently published study compared the subjective and physiologic effects of another brand of electronic cigarette, the Ruyan electronic cigarette, to that of a pharmaceutical nicotine inhalator and found that the two products were associated with similar low levels of nicotine exposure, incomplete withdrawal suppression, and moderate acceptability (20). In addition, behavioral studies that assess the reinforcing/rewarding properties of electronic cigarettes could reveal the extent to which electronic cigarettes might be used or abused (42). Finally, the adverse event profile associated with long-term use of electronic cigarettes is uncertain, and must be explored empirically. Many of these methodologic issues and research questions can be addressed parametrically and conveniently in the clinical laboratory, highlighting the strength of these efficient and reliable evaluation methods (see also ref. 24).

In sum, this study revealed that two electronic cigarette brands do not expose electronic cigarette–naïve users to nicotine or carbon monoxide under the acute testing procedures described here, but do produce some tobacco abstinence symptom suppression and positive ratings of product acceptability. Although these results are necessarily a function of the products tested and procedures used, they suggest that electronic cigarette–naïve individuals may require substantial motivation if they are to learn whatever product/procedure combination maximizes these outcomes for them. Future clinical laboratory evaluation can build on these methods and results to establish the extent to which electronic cigarettes might be expected to substitute for tobacco cigarettes, and help to identify under what conditions this substitution might occur. Parallel studies addressing the abuse liability and long-term adverse event profile of electronic cigarettes are also required to ensure safety and appropriate labeling and marketing of these products.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We acknowledge the expert technical and medical assistance provided by the staff of VCU's Behavioral Pharmacology Laboratory (Janet Austin, M.S. and Barbara Kilgalen, R.N.) as well as the staff at VCU's Bioanalytical Core Laboratory Service Center.

Grant Support

US NCI. This research was supported by USPHS grants R01CA103827, R01CA120142, and T32DA007027-34.

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Received 03/31/2010; revised 05/12/2010; accepted 05/17/2010; published OnlineFirst 07/20/2010.

References

1. Stratton K, Shetty P, Wallace R, Bondurant S. Clearing the smoke: the science base for tobacco harm reduction—executive summary. Tob Control 2001;10:189–95.
2. O'Connor RJ, Cummings KM, Rees VW, et al. Surveillance methods for identifying, characterizing, and monitoring tobacco products: potential reduced exposure products as an example. Cancer Epidemiol Biomarkers Prev 2009;18:3334–48.
3. Pederson LL, Nelson DE. Literature review and summary of perceptions, attitudes, beliefs, and marketing of potentially reduced exposure products: communication implications. Nicotine Tob Res 2007;9:525–34.

4. O'Connor RJ, Hyland A, Giovino GA, Fong GT, Cummings KM. Smoker awareness of and beliefs about supposedly less-harmful tobacco products. Am J Prev Med 2005;29:85–90.
5. Caraballo RS, Pederson LL, Gupta N. New tobacco products: do smokers like them? Tob Control 2006;15:39–44.
6. Rogers JD, Biener L, Clark PI. Test marketing of new smokeless tobacco products in four U S. cities. Nicotine Tob Res 2010;12:69–72.
7. Eissenberg T. The time for tobacco industry sponsored PREP evaluation has arrived. Tob Control 2006;15:1–2.
8. Hanson K, O'Connor R, Hatsukami D. Measures for assessing the subjective effects of potential reduced-exposure products. Cancer Epidemiol Biomarkers Prev 2009;18:3209–24.
9. Buchhalter AR, Schrine L, Eissenberg T. Withdrawal-suppressing effects of a novel smoking system: comparison with own brand, not own brand, and de-nicotinized cigarettes. Nicotine Tob Res 2001; 3:111–8.
10. Cobb CO, Weaver MF, Eissenberg T. _LINK1_LINK2Evaluating the acute effects of oral, non-combustible potential reduced exposure products marketed to smokers. Tob Control. Epub 2010 Apr 2. doi: 10.1136/tc.2008.028993.
11. Heavner K, Dunworth J, Bergen P, Nissen C, Phillips CV. Electronic cigarettes (e-cigarettes) as potential tobacco harm reduction products: results of an online survey of e-cigarette users. Tob Harm Reduction [Internet]. 2009 [cited 2010 Feb 9]; working paper 011: [about 15 pp]. Available from: http://www.tobaccoharmreduction.org/wpapers/011.htm.
12. Zezima K. Cigarettes without smoke, or regulation. The New York Times. 2009 [cited 2009 Dec 23]. Available from: http://nytimes.com/2009/06/02/us/02cigarette.html?_r=1.
13. U.S. Food and Drug Administration. Evaluation of e-cigarettes. FDA. 2009 May 4 [Cited 2009 Dec 10]. Available from: http://www.fda.gov/downloads/Drugs/ScienceResearch/UCM173250.pdf.
14. Wollsheid KA, Kremzner ME. Electronic cigarettes: safety concerns and regulatory issues. Am J Health Syst Pharm 2009;66:1740–2.
15. Laugesen M. Second safety report on the Ruyan e-cigarette. Health New Zealand. 2008 Apr [cited 2009 Dec 10]; version 8: [about 28 pp]. Available from: http://www.healthnz.co.nz/2ndSafetyReport_9Apr08.pdf.
16. Crown Seven Hydro [cited 2010 Jan 26]. Available from: http://www.crown7.com/.
17. E-puffer® International Inc.: Extending Smoker's Life!™ c 2007–2010[cited 2010 Jan 27]. Available from: http://www.epuffer.com/.
18. Njoy Electronic Cigarettes-Electronic Cigarettes and Accessories [cited 2010 Jan 26]. Available from: http://www.njoycigarettestore.com/faq.
19. Laugessen M. Ruyan e-cigarette bench-top tests. Poster presented to the joint conference of the Society for Research on Nicotine and Tobacco and Society for Research on Nicotine and Tobacco-Europe. 2009.
20. Bullen C, McRobie H, Thornley S, Glover M, Laugesen M. Effect of an electronic cigarette on desire to smoke and withdrawal, user preferences and nicotine delivery: randomized cross-over trial. Tob Control 2010;19:98–103.
21. Eissenberg T. Electronic nicotine delivery devices: ineffective nicotine delivery and craving suppression after acute administration. Tob Control 2010;19:87–8.
22. Gray JN, Breland AB, Weaver M, Eissenberg T. Potential reduced exposure products (PREPs) for smokeless tobacco users: clinical evaluation methodology. Nicotine Tob Res 2008;10:1441–8.
23. Breland AB, Buchhalter AR, Evans SE, Eissenberg T. Evaluating acute effects of potential reduced-exposure products for smokers: clinical laboratory methodology. Nicotine Tob Res 2002;4 Suppl 2: S131–40.
24. Breland AB, Kleykamp BA, Eissenberg T. Clinical laboratory evaluation of potential reduced exposure products for smokers. Nicotine Tob Res 2006;8:727–38.
25. NJOY [Internet]. Scottsdale, AZ: NJOY®; c 2010 [cited 2010 Jan 26]. Available from: http://www.njoythefreedom.com/index.php.
26. Crown Seven [Internet]. Scottsdale, AZ: Crown Seven; c 2010 [cited 2010 Jan 26]. Available from: http://crown7.com/index.shtml.
27. Federal Trade Commission. Tar, nicotine, and carbon monoxide of the smoke of 1294 varieties of domestic cigarettes for the year 1998. [Cited 2010, Jan 26]. Available from: http://www.ftc.gov/reports/tobacco/1998tar&nicotinereport.pdf%22.
28. Cox LS, Tiffany ST, Christen AG. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. Nicotine Tob Res 2001;3:7–16.
29. Eissenberg T, Griffiths RR, Stitzer ML. Mecamylamine does not precipitate withdrawal in cigarette smokers. Psychopharmacology 1996; 127:328–36.
30. Gourlay SG, Forbes A, Marriner T, Pethica D, McNeil JJ. Double blind trial of repeated treatment with transdermal nicotine for relapsed smokers. BMJ 1995;311:363–6.
31. Kleykamp BA, Jennings JM, Sams C, Weaver MF, Eissenberg T. The influence of transdermal nicotine on tobacco/nicotine abstinence and the effects of a concurrently administered cigarette in women and men. Exp Clin Psychopharmacol 2008;16:99–112.
32. Eissenberg T, Shihadeh A. Waterpipe tobacco and cigarette smoking: direct comparison of toxicant exposure. Am J Prev Med 2009; 37:518–23.
33. Blank MD, Sams C, Weaver MF, Eissenberg T. Nicotine delivery, cardiovascular profile, and subjective effects of an oral tobacco product for smokers. Nicotine Tob Res 2008;10:417–21.
34. Perkins KA, Epstein LH, Stiller RL, Marks BL, Jacob RG. Acute effects of nicotine on resting metabolic rate in cigarette smokers. Am J Clin Nutr 1989;50:545–50.
35. Evans SE, Blank MD, Sams C, Weaver MF, Eissenberg T. Transdermal nicotine-induced tobacco abstinence symptom suppression: nicotine dose and smokers' gender. Exp Clin Psychopharmacol 2006;14:121–35.
36. Fant RV, Henningfield JE, Nelson RA, Pickworth WB. Pharmacokinetics and pharmacodynamics of moist snuff in humans. Tob Control 1999;8:387–92.
37. Buchhalter AR, Acosta MC, Evans SE, Breland AB, Eissenberg T. Tobacco abstinence symptom suppression: the role played by the smoking-related stimuli that are delivered by denicotinized cigarettes. Addiction 2005;100:550–9.
38. Hughes JR, Keely JP. The effect of a novel smoking system—Accord—on ongoing smoking and toxin exposure. Nicotine Tob Res 2004; 6:1021–7.
39. Lakier JB. Smoking and cardiovascular disease. Am J Med 1992;93 Suppl 1:S8–12.
40. Hatsukami DK, Henningfield JE, Kotlyar M. Harm reduction approaches to reducing tobacco-related mortality. Annu Rev Public Health 2004;25:377–95.
41. Blank MD, Eissenberg T. Evaluating oral noncombustible potential reduced exposure products for smokers. Nicotine Tob Res 2010; 12:336–43.
42. Carter LP, Stitzer ML, Henningfield JE, O'Connor RJ, Cummings KM, Hatsukami DK. Abuse liability assessment of tobacco products including potential reduced exposure products. Cancer Epidemiol Biomarkers Prev 2009;18:3241–62.