Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes

X. Sherwin Yan*, Carl D’Ruiz

*Corresponding author at: 420 English Street, Greensboro, NC 27420, United States.
E-mail address: syan@lortobco.com (X.S. Yan).

Contents lists available at ScienceDirect
Regulatory Toxicology and Pharmacology
journal homepage: www.elsevier.com/locate/yrtph

ARTICLE INFO

Article history:
Received 8 September 2014
Available online 22 November 2014

Keywords:
Electronic cigarette
Nicotine
Cardiovascular function
Carbon monoxide
Smoking

ABSTRACT

The development of electronic cigarettes (e-cigs) has the potential to offer a less harmful alternative for tobacco users. This clinical study was designed to characterize e-cig users’ exposure to nicotine, and to investigate the acute effects of e-cigs on the hemodynamic measurements (blood pressure and heart rate) in comparison with the effects of regular smoking. Five e-cigs and one Marlboro® cigarette were randomized for twenty-three participants under two exposure scenarios from Day 1 to Day 11: half-hour controlled administration and one hour ad lib use. The nicotine plasma concentrations after 1.5 h of product use (C_{0.5}) were significantly lower in the users of e-cigs than of Marlboro® cigarettes. The combination of glycerin and propylene glycol as the vehicle facilitated delivery of more nicotine than glycerin alone. The heart rate, systolic and diastolic blood pressure were significantly elevated after use of Marlboro® cigarettes, but the elevation was less after use of most of the e-cigs. Use of e-cigs had no impact on the exhaled CO levels, whereas the Marlboro® cigarette significantly increased the exhaled CO more than 8 times above the baseline. In conclusion, e-cigs could be a less harmful alternative for tobacco users.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Cigarette smoking is a major health hazard, and contributes significantly to cardiovascular morbidity and mortality (Ambrose and Barua, 2004). According to the World Health Organization (WHO), smoking is the most preventable risk factor for cardiac and lung disease and is expected to cause 1 billion deaths during the 21st century. Studies have shown that acute smoking inhalation has significant adverse effects on left ventricular function in healthy smokers (Lichodziejewska et al., 2007). Smoking inhalation increases inflammation, thrombosis, and oxidation of low-density lipoprotein cholesterol that contributes to the cardiovascular dysfunction, such as defects in myocardial function (Farsalinos et al., 2013). Smoking also increases the risk of developing atherosclerosis, a disease which can cause heart attacks, strokes, and can even lead to death. The mechanisms by which cigarette smoking contributes to acute cardiovascular effects include (1) induction of a hypercoagulable state; (2) increased myocardial work; (3) CO mediated reduction in the oxygen-carrying capacity of the blood; (4) induction of endothelial dysfunction; (5) coronary vasoconstriction; and (6) catecholamine (Benowitz and Gourlay, 1997; Benowitz et al., 2002). There are many toxicants in cigarette smoke, such as CO, α, β-unsaturated aldehydes, superoxide, N₂O, and other oxidant gases, which could contribute to heart disease. Heart disease is the main cause of morbidity and mortality in smokers, with 40% of deaths in smokers due to coronary artery disease alone (Deanfield et al., 1986).

Thus, Tobacco Harm Reduction strategies and products have been developed to reduce the amount of toxic substances that a smoker is exposed to while smoking. As an alternative for smokers, e-cigs are rapidly growing worldwide and are gaining significant attention as potentially reduced exposure products and smoking cessation products (Etter and Bullen, 2013; Polosa et al., 2011; Farsalinos and Polosa, 2014). Though only developed and marketed in recent years, e-cigs are already used by several millions of people worldwide. The device consists of a battery, a cartridge containing liquid and a heating element which is heated by the battery and evaporates the liquid. The liquid usually contains water, nicotine, glycerin, propylene glycol, and a variety of flavors. E-cigs simulate the effect of smoking by producing an inhaled aerosol and satisfy the behavioral aspects associated with smoking. Because e-cigs does not involve the combustion of the chemical components commonly found in tobacco cigarettes, it is expected that user exposure to the toxicants may be less so use of e-cigs could avoid many of the detrimental health effects attributed to cigarette smoking. Laboratory analyses of the e-liquids show that there are less harmful and potentially harmful constituents (HPHCs) than regular cigarettes (Burstyn, 2013). Most studies have found no
nitrosamines in the vapor, but even in studies where nitrosamines were found, the levels detected were 500–1400 times less than the amount present in one tobacco cigarette (Cobb et al., 2010; Burstyn, 2013; Goniewicz et al., 2013; Kim and Shin, 2013).

As an emerging product developed recently, public health concerns have been raised globally about using of this smoking alternative. To date, there is little objective data that provide sound information on e-cigs toxicant content, the toxicant exposure level and the potential health effects to end users. Studies provided mixed data on plasma nicotine levels after use of e-cigs. One study found that in contrast to use of usual-brand cigarettes, use of e-cigs containing 16–18 mg/mL of nicotine did not increase nicotine plasma concentrations significantly from baseline (Vansickel et al., 2010). Other studies reported significant increases from baseline in nicotine plasma concentration after use of the usual-brand electronic devices (containing 9–24 mg/mL of nicotine), similar to conventional cigarette smoking (Vansickel and Eisenberg, 2013; Dawkins et al., 2013). These mixed results may reflect the differences in study design, or device characteristics, or suggest an acclimation to the product for new users.

Extremely limited studies have evaluated the effects of e-cigs on the hematological and cardiovascular system in relation to nicotine delivery. A study evaluated the acute effects of e-cigs and cigarette smoking on complete blood count markers in 30 human subjects and found cigarette smoking increased white blood cell, lymphocyte, and granulocyte counts for at least 1 h in smokers and never smokers, but the e-cigs smoking did not influence the complete blood count (Flouris et al., 2012). A clinical study examined the acute effects of e-cigs and regular cigarettes on nicotine delivery profile and cardiovascular function. It was observed that regular cigarettes significantly increased the plasma nicotine and CO concentration and heart rate within the first 5 min of administration, whereas e-cigs did not (Vansickel et al., 2014). Farsalinos et al. (2014) found that smoking one tobacco cigarette led to significant acute myocardial dysfunction but e-cigs had no acute adverse effects on cardiac function. The researchers reported that smoking a tobacco cigarette had important hemodynamic consequences, with significant increases in heart rate, systolic and diastolic blood pressure. In contrast, e-cigs produced only a slight elevation in diastolic blood pressure. The nicotine level in the e-cigs reported in the Farsalinos study was 1.1% in the liquid. The authors concluded nicotine in e-cigs was absorbed at a lower rate compared to regular cigarette smoking and e-cigs did not show to have adverse effects on the heart (Farsalinos et al., 2014).

The nicotine concentrations tested in above studies, were relatively considered to be “low-medium”, with short duration of exposure. Given the fact that there is wide range of nicotine concentrations in the e-liquid of currently marketed e-cigs, and the amount of regular and electronic cigarettes consumed can be very different from smoker to smoker and from day to day, this clinical study was designed to characterize blu e-cigs users’ nicotine exposure, and to investigate the acute effects of blu e-cigs with higher nicotine level (up to 2.4% in the e-liquid) and longer duration (up to 1.5 h) on the hemodynamic effects (blood pressure and heart rate) in relation to internal nicotine dose, compared to the adverse effects of regular smoking.

2. Methods

2.1. Participants

The study was approved by the Institutional Review Board (IRB) of Chesapeake Research Review Inc. (CRR, Columbia, MD). Thirty-eight subjects underwent the screening procedures to ensure that they met the requirements for inclusion within 28 days prior to participation in the study. The IRB-approved informed consent form (ICF) was collected from all participants prior to completion of the screening or other study procedures. Fourteen subjects withdrew from the study. The remaining 23 participants (11 male and 12 female) properly completed the study and were included in the analyses. All participants were between 21 and 65 years of age, smoked an average of 10 or more manufactured cigarettes per day for at least 12 months prior to the study. All provided positive urine cotinine at screening (>500 ng/mL). Exclusion criteria included self-reported history of any chronic mental or physical health condition, pregnancy or breastfeeding, systolic blood pressure >150 mmHg, diastolic blood pressure >95 mmHg, or heart rate >99 bpm at screening, use of tobacco or nicotine-containing products other than manufactured cigarettes and e-cigs, and use of any prescription smoking cessation treatments within 3 months prior to Day 1 product administration and throughout the study, use of prescription anti-diabetic medication and/or insulin therapy within 12 months of Day 1 product administration, and use of medications known to interact with cytochrome P450 2A6 within 3 months prior to Day 1 product administration.

2.2. Test articles

The blu e-cigs are currently sold in retail outlets across the United States (US) in both disposable and re-useable forms. The blu e-cigs prepared for use in the current study were 2 commercial products (Product D and E) that contain 16 mg/mL (1.6%) nicotine (USP grade), and 3 non-commercial products (Product A, B and C) that contain 24 mg/mL (2.4%) nicotine (USP grade), in the cartomizer device format attached to rechargeable batteries. In comparison, the nicotine yield of the market-leading conventional cigarette (Marlboro® Gold King Size) is approximately 0.8 mg per cigarette (FTC 2007). As the blu e-cigs may yield from 250 to 400 puffs per cartridge, a single cartridge may equate to approximately 1–2 packs of conventional cigarettes.

The following investigational and comparator product designations were used in this study.

| Product A: | Classic Tobacco e-cigarette in rechargeable cartomizer (2.4% nicotine, ~75% glycerin vehicle), or Product A Classic e-cig (2.4% Nic in Gly) |
| Product B: | Classic Tobacco e-cigarette in rechargeable cartomizer (2.4% nicotine, ~50% glycerin/ ~20% propylene glycol vehicle), or Product B Classic e-cig (2.4% Nic in Gly/PG) |
| Product C: | Magnificent Menthol e-cigarette in rechargeable cartomizer (2.4% nicotine, ~75% glycerin vehicle), or Product C Menthol e-cig (2.4% Nic in Gly) |
| Product D: | Classic Tobacco e-cigarette in rechargeable cartomizer (1.6% nicotine, ~75% glycerin vehicle), or Product D Classic e-cig (1.6% Nic in Gly) |
| Product E: | Classic Tobacco e-cigarette in rechargeable cartomizer (1.6% nicotine, ~50% glycerin/ ~20% propylene glycol vehicle), or Product E Classic e-cig (1.6% Nic in Gly/PG) |
| Product F: | Marlboro® Gold King Size, or Product F Marlboro® cigarette |

In addition to nicotine, the blu e-cigs prepared for use in this study contain vegetable glycerin, natural and artificial flavors, distilled water, citric acid, and propylene glycol.
2.3. Study design and procedures

This study served as the initial characterization of the e-cig products, gaining an understanding of the exposure to nicotine, and potential cardiovascular effects following use of the e-cigarettes by adult smokers. It was a randomized, partially single-blinded, six-period crossover study. 38 potential subjects participated in a lead-in period from approximately Day -9 to Day -2 during which they were asked to become accustomed to using the e-cig products. All subjects participating in the lead-in were checked in on Day -2 and remained in the clinic to better ensure that up to 23 subjects are randomized to receive study products on Day 1. Subjects abstained from use of nicotine-containing products for a period of at least 36 h prior to each product administration (Days 1, 3, 5, 7, 9, and 11). Days 1, 2, 4, 6, 8, and 10 were designated wash-out days in order to obtain the required 36-h nicotine-free period between product administrations.

Each product administration day included a controlled product administration and a 1-hour ad lib use of the study product. The controlled product administration consisted of 50 puffs of the assigned e-cig product (5-s puffs at 30-s intervals) or smoking one Marlboro Gold King Size cigarette (30-s intervals with the subjects’ normal puff duration) with puff counts monitored by the clinical staff. During ad lib use, subjects assigned to an e-cig product self-administered the product as desired for the entire hour and maintained their own puff counts. Subjects assigned to the cigarette product requested the product from the staff as desired during ad lib use and maintained their own puff counts. The ad lib product use started 30 min following the start of the controlled product use. Blood samples for plasma nicotine, blood pressure, pulse rate, and exhaled CO measurements were obtained at scheduled time points on each product administration day. Comparisons were made to evaluate differences between the e-cig formulations as well as to the market-leading conventional cigarette, Marlboro Gold King Size.

2.4. Plasma nicotine

Serial blood samples were collected at approximately 10 min prior to and at 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90 min following the start of the controlled product administration.

Blood samples for plasma nicotine analysis were taken by direct venipuncture into a 4 ml plastic K2 EDTA (lavender top) vacutainer tube. Approximately 264 mL of blood were required for pharmacokinetic assessments. The samples were kept at room temperature prior to centrifuge, and centrifuged at 2500 RPM for 15 min at 4 °C within 60 min of collection. After centrifugation, the plasma was transferred to two methanol-prewashed polypropylene screw cap tubes, properly labeled, and then stored at −20 °C (±10 °C) or below (within 90 min from collection) until analysis. Plasma nicotine was analyzed by LC-MS/MS using validated analytical methods with appropriate quality controls in accordance with FDA Good Laboratory Practice (GLP) regulations (Title 21 CFR Part 58).

2.5. Cardiovascular responses

Cardiovascular vital signs, e.g., systolic blood pressure, diastolic blood pressure, and heart rate, were measured at approximately 30 min prior to the start of the controlled product use and approximately 20 min following the end of the ad lib product use on Days 1, 3, 5, 7, 9, and 11. All were measured in the sitting position after at least 5 min of rest.

2.6. Exhaled carbon monoxide (CO)

Exhaled CO was assessed at approximately 20 min prior to the start of the controlled product use and at approximately 15 min following the end of the ad lib product use. Exhaled CO levels were measured using a Bedfont Micro+ Smokerlyzer. Pre-product administration values greater than 12 ppm may indicate that illicit smoking has occurred and may result in subject removal from the study at the discretion of the Investigator.

2.7. Data analyses

The statistical analyses were performed using the appropriate SAS procedures. Differences will be considered statistically significant at an alpha level of 5%.

Nicotine concentrations were listed by subject and summarized by study product using descriptive statistics. Repeated measures ANOVA with simple contrasts were used to compare the maximum observed concentrations of plasma nicotine within the first 30 min and at 90 min (Cmax0–30 and C90) to the pre-product administration concentration for each product and the changes from baseline between products.

Cardiovascular vital sign data (e.g., blood pressure and heart rate) were summarized by study product and time point of collection. Descriptive statistics, including a mean change-from-baseline table was provided for all data. Comparisons between pre- and post-product administration values were assessed using t-tests for each product while pre-to-post administration differences between products were be compared using an ANOVA model.

Exhaled CO comparisons between pre- and post-product administration values were assessed using t-tests for each product while pre-to-post administration differences between products were be compared using an ANOVA model.

3. Results

3.1. Study population

The demographic characteristics of the study population are presented in Table 1. Twenty-three subjects (11 male and 12 female) properly completed the study and their data were included in the analyses. The average age of this study population was 39 years with a standard deviation of about 11. The youngest was 23 and oldest was 58 years old. Most of subjects were white (83%), with 13% African American, and 4% of American Indian. Their body weight averaged at 78.8 kg with a standard deviation of about 15 kg. The lightest body weight was 61 kg, whereas the heaviest was 116 kg. None of the subjects reported any history of chronic mental or physical health condition. None of them had systolic blood pressure > 150 mmHg, diastolic blood pressure > 95 mmHg, or pulse rate > 99 bpm at Screening. None of the female subjects were pregnant or breastfeeding. There were no individual clinically significant findings at screening in clinical laboratory serum chemistry, hematology, urinalysis, and virology measurements. All the 23 subjects met the study inclusion and exclusion criteria thus qualified to enter the study.

3.2. Product use

The product use is summarized in Table 2. In the controlled phase, the mean cartomizer weight differences were comparable across blu e-cigs (0.2338 – 0.2570 g). The nicotine amounts in the 50 puffs were in a range from 0.53 to 1.30 mg based on internal analytical tests which measured the amount of nicotine delivered by the blu™ products under Canadian-Intense regime machine smoking parameters. In the ad lib phase, the mean total puffs varied between 49.5 and 60.3, with the highest number of puffs (60.3) observed during administration of Product D 1.8% nicotine glycerin vehicle Classic Tobacco product and the lowest number of puffs...
more, during ad lib product use. This was likely due to shorter puff durations during ad lib product use.

For Marlboro® Gold cigarettes, subjects smoked only a single cigarette during the controlled product administration, with an average of 10.5 puffs per cigarette taken. During the ad lib phase subjects smoked an average of 3.6 cigarettes, with approximately the same number of puffs taken per cigarette. The nicotine amount was estimated up to 2.88 mg in the 38.6 puffs of cigarette according to nicotine yield of Marlboro® Gold King Size: approximately 0.8 mg per cigarette (FTC 2007).

### 3.3. Plasma nicotine levels

Nicotine plasma levels were measured at approximately 10 min prior to and at 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90 min following the start of the controlled product administration. The mean values and standard deviations of nicotine concentrations at each time point for each product are shown in Table 3, and Fig. 1. The plasma nicotine $C_{\text{max,0–30}}$ and $C_{90}$ values of each product are presented in Table 4.

In the controlled phase of product use, all e-cig products (A–E) showed steady increase of plasma level of nicotine. Except for Product D Classic e-cig (1.6% nicotine in Gly) that only displayed an insignificant elevation of plasma nicotine level at 5 min after start of the controlled use (1.98 ng/mL, $p = 0.088$), all rest e-cig products increased the nicotine level significantly higher than their respective baseline values ($p < 0.01$). This pattern of increase of nicotine plasma level continues into the ad lib phase for each e-cig, but with much lower slope.

Product F Marlboro® cigarette also significantly increased the plasma nicotine levels at all time points in both controlled and ad lib phases, when compared to the baseline level ($p < 0.0001$). In the controlled phase, the nicotine reached a peak level at 5 min after use of Marlboro® cigarette which is approximately 20 min quicker than any other blu e-cigs in this test. In the ad lib phase, Marlboro® cigarette exhibited a steeper slope of elevation of plasma nicotine. At 60 min following the start of the controlled product administration till the end of ad lib, the plasma nicotine levels after use of Marlboro® cigarette were higher than all the levels after use of any of the blu e-cigs (Fig. 1).

### Table 1
Demographic summary of study population.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Characteristics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>12 (52%)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>11 (48%)</td>
</tr>
<tr>
<td>Race</td>
<td>American Undian/Alaska Native</td>
<td>1 (4%)</td>
</tr>
<tr>
<td></td>
<td>Black or African American</td>
<td>3 (13%)</td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>20 (83%)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>N</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>38.7</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>10.77</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>36.5</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>58</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>N</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>170.54</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>7.33</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>170.5</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>185</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>N</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>78.8</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>14.91</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>74.1</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>116</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>N</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>27.06</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>4.85</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>25.51</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>21.11</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>38.72</td>
</tr>
</tbody>
</table>

(49.5) observed during administration of Product E 1.6% nicotine, glycerin/PG vehicle Classic Tobacco product. Cartomizer weight differences followed the same pattern. The nicotine amounts in these puffs were in a range from 0.64 to 1.44 mg based on internal analytical tests. The mean differences in cartomizer weights were observed to be smaller for each e-cigarette (~22% to 54%) following ad lib product use compared to the controlled product administration despite the subjects having taken similar numbers of puffs, or

### Table 2
Product use.

<table>
<thead>
<tr>
<th>Study product</th>
<th>Controlled product administration</th>
<th>Ad lib product use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cart weight difference (g)</td>
<td>Puffs</td>
</tr>
<tr>
<td>A</td>
<td>0.2251 ± 0.00408 (50–51)</td>
<td>50 (50–100)</td>
</tr>
<tr>
<td>B</td>
<td>0.2349 ± 0.0633 (50–51)</td>
<td>50 (50–100)</td>
</tr>
<tr>
<td>C</td>
<td>0.2421 ± 0.0477 (50–51)</td>
<td>50 (50–100)</td>
</tr>
<tr>
<td>D</td>
<td>0.2238 ± 0.0399 (50–51)</td>
<td>50 (50–100)</td>
</tr>
<tr>
<td>E</td>
<td>0.2570 ± 0.043 (50–51)</td>
<td>50 (50–100)</td>
</tr>
<tr>
<td>F</td>
<td>Cigarettes Smoked</td>
<td></td>
</tr>
<tr>
<td>(N = 24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (1, 1)</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>(9–13)</td>
<td></td>
</tr>
</tbody>
</table>

A: blu® Classic Tobacco e-cigarette (2.4% nicotine, glycerin vehicle).

B: blu® Classic Tobacco e-cigarette (2.4% nicotine, glycerin/propylene glycol vehicle).

C: blu® Magnificent Menthol e-cigarette (2.4% nicotine, glycerin vehicle).

D: blu® Classic Tobacco e-cigarette (1.6% nicotine, glycerin vehicle).

E: blu® Classic Tobacco e-cigarette (1.6% nicotine, glycerin / propylene glycol vehicle).

F: Marlboro® Gold King Size.

Weight values presented as mean ± SD; Nicotine in puffs presented as mean. Cigarettes smoked and puffs presented as mean (range).

* Based on internal testing of nicotine amount in the puffs.

* Based on FTC 2007 data: approximately 0.8 mg per cigarette yield of Marlboro® Gold King Size.
The maximum observed concentrations of plasma nicotine ($C_{\text{max-30}}$) from time zero to 30 min are shown in Table 4. Product B Classic e-cig (2.4% Nic in Gly/PG) had the highest $C_{\text{max-30}}$ (19.80 ng/mL), and Product D Classic e-cig (1.6% Nic in Gly) had the lowest $C_{\text{max-30}}$ (10.34 ng/mL). The $C_{\text{max-30}}$ rank from highest to lowest are:

- B: 19.80 ng/mL
- D: 10.34 ng/mL
- C: 15.14 ng/mL
- E: 13.09 ng/mL
- F: 8.64 ng/mL

The maximum observed concentrations of plasma nicotine at 90 min ($C_{90}$) had a rank order from highest to lowest among the 6 products:

- F: 29.23 ng/mL
- B: 22.42 ng/mL
- A: 19.67 ng/mL
- D: 13.70 ng/mL

The $C_{90}$ value resulting from use of Marlboro® cigarettes was significantly higher than the $C_{90}$ values from any of the e-cig products ($p < 0.0001$). Among the e-cigs, Product B Classic e-cig (2.4% Nic in Gly/PG) induced significantly higher $C_{90}$ than Product C Menthol e-cig (2.4% Nic in Gly), Product E Classic e-cig (1.6% Nic in Gly/PG), and Product D Classic e-cig (1.6% Nic in Gly) ($p < 0.03$).

### 3.4. Blood pressure

Systolic blood pressure (BP) and diastolic blood pressure were measured at approximately 30 min prior to the start of the controlled product use and approximately 20 min following the end of the ad lib product use on Days 1, 3, 5, 7, 9, and 11 corresponding to use of different product each day. The baseline BP, after use BP, and the mean changes from baseline are shown in Table 5, and Fig. 2. Overall all products increased the systolic and diastolic BPs. The increase in systolic BP was statistically significant compared to baseline for Product D Classic e-cig (1.6% Nic in Gly) ($p = 0.02$), and Product F Marlboro® cigarette ($p = 0.04$), but not for Product A Classic e-cig (2.4% Nic in Gly), Product B Classic e-cig (2.4% Nic in Gly/PG), Product C Menthol e-cig (2.4% Nic in Gly), and Product E Classic e-cig (1.6% Nic in Gly/PG). All products significantly increased the diastolic BPs when compared to their respective baseline values ($p < 0.05$).

Though Product D Classic e-cig (1.6% Nic in Gly) and Product F Marlboro® cigarette increased the systolic BP in a higher degree than other products, the increase in systolic BP from baseline was not statistically significant among the products. However regarding the diastolic BP changes from baseline, Product C Menthol e-cig (2.4% Nic in Gly) had significantly less increase than Marlboro® cigarette ($p = 0.048$), no other changes from baseline in diastolic BP were statistically significant among other pairs of products.

### 3.5. Heart rate

Heart rate was measured at approximately 30 min prior to the start of the controlled product use and approximately 20 min...
following the end of the ad lib product use on Days 1, 3, 5, 7, 9, and 11. As expected due to presence of nicotine, all products increased the heart rates after the controlled and ad lib product uses (Table 5, Fig. 3). Marlboro® cigarette induced the highest pulse increase, approximately 4.26 beats per minute (bpm) increase compared to baseline ($p = 0.001$). Product C Menthol e-cig (2.4% Nic in Gly)
showed the second highest increase, approximately 4.09 bpm above its baseline ($p = 0.002$), and Product B Classic e-cig (2.4% nicotine, glycerin vehicle) had the third highest increase, approximately 3.61 bpm above its baseline ($p = 0.008$). Though there were no statistically significant differences in the heart rate increase among the products, the data trend implied a good correlation between the nicotine plasma level and the increased heart rate ($p < 0.05$, Fig. 4).

3.6. Exhaled carbon monoxide (CO)

Exhaled CO was assessed at approximately 20 min prior to the start of the controlled product use and at approximately 15 min following the end of the ad lib product use. There were no significant changes in exhaled CO after ad lib use from baseline (2.86–3.52 ppm). However Marlboro® Gold King Size cigarette (30-s intervals with the subjects’ normal puff duration). As smoking behaviors vary from smoker to smoker, a controlled administration allowed for some standardization of nicotine ‘dose’ for each of the study products to better understand their uptake characteristics as well as other parameters studied with that controlled “dose”. In this study, knowing the concentration of nicotine in blu e-cig puffs, 50 puffs were required to deliver

4. Discussion

The key objectives of this study was to characterize e-cig users’ plasma nicotine levels, and to investigate and compare the acute effects of blu e-cigs with high nicotine level (up to 2.4% in e-liquid) and long duration (up to 1.5 h) on hemodynamic effects, with those of regular cigarette smoking.

Two types of exposures were utilized: a single controlled administration and a short-term ad lib use. The controlled administration consisted of 50 puffs of the assigned e-cig product (5-s puffs at 30-s intervals) or smoking one Marlboro® Gold King Size cigarette (30-s intervals with the subjects’ normal puff duration). As smoking behaviors vary from smoker to smoker, a controlled administration allowed for some standardization of nicotine “dose” for each of the study products to better understand their uptake characteristics as well as other parameters studied with that controlled “dose”. In this study, knowing the concentration of nicotine in blu e-cig puffs, 50 puffs were required to deliver
similar dose of nicotine (~0.8 mg) as smoking one Marlboro® Gold King Size cigarette (Table 2). This controlled “dose” did not necessarily indicate the “dose” that the usual user of e-cigs would experience. During ad lib use, subjects assigned to an e-cig product self-administered the product as desired for the entire hour. Evaluation under ad lib product use conditions provided insight into product self-administration behaviors that allowed subjects to achieve acceptable levels of urge reduction.

The present study observed that all products, including blu e-cigs and Marlboro® cigarette, significantly increased the plasma nicotine levels during the controlled and ad lib product use, however use of Marlboro® cigarette resulted in quicker peak level
of nicotine (at 5 min) and higher levels than any of the blu e-cigs at 60 min following the start of the controlled product administration and thereafter. At the end of \textit{ad lib}, use of Marlboro\textsuperscript{®} cigarette showed significantly higher nicotine in plasma (C\textsubscript{90}) than all the blu e-cigs. In general, Product B Classic e-cig (2.4\% Nic in Gly/PG), Product A Classic e-cig (2.4\% Nic in Gly), and Product C Menthol e-cig (2.4\% Nic in Gly) displayed higher plasma nicotine levels, e.g., C\textsubscript{max0–30}, and C\textsubscript{90}, than Product E Classic e-cig (1.6\% Nic in Gly/PG), and Product D Classic e-cig (1.6\% Nic in Gly). This observation can be reasonably explained by the higher concentrations of Nicotine in the e-liquids of Products B, A, and C (2.4\%) compared to the concentrations in products E and D (1.6\%). Among the blu e-cigs with the same level of nicotine in the e-formula, use of Product B Classic e-cig (2.4\% Nic in Gly/PG) resulted in higher C\textsubscript{max0–30}, and C\textsubscript{90} than Product A Classic e-cig (2.4\% Nic in Gly), Product C Menthol e-cig (2.4\% Nic in Gly). Between the 2 blu e-cigs with 1.6\% nicotine in the vehicle, use of Product E Classic e-cig (1.6\% Nic in Gly/PG) resulted in higher C\textsubscript{max0–30}, and C\textsubscript{90} than Product C Menthol e-cig (2.4\% Nic in Gly). The major difference between the same nicotine level e-cigs is the vehicle. The findings from this study indicate that the combination of glycerin and propylene glycol as the vehicle helps to deliver more nicotine to human body than glycerin alone. The nicotine delivery difference between the 2 types of vehicles is probably due to their characteristic physical properties. Propylene glycol has a vapor pressure 0.129 mmHg and boiling point 187.6 °C, whereas glycerin has a vapor pressure 1.68E-04 mmHg and boiling point 290 °C. This suggests that propylene glycol’s vaporization rate, or its tendency to form vapor during heating is higher than glycerin when heated at the same temperature. Thus more nicotine can be carried over to the mainstream smoke by propylene glycol vapor and then inhaled by the smoker compared to the glycerin alone. Our internal analytical studies collected 150 puffs and measured the amounts of nicotine in the 150 puffs and the cartridge weight changes from a single cartomizer for each of the 5 blu e-cigs under Canadian-Intense regime machine smoking parameters. Data confirmed that the amount of nicotine in 150 puffs and its ratio out of cartridge weight change was higher from the e-cig with propylene glycol/glycerin combined vehicle than from the e-cig with glycerin vehicle alone.

Another observation from this study was that for Classic Tobacco e-cigs containing 2.4\% nicotine, use of the Product C Magnificent Menthol product with only glycerin in the vehicle resulted in a small, but significantly lower exposure during both controlled product administration (∼15\% lower C\textsubscript{max0–30}) and \textit{ad lib} use (−14\% lower C\textsubscript{90}) compared to product B Classic Tobacco product with both glycerin and PG in the vehicle. When compared to product A Classic Tobacco product with glycerin only in the vehicle, use of the Product C Magnificent Menthol product with only glycerin in the vehicle resulted in a lower exposure during the controlled product administration (∼11\% lower) but comparable exposure at \textit{ad lib} use. While smokers typically have a strong taste preference when it comes to menthol flavoring, personal taste preference did not appear to play a primary role in the nicotine exposure differences. During both the controlled product administration and \textit{ad lib} use, the total number of puffs, as well as changes in cartomizer weights (Table 2), were comparable between Classic Tobacco and Magnificent Menthol e-cigarettes. It is possible that the combination of formulation (inclusion of menthol and exclusion of PG) may have impacted nicotine absorption in some unknown way. At least the data from this study did not indicate that menthol flavor in the e-formula promotes more nicotine delivered to human body.

Most blu e-cigs increased the systolic blood pressure (SBP), but the magnitude of increase was less than Marlboro\textsuperscript{®} cigarette, except Product D Classic e-cig (1.6\% Nic in Gly). Both Marlboro\textsuperscript{®} cigarette and Product D Classic e-cig (1.6\% Nic in Gly) resulted in significant increase in SBP compared to baseline (p < 0.05). However, there were no statistically significant differences in the degrees of increase among all the products. All test products increased the diastolic blood pressure (DSP) significantly above baseline, only Product C Menthol e-cig (2.4\% Nic in Gly) exhibited a significant less increase in DSP than Marlboro\textsuperscript{®} cigarette. All test products increased the heart rates, with Marlboro\textsuperscript{®} cigarette at the highest (4.26 bpm, p = 0.001), Product C Menthol e-cig (2.4\% Nic in Gly) at second (4.09 bpm, p = 0.002), and Product B Menthol e-cig (2.4\% Nic in Gly) at third highest (3.6 bpm, p = 0.008). The data trend revealed a correlation between the nicotine plasma level and the increased heart rate (Fig. 4, p < 0.05), though the increases in heart rate is not statistically significant among all products.

The mechanism of heart rate and blood pressure elevation by nicotine is believed to be by activation of the sympathetic nervous system with release of norepinephrine and epinephrine (Cryer et al., 1976). Cigarette smoking results in sympathetic neural arousal that can last for up to 24 h of the day (Benowitz et al., 1984). The renin-angiotensin system is involved in blood pressure regulation. Nicotine has been reported to inhibit aldosterone synthesis in the adrenal gland with decreased excretion of aldosterone (Zevin et al., 1998). More published studies have reported that cigarette smoking increased the plasma nicotine levels, the blood pressure and the heart rate (Benowitz et al., 2002; Mikkelsen et al., 1997; Minami et al., 1999). Our study further confirmed that use of Marlboro\textsuperscript{®} cigarettes, under the conditions of this study design, increase the systolic, diastolic blood pressure, and the heart rate. Further, the data trend from this study showed that blu e-cigs had less magnitude of increase in heart rate and systolic blood pressure compared to Marlboro\textsuperscript{®} cigarette. Since the nicotine plasma concentration at 1 h after cigarette smoking was higher and reached statistical significance at 1.5 h (C\textsubscript{90}) compared to all blu e-cigs, the dose–response relations between plasma nicotine levels and the blood pressure and the heart rate were assessed. Previous studies have shown that the dose–response relationship between nicotine plasma level and the cardiovascular parameters is flat (Benowitz et al., 1984; Zevin et al., 1998). The data from this study did suggest a trend of correlation between nicotine level and heart rate only (Fig. 4), but such trend of correlation did not appear between nicotine level and the blood pressures, also did not appear between regular and electronic cigarette, and among all electronic cigarettes tested. The trend of correlation between nicotine level and heart rate could be due to the immediate direct sympathetic arousal effects on the heart, but the flat dose–response between plasma nicotine and blood pressure could reflect the probable development of tolerance after 1.5 h of controlled and \textit{ad lib} use of the products involving the renin–angiotensin–aldosterone system adjustment. However, surprisingly no significant bigger magnitude of increase in HR was observed following the use of Marlboro\textsuperscript{®} cigarettes compared to the use of blu e-cigs, though the plasma level of nicotine (C\textsubscript{90}) of cigarette smokers was significantly higher at the end of \textit{ad lib} phase. It could reflect one of the limitations of this study, i.e., there were no significant observed differences of cardiovascular vital signs noted between test products in this small pilot study, and the minimal changes from baseline reported were considered to be not clinically significant.

There were basically no changes in exhaled CO after use of any of the 5 blu e-cigs. Only Marlboro\textsuperscript{®} cigarette significantly increased the exhaled CO more than 8 times above baseline prior to smoking. CO takes approximately 4.5\% in the gas component of cigarette smoking, and the CO concentration of inhaled cigarette smoke may reach as high as 500 ppm (WHO, 1999). CO is considered a toxic chemical at high concentrations, leading to a severe hypoxic condition by displacing oxygen from hemoglobin (Hb), leftward shift of the oxyhemoglobin dissociation curve, and binding to intracellular enzymes. Epidemiological studies have shown that
ambient CO levels correlate with onset of heart diseases, increased mortality rates, and hospital admission for cardiovascular diseases (Stenn et al., 1988; Kleinman et al., 1989; Burnett et al., 1997). Animal studies have shown that inhalation of CO at doses corresponding to tobacco smoking increases cardiac weight that could result from blood volume overload (Serhaug et al., 2006), worsens cardiac failure both in rats with experimental myocardial infarction and pre-existing hypertrophic cardiomyopathies (Melin et al., 2005; Mirza et al., 2005). Clinical studies have shown smoking increases carboxyhemoglobin (COHb) levels from 1% to 2% in nonsmokers up to 15% in heavy smokers (Omaye, 2002). All above weight of evidence indicates the exposure to CO is an important risk factor for cardiovascular dysfunction. Our study confirms that the vaporization process on heating due to use of e-cigs does not produce CO, whereas, use of regular cigarettes exhibited an 8-fold increase in CO exposure that could contribute to the detrimental health effects to cardiovascular function.

The obvious limitations of this study are the small sample size and acute effects studied. The results showed blu e-cigs delivered less exposure of nicotine and less cardiovascular effects (e.g., blood pressure and heart rate) compared to regular cigarette smoking. But long-term studies are necessary to confirm the findings from this study and to provide additional support that e-cigs are less harmful than cigarette smoking for cardiovascular system. Besides the hemodynamic measurements taken in the study, other parameters, such as dimension and wall thickness of the heart, cardiac output, blood flow velocity, ejection time etc., may be included in the long-term study.

5. Conclusion

This study showed that after half hour controlled and 1 h ad lib product use, the nicotine plasma concentrations (CnP) were statistically significantly lower in e-cigarette users than in Marlboro® cigarette users. Among the blu e-cigarettes, the concentrations of nicotine in the e-liquid correspond to the nicotine plasma nicotine levels (CnP) after 1.5 h of product use. 2.4% nicotine in the e-liquid of e-cigarettes delivered higher nicotine in plasma that 1.6% (e.g., CnP = 22.41 ng/mL of e-cig 2.4% Nic in Glycerin) vs. CnP = 16.78 ng/mL of e-cig 1.6% Nic in Glycerin). It’s also found the combination of tobacco cigarette substitutes: a systematic review. Ther. Adv. Drug Saf. 5 (2), 67–86.


This study was conducted in Celerion (Lincoln, Nebraska), funded by the LOEC, Inc. d/b/a blu ecigs.

References


