Research Article

Cancer Prevention Research

Effects of Switching to Electronic Cigarettes with and without Concurrent Smoking on Exposure to Nicotine, Carbon Monoxide, and Acrolein

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Abstract

Concern has been raised about the presence of toxicants in electronic cigarette (EC) aerosol, particularly carbonyl compounds (e.g., acrolein) that can be produced by heating glycerol and glycols used in e-liquids. We investigated exposure to carbon monoxide (CO), nicotine (by measuring cotinine in urine), and to acrolein (by measuring its primary metabolite, S-(3-hydroxypropyl)mercapturic acid (3-HPMA) in urine) before and after 4 weeks of EC (green smoke, a "cig-a-like" EC, labeled 2.4% nicotine by volume) use, in 40 smokers. Thirty-three participants were using EC at 4 weeks after quitting, 16 (48%) were abstinent (CO-validated) from smoking during the previous week (EC only users), and 17 (52%) were "dual users." A significant reduction in CO was observed in EC-only users [−12 ppm, 95% confidence interval (CI), −16 to −7, 80% decrease) and dual users (−12 ppm, 95%CI, −19 to −6, 52% decrease). Cotinine levels also declined, but to a lesser extent (EC-only users: −184 ng/mg creatinine; 95% CI, −733 to −365, 17% decrease; and dual users: −976 ng/mg creatinine; 95%CI, −1,682 to −270, 44% decrease). Mean 3-HPMA levels had decreased at 4 weeks by 1,280 ng/mg creatinine (95%CI, −1,699 to −861, 79% decrease) in EC-only users and by 1,474 ng/mg creatinine (95%CI, −2,101 to −847, 60% decrease) in dual users. In dual users, EC use significantly reduced exposure to CO and acrolein because of a reduction in smoke intake. EC may reduce harm even in smokers who continue to smoke, but long-term follow-up studies are needed to confirm this. *Cancer Prev Res; 8(9); 873–8. ©2015 AACR.*

Introduction

Cigarette smoke contains a number of carcinogens. Tobacco-specific nitrosamines are among the most recognized, but some of the carbonyl compounds that are formed during the combustion process, such as formaldehyde, acetaldehyde, and acrolein, are also considered to be carcinogenic (1).

Electronic cigarettes (EC) may have a potential for public health benefit, as EC use does not involve tobacco combustion, which is the primary source of the dangerous chemicals to which smokers of conventional cigarettes are exposed. However, heating the liquid used in EC, which typically contains nicotine, flavorings, propylene glycol, and/or glycerine, can also result in the formation of new compounds, and previous studies found small amounts of formaldehyde and acetaldehyde in EC cartridges and aerosol (2). The presence of acrolein in aerosol has also been found (3–5).

Acrolein (2-propenal) is present in cigarette smoke at levels between 60 and 100 μg/cigarette (6). Its adverse effects are dose- and cell type–dependent and influenced by experimental conditions (7). Animal experiments showed that acrolein can have a range of adverse effects, including a role in carcinogenesis (8, 9); excessive mucus production and macrophage and neutrophil accumulation with consequent production of proinflammatory cytokines and proteases (10); damage to neurons and myelin disruption (11); and may play a role in the progression of atherosclerosis (12) and cardiovascular disease (13). The main pathway for elimination of acrolein is conjugation with glutathione (GSH) in the liver, followed by enzymatic cleavage of the γ-glutamic acid and glycine residues, respectively, in the liver and in the kidney and N-acetylation of the resultant cysteine conjugate to form S-(3-oxopropyl)-N-acetylcysteine (OPMA) in the kidney. Reduction of this aldehyde yields S-(3-hydroxypropyl)mercapturic acid (3-HPMA; other name S-(3-hydroxypropyl)-N-acetylcysteine), the main metabolite of acrolein found in urine (9).

As acrolein is found in both tobacco smoke and EC aerosol, there is concern that people who use EC and continue to smoke tobacco (so-called dual users) might be exposed to higher levels than those who smoke only conventional cigarettes. To help consider the potential for EC in harm reduction, data are needed comparing the concentration of toxicants in smokers of conventional cigarettes, users of EC, and dual users. We investigated exposure to acrolein (as measured by its primary metabolite, S-(3-hydroxypropyl)mercapturic acid (3-HPMA; other name N-Acetyl-S-(3-hydroxypropyl)-L-cysteine), in urine; Fig. 1) together with exposure to nicotine and carbon monoxide (CO) in a cohort of

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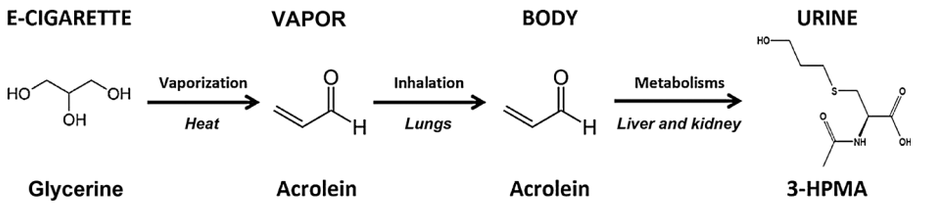

Figure 1.
The process by which acrolein is metabolized.

40 smokers before and after 4 weeks of EC use, both in exclusive EC users and dual users.

Materials and Methods

Participants

Forty adult smokers wanting to stop smoking were recruited through advertisements in free London newspapers. We excluded women who were pregnant or breastfeeding, smokers with any current serious illness, and those who had used EC for more than 1 week in the past.

Study procedures

Participants were screened over the telephone and attended a baseline session 1 week prior to their target quit date (TQD), where they provided written informed consent and baseline measures. Participants were advised to smoke ad lib for the following week. On the TQD, participants were provided with their EC and received instructions on its use. They were instructed to use EC ad-lib. Two cartridges per day were supplied initially, with the supply adjusted to actual use later. Participants received standard withdrawal-oriented behavioral support (14) at baseline, TQD, and at four further weekly sessions. A subsample of 10 participants also provided pharmacokinetic (PK) data on nicotine delivery from EC. The PK part of the study is covered in a separate report (15).

The study was approved by the NHS Health Research Authority, NRES Committee London (12/LO/1987) and registered on ClinicalTrials.gov (NCT01714778).

Study product

The study used a Green Smoke EC (labeled 2.4% nicotine), a first-generation "cig-a-like" device, purchased directly from the manufacturer's website. At the time of the study, the company produced only one model. In our previous study, the model provided a consistent nicotine content and delivered 9 mg of nicotine in aerosol over 300 puffs (16), which was in the middle range of the products tested. Peak mean plasma nicotine concentration achieved after 5 minutes of ad lib use, after overnight abstinence, was 5.7 ng/mL (15). While many EC include propylene glycol only, Green Smoke includes propylene glycol and vegetable glycerine, the latter being the precursor to acrolein (17).

We tested aerosol generated from 5 Green Smoke cartridges for acrolein content using a smoking machine as described previously

Table 1. Participant characteristics

Characteristic	Abstinent at 4 weeks (EC use only) ($n = 16$)	Smoking at 4 weeks (dual users) ($n = 17$)
Age, mean (SD)	44.8 (13.22)	48.2 (12.37)
Gender: *N* (%) male	8 (50%)	9 (52.9%)
Cigarettes per day, mean (SD)	16.3 (8.68)	21.0 (11.87)
FTCD[a], mean (SD)	3.88 (2.28)	4.65 (2.09)
Previous quit attempts, mean (SD)	2.63 (1.02)	2.29 (1.05)
In full time employment, *N* (%)	10 (62.5%)	11 (64.7%)
White British, *N* (%)	10 (62.5%)	9 (52.9%)

[a]FTCD = Fagerstrom Test for Cigarette Dependence (33).

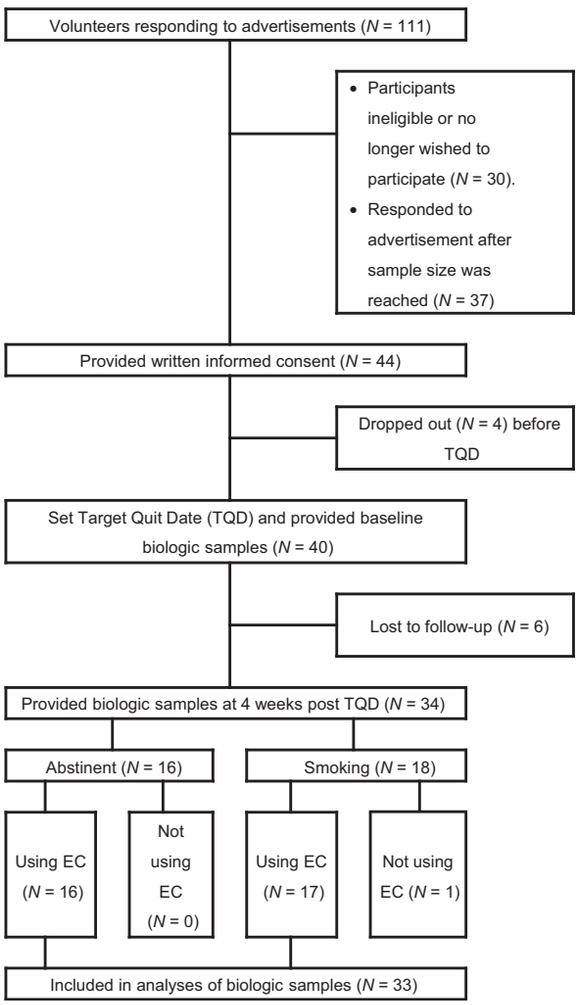

Figure 2.
The flow of participants throughout the study. *No smoking at all during the last week of treatment validated with a CO reading < 10 ppm.

(3). The average acrolein yield in aerosol delivered in 15 puffs was 19.4 ng (SD 1.5).

Measures

Demographic and smoking history data were collected at baseline. At each visit, participants completed the Mood and Physical Symptoms Scale (MPSS; ref. 18), a commonly used assessment of tobacco withdrawal symptoms and urges to smoke; reported on their cigarette and EC use; and provided an end-expired CO reading.

Urine samples were collected at TQD and at 4 weeks after TQD to assay for 3-HPMA and cotinine. The urine samples were stored at −20°C before being couriered to ABS Laboratories, Ltd. (19) who performed the assays.

The urine was assayed for 3-HPMA using an LC-MS/MS assay developed and validated by ABS Laboratories using 3-HPMA-D3 as the internal standard. The inter-batch precision (CV) and accuracy results for the QC samples analyzed during the analysis of the samples from this study had precision less than or equal to 4.9% and a mean accuracy that ranged from 93.1% to 107.1%.

The urine samples were assayed for free cotinine using an LC-MS/MS assay over the calibration range of 1 to 1,000 ng/mL. The inter-batch precision (CV) and accuracy results for the QC samples analyzed during the analysis of the samples from this study had precision below or equal to 9.3% and an average accuracy that ranged from 95.8% to 102.3%.

The urinary creatinine levels were assayed in a clinical laboratory using the standard Jaffe's reaction.

All the analytical methods were validated, and the analysis of the samples from this study was performed in accordance with the FDA Guidance for Industry (20) and the EMA Guideline on bioanalytical method validation (21).

Participants were asked to report any adverse events (AE) on a weekly basis. All AEs were assessed for their seriousness, causality, and severity in accordance with the ICH Guideline for Good Clinical Practice (22).

Statistical considerations

In our previous study, use of conventional cigarettes was associated with 3-HPMA levels of 1,614 ng/mL (SD = 1,141), whereas in users of EC, this was 616 ng/mL (SD = 509; ref. 23). Eleven participants would be needed to have 85% probability of detecting this difference ($P < 0.05$, two-sided). We recruited 40 participants with the expectation that at the end of the 4-week course, 10 would be lost to follow-up, 15 would be smoking EC only, and 15 would be using both EC and conventional cigarettes.

Participant characteristics were summarized with descriptive statistics. Changes in CO, cotinine, and 3-HPMA from baseline to 4 weeks after TQD were assessed using paired sample t-test. At end of treatment, one sample had a cotinine concentration below limits of quantification, and so we used a value of LLOQ/10 in the analysis. All samples had detectable levels of 3-HPMA. Differences in average change scores between participants who did and did not smoke at all in the week prior to the follow-up testing were examined using t-tests. For these analyses, we considered participants to be abstinent if they reported not smoking (not even a puff) over the previous week validated by a CO reading of <10 ppm. In reporting 4-week abstinence rates, we considered those lost to follow-up as nonabstainers.

Table 2. Changes in CO in expired breath (ppm), urinary cotinine and 3-HPMA levels (ng/mg creatinine) at baseline and after 4 weeks of EC use

	Total sample ($n = 33$)				Abstinent at 4 weeks ($n = 16$; EC use only)				Smoking at 4 weeks ($n = 17$; dual users)			
	Baseline Mean (SD)	4 weeks Mean (SD)	Change Mean (95% CI) % reduction[a]	Sig	Baseline Mean (SD)	4 weeks Mean (SD)	Change Mean (95% CI) % reduction[a]	Sig	Baseline Mean (SD)	4 weeks Mean (SD)	Change Mean (95% CI) % reduction[a]	Sig
CO (ppm)	19 (11)	7 (7)	−12 (−16 to −8) 63%	$P < 0.001$	15 (8)	3 (2)	−12 (−16 to −7) 80%	$P < 0.001$	23 (11)	11 (8)	−12 (−19 to −6) 52%	$P = 0.001$
Urinary cotinine (ng/mg creatinine)	1,655 (1469)	1,063 (831)	−592 (−1,041 to −143) 36%	$P = 0.011$	1,073 (832)	889 (959)	−184 (−733 to 365) 17%	$P = 0.486$	2,203 (1734)	1,227 (679)	−976 (−1,682 to −270) 44%	$P = 0.010$
3-HPMA (ng/mg creatinine)	2,046 (1,060)	666 (664)	−1,380 (−1,742 to −1,018) 67%	$P < 0.001$	1,623 (850)	343 (178)	−1,280 (−1,699 to −861) 79%	$P < 0.001$	2,443 (1,105)	969 (807)	−1,474 (−2,101 to −847) 60%	$P < 0.001$

Abbreviation: Sig, statistical significance.

[a]Percentage reduction from baseline, calculated as the mean change/baseline mean × 100.

Table 3. EC use at week 4

	Total sample ($n = 33$)	Abstinent at 4 weeks ($n = 16$; EC use only)	Smoking at 4 weeks ($n = 17$; dual users)	Sig
Mean number of cartridges used/day[a] (SD)	1.54 (0.85)	1.51 (0.89)	1.56 (0.83)	$P = 0.878$
Mean number of days EC used[a] (SD)	6.24 (1.73)	6.31 (1.89)	6.18 (1.63)	$P = 0.826$

Abbreviation: Sig, statistical significance.
[a]Use over the previous week at week 4.

Results

Table 1 shows participant characteristics. Participant flow is shown in Fig. 2. Six participants were lost to follow-up, 33 were using EC at the end of treatment, and one was not using EC anymore. The 33 participants using EC were included in the analyses below.

Changes in CO, urinary cotinine, and 3-HPMA levels are shown in Table 2. Average daily EC cartridges use and number of days EC were used in the last week of treatment are shown in Table 3.

Of participants who reported smoking at 4 weeks after TQD, seven had smoked one to five cigarettes in the last week and 10 had smoked more than 5. The average CO levels for these two groups were 7 ppm (SD, 4; range, 1–15) and 14 ppm (SD, 8; range, 5–33), respectively.

There was a significant decrease in 3-HPMA levels in abstainers, but also in participants who continued to smoke ($P < 0.05$). Three participants showed an increase in 3-HPMA; one by 34% and another by 30% which was associated with a 50% and 20% increase in end-expired CO level, respectively, indicating an increase in smoke intake. Another participant who also continued to smoke had a marginal 6% increase in 3-HMPA levels accompanied by an 11% decrease in CO levels. All other participants recorded a decrease in 3-HPMA levels (from 9% to 94% of baseline).

Urinary 3-HPMA levels at 4 weeks were highly correlated with the concentration of CO in expired breath, i.e., with the intake of smoke from cigarettes ($r = 0.82$; $P < 0.001$).

Participants who did not manage to stop smoking had significantly higher baseline cotinine (2,203 vs. 1,073 ng/mg creatinine, $t = 2.36$, $P = 0.025$) and CO (23 vs. 15 ppm, $t = 2.39$, $P = 0.023$) levels than abstainers.

Overall, 16 of the 40 participants (40%) who started treatment achieved CO-validated abstinence in the last week of treatment. In addition to this, 11 nonabstainers (an additional 28%) reduced their CO readings by at least 50% compared with baseline.

There were no serious adverse effects from EC use. Transient mild to moderate events were recorded in 12 participants (all recorded by a single participant unless stated otherwise) and included chest discomfort ($n = 2$), nausea, vomiting, dizziness, anxiety, flatulence, upper airways secretion, vertigo, oral discomfort ($n = 2$), dry nose, throat or mouth ($n = 2$), headache ($n = 2$), and throat irritation.

Discussion

The headline finding concerning acrolein is that in dual users, EC use significantly reduces rather than increases exposure to this toxicant. As expected, dual use also reduced smoke intake generally, as indexed by expired CO levels.

Acrolein levels of 337 ± 383 μg/24h were reported for nonsmokers previously ($N = 100$; ref. 24). Assuming an average creatinine excretion rate of 1,400 mg/24h, these levels correspond to 259 ± 295 ng/mg creatinine. This is somewhat lower than the levels we found among EC users who abstained from smoking but used EC (343 ± 178 ng/mg), but these are still 4- to 6-fold lower than concentrations in smokers (25). There is a similar relationship between the 5-fold reduction in acrolein intake in smokers who stopped smoking while using EC in our study and the results of a previous study of 17 smokers abstinent for 4 weeks (and not using EC) in whom the acrolein biomarker (HPMA) was reduced 7-fold (26).

Participants who managed to achieve complete abstinence from smoking had lower baseline smoke intake as measured by CO, cotinine, and HPMA than those who continued to smoke. No other baseline differences between these two groups were detected. Interestingly, in the successful abstainers, EC were used with sufficient frequency to provide accumulated cotinine levels similar to those recorded at baseline. Participants who did not manage to stop smoking altogether (dual users) and who started with higher cotinine levels experienced a significant reduction in cotinine levels despite dual use. In fact, there was a trend for dual users to reduce their cotinine levels more than abstainers, although the difference between the two groups did not reach statistical significance ($P = 0.072$). Dual users were trying to limit their smoking and reduced substantially their smoke intake, as shown by the reduction in their expired CO. Although they were vaping with the same frequency and used a similar number of cartridges per day as abstainers, they did less well in maintaining their high baseline cotinine levels. The inability to obtain sufficient nicotine levels from the relatively weak cig-a-like EC product used in this study may have contributed to their inability to switch to EC fully.

The validated quit rate observed in this study (40%) is comparable with that achieved by the UK Stop Smoking Services which use a combination of behavioral support and pharmacotherapy (NRT, bupropion, or varenicline) and have reported over the past 5 years validated 4-week quit rates of 31% to 37% (27).

It is interesting to note that only 15% of participants dropped out during the first 4 weeks of treatment, which is less than what we normally observe in studies using traditional stop-smoking medications. For instance, in a recently completed trial where participants received the standard UK Stop Smoking Service treatment with support identical to the current study, 46% of participants dropped-out by 4 weeks (28). EC may thus improve treatment retention compared with traditional treatments. They may also have another potentially important attribute if patients who do not succeed in stopping smoking continue to use EC and reduce their toxin intake in the long term; the extent to which this happens is currently not known.

Dual use of EC and conventional cigarettes is often cited as a concern because of a possibility that it may expose dual users to greater health risks than smoking alone (5). Theoretically, if smokers are getting nicotine from an alternative "cleaner" source, then their need for nicotine from cigarettes should decrease,

which in turn should generate a reduction in smoking and an accompanying reduction in toxicant intake. In some countries, NRT is licensed for this purpose (29). Results of this study support findings from other cohort studies that show that EC use can help those who do not manage to stop smoking altogether to reduce cigarette consumption, with an accompanying decrease in biomarkers of tobacco exposure (15, 30, 31).

The study had several limitations. Our cohort consisted of people who wanted to stop smoking, and the results may not generalize to smokers only interested in cutting down. We only monitored EC use over 4 weeks—vaping behavior may change further with time. It is possible that Green Smoke is on the lower end of the spectrum of acrolein delivery and that other brands may deliver higher levels, although all EC brands tested so far released levels of acrolein in the aerosol at much lower levels than cigarettes (3). Our study included a "cig-a-like" EC. In a recent lab study, an advanced EC with a tank system and variable voltage also released only very low levels of carbonyl compounds in normal use (32), but future EC products may have different potential for exposure. We only used biomarkers of two toxicants, CO and acrolein, but given the significant decrease in smoke intake, the finding is likely to concern toxicants from cigarette smoke generally. Finally, we did not include nonsmoking controls to establish environmental acrolein exposure.

In conclusion, EC use in both smokers who quit smoking and in dual users was accompanied by a significant decrease in tobacco smoke toxicant exposure.

Disclosure of Potential Conflicts of Interest

H. McRobbie is Clincal Director at The Dragon Institute; reports receiving commercial research grant from Pfizer; and has received speakers bureau honoraria from Johnson & Johnson and Pfizer. M.L. Goniewicz reports receiving commercial research grant from Pfizer. P. Hajek has received speakers bureau honoraria from and is a consultant/advisory board member for the manufacturers of stop-smoking medications. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: H. McRobbie, P. Hajek, M.L. Goniewicz
Development of methodology: H. McRobbie, M.L. Goniewicz, P. Hajek
Acquisition of data (acquired and managed patients, provided facilities, etc.): H. McRobbie, A. Phillips, K.M. Smith, O. Knight-West, D. Przulj
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. McRobbie, A. Phillips, M.L. Goniewicz, O. Knight-West, P. Hajek
Writing, review, and/or revision of the manuscript: H. McRobbie, P. Hajek, A. Phillips, M.L. Goniewicz, K.M. Smith, O. Knight-West, D. Przulj
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. McRobbie, A. Phillips, K.M. Smith
Study supervision: A. Phillips, K.M. Smith, P. Hajek

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