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Evaluation of Toxicant and Carcinogen Metabolites in the Urine of E-Cigarette Users Versus Cigarette Smokers

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Abstract

Introduction: Electronic cigarettes (e-cigarettes) are rapidly increasing in popularity but little information is available on their potential toxic or carcinogenic effects.
Methods: Twenty-eight e-cigarette smokers who had not smoked tobacco cigarettes for at least 2 months provided urine samples which were analyzed by validated methods for a suite of toxicant and carcinogen metabolites including 1-hydroxypyrene (1-HOP), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL), 3-hydroxypropylmercapturic acid (3-HPMA), 2-hydroxypropylmercapturic acid (2-HPMA), 3-hydroxy-1-methylpropylmercapturic acid (HMPMA), *S*-phenylmercapturic acid (SPMA), nicotine, and cotinine. Levels of these compounds were compared to those found in cigarette smokers from three previous studies.
Results: Levels of 1-HOP, total NNAL, 3-HPMA, 2-HPMA, HMPMA, and SPMA were significantly lower in the urine of e-cigarette users compared to cigarette smokers. Levels of nicotine and cotinine were significantly lower in e-cigarette users compared to cigarette smokers in one study but not in another.
Conclusions: With respect to the compounds analyzed here, e-cigarettes have a more favorable toxicity profile than tobacco cigarettes.

Introduction

Electronic cigarettes (e-cigarettes) and other electronic nicotine delivery systems (ENDS) have rapidly emerged onto the marketplace, challenging conventional tobacco products in popularity. In one online survey reported 2 years ago, 40.2% of Americans aged 18 years and older had heard of ENDS, and 11.4% of current smokers had used them.[1] Predictably, major tobacco companies have now joined the legions of e-cigarette manufacturers. A typical e-cigarette has a battery, an atomizer or heating element, and a container that holds a propylene glycol or glycerin solution of nicotine and one or more of a seemingly limitless variety of flavor substances. The lack of tobacco in these devices strongly suggests that they should have a more favorable toxicology profile than conventional tobacco products, but so far there are limited published data, restricted primarily to analyses of the nicotine solutions and the aerosols produced upon heating of these solutions. These studies have been reviewed.[2–5]

A variety of compounds in addition to nicotine have been detected in the refill solutions and aerosols of e-cigarettes. These include other tobacco alkaloids, tobacco-specific nitrosamines, formaldehyde, acetaldehyde, acrolein, metals, polycyclic aromatic hydrocarbons (PAH), and propylene glycol or glycerin.[2,6–9] With the exception of nicotine,

propylene glycol, and glycerin, most of these compounds are present in amounts far less than in the smoke of conventional tobacco cigarettes. However, there is no agreed upon set of standard conditions for measurement of constituents of e-cigarette liquids or aerosols. The huge variety of products of different origin and design, the rapid appearance of new products, and the varied ways in which consumers use these products makes the development of standard measurement conditions challenging. A more relevant approach to assessing the potential toxic effects of e-cigarettes could be measurement of actual constituent uptake in their users, but we are not aware of any published study that has addressed this topic. Therefore, in the study presented here, we quantified urinary toxicant and carcinogen metabolites in people using e-cigarettes and compared their levels to those found in cigarette smokers. The compounds quantified were 1-hydroxypyrene (1-HOP), a biomarker of carcinogenic PAH exposure; 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL), metabolites of the tobacco-specific nitrosamine and lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); 3-hydroxypropylmercapturic acid (3-HPMA), a metabolite of the toxicant acrolein; 2-hydroxypropylmercapturic acid (2-HPMA), a metabolite of the carcinogen propylene oxide; 3-hydroxy-1-methylpropylmercapturic acid (HMPMA), a metabolite of the carcinogen crotonaldehyde; *S*-phenylmercapturic acid, a metabolite of the carcinogen benzene; and nicotine and cotinine.

Methods

Study Design

This study was approved by the University of Minnesota Institutional Review Board. Subjects were recruited by a member of the research staff of the University of Minnesota Tobacco Research Programs and initially screened over the telephone. Inclusion criteria were as follows: 18 years or older, in good physical and mental health with no unstable medical condition or current infection as determined by medical history and investigator assessment, stable on psychiatric medications if taking them, not smoking cigarettes for at least 2 months, using e-cigarettes for at least 1 month and at least 4 days per week, no current use of medicinal nicotine or other tobacco products, and not pregnant. Smoking status was confirmed by determination of exhaled carbon monoxide (CO).[10] Participants attended a clinic visit where they completed a tobacco and e-cigarette use history questionnaire and a spot urine sample was collected.

Analysis of Urine

Analyses were carried out by validated methods as follows: 1-HOP,[11] total NNAL,[12] 3-HPMA,[13] 2-HPMA,[14] HMPMA,[13] SPMA,[15] total nicotine, and total cotinine.[16]

Comparison Studies

The results from the analyses of urine samples from e-cigarette users were compared to those obtained from previous analyses of cigarette smokers' urine using essentially identical assay methods. These smokers were participants in three previous studies. In one study, 17 smokers who wanted to quit were recruited and provided urine samples at baseline prior to 8 weeks of refraining from smoking; baseline data were used here.[15] A second study recruited 165 smokers of "light" cigarettes who were interested in quitting smoking and were assigned to either low nicotine cigarettes or nicotine lozenges; their baseline first morning urine samples were analyzed for the data reported here.[17] The third study analyzed 40 samples from cigarette smokers who provided spot urine samples to the Tobacco Research Programs Repository.[14]

Statistical Analysis

The patterns of e-cigarette use were summarized. e-Cigarette users who had a CO level greater than or equal to 6 ppm (indicating use of cigarettes), reported dual use of e-cigarettes and tobacco cigarettes, or did not provide a urine sample were excluded. Demographic and smoking history data were summarized for the e-cigarette users and all participants from each of the three cigarette smoking studies. When available, the baseline biomarker data from each of the studies representing smokers were compared with the biomarkers found in e-cigarette users using linear regression models, adjusting for age and gender. For biomarker values determined to be below the limit of detection (LOD), one half the LOD was used. All biomarkers had skewed distributions and therefore were transformed using the natural logarithm to approximate normality and were summarized using geometric means and 95% confidence intervals. Reported p values are adjusted for multiple comparisons using Dunnett's method.[18] Analyses were carried out in SAS Version 9.3 (SAS Institute, Inc.) and p values <.05 were considered statistically significant.

Results

A total of 35 e-cigarette users participated in the study; of those, four were excluded due to CO values greater than or equal to 6 ppm and three were additionally excluded due to lack of biomarker data. Of the 28 participants eligible for this analysis, e-cigarette use was for a median of 9 months (range 3–36) and they quit smoking 9 months (range 2–36) before study entry. Most used e-cigarettes daily (96.2%) and the average nicotine concentrations were 12.5 ± 7.0 mg/ml. All e-cigarette users used refillable e-cigarettes and refilled an average of one time (range 0.3–5) per day. The brands of e-cigarettes used are summarized in Table 1.

Demographics and smoking history are summarized in Table 2. The e-cigarette users were significantly younger than the smokers in two of the studies; however, there were no other significant differences between the study groups.

The results of the analyses of urine samples from e-cigarette users and cigarette smokers are summarized in Table 3. Levels of 1-HOP, total NNAL, 3-HPMA, 2-HPMA, HMPMA, and SPMA were significantly lower in the users of e-cigarettes than in cigarette smokers across all studies with available data (all $p < .05$). Nicotine and cotinine were statistically significantly lower in e-cigarette users compared to one group of cigarette smokers[17] but not another.[14] Four e-cigarette users had higher than expected levels of total NNAL—0.613, 0.261, 0.789, and 0.953 pmol/ml. Total NNAL was below the LOD (0.015 pmol/ml) in 16 e-cigarette users.

Discussion

The results of this study clearly demonstrate that levels of a suite of toxicant and carcinogen metabolites were significantly lower in the urine of e-cigarette users than in cigarette smokers, while nicotine and cotinine levels were comparable in one study and lower in e-cigarette users in another. These results support the assumption that e-cigarettes may be less harmful than conventional tobacco cigarettes, at least with respect to the metabolites analyzed here. We note however that four e-cigarette users had higher than expected levels

of total NNAL, albeit lower than typically seen in smokers (Table 3). Total NNAL is generally not detected in nonsmokers unless they are exposed to secondhand smoke, in which case levels typically range from 0.03 to 0.06 pmol/ml urine in adults, which is lower than seen in these four subjects.[19]

Levels of 1-HOP were significantly lower in e-cigarette users than in cigarette smokers, and in the range observed in nonsmokers.[20] 1-HOP is a metabolite of pyrene and a widely employed urinary biomarker of exposure to PAH, ubiquitous environmental and dietary contaminants resulting from incomplete combustion of organic matter.[21] If e-cigarette heating sources reached high temperatures, PAH formation would be possible. While pyrene itself is not carcinogenic, it always occurs as part of a mixture of PAH, many of which, including the prototypic benzo[*a*]pyrene, considered carcinogenic to humans by the International Agency for Research on Cancer, are potent carcinogens.[21] Virtually all humans have 1-HOP and other PAH metabolites in their urine; levels of 1-HOP in the urine of smokers are generally 2–3 times higher than in nonsmokers, consistent with the results presented here.[20,22,23] 1-HOP was present in more than 99% of the urine samples collected in 1999 and 2000 as part of the National Health and Nutrition Examination Survey in the United States, with a geometric mean for the entire population of 0.37 pmol/ml urine, close to the level reported here for e-cigarette users.[20,22,23]

Urinary total NNAL concentrations were also significantly lower in e-cigarette users than in cigarette smokers. This is a reflection of the fact that cigarette tobacco and its smoke contain NNK while the amounts of NNK so far reported in e-liquids are considerably lower than found in tobacco products.[3] The reduction in exposure to NNK in e-cigarette users compared to smokers is a favorable sign, and total NNAL was below the LOD in 16 of our subjects, consistent with its tobacco specificity. However, four of our e-cigarette users did not have such low levels of urinary total NNAL. Since their other toxicant profiles were consistent with the generally reduced levels in e-cigarette users, this suggests that the nicotine in their products was contaminated with NNK in levels higher than previously reported. Arguing against this interpretation is the fact that they all used different brands of e-cigarettes. Alternatively, they may have cheated and smoked a few cigarettes on the days before donating their urine sample. This might not have been detected by the CO analysis, as the half-life of exhaled CO is short compared to that of NNAL.[24]

The mercapturic acids measured in this study are formed by an initial reaction of each compound or metabolite with glutathione, followed by normal metabolic processing and excretion in the urine. Virtually all human urine samples contain these compounds, resulting from environmental or endogenous exposure to acrolein, crotonaldehyde, propylene oxide, or benzene.[20] Their levels are generally higher in smokers than in nonsmokers and decrease rapidly upon smoking cessation.[14,15,25] In the study reported here, levels of all of these mercapturic acids were significantly lower in the urine of e-cigarette users than in smokers. Although we did not formally compare levels of the mercapturic acids to those of nonsmokers, they were generally in the same range as previously reported.[15,20]

3-HPMA is produced from acrolein, which has been detected at low levels in vapor from e-cigarettes.[8] Acrolein is a powerful toxicant which can cause intense eye and respiratory tract irritation. Exposure of laboratory animals to acrolein by inhalation consistently produces irritation, inflammation, cell proliferation, and other toxic effects, but not tumors.[26] Nevertheless, acrolein reacts with DNA to form well-characterized adducts and produces mutations in

Table 1. e-Cigarette Brands Used by Study Subjects

e-cigarette brand	Number of users[a]
Aqua	2
Aspire	2
Buck Naked Express	1
eGo	8
eQ	1
Green Smoke[b]	1
Green Smart Living[b]	1
Hades	1
iGo	1
Itazte	5
JDTech	1
Kanger	7
MyVape	1
Origin	1
Provari	4
Sigelei	1
SMOKTech	2
V2[b]	1
Vapor4Life	1
Vision Spinner	3
Vmax	1

[a]Some users used more than one brand.
[b]Two subjects used cartridges (Green Smoke, V2, and Green Smart Living); all others used tank systems.

Table 2. Demographics and Smoking History of Subjects by Study

	e-Cigarettes	Cigarette smokers		
	e-Cigarette users, *N* = 28	Carmella *et al.*[15] (*N* = 17)	Hatsukami *et al.*[17] (*N* = 165)	Zarth *et al.*[14] (*N* = 40)
Age (years)	34.0 ± 12.7	43.3 ± 10.8	41.3 ± 13.2	34.4 ± 9.5
Female	42.9%	64.7%	47.3%	57.5%
Non-Hispanic Whites	92.6%	88.2%	85.9%	–
Education				
Some high school	0.0%	5.9%	4.3%	–
High school graduate	11.1%	17.7%	24.5%	–
Some college or more	88.9%	76.5%	71.2%	–
Cigarettes per day	21.1 ± 10.3[a]	22.2 ± 6.4	20.7 ± 8.5	17.5 ± 5.7
Age smoking first (years)	15.3 ± 2.9	14.0 ± 3.4	14.8 ± 3.7	–

[a]Reported number of cigarettes per day before switching to e-cigarettes

Table 3. Geometric Means and 95% Confidence Intervals of Levels of Toxicant and Carcinogen Metabolites in the Urine of e-Cigarette Users and Cigarette Smokers by Study, Adjusted for Age and Gender

	e-Cigarettes	Cigarette smokers					
	e-Cigarette users, *N* = 28	Carmella *et al.*[15] (*N* = 17)		Hatsukami *et al.*[17] (*N* = 165)		Zarth *et al.*[14] (*N* = 40)	
Metabolite	Geometric mean (95% CI)	Geometric mean (95% CI)	*p* value[a]	Geometric mean (95% CI)	*p* value[a]	Geometric mean (95% CI)	*p* value[a]
1-HOP (pmol/ml)	0.38 (0.26–0.55)	0.88 (0.55–1.41)	.013	0.97 (0.80–1.17)	<.0001	Not analyzed	
Total NNAL (pmol/ml)	0.02 (0.02–0.03)	1.48 (0.90–2.43)	<.0001	1.21 (0.99–1.47)	<.0001	Not analyzed	
3-HPMA (pmol/ml)	1,100 (766–1,590)	5,800 (3,730–9,030)	<.0001	4,040 (3,380–4,830)	<.0001	6,070 (4,580–8,050)	<.0001
2-HPMA (pmol/ml)	141 (80–252)	Not analyzed		Not analyzed		399 (255–626)	.006
HMPMA (pmol/ml)	705 (456–1,090)	4,990 (2,930–8,490)	<.0001	Not analyzed		Not analyzed	
SPMA (pmol/ml)	0.29 (0.18–0.46)	1.11 (0.61–2.08)	0.001	2.85 (2.24–3.63)	<.0001	Not analyzed	
Nicotine (ng/ml)	869 (604–1,250)	Not analyzed		1,380 (1190–1,600)	.035	1,270 (834–1,710)	.182
Cotinine (ng/ml)	1,880 (1,420–2,480)	Not analyzed		3,930 (3,500–4,400)	<.0001	1,930 (1,530–2,440)	.981

1-HOP = 1-hydroxypyrene; 2-HPMA = 2-hydroxypropylmercapturic acid; 3-HPMA = 3-hydroxypropylmercapturic acid; CI = confidence interval; HMPMA = 3-hydroxy-1-methylpropylmercapturic acid; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; SPMA = *S*-phenylmercapturic acid.

[a]Compared to e-cigarette users, adjusted for age and gender.

the *p53* tumor suppressor gene similar to those found in lung tumors from smokers.[27–29] These observations support a role for acrolein in the toxic and possibly carcinogenic effects of cigarette smoking, but levels of 3-HPMA were significantly decreased in e-cigarette users.

HMPMA is a metabolite of crotonaldehyde, a homolog of acrolein with similar strong irritant and toxic properties.[30] Crotonaldehyde reacts with DNA to form 1,N^2-cyclic deoxyguanosine adducts, which have been detected in human lung samples.[27,31] Crotonaldehyde caused altered liver cell foci, liver damage, and neoplastic nodules when administered in the drinking water to rats.[32] Levels of HMPMA were also lower in the urine of e-cigarette users than in cigarette smokers.

2-HPMA is a metabolite of propylene oxide, a strong irritant which produced nasal cavity tumors in mice and rats when administered by inhalation.[33] The U.S. National Toxicology Program classifies propylene oxide as "reasonably anticipated to be a human carcinogen" while the International Agency for Research on Cancer evaluated it as "possibly carcinogenic to humans."[33,34] At high temperatures, which might be encountered in some ENDS products, propylene oxide could be formed from propylene glycol, but this would be unlikely at lower temperatures.[35] Our data do not indicate that formation of propylene oxide from propylene glycol was a significant occurrence in the e-cigarette users studied here.

SPMA is an established biomarker of benzene exposure, ultimately resulting from the reaction of its metabolite benzene oxide with glutathione.[20] Benzene is universally recognized as a cause of acute myeloid leukemia in humans and has also been associated with acute lymphocytic leukemia, multiple myeloma, and non-Hodgkins lymphoma.[33,36] Some studies implicate benzene as a possible cause of lung cancer.[33,36] Benzene causes multiple types of tumors in rats and mice.[33,36] As was the case with the other mercapturic acids, SPMA levels were significantly lower in e-cigarette users than in smokers.

Our study had certain limitations. The sample size of e-cigarette users was relatively small and they were sampled at only one time point. The comparison studies of cigarette smokers were carried out at a different time with different recruiting methods and aims. Nevertheless, the toxicant data for the cigarette smokers in this study were quite consistent with literature data.

In summary, the results of this study demonstrate that levels of a suite of urinary toxicant and carcinogen metabolites were significantly lower in e-cigarette users than in cigarette smokers. These results suggest that e-cigarette use may be safer than cigarette smoking, at least with respect to the compounds studied here, which represent typical carcinogens and toxicants believed to be involved in causing cancer in cigarette smokers.

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Declaration of Interests

None declared.

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