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Authors

Liakoni, Evangelia
Nardone, Natalie
St Helen, Gideon
et al.

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Brief Report

Effects of Nicotine Metabolic Rate on Cigarette Reinforcement

Evangelia Liakoni MD[1,2,*], Natalie Nardone PhD[1,*], Gideon St.Helen PhD[1,3], Delia A. Dempsey MD[1], Rachel F. Tyndale PhD[4,5,6], Neal L. Benowitz, MD[1,3,7]

[1]Program in Clinical Pharmacology, Division of Cardiology, Department of Medicine, University of California San Fransisco, San Francisco, CA; [2]Clinical Pharmacology and Toxicology, Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland; [3]Center for Tobacco Control Research and Education (CTCRE), University of California San Fransisco, San Francisco, CA; [4]Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada; [5]Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada; [6]Department of Psychiatry, University of Toronto, Toronto, ON, Canada; [7]Department of Bioengineering and Therapeutic Sciences, University of California San Fransisco, San Francisco, CA

Corresponding Author: Natalie Nardone, PhD, 1001 Potrero Ave, San Francisco, CA, 94110, USA. Tel: 415-514-1450; Fax: 415-502-4948; E-mail: natalie.nardone@ucsf.edu

*These authors contributed equally to this work.

Abstract

Introduction: The rate of nicotine metabolism, estimated by the nicotine metabolite ratio (NMR), is an important determinant of tobacco dependence. This study investigated the effect of NMR on smoking behavior due to nicotine reinforcement during ad libitum smoking.

Aims and Methods: As part of a larger study, participants were stratified based on saliva NMR as fast and slow metabolizers. After smoking a cigarette and measuring nicotine blood concentrations, participants smoked as desired over a 90-minute period. Analysis included time to first cigarette, total number of cigarettes, total number of puffs, and weight of tobacco consumed.

Results: Sixty-one (48%) participants were fast metabolizers and 66 (52%) slow metabolizers by NMR. No significant differences were found regarding the smoking topography variables by NMR. Normal metabolizers by genotype (n = 79) had a shorter time to first cigarette than reduced metabolizers (n = 39; p = .032). Blacks smoked fewer cigarettes (p = .008) and took fewer total puffs (p = .002) compared with Whites. Among Whites, fast metabolizers by NMR had a shorter time to first cigarette compared with slow metabolizers (p = .014). Among fast metabolizers, Whites had, compared with Blacks, shorter latency to first cigarette (p = .003) and higher number of total puffs (p = .014) and cigarettes smoked (p = .014). Baseline cigarettes per day and nicotine elimination half-life significantly predicted topography outcomes.

Conclusions: Saliva NMR did not predict cigarette reinforcement during a relatively brief period of ad libitum smoking. Differences were seen by race, with White fast metabolizers by NMR having shorter time to first cigarettes compared with slow metabolizers.

Implications: After a 90-minute period of nicotine abstinence, NMR was not significantly associated with smoking reinforcement. Slow and fast metabolizers had similar time to first cigarette, number of cigarettes smoked, total number of puffs, and tobacco consumed; however, within-race differences show that within Whites, fast metabolizers had a faster time to first cigarette than slow metabolizers.

Introduction

Variability in smoking behavior is attributable, in part, to genetic variation in the rate of nicotine metabolism.[1] The hepatic cytochrome P450 enzyme CYP2A6 converts approximately 80% of nicotine to its inactive metabolite cotinine, which is further metabolized by the same enzyme to 3′-hydroxycotinine.[2] The clearance of nicotine is also affected by nongenetic environmental and hormonal factors that can induce (eg, estrogen) or inhibit (eg, grapefruit) the activity of CYP2A6.[1] The 3′-hydroxycotinine/cotinine ratio, also called the nicotine metabolite ratio (NMR), accounts for both genetic and nongenetic influences of CYP2A6 activity and provides a noninvasive marker for the rate of nicotine clearance.[3]

The rate of nicotine metabolism is an important determinant of smoking behavior and nicotine dependence. In many (but not all) studies, NMR has been associated with greater dependence and lower rates of quitting,[4–6] more intense withdrawal and craving symptoms,[7,8] and greater reward after administration of nicotine intravenously.[7] Additionally, faster metabolizers adjust their nicotine intake by smoking more cigarettes per day (CPD)[9] and inhaling a greater puff volume during ad libitum smoking.[10]

Nicotine metabolism and smoking behavior differ across race.[11,12] On average Black smokers metabolize nicotine more slowly,[1,5,13] and smoke fewer CPD,[14] but report greater difficulty quitting than Whites,[12] suggesting higher dependence, contrary to what one would expect. Blacks take in more nicotine per cigarette, consistent with more intense smoking.[13] The intake of nicotine, assessed by urine total nicotine equivalents (TNE), is determined in part by CYP2A6 activity in Blacks as well as Whites.[15] Blacks appear to be less likely to titrate their nicotine intake based on NMR compared with Whites,[16] thus indicating possible differences in underlying smoking behavior mechanisms.

The primary aim of this study was to investigate the effect of NMR on smoking behavior during an ad libitum smoking period of 90 minutes that followed a single cigarette after 6 hours of abstinence. Our hypothesis was that fast metabolizers based on NMR, compared with slow metabolizers, would have a shorter latency to smoke and would smoke more cigarettes and/or consume more tobacco during the ad libitum period. In exploratory analyses, we examined possible between and within racial differences of these relationships and compared NMR to *CYP2A6* genotype as biomarker of nicotine reinforcement. We also explored other factors associated with smoking behavior including sex, CPD, dependence via the Fagerstrom test for cigarette dependence, usual time to first cigarette, plasma nicotine level following last cigarette, TNE, and nicotine elimination half-life.

Methods

The results of the ad libitum smoking session presented here were part of a study with prospectively stratified design investigating the NMR effect on withdrawal/craving and reward after smoking abstinence. As part of the parent study, participants in a sequestered, hospital environment smoked two "loading cigarettes" with a standardized puffing protocol after 12 hours overnight abstinence, and then after 6 hours abstinence a third "reward cigarette" of their own brand in their usual way, immediately followed by a period of ad libitum smoking. The results of the first part of the study are described in another publication.[8] Here, we describe the 90-minute period of monitored ad libitum smoking starting directly after the "reward cigarette." All participants completed both parts of the study.

Participants and Recruitment

Recruitment procedures are described in detail in the primary paper.[8] Based on prior studies,[17] the saliva NMR cut points were ≤0.20 and ≥0.37 in Blacks, and ≤0.26 and ≥0.45 in Whites. Saliva and plasma NMR are highly correlated and are stable within-individuals over time.[18] Participants with NMR values between those cut points were not included in the study.

Ad Libitum Smoking Procedures

Starting at approximately 4 p.m. and after collecting a blood sample for plasma nicotine concentrations following the "reward cigarette," participants were given a pack of their usual brand cigarettes and instructed to smoke as desired over a 90-minute period. We recorded smoking behaviors using a high definition video camera as reported in previous studies.[19,20] One of the usual brand cigarettes was weighed before distributing to participants, and cigarette butts were weighed upon completion of the 90-minute period. This was to capture variability in the amount of each cigarette consumed, as this is a strong predictor of smoke generation.[21] At the end of the 90-minute period, participants were discharged from the hospital unit.

Laboratory Methods

The analytical methods used for saliva 3′-hydroxycotinine and cotinine, genotyping and baseline urine TNE (normalized by urine creatinine) are described in detail in the primary paper.[8] For analyses using the *CYP2A6* genotype, variant alleles associated with slow or intermediate activity were grouped together as reduced metabolizers.

Data Analysis

Videos were analyzed for time to first cigarette, total number of cigarettes smoked, and total number of puffs. The estimated amount of tobacco consumed was calculated by the [total number of cigarettes smoked] × [average amount of tobacco consumed per cigarette], where the latter was computed as the weight of the cigarette prior to smoking minus the average post-smoking (butt) weight per cigarette smoked. Nicotine elimination half-lives were estimated within the parent study.[8]

Our sample size was based on a power analysis of change in withdrawal scores utilizing a three-way analysis of variance, as used for the primary paper.[8] We did not power the study specifically to investigate the ad libitum period data. Numerical data are presented as arithmetic mean and *SD* if normally distributed or median and range if not normally distributed, and nominal data as proportion (%). Differences were tested using the chi-square test for categorical variables, the *t*-test for normally distributed continuous variables, and the Mann–Whitney test for nonparametric variables. The correlations between smoking topography parameters and other variables were investigated using the Pearson and the Spearman's nonparametric correlation test for normally and not normally distributed data, respectively. Skewed variables were log transformed including: time to first cigarette, total number of puffs, total number of cigarettes, and amount of tobacco consumed. A multiple regression analysis was performed with three selected predictor variables (CPD, nicotine elimination half-life, and plasma nicotine levels) based on biological plausibility and a significant univariate correlation with topography outcomes. These predictors were entered in the regression model simultaneously as continuous variables predicting the outcomes of time to first cigarette, total number of puffs, total number of cigarettes, and amount of tobacco consumed. In total, four models were conducted.

A $p < .05$ was considered statistically significant for all analyses. Analyses were conducted using SPSS statistical software (IBM SPSS Statistics 23.0).

Results

Among the 137 participants who completed the ad libitum session, 10 participants smoked no cigarettes during the 90 minutes and were not included in further analyses. The baseline demographics and smoking history of the remaining 127 participants are provided in Table 1. There were more women among the fast compared with the slow metabolizers ($p = .042$) and Blacks smoked fewer CPD than Whites ($p = .004$) and had lower NMR ($p = .006$) and baseline TNE ($p = .008$). Sixty-one (48%) participants were fast metabolizers and 66 (52%) slow metabolizers by NMR, and 79 (62%) normal metabolizers and 39 (31%) reduced metabolizers by *CYP2A6* genotype, while no genotype data were available in nine cases (7%). Thirty (77%) of the genetically reduced metabolizers were slow, whereas 47 (59%) of the normal metabolizers were fast by NMR.

The unadjusted smoking topography variables grouped by NMR, *CYP2A6* genotype, and race as well as comparisons within race by NMR and within NMR by race are shown in Table 2. No significant differences were found between fast and slow metabolizers by NMR, whereas normal metabolizers by genotype had a shorter latency to smoking the first cigarette compared to reduced metabolizers ($p = .032$). Blacks smoked fewer cigarettes ($p = .008$) and total puffs ($p = .002$). Significant differences within NMR and by race were seen only among the fast metabolizers, with Whites having shorter latency to the first cigarette ($p = .003$) and higher number of total puffs ($p = .014$) and cigarettes smoked ($p = .014$). Within race, the only significant difference was seen among Whites, with fast metabolizers by NMR having, compared with slow metabolizers, a shorter time to first cigarette ($p = .014$).

Univariate regression results are shown in Supplementary Table 1, with CPD emerging with the most consistent, significant relationship to our outcomes. In our multiple regression analysis, CPD was a significant, positive predictor for all outcomes; time to first cigarette ($p = .008$), number of cigarettes smoked ($p < .001$), total number of puffs ($p = .009$), and total tobacco consumed ($p = .001$). Nicotine elimination half-life was a negative predictor for number of cigarettes smoked ($p = .014$), total number of puffs ($p < .001$), and total tobacco consumed ($p = .006$). Plasma nicotine levels after smoking a cigarette were significantly associated with total tobacco consumed during ad lib use ($p = .028$).

Discussion

Overall, NMR had no significant effect on smoking topography parameters during a 90-minutes ad libitum smoking period. Thus, our general hypothesis that fast metabolizers by NMR, compared with slow metabolizers, will smoke more cigarettes and have a shorter latency to smoke was not confirmed. The *CYP2A6* genotype was associated with a significant effect on the time to first cigarette. In our within-race analysis, in Whites, fast metabolizers by NMR had, compared with slow metabolizers, significantly shorter time to first cigarette, and among fast metabolizers, Whites had shorter time to first cigarette, smoked more cigarettes and took more puffs than Blacks.

Overall, Whites smoked more cigarettes and consumed more tobacco than Blacks. The difference in cigarettes and puffs was

Table 1. Baseline Demographics and Smoking History Information (Mean [*SD*], Median [Range], or *n* [%])

	All ($n = 127$)	Fast by NMR ($n = 61$)	Slow by NMR ($n = 66$)	*p*	Whites ($n = 94$)	Blacks ($n = 33$)	*p*
Age (years; mean [*SD*])	36.1 (12.1)	37.6 (11.5)	34.7 (12.6)	.17	35.0 (12.1)	39.2 (11.8)	.087
Sex (female; *n* [%])	46 (36%)	28 (46%)	18 (27%)	**.042**	31 (33%)	15 (46%)	.21
Race (Blacks; *n* [%])	33 (26%)	14 (23%)	19 (29%)	.55	n.a.	n.a.	n.a.
FTCD (median [range])	5.0 (0–10.0)	5.0 (0–10.0)	5.0 (0–8.0)	.60	5.0 (0–10.0)	4.0 (0–8.0)	.23
CPD (median [range])	12.0 (5.0–40.0)	13.0 (5.0–35.0)	12.0 (5.0–40.0)	.46	13.0 (5.0–40.0)	10.0 (5.0–20.0)	**.004**
Saliva NMR (mean [*SD*])	0.25 (0.06–1.32)	0.55 (0.37–1.32)	0.16 (0.06–0.27)	**<.001**	0.36 (0.07–1.32)	0.19 (0.06–0.57)	**.006**
CYP2A6 genotype (normal; *n* [%])	79 (62%)	47 (77%)	32 (48%)	**<.001**	60 (64%)	19 (58%)	.52
Nicotine elimination half-life (min; median [range])	113.9 (41.1–272.4)	93.6 (41.1–272.4)	133.5 (50.3–255.5)	**<.001**	107.6 (41.1–272.4)	152.3 (50.3–255.5)	**<.001**
Plasma nicotine (ng/mL; median [range])	17.3 (3.6–51.8)	16.2 (3.6–51.8)	17.6 (6.1–51.0)	.098	17.3 (3.6–51.0)	17.5 (8.3–51.8)	.67
Baseline creatinine-corrected urine TNE (nmol/mg; median [range])	57.0 (0.9–195.5)	57.2 (0.9–173.8)	56.8 (1.0–195.5)	.84	66.2 (1.0–195.5)	37.1 (0.9–116.2)	**.008**

CPD = cigarettes per day; FTCD = Fagerstrom test for cigarette dependence; NMR = nicotine metabolite ratio; TNE = total nicotine equivalents. Bold *p* values indicate significant differences between fast and slow by NMR and between Whites and Blacks.

Table 2. Smoking Topography Variables Grouped by NMR, *CYP2A6* Genotype and Race, Within Race by NMR, and Within NMR by Race (Median [Range])

		Time to first cigarette (min)	Number of total cigarettes smoked	Number of total puffs	Total tobacco consumed (g)
NMR	Fast (n = 61)	26.0 (8–82)	2.0 (1–5)	26.0 (8–129)	1.31 (0.20–3.65)
	Slow (n = 66)	30.5 (10–67)	2.0 (1–5)	25.5 (7–111)	1.26 (0.37–3.80)
	p	.13	.51	.27	.62
Genotype	Normal (n = 79)	25.0 (8–66)	2.0 (1–5)	27.0 (7–129)	1.33 (0.20–3.80)
	Reduced (n = 39)	31.0 (11–74)	2.0 (1–4)	25.0 (8–51)	1.22 (0.37–2.22)
	p	**.032**	.17	.08	.12
Race	Whites (n = 94)	26.0 (8–82)	2.0 (1–5)	27.5 (8–129)	1.30 (0.37–3.80)
	Blacks (n = 33)	31.0 (11–66)	1.0 (1–3)	17.0 (7–53)	0.90 (0.20–2.27)
	p	.08	**.008**	**.002**	.08
Fast NMR	Whites (n = 47)	22.0 (8–82)	2.0 (1–5)	27.0 (9–129)	1.38 (0.45–3.65)
	Black (n = 14)	36.5 (21–51)	1.5 (1–3)	18.5 (8–50)	1.04 (0.20–2.14)
	p	**.003**	**.014**	**.014**	.09
Slow NMR	Whites (n = 47)	31.0 (10–67)	2.0 (1–5)	28.0 (8–111)	1.28 (0.37–3.80)
	Black (n = 19)	30.0 (11–66)	1.0 (1–3)	15.0 (7–53)	0.82 (0.52–2.27)
	p	.62	.22	.09	.54
Whites	Fast NMR (n = 47)	22.0 (8–82)	2.0 (1–5)	27.0 (9–129)	1.38 (0.45–3.65)
	Slow NMR (n = 47)	31.0 (10–67)	2.0 (1–5)	28.0 (8–111)	1.28 (0.37–3.80)
	p	**.014**	.33	.25	.45
Blacks	Fast NMR (n = 14)	36.5 (21–51)	1.5 (1–3)	18.5 (8–50)	1.04 (0.20–2.14)
	Slow NMR (n = 19)	30.0 (11–66)	1.0 (1–3)	15.0 (7–53)	0.82 (0.52–2.27)
	p	.20	.68	.73	.46

NMR = nicotine metabolite ratio. Bold p values indicate significant differences.

significant when looking at the whole study population and in fast metabolizers by NMR, with the same numerical trend in slow metabolizers. Other studies have also observed that Blacks smoke fewer cigarettes[14,22] and titrate their nicotine intake based on NMR less compared to Whites.[16] At the same time, Black smokers have higher increases in carbon monoxide levels post-smoking[23] and take in more nicotine per cigarette,[13] indicative of more intensive smoking. In our study, Blacks smoked significantly fewer cigarettes and puffs compared to Whites, but there was no significant difference regarding the consumed tobacco amount, which is consistent with more intense smoking in Blacks compared to Whites. Thus, CPD alone is not as a useful indicator of nicotine self-administration in Black as in White smokers.

CPD was positively associated with all of our topography outcomes. This might be expected as the higher one's CPD, the more likely they are to smoke at shorter time intervals and the more cigarettes they will smoke. Participants with long nicotine half-lives smoked fewer cigarettes and consumed less tobacco, consistent with greater persistence of nicotine in the body over time and less need to self-administer nicotine to maintained desired effects. Nicotine half-life was a more significant predictor than nicotine levels following the standardized cigarette, showing the importance of metabolism to smoking behavior. Of note is that nicotine half-life predicted outcomes when no significant differences were found by NMR. The likely explanation for the discrepancy in our study is that NMR is a less precise measure of nicotine clearance than nicotine half-life. It is unclear why the genotype should predict time to first cigarette where overall the phenotypic marker NMR does not, although a similar significant NMR effect was seen among White smokers.

Limitations of our study include inadequate power to detect an effect of NMR. A univariate analysis of NMR's observed influence on the four topography outcomes demonstrated low power, all <0.70. We had a relatively small number of Blacks and women. There may have been a confounding effect of provision of a "reward cigarette" preceding ad libitum smoking. Additionally, we did not measure withdrawal/craving symptoms, which may have elucidated our findings.

In conclusion, we found that saliva NMR did not predict nicotine reinforcement during a relative brief ad libitum smoking period that followed 6 hours of not smoking followed by a single cigarette. However, nicotine half-life, which presumably mediates the NMR effect, did predict cigarette self-administration. In Whites, time to first cigarette was shorter in fast metabolizers, suggesting race differences in the effects of nicotine metabolism on cigarette reinforcement. Blacks smoked fewer cigarettes and took fewer puffs, but puffed more intensively. Baseline CPD was associated with the smoking topography parameters during the ad libitum smoking period.

Supplementary Material

Supplementary data are available at *Nicotine and Tobacco Research* online.

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Declaration of Interests

Dr. Benowitz is a consultant to Pfizer and Achieve Life Sciences, companies that market or are developing smoking cessation medications, and has been a paid expert witness in litigation against tobacco companies. Dr. Tyndale has served as a paid consultant to Apotex, Quinn Emmanuel, and Ethismos, and has received unrestricted research funding from Pfizer as part of the Global Research Awards for Nicotine Dependence (GRAND), an independently reviewed competitive grants program.

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