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Relationship between skin melanin index and nicotine pharmacokinetics in African American smokers

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ABSTRACT

Background: Blacks bear a disproportionate burden of smoking-related diseases and experience greater difficulty quitting smoking than Whites. Nicotine has a high affinity for melanin, and it has been hypothesized that melanin levels might influence nicotine pharmacokinetics and enhance dependence. The aim of this study was to evaluate the hypothesis that melanin affects nicotine disposition kinetics in humans.

Methods: Forty-four Black participants were administered intravenous infusions of deuterium-labeled nicotine and cotinine. Plasma concentrations of nicotine and cotinine were measured, and pharmacokinetic parameters were estimated. The constitutive and facultative melanin indexes were measured using a dermaspectrophotometer.

Results: The median constitutive melanin index was 60.7 (32.8–134.7) and the median facultative melanin index 68.1 (38.6–127.1). The mean (± SD) nicotine elimination half-life was 136 min (± 33.5), clearance was 1237 mL/min (± 331), and V_{ss} was 204 L (± 66), or 2.6 L/kg (± 0.7). No evidence of significant differences was found in nicotine pharmacokinetic parameters by comparing participants in different melanin index quartiles (outliers with very high melanin index had similar pharmacokinetic values to others). Differences were not statistically significant when adjusted for age, BMI, sex and CYP2A6 genotype or the nicotine metabolite ratio (NMR), and no evidence of significant correlations were found between melanin (facultative or constitutive) and the pharmacokinetic parameters of nicotine or cotinine or tobacco dependence measures.

Conclusions: Based on our finding in this group of Black smokers, we could not confirm the hypothesis that melanin significantly affects nicotine disposition kinetics or measures of tobacco dependence.

1. Introduction

African Americans (Blacks) bear a disproportionate burden of smoking-related diseases (CDC, 2007) and experience greater difficulty quitting smoking than Whites (Kulak et al., 2016). Since nicotine, the primary addictive chemical that sustains tobacco dependence (Benowitz, 2010), has a high affinity for melanin (Uematsu et al., 1995), it has been hypothesized that melanin levels might influence nicotine pharmacokinetics and dependence.

Melanin, the primary source of skin and hair color, mainly includes the yellow-red pheomelanin and the black-brown eumelanin (Alaluf et al., 2001). Melanin can further be classified as genetically-determined constitutive melanin and facultative melanin, or "tan", comprised of constitutive plus melanin induced by exposure to ultraviolet radiation from the sun or other sources (Kollias et al., 1991).

Approximately 80% of nicotine is converted by the hepatic cytochrome P450 enzyme CYP2A6 to cotinine (COT), which is further metabolized by the same enzyme to 3′-hydroxycotinine (3HC) (Benowitz, 2009). There is wide variability in nicotine clearance, due to genetic, environmental and hormonal factors. The ratio of 3HC/COT,

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called the nicotine metabolite ratio (NMR), is a phenotypic biomarker for the rate of nicotine clearance (Dempsey et al., 2004).

Nicotine has a moderately large volume of distribution (2–3 L/kg), reflecting binding to various body tissues (Hukkanen et al., 2005). Several studies have shown accumulation of nicotine and carcinogenic tobacco-specific nitrosamines in melanin-containing animal tissues (Castonguay et al., 1985, 1984; Tjälve and Castonguay, 1983). It has been theorized that individuals with higher melanin concentrations may accumulate and then slowly release nicotine/nicotine-derived carcinogens, resulting in prolonged systemic exposure that could influence nicotine and nitrosamine pharmacokinetics, smoking behavior, and increase adverse health outcomes (Yerger and Malone, 2006). Furthermore, previous studies have found an association between skin melanin and cigarettes smoked per day (CPD) and Fagerström Test for Cigarette Dependence (FTCD) scores among Black smokers (King et al., 2009) and between skin melanin and cigarette consumption among Japanese women (Tamai et al., 2014).

Despite these reports, there has been no explicit investigation of the effect of skin melanin on nicotine pharmacokinetics. If melanin binds substantial amounts of nicotine and slowly releases it over time, this should be reflected in increased volume of distribution and half-life of nicotine. The aim of the present study was to evaluate the hypothesis that melanin affects nicotine disposition kinetics in Black smokers, a racial group with characteristically higher skin melanin levels, more severe nicotine dependence and higher risk of smoking-related adverse health outcomes.

2. Materials and methods

Forty-four Black smokers (self-identified as having four Black grandparents) were recruited from newspaper advertisements. Inclusion criteria were: aged 18–65 years, healthy based on medical history, physical exam, and blood exam, and expired carbon monoxide ≥8 ppm. Exclusion criteria included pregnancy/breast feeding, current alcohol/drug abuse, current use of smokeless tobacco, pipes or cigars, nicotine replacement therapy, and regular use of medications other than vitamins, oral contraceptives, hormone replacements, or aspirin.

At baseline, participants completed the FTCD, including time to first cigarette after waking (TFC) and CPD (Heatherton et al., 1991). Participants came to the General Clinical Research Center at San Francisco General Hospital in the morning, with instructions not to eat or use tobacco starting at 10 PM on the previous night, and refrain from grapefruit/grapefruit juice for 48 h prior to and during the study. At 8 AM, participants received a 30-minute intravenous infusion of deuterium-labelled nicotine (nicotine-3′,3′-d_2) and cotinine (cotinine-2,4,5,6-d_4), each dosed at 1.5 mg/kg/min. These compounds were synthesized in our laboratory as described previously (Benowitz and Jacob, 1994). Blood was collected at 10, 20, 30, 45, 60, 90 min and 2, 3, 4, 6, 8, 12, 16, 23, 47, and 71 h after dosing, and for CYP2A6 genotyping. A baseline sample was used for the calculation of NMR.

Constitutive (inner forearm) and facultative melanin (central area of forehead) were measured using the second-generation Dermaspectrophotometer DSM II ColorMeter (Cortex Technology, Hadsund, Denmark) (Diffey, 1983). For each measurement, the average of three readings was recorded.

Measurements of nicotine and metabolites in plasma were performed by gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry using previously published methods (Dempsey et al., 2004; Jacob et al., 1991, 2011). The limit of quantitation for nicotine (d_0, d_2) was 0.1 ng/mL and for cotinine (d_0, d_2, d_4) 1.0 ng/mL. CYP2A6 genotyping was performed using methods previously described (Wassenaar et al., 2016). Participants without variants (or with the duplication CYP2A6*1 × 2 variant) were characterized as normal metabolizers, while those with one or more decreased or loss of function variants (e.g., CYP2A6*9, CYP2A6*17) were grouped together as reduced metabolizers (Liakoni et al., 2018).

The measure of the rate of nicotine metabolism was total clearance of nicotine-d_2, determined as the dose divided by the area under the plasma nicotine concentration–time curve extrapolated to infinity ($AUC_{0\rightarrow\infty}$). Clearance of cotinine-d_4 was computed in a similar manner. Elimination half-lives were determined using Phoenix WinNonlin (Pharsight Corporation, St.Louis, MO). The volume of distribution at steady state (V_{ss}) was estimated as the mean residence time extrapolated to infinity multiplied by clearance.

Numerical data are presented as mean and standard deviation (± SD) if normally distributed or median and range if not normally distributed, nominal data as proportion (%). Melanin quartile 4 represents highest melanin levels. Differences were tested using the *t* test or one-way analysis of variance (ANOVA) for normally distributed variables and the Mann Whitney or Kruskal-Wallis test for not normally distributed variables with correlations using the Pearson and the Spearman's correlation tests for normally and not normally distributed data, respectively. Investigations included univariate general linear models with pharmacokinetic parameters as dependent variables (skewed values were first log-transformed) and constitutive or facultative melanin as independent variable. Variables that might influence the nicotine metabolism such as CYP2A6 genotype/NMR, sex (Benowitz et al., 2006a), age (Ho et al., 2009; Tanner and Tyndale, 2017), and body mass index (BMI) (Ho et al., 2009) were entered as covariates, with the exception of pharmacokinetic parameters with kilograms (kg) in their units for which BMI was not included. A $p < 0.05$ was considered statistically significant. Analyses were conducted using SPSS statistical software (IBM SPSS Statistics 23.0).

3. Results

The mean age was 33.2 years (± 9.8, range 20–60), mean BMI was 27.2 ± 4.8, and 27 participants (61.4%) were females. The median CPD was 12.5 (5–30), median TFC was 10 (1–60) minutes, and mean FTCD score was 5.4 ± 1.8. The median administered deuterium-labelled nicotine or cotinine dose was 3505 (1146–4050) μg. The median constitutive and facultative melanin indexes were 60.7 (32.8–134.7) and 68.1 (38.6–127.1), respectively. Omitting three participants with outlying melanin measurements (i.e., > 118) resulted in normal distribution of these variables with mean levels of 60.7 (± 12.3, range 32.8–87.8) and 67.5 (± 13.3, range 38.6–92.7) for constitutive and facultative melanin, respectively.

The median NMR was 0.32 (0.13–1.74), mean nicotine elimination half-life was 136 ± 33.5 min, mean nicotine clearance was 1237 ± 331 mL/min, or 15.9 ± 4.1 mL/min/kg, and mean nicotine V_{ss} was 204 ± 66 L, or 2.6 ± 0.67 L/kg. The mean cotinine elimination half-life was 1039 min (± 238), median cotinine clearance was 35.8 (14.6–82.7) mL/min, or 0.47 (0.22–1.53) mL/min/kg, and median cotinine V_{ss} was 48.3 (34.4–88.9) L, or 0.62 (0.43–1.0) L/kg.

Table 1 shows the nicotine and cotinine pharmacokinetic parameters and NMR in the four constitutive (Q_1 = 52.53, Q_2 = 60.68, Q_3 = 71.99) and facultative melanin quartiles (Q_1 = 59.40, Q_2 = 68.07, Q_3 = 79.57); no evidence of significant differences was found by melanin quartile. The values of nicotine and cotinine pharmacokinetic parameters and NMR of the three outliers with high melanin index were very similar to the rest of the participants (Table 2).

Twenty-six (59%) participants were genetically normal and 18 (41%) reduced metabolizers. The median constitutive melanin index among normal metabolizers was 65.9 (37.1–134.7) compared to 58.3 (32.8–125.1) among reduced metabolizers ($p = 0.22$). Regarding facultative melanin, the median values were 75.3 (38.6–122.9) and 67.2 (44.6–127.1) among normal and reduced metabolizers, respectively ($p = 0.92$).

A significant correlation ($r = 0.76$, $p < 0.001$) was found between facultative and constitutive melanin indices but not between melanin index (constitutive or facultative) and the various pharmacokinetic parameters, CPD, TFC, and FTCD (all $p > 0.05$). Specifically, there was

Table 1
NMR, nicotine and cotinine pharmacokinetic parameters by melanin index quartiles (Q) (mean (SD) or median (range)).

	NMR	Nicotine elimination half-life (min)	Nicotine clearance (mL/min/kg)	Nicotine Vss (L/kg)	Cotinine elimination half-life (min)	Cotinine clearance (mL/min/kg)	Cotinine Vss (L/kg)
A. Constitutive melanin							
Q1	0.29 (0.13-0.71)	134.6 (30.0)	15.9 (3.3)	2.54 (0.54)	1081.4 (182.7)	0.46 (0.31-0.81)	0.72 (0.53-1.00)
Q2	0.27 (0.14-0.56)	137.7 (27.4)	15.9 (3.0)	2.72 (0.46)	1041.8 (211.9)	0.39 (0.28-0.57)	0.58 (0.50-0.65)
Q3	0.35 (0.20-1.74)	133.0 (44.3)	16.0 (5.3)	2.54 (1.09)	1018.6 (267.7)	0.54 (0.22-1.53)	0.70 (0.43-0.91)
Q4	0.31 (0.20-0.74)	135.9 (34.0)	16.4 (4.9)	2.64 (0.44)	1013.3 (300.3)	0.50 (0.22-0.84)	0.61 (0.50-0.95)
p value	0.59	0.99	0.99	0.91	0.91	0.24	0.09
B. Facultative melanin							
Q1	0.38 (0.15-0.71)	126.3 (31.2)	16.8 (3.5)	2.44 (0.28)	1025.9 (217.1)	0.48 (0.28-0.81)	0.61 (0.57-1.00)
Q2	0.29 (0.14-0.51)	138.0 (32.6)	16.3 (3.4)	2.86 (0.55)	1100.6 (185.5)	0.37 (0.31-0.69)	0.59 (0.50-0.91)
Q3	0.35 (0.20-1.74)	130.6 (38.9)	16.3 (5.0)	2.64 (1.07)	895.7 (230.3)	0.57 (0.30-1.53)	0.66 (0.58-0.92)
Q4	0.27 (0.13-0.74)	146.3 (31.3)	14.9 (4.6)	2.52 (0.51)	1131.4 (269.6)	0.41 (0.22-0.81)	0.60 (0.43-0.95)
p value	0.24	0.53	0.76	0.50	0.09	0.06	0.46

Note: NMR = nicotine metabolite ratio; V_{ss} = volume of distribution at steady state.

no significant correlation between either melanin index (constitutive or facultative) and the volume of distribution ($r = 0.06$, $p = 0.69$ and $r = 0.05$, $p = 0.73$, respectively) or half-life of nicotine ($r = -0.03$, $p = 0.84$ and $r = 0.11$, $p = 0.49$, respectively).

In the general linear models, neither constitutive nor facultative melanin was significantly associated with nicotine or cotinine pharmacokinetic parameters before and after adjusting for age, BMI, sex, and NMR or genotype. The relationships between melanin indices and PK parameters did not differ across sex. In the same models, nicotine half-life and clearance and V_{ss} normalized by body weight were not significantly different across sex. For cotinine, V_{ss} normalized by body weight was significantly lower in females compared to males, but half-life and clearance normalized by body weight were not significantly different.

4. Discussion

Although melanin binding of nicotine has been proposed as an underlying explanation for greater tobacco dependence of Blacks compared to Whites, we found no evidence to support the hypothesis that skin melanin content influences nicotine pharmacokinetic parameters or tobacco dependence measures among Black smokers. Our analysis included adjustments for the effects of age, BMI, sex, and genotype or NMR. Significant sex differences in nicotine/cotinine PK, which are thought to be driven by sex hormones, have been shown previously (Benowitz et al., 2006a). However, as expected, we saw no influence of sex on the relationships between melanin indices and nicotine/cotinine PK parameters.

Previous human studies reported significant intra-oral mucosal melanin pigmentation in smokers (Axéll and Hedin, 1982; Sarswathi et al., 2003), an association between smoking and darker skin color (Tamai et al., 2014), and higher nicotine accumulation in black compared to white hair from the same subject (Uematsu et al., 1995). Hair melanin content is among the proposed factors influencing the hair nicotine concentration of newborns (Pichini et al., 2003). Although these observations might indicate that nicotine plays a role in melanin formation or that it accumulates in melanin-containing tissues, we found no evidence of a significant increase in nicotine V_{ss} as a function of melanin index, which would be expected if a substantial nicotine amount was bound to melanin. The non-significant effect of melanin on nicotine pharmacokinetic parameters suggests that nicotine accumulation in skin was negligible rather than substantial. In addition, despite considerable evidence that nicotine binds to melanin, it has been suggested that this relationship may stem from irreversible covalent binding (Claffey et al., 2001; Dehn et al., 2001). Since only free drug acts on receptors, no alteration in the effects of nicotine would be expected in this case, regardless of the amount bound.

A previous pharmacokinetic study by our research group in predominantly White smokers (Benowitz et al., 2006b), which followed a similar method of nicotine intravenous administration, reported slightly lower values of nicotine clearance (11.7–18.8 mL/min/kg) and longer nicotine elimination half-life (113–169 min) but similar nicotine V_{ss} estimates (2.3–2.5 L/kg) to the current study. This also supports the conclusion that there is no substantive increase of the V_{ss} due to higher skin melanin levels.

Previous studies reported a relationship between facultative melanin and cigarette consumption and nicotine dependence among Black smokers (King et al., 2009) and between tanning capacity (i.e., difference between facultative and constitutive melanin) and morning smoking urgency (King et al., 2018). Although the larger sample size and differences in dependence level (lower CPD and longer TFC in the current study) might contribute to the different results, the previous studies did not investigate disposition kinetics of nicotine. Also, the data were collected previously during summer in the Northeast of the United States, where differences between facultative and constitutive melanin might be more pronounced compared to the Northern

Table 2
NMR, nicotine and cotinine pharmacokinetic parameters of the high melanin index outliers and the rest of the participants (mean (SD) or median (range)).

	High melanin index outliers (n = 3)	Participants with normally distributed melanin index (n = 41)
NMR	0.37 (0.31-0.74)	0.30 (0.13-1.74)
Nicotine elimination half-life (min)	118.2 (20.7)	136.5 (33.9)
Nicotine clearance (mL/min)	1571.2 (372.1)	1215.2 (315.8)
Nicotine clearance (mL/min/kg)	19.6 (5.9)	15.8 (3.9)
Nicotine V_{ss} (L)	209.3 (67.3)	204.0 (66.7)
Nicotine V_{ss} (L/kg)	2.56 (0.7)	2.62 (0.7)
Cotinine elimination half-life (min)	967.7 (108.2)	1044.0 (245.4)
Cotinine clearance (mL/min)	35.81 (33.4-56.7)	35.7 (14.6-82.7)
Cotinine clearance (mL/min/kg)	0.40 (0.39-0.81)	0.48 (0.22-1.53)
Cotinine V_{ss} (L)	54.0 (41.2-66.1)	48.2 (34.4-88.9)
Cotinine V_{ss} (L/kg)	0.59 (0.50-0.95)	0.62 (0.43-1.00)

California where the current study was performed.

One limitation of our study is the inclusion of Black smokers only, thus not allowing comparisons with a wider range of skin melanin levels. However, the range was fairly wide among our participants. Furthermore, although the same device has been used in several previous studies to estimate skin melanin content, limitations include any possible measurement inaccuracies. Our study is one of the largest detailed pharmacokinetic studies in Black smokers and the first investigating the relationship of melanin index and nicotine disposition kinetics in humans. Thus, our findings, albeit negative, can be used to guide future investigations in this area.

5. Conclusions

Our study in Black smokers found no evidence of significant differences in nicotine pharmacokinetics based on melanin index and no evidence of significant correlations between pharmacokinetic parameters of nicotine or cotinine, or tobacco dependence measures and melanin levels. Furthermore, the nicotine V_{ss} was quite similar to that of White smokers in an earlier study using similar methods, suggesting that melanin does not have a substantial effect on nicotine disposition.

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Contributors

E. Liakoni and G. St. Helen contributed equally to this manuscript as co-first authors. Dempsey, Tyndale and Benowitz were responsible for the study concept and design. Benowitz and Dempsey oversaw the study design and recruitment. Liakoni, St. Helen, Jacob, Benowitz and Tyndale were responsible for acquisition of data and analysis of results. Liakoni and St. Helen were responsible for the first draft of the article. All authors critically reviewed content and approved final version of the article for publication.

Declaration of Competing Interest

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 paid consultant to pharmaceutical companies on unrelated topics. The other authors have no conflicts to disclose.

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