0022-3565/99/2913-1196$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 291:1196–1203, 1999

Vol. 291, No. 3
Printed in U.S.A.

Ethnic Differences in *N*-Glucuronidation of Nicotine and Cotinine[1]

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Accepted for publication August 2, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

We previously reported that the metabolism of cotinine, the proximate metabolite of nicotine, is significantly slower in black than in white cigarette smokers. To understand why the metabolism of nicotine and cotinine might differ between blacks and whites, we studied the pattern of nicotine metabolism in blacks and whites. One hundred eight healthy smokers (51 blacks and 57 whites), of similar age, gender distribution, and smoking history, received an i.v. infusion of deuterium-labeled nicotine and cotinine. The clearance of cotinine, the fractional conversion of nicotine to cotinine, and the metabolic clearance of nicotine to cotinine were significantly lower in blacks than in whites. Blacks excreted significantly less nicotine as nicotine-*N*-glucuronide and less cotinine as cotinine-*N*-glucuronide than whites, but there was no difference in the excretion of 3′-hydroxycotinine-*O*-glucuronide. Nicotine and cotinine glucuronidation appeared to be polymorphic, with evidence of slow and fast *N*-glucuronide formers among blacks but was unimodal with fast conjugators only among whites. Other findings of note included the demonstration of a significant correlation between the distribution volumes of nicotine and cotinine with lean body mass: there was a smaller distribution volume and a shorter half-life for cotinine in women than in men and a smaller volume of distribution of cotinine in blacks than in whites. We conclude that the metabolism of cotinine is slower in blacks than in whites because of both slower oxidative metabolism of nicotine to cotinine (presumably via cytochrome P-450 2A6) and slower *N*-glucuronidation. Ethnic differences in the metabolism of other drugs undergoing *N*-glucuronidation should be studied.

We recently reported that the metabolism of cotinine, the proximate metabolite of nicotine, is significantly slower in black than in white cigarette smokers (Perez-Stable et al., 1998). Nicotine clearance was on average lower in black smokers as well, but the difference was not statistically significant.

Nicotine is metabolized primarily by C-oxidation to cotinine but is also metabolized by *N*-oxidation to nicotine *N*′-oxide and by *N*-glucuronidation (Fig. 1) (Benowitz et al., 1994). Cotinine is hydroxylated to *trans*-3′-hydroxycotinine and is metabolized by *N*-oxidation to cotinine *N*-oxide. Cotinine is also metabolized by *N*-glucuronidation, and 3′-hydroxycotinine is metabolized by *O*-glucuronidation.

To understand why the metabolism of nicotine and cotinine might differ in blacks and whites, we studied the pattern of nicotine metabolite excretion in blacks and whites who received i.v. infusions of deuterium-labeled nicotine and cotinine.

Materials and Methods

Subjects. One hundred eight volunteer cigarette smokers were recruited through advertisements in local newspapers and from local community colleges. The subjects included those whose clearance data have been previously reported (Perez-Stable et al., 1998), plus an additional 29 subjects studied subsequently. The demographic and smoking characteristics of the subjects are given in Table 1. Of the group, 37% were women (average age, 34 years). By self-reports, the subjects smoked an average of 19 cigarettes per day and had smoked for an average of 18 years. Eligibility criteria included 1) being in good health on the basis of history, physical examination, electrocardiogram, and blood chemistries; liver and kidney function

Received for publication April 27, 1999.
[1] This work was supported by State of California Tobacco-Related Disease Research Program Grant 1RT-0521, United States Public Health Service Grants DA02277 and DA01696 awarded by the National Institute on Drug Abuse, Grants CA39260 and CA32389 awarded by the National Cancer Institute, Grant HS07373 awarded by the Agency for Health Care Policy and Research, and Grant 1P30-AG15272 awarded by the National Institute on Aging, the National Institute of Nursing Research, and the Office of Research on Minority Health and carried out in part at the General Clinical Research Center at San Francisco General Hospital with support of the Division of Research Resources, National Institutes of Health (Grant RR00083). E.J.P.-S. was a Henry J. Kaiser Family Foundation Faculty Scholar in General Medicine during the completion of the study.

ABBREVIATIONS: CL, clearance; AUC, area under the plasma concentration-time curve; CYP, cytochrome P-450; f, fractional conversion of nicotine to cotinine.

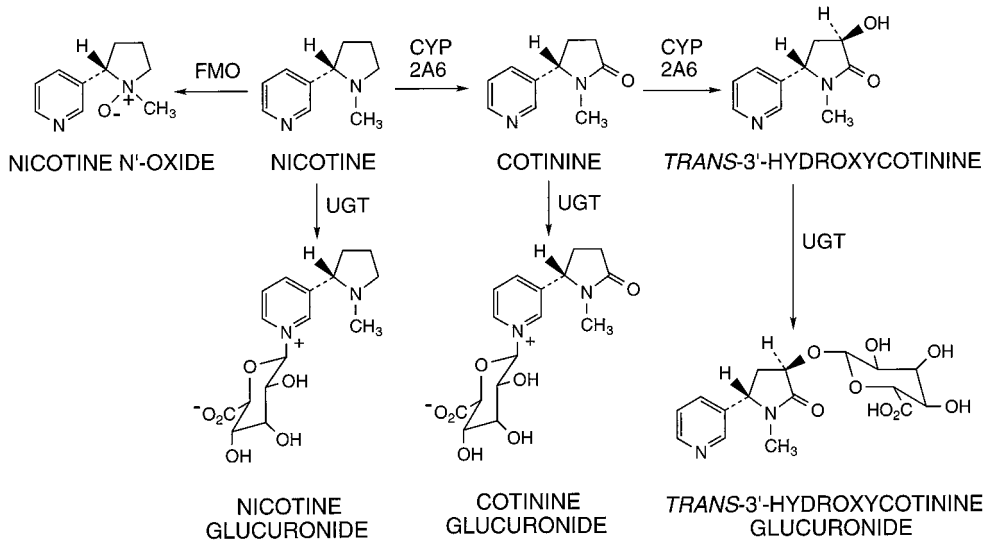

Fig. 1. Nicotine metabolic pathways and chemical structures of nicotine, cotinine, *trans*-3′-hydroxycotinine, and their glucuronide conjugates. UGT isoenzymes involved in nicotine metabolism have not yet been identified.

TABLE 1
Characteristics of subjects
FTC, U.S. Federal Trade Commission test method for cigarette yield.

	African-Americans (n = 51)		Caucasians (n = 57)	
Age	33.5	(31.0–36.0)	35.1	(32.2–38.1)
Female (%)	37	(24–50)	37	(25–49)
Cigarettes per day (CPD)	17.9	(14.8–21.0)	20.4	(17.2–23.6)
No. of years smoking	15.9	(13.4–18.4)	18.9	(15.4–22.3)
Menthol brands (%)	76*	(62–86)	9	(3–20)
FTC nicotine (mg)	1.10	(1.06–1.15)	1.08	(1.00–1.16)
FTC tar (mg)	16.0	(15.3–16.7)	15.8	(14.5–17.1)
Body weight (kg)	74.2	(70.6–77.8)	72.4	(68.7–76.2)
Lean body mass (kg)	56.2	(52.9–59.5)	55.6	(52.2–59.1)
Screening plasma cotinine (ng/ml)	250	(202–298)	237	(197–277)
Plasma cotinine/CPD (ng/ml)	17.0**	(13.7–20.2)	12.6	(10.6–14.5)

* $P < .001$, ** $P < .05$; mean (95% CI).

tests were normal in all subjects; 2) age of 21 to 64 years; 3) women had to be nonpregnant based on surgical sterilization or negative pregnancy test; and 4) self-identified as non-Latino, white, or black. Subjects were excluded if there was habitual use of any prescription medication, narcotic or sedative drug addiction, or chronic alcoholism. At the time of eligibility evaluation, blood was collected and analyzed for concentration of cotinine, the proximate metabolite of nicotine, which is often used as a marker of daily nicotine intake (Benowitz, 1996).

Experimental Procedure. Eligible subjects were asked to come to the Clinical Study Center at San Francisco General Hospital between 7 and 8 AM. Subjects were asked to abstain from food and cigarette smoking from 10 PM the previous night until arrival at the Clinical Study Center. Venous catheters were placed in both forearms. Subjects received a simultaneous infusion of deuterium-labeled nicotine-d_2 (3′,3′-dideuteronicotine) and cotinine-d_4 (2,4,5,6-tetradeuterocotinine) for 30 min. The syntheses of these deuterium-labeled compounds have been described previously (Jacob et al., 1988; Jacob and Benowitz, 1993). Subjects received 1.5 or 2.0 μg/kg/min for 30 min of nicotine-d_2 and cotinine-d_4 (calculated as the free base). During all infusions, subjects were monitored by continuous electrocardiogram and frequent blood pressure measurement. Two hours after the end of the infusion, subjects were given a light breakfast. Subjects were allowed to smoke their cigarettes freely after 1 PM.

Blood samples for measurement of nicotine and cotinine levels were collected at 0, 10, 20, 30, 45, 60, 90, 120, 240, 360, and 480 min and then at 24, 48, 72, and 96 h after the infusion, to include at least three half-lives for cotinine. Urine was collected during the infusion and up to 480 min after the start of the infusion. The study had the approval of the University of California, San Francisco, Committee on Human Research.

Body Fat Measurement. Body fat was estimated using the skinfold thickness technique (Gibson, 1990). This method involves measuring skinfold thickness at four sites: triceps, biceps, subscapular, and suprailiac regions. Skinfold thickness measurements were used to estimate body density, which is then used to calculate percent body fat.

Analysis of Nicotine and Metabolites in Biological Fluids. Nicotine and metabolite concentrations were determined by gas chromatography-mass spectrometry. Nicotine, nicotine-3′-3′-d_2, cotinine, cotinine-2,4,5,6-d_4, *trans*-3′-hydroxycotinine, *trans*-3′-hydroxycotinine-4′,4′-d_2, and *trans*-3′-hydroxycotinine-2,4,5,6-d_4 were determined according to published methods (Jacob et al., 1992, 1991).

Glucuronide-conjugated nicotine, cotinine, and *trans*-3′-hydroxycotinine were measured as the difference in the total concentration before and after hydrolysis by incubation with β-glucuronidase, as described previously (Benowitz et al., 1994). Enzymatic hydrolysis was performed using 6000 Sigma units of β-glucuronidase (EC 3.2.1.31; Helix Pomita, Fluka Chemical AG, Milwaukee, WI).

Pharmacokinetic Analysis. Pharmacokinetic parameters were estimated from blood concentration and urinary excretion data using model-independent methods as described previously (Benowitz and Jacob, 1994). Total clearance was computed as:

$$CL_{nic} = \frac{\text{Dose (nic-d}_2)}{\text{AUC (nic-d}_2)} \text{ and } CL_{cot} = \frac{\text{Dose (cot-d}_4)}{\text{AUC (cot-d}_4)}$$

where CL is clearance, AUC is area under the plasma concentration-time curve extrapolated to infinity, nic is nicotine, and cot is cotinine. Renal clearances were calculated as:

$$\frac{\text{Urinary excretion nic or cot}}{\text{AUC}}$$

based on urine collected and the AUC for the 8 h after the infusion while subjects were on the research ward. Nonrenal clearance was estimated as total minus renal clearance.

Fractional conversion of nicotine to cotinine (f) was estimated using blood levels of cotinine generated from infused nicotine and the clearance of cotinine itself, determined by infusion of cotinine, as follows:

$$f = \frac{AUC_{cot\text{-}d_2}}{Dose_{nic\text{-}d_2}} \times CL_{cot\text{-}d_4}$$

The metabolic clearance of nicotine via the cotinine pathway ($CL_{nic \rightarrow cot}$) was computed as $CL_{nic} \times f$.

Urine metabolite data were analyzed based on the urine collection over 8 h after the beginning of the nicotine and cotinine infusion. Urine metabolite concentrations were expressed as a fraction of the total nicotine plus metabolites recovered. The conjugates of nicotine and its metabolites were also analyzed as ratios compared with the unconjugated parent compound and as a fraction of parent compound plus the conjugate.

Data Analysis. Group differences were compared with the use of ANOVA (race × gender). Correlation between the volume of distribution and body weight was determined by linear regression.

The distribution of urine metabolite ratios is shown using frequency histograms and probit plots. In the probit plot, the correlation frequency is expressed as the probit, with each probit number representing the width of 1 S.D. in the distribution from the mean (probit value at the mean = 0; Jackson et al., 1989). Linearity in a probit plot suggests a normally distributed observation, whereas nonlinearity suggests groups with different distributional characteristics within a population. Histograms and probability plots were generated using the graphic procedures in SYSTAT (1997). The grouping of the black subjects by urine ratio was obtained with the method of K-means clustering. K-means clustering splits a set of subjects, in this case, the two groups, by maximizing the between-cluster variation to the within-cluster variation. The algorithm starts by picking the value farthest from the mean as a seed for the second cluster. Then cases are reassigned until the within-group sum of squares is minimized. The statistical program SYSTAT was used to perform this procedure.

Results

Subjects. Blacks and whites on average were of similar age, body weight, and lean body mass (Table 1). Blacks were much more likely to smoke menthol cigarettes (76%) than were whites (9%), although the average FTC (machine-determined) yields of nicotine and tar were similar. Blacks on average smoked fewer cigarettes per day but had higher average plasma cotinine concentrations during ad libitum smoking (P = NS). Blacks had significantly higher plasma cotinine normalized for cigarette consumption (17.0 ng/ml/cigarette) than whites (12.6 ng/ml/cigarette).

Disposition Kinetics. Blacks on average metabolized nicotine more slowly than whites, and blacks on average had a smaller volume of distribution of nicotine compared with whites, but the differences were not significant (Table 2). Blacks did convert significantly less nicotine to cotinine (81%) than whites (86%), and the clearance of nicotine via the cotinine pathway was significantly lower in blacks (14.8 ml/min/kg) than in whites (17.7 ml/min/kg).

Blacks had significantly lower total and nonrenal clearance of cotinine (0.57 and 0.49 ml/min/kg, respectively) compared with whites (0.76 and 0.72 ml/min/kg, respectively) but had greater renal clearance of cotinine (0.08 versus 0.05 ml/min/kg). The distribution volume of cotinine was significantly smaller in blacks (0.81 liters/kg) than in whites (0.93 liters/kg). Cotinine half-life was significantly longer in blacks (1085 min) than in whites (961 min). Significant gender differences were noted in the volumes of distribution of cotinine [men, 0.93 liters/kg, 95% confidence interval (CI), 0.88–0.99; women, 0.78 liters/kg, 95% CI, 074–0.83; $P < .01$] and for the half-life of cotinine (men, 1107 min, 95% CI, 1050–1165; women, 870 min, 95% CI, 772–769 min; $P < .001$).

Volumes of distribution of nicotine and cotinine were highly correlated with total body weight, although the correlation was less strong for nicotine than for cotinine. For nicotine, the correlation coefficient was 0.23 ($P < .05$) for V_{ss} versus total body weight and 0.36 for lean body mass ($P < .001$). For cotinine, the correlation coefficient between V_{ss} and total body mass was 0.53, and that for lean body mass was 0.67 (both $P < .0001$). There was no significant correlation between mass of adipose and the distribution volumes of either nicotine or cotinine.

Urine Metabolite Excretion. Metabolite excretion over the 8 h during and after infusion of nicotine, expressed as percent of total nicotine plus metabolite recovery, is shown in Table 3. Blacks excreted a significantly lower percentage of nicotine and metabolites as nicotine *N*-glucuronide and as cotinine *N*-glucuronide than did whites. This difference was seen both with deuterium-labeled nicotine (from the infusion) and nonlabeled nicotine (residual from prior cigarette smoking). Blacks excreted a significantly greater percentage of unlabeled alkaloids as cotinine compared with whites. The

TABLE 2

Disposition kinetics of deuterium-labeled nicotine and cotinine

CL_T, total plasma clearance; CL_{NR}, nonrenal clearance; CL_R, renal clearance; V_{ss}, steady-state volume of distribution; $T_{1/2}$, half-life; $f_{NIC \rightarrow COT}$, fractional conversion of nicotine to cotinine; $CL_{NIC \rightarrow COT}$, metabolic clearance of nicotine to cotinine.

	Nicotine		Cotinine	
	Blacks (n = 51)	Whites (n = 57)	Blacks (n = 51)	Whites (n = 57)
CL_T (ml/min/kg)	18.1 (16.7–19.5)	20.5 (18.0–22.9)	0.57** (0.53–0.62)	0.76 (0.67–0.85)
CL_{NR} (ml/min/kg)	17.5 (16.1–18.8)	20.0 (17.5–22.4)	0.49*** (0.44–0.53)	0.72 (0.63–0.80)
CL_R (ml/min/kg)	0.64 (0.46–0.83)	0.49 (0.35–0.63)	0.08*** (0.07–1.00)	0.05 (0.04–0.06)
V_{ss} (liters/kg)	2.9 (2.7–3.2)	3.3 (3.0–3.6)	0.81** (0.78–0.85)	0.93 (0.87–1.00)
$T_{1/2}$ (min)	131 (123–139)	136 (124–147)	1085* (1013–1156)	961 (881–1042)
$f_{NIC \rightarrow COT}$	0.81* (0.79–0.83)	0.86 (0.83–0.88)		
$CL_{NIC \rightarrow COT}$ (ml/min/kg)	14.8* (13.5–16.0)	17.7 (15.5–20.0)		

* $P < .05$, ** $P < .01$ for blacks versus whites, *** $P < .001$; mean (95% CI).

TABLE 3

Urine metabolite excretion as molar percentage of total nicotine plus metabolite recovery

Based on recovery in 8-h collections during and after infusion of labeled nicotine. The minor metabolites nicotine *N*′-oxide, cotinine-*N*-oxide, and nornicotine were not measured.

	Deuterium-Labeled		Unlabeled	
	Blacks (n = 51)	Whites (n = 57)	Blacks (n = 51)	Whites (n = 57)
Nicotine	28.3 (23.5–33.2)	22.7 (18.1–27.2)	3.6 (2.6–4.7)	4.1 (2.6–5.5)
Nicotine *N*-glucuronide	8.0** (6.5–9.6)	12.2 (10.5–13.9)	1.7* (1.2–2.2)	2.6 (2.0–3.3)
Cotinine	26.7 (23.9–29.4)	24.1 (22.2–26.0)	20.2*** (17.7–22.7)	13.8 (12.1–15.6)
Cotinine *N*-glucuronide	7.2*** (5.7–8.6)	12.8 (11.1–14.4)	12.1*** (9.9–14.4)	22.5 (20.2–24.9)
3′-Hydroxycotinine (3′HC)	22.2 (19.0–25.4)	21.6 (18.7–24.6)	49.0 (45.6–52.3)	44.2 (40.7–47.6)
3′HC-*O*-glucuronide	6.5 (5.2–7.8)	7.3 (5.9–8.7)	12.5 (10.6–14.2)	12.3 (10.6–14.0)

* P = .05, ** P < .01, *** P < .001; mean (95% CI).

excretion of 3′-hydroxycotinine or 3′-hydroxycotinine glucuronide was similar in blacks and whites.

Conjugation of Nicotine and Metabolites. The percentage of labeled nicotine excreted as the *N*-glucuronide was significantly lower in blacks (25%) than in whites (40%; P < .01; Table 4). Similarly, the percentage of labeled cotinine excreted as the *N*-glucuronide was lower in blacks (21%) than in whites (35%; P < .001). A similar observation was made for unlabeled metabolite excretion. No ethnic differences were seen in the conjugation of 3′-hydroxycotinine (Table 4).

Frequency histograms based on deuterium-labeled and unlabeled compounds were similar, but the polymorphisms were more clearly seen with the unlabeled compound data. There was an apparent trimodal distribution of nicotine *N*-glucuronide/nicotine urine ratios in blacks, which was not seen in whites (Fig. 2). There was an apparent bimodal distribution of urine ratios of cotinine *N*-glucuronide to cotinine in blacks but a unimodal distribution in whites (Fig. 3). Frequency histograms suggested a unimodal distribution of 3′-hydroxycotinine glucuronide/3′-hydroxycotinine ratios in blacks and whites (Fig. 4). Probit analysis of the nicotine-glucuronide/nicotine ratio data showed a difference in the population means (seen as a separation of the curves for blacks versus whites) and a discontinuity in linearity of the curve in blacks (Fig. 5). Probit plots of all whites and blacks grouped as rapid, slow, and very slow glucuronide formers indicate parallel curves where white and black rapid glucuronide formation looks similar, and there are two linear curves suggesting distinct populations for slower glucuronide formers among blacks. Probit analysis of the cotinine glucuronide/cotinine ratios (Fig. 6) shows a separation of curves consistent with the population mean difference in blacks and whites and shows a definite discontinuity. Further probit analysis suggests that there is a distinct normally distributed population of slow conjugators, whereas the remainder of blacks and whites appear to be members of a similarly distributed population (Fig. 6).

Discussion

This study provides novel findings in several areas. First, we expanded our recent observations on ethnic differences in nicotine and cotinine metabolism, providing data on a larger group of subjects. As previously reported (Perez-Stable et al., 1998), blacks metabolize cotinine significantly more slowly than do whites. We now find in the larger group that the percent conversion of nicotine to cotinine and the metabolic clearance of nicotine via the cotinine pathway are significantly greater in whites than in blacks. We have also observed gender differences, independent of ethnicity, in the volume of distribution of cotinine and half-life; both are significantly lower in women. Further analyses show that the volume of distribution of cotinine is primarily related to lean body mass.

Second, we report that the extent of *N*-glucuronidation of nicotine and cotinine is on average less in blacks than in

TABLE 4

Urine conjugate excretion ratios

Based on recovery in 8-h collections during and after infusion of labeled nicotine and cotinine. The minor metabolites nicotine *N*′-oxide, cotinine *N*-oxide, and nornicotine were not measured.

Ratio	Deuterium-Labeled		Unlabeled	
	Blacks (n = 51)	Whites (n = 57)	Blacks (n = 51)	Whites (n = 57)
$\frac{\text{NicG}}{\text{Nicotine}}$	0.44** (0.30–0.59)	1.11 (0.74–1.47)	0.94 (0.53–1.34)	1.52 (0.93–2.10)
$\frac{\text{NicG}}{\text{Nicotine + NicG}}$	0.25** (0.20–0.30)	0.40 (0.33–0.46)	0.35 (0.29–0.42)	0.46 (0.40–0.52)
$\frac{\text{CotG}}{\text{Cotinine}}$	0.31*** (0.24–0.39)	0.74 (0.37–1.11)	0.71*** (0.55–0.87)	1.90 (1.57–2.22)
$\frac{\text{CotG}}{\text{Cotinine + CotG}}$	0.21*** (0.17–0.25)	0.35 (0.31–0.38)	0.35*** (0.29–0.41)	0.61 (0.57–0.65)
$\frac{\text{3HCotG}}{\text{3HCotinine}}$	0.38 (0.26–0.51)	0.35 (0.30–0.40)	0.26 (0.22–0.31)	0.28 (0.24–0.33)
$\frac{\text{3HCotG}}{\text{3HCotinine + 3HCotG}}$	0.24 (0.20–0.28)	0.25 (0.22–0.28)	0.20 (0.17–0.22)	0.21 (0.19–0.24)

** P < .01, *** P < .001; mean (95% CI).

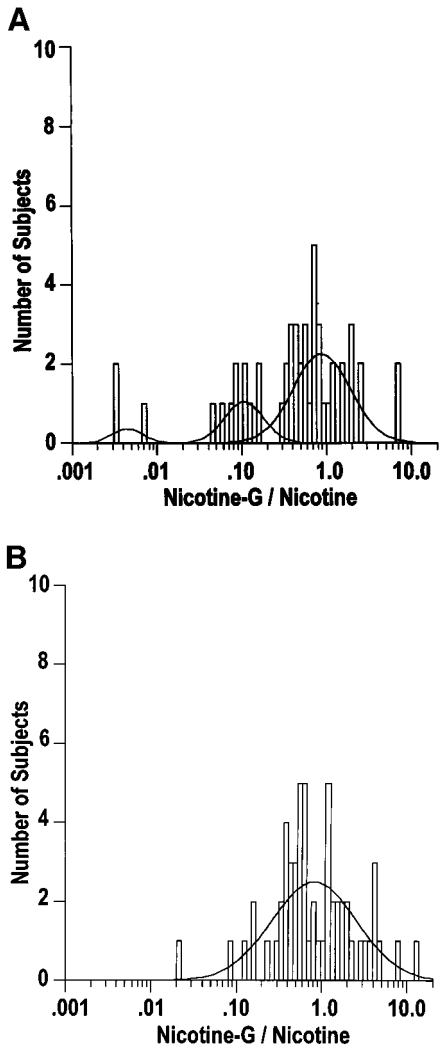

Fig. 2. Frequency histograms of urine nicotine-glucuronide/nicotine ratios in blacks (A) and whites (B). Data are based on unlabeled nicotine excretion.

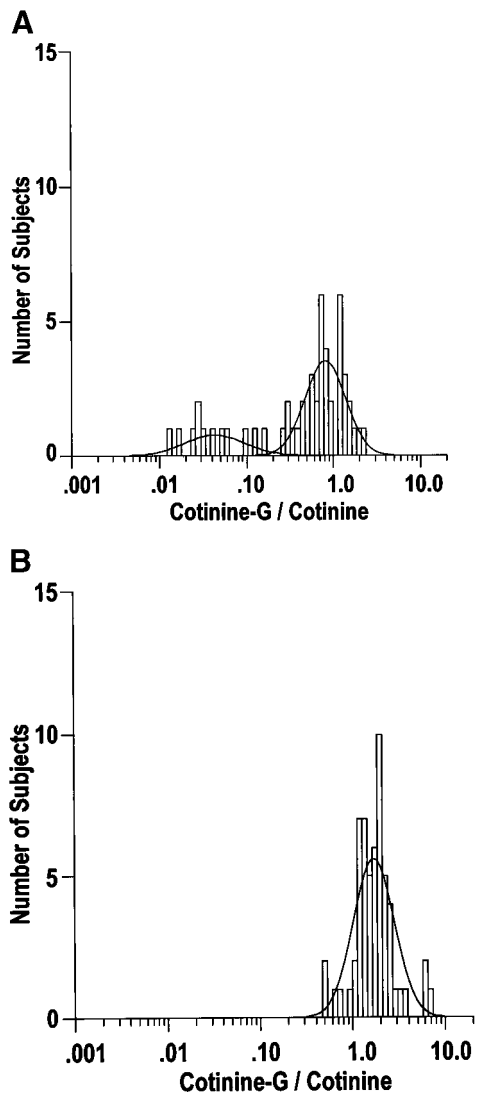

Fig. 3. Frequency histograms of urine cotinine-glucuronide/cotinine ratios for blacks (A) and whites (B). Based on unlabeled cotinine excretion data.

whites, which accounts for some but not all of the observed ethnic difference in the rate of metabolism of nicotine and cotinine. Third, we provide the first data on *N*-glucuronidation of nicotine and cotinine, as well as *O*-glucuronidation of 3′-hydroxycotinine, in groups of people. We find evidence of a polymorphic distribution of *N*-glucuronidation, with the slow metabolizers almost exclusively blacks. The population distribution of *O*-glucuronidation appears to be unimodal, with no ethnic differences.

The main difference between the present study and our previously published study is the detailed metabolic profile of nicotine in blacks and whites. This provides insight into the mechanisms of differences in nicotine and cotinine metabolism. As noted previously, blacks on average metabolize nicotine more slowly than whites, although this difference is not statistically significant. In the present study, we did find differences in the pattern of nicotine metabolism. Nicotine metabolism via the cotinine pathway, which is known to be mediated primarily by cytochrome P-450 (CYP) 2A6 (Nakajima et al., 1996b), is significantly slower, as was metabolism via *N*-glucuronidation, in blacks than in whites.

Blacks were found to metabolize cotinine substantially more slowly than whites, as seen in measurements of both total and nonrenal clearance. Cotinine is metabolized primarily by CYP2A6 (Nakajima et al., 1996a) to *trans*-3′-hydroxycotinine and by conjugation to cotinine-*N*-glucuronide. The observed slow metabolism of cotinine in blacks appears to be due to both reduced CYP2A6 activity and slower *N*-glucuronidation of cotinine. Of note, although nonrenal clearance is slower, renal clearance of cotinine is faster in blacks than in whites, for unclear reasons.

The finding that the distribution volume of cotinine is significantly smaller in blacks than in whites (the distribution volume of nicotine was smaller by a similar 12% but was not significant) is a novel finding for which we have no explanation. We did explore various possible physiological factors that might explain distribution volume. We found that the distribution volume of cotinine was most highly correlated with lean body mass and was smaller in women than men. However, lean body mass and gender distribution were similar in blacks and whites, so these cannot explain the ethnic difference.

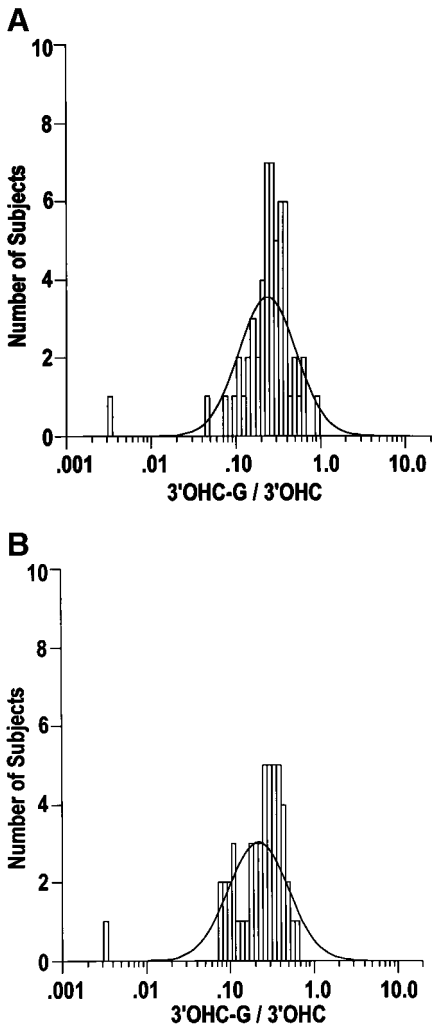

Fig. 4. Frequency histogram of urine *trans*-3′-hydroxycotinine-glucuronide and *trans*-3′-hydroxycotinine ratio for blacks (A) and whites (B). Based on unlabeled 3′-hydroxycotinine excretion data.

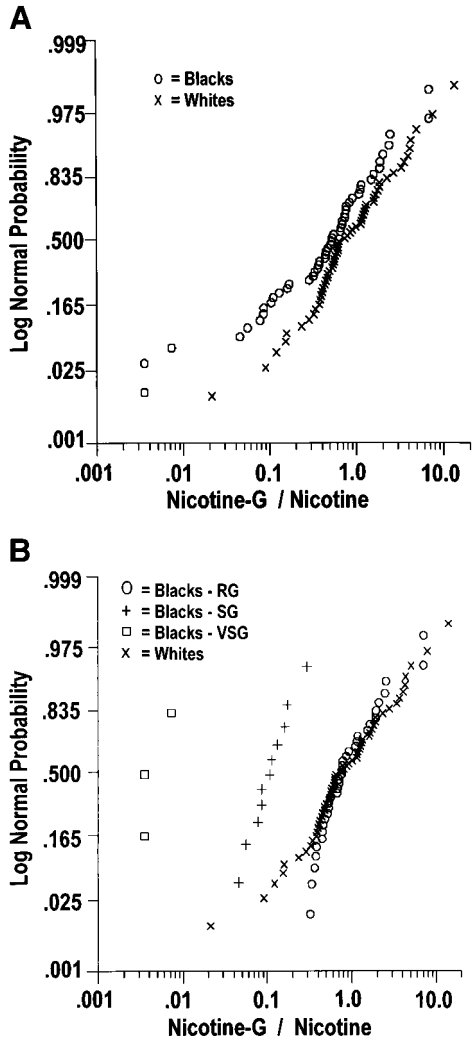

Fig. 5. Probit plot of the distribution of nicotine-glucuronide/nicotine in blacks and whites. The abscissa indicates logarithmic values of urine metabolite ratios; the ordinate, the cumulative frequency in the form of probits. A, plots for all blacks and all whites. B, plots for all whites, and blacks grouped as rapid (RG), slow (SG), and very slow (VSG) glucuronide formers.

We report the novel observations that the half-life of cotinine is significantly longer in blacks than in whites and shorter in women than in men. The explanations for half-life differences are not the same. The half-life of cotinine was significantly longer on average because of lower clearance of cotinine in blacks than in whites. The half-life of cotinine was significantly shorter on average because of the smaller distribution volume of cotinine in women than in men.

Relatively little research has been done on *N*-glucuronidation of drugs in general. Glucuronide formation is the most widespread among phase 2 reactions in drug metabolism, but for the most part these involve formation of acyl, hydroxyl, or phenolic glucuronides (Miners and Mackenzie, 1991; Kroemer and Klotz, 1992). The formation of *N*-glucuronides is described as a significant metabolic pathway in humans for relatively few drugs (Hawes, 1998). Nicotine and cotinine have been shown to form *N*-glucuronides (Curvall et al., 1991; Byrd et al., 1992; Caldwell et al., 1992; Benowitz et al., 1994), as shown in Fig. 1. We have previously shown a high correlation in the extent of *N*-glucuronide formation between nicotine and cotinine within individuals (Benowitz et al., 1994), suggesting that the same enzyme is involved in each. In contrast, the extent of *O*-glucuronidation of 3′-hydroxycotinine is not correlated with the conjugation of nicotine and cotinine, indicating that another enzyme is involved. Glucuronidation is catalyzed by UDP glucuronosyltransferase (UGT) enzymes (Miners and Mackenzie, 1991; Kroemer and Klotz, 1992). In humans, only UGT1A3 and UGT1A4 isoenzymes have been shown to catalyze quaternary ammonium glucuronide formation (Green and Tephly, 1998). To date, the particular UGT isoenzyme involved in *N*-glucuronidation of nicotine and cotinine or *O*-conjugation of 3′-hydroxycotinine has not been determined.

We provide evidence that *N*-glucuronidation of nicotine and cotinine is polymorphic, with a small group of slow metabolizers, most of whom are black. Although the distribution of glucuronide ratios appeared to be unimodal in whites, there was one slow metabolizer outlier (Figs. 2 and 5) who could represent a low prevalence slow-metabolizing polymorphism in the white population. Another recent report on the glucuronidation of the nicotine-derived nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) showed a lesser average extent of glucuronidation in blacks

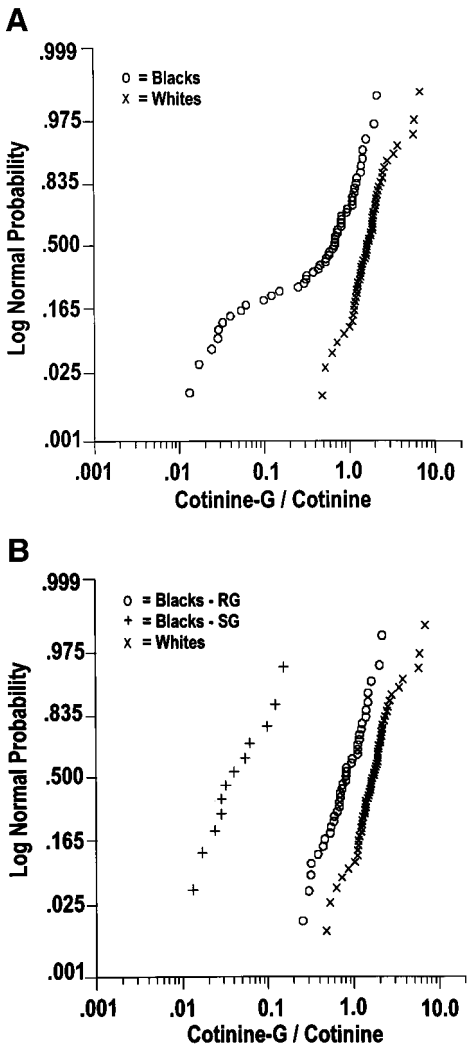

Fig. 6. Probit plot of the distribution of cotinine-glucuronide/cotinine urine ratios in blacks and whites. See legend for Fig. 5 for description of probit plot. A, plots for all blacks and all whites. B, plots for all whites and blacks grouped as rapid (RG) and slow (SG) glucuronide formers.

and evidence that blacks predominated among slow metabolizers (Richie et al., 1997). It is not known whether NNAL forms an *N*-glucuronide or an *O*-glucuronide, or both. However, the similarity in our findings and those with NNAL suggests that ethnic differences among NNAL, nicotine, and cotinine glucuronide formation reflect ethnic differences in the same UGT isoenzyme.

Ethnic differences in *N*-glucuronidation of nicotine and cotinine could be a result of genetic or environmental influences. The finding that *O*-glucuronidation of 3′-hydroxycotinine is similar in blacks and whites provides evidence that substrate availability is not a determinant of ethnic differences in glucuronidation. A possible effect of menthol in influencing the extent of *N*-glucuronidation must be considered because most blacks, but very few whites, smoke mentholated cigarettes. However, Richie et al. (1997) found in rats that menthol feeding does not decrease NNAL glucuronidation, which presumably involves the same enzyme as nicotine and cotinine glucuronidation, as discussed previously. The lack of apparent environmental explanation suggests a genetic basis for the ethnic difference in *N*-glucuronidation, but specific genetic analyses are necessary to confirm this proposition.

Our data enable us to examine the quantitative importance of differences in *N*-glucuronidation as the explanation for differences in the metabolism of cotinine in blacks versus whites. In the present study, we found that the total clearance of cotinine was 35% greater and the nonrenal clearance of cotinine was 46% greater in whites than in blacks. In a previous study, we found that on average 12.6% of cotinine was excreted as cotinine glucuronide (Benowitz et al., 1994). Thus, even if blacks had no *N*-glucuronidation of cotinine, 20% of the difference in total cotinine clearance would still be unaccounted for. We did not measure cotinine *N*-oxide or norcotinine, but these are minor metabolites, accounting for 5% or less of cotinine clearance in combination (Benowitz et al., 1994). Thus, it is most likely that blacks metabolize cotinine more slowly primarily because of lower CYP2A6 activity. Consistent with this hypothesis is the observation that the clearance of nicotine via the cotinine pathway, which is also believed to be determined by CYP2A6, was 20% greater in whites than in blacks.

The implications of our study are as follows. Cotinine clearance is slower in blacks than in whites and in part explains why plasma and urinary cotinine levels per cigarette smoked were significantly higher in blacks than in whites in our study, as well as in a number of other studies (Wagenknecht et al., 1990; Carabello et al., 1998; Perez-Stable et al., 1998). We also recently showed that blacks take in more nicotine per cigarette, presumably through more intensive inhalation, than whites, which also contributes to higher cotinine levels per cigarette (Perez-Stable et al., 1998). Ethnic differences, as well as gender differences, in the half-life of cotinine could affect temporal considerations in monitoring cotinine levels in smokers after cessation.

Our study also has implications regarding ethnic differences in drug metabolism in general. Other researchers have reported black/white differences in the metabolism of some drugs, with most research done on the drug-metabolizing enzyme CYP2D6. CYP2D6 activity is known to be polymorphically distributed, and a lower prevalence of slow metabolizers due to a lower prevalence of a particular CYP2D6 mutant gene has been reported in blacks than in whites (Evans et al., 1993). Conceivably, the opposite could be true for isozymes involved in *N*-glucuronidation, with a greater prevalence of a mutant UGT isomer gene in blacks. As noted previously, blacks form less NNAL-glucuronide than do whites (Richie et al., 1997). Ethnic differences have also been reported for hydroxyglucuronide formation of codeine between Chinese and whites of Swedish background (Yue et al., 1989).

Acknowledgments

We thank Patricia Buley and Sandra Tinetti for assistance in conducting the study and Kaye Welch for editorial assistance.

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