Trans-3′-hydroxycotinine: Disposition kinetics, effects and plasma levels during cigarette smoking

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Aims (3′R,5′S)-*trans*-3′-hydroxycotinine (3-HC) is a major metabolite of nicotine. The aim of this study was to characterize the disposition kinetics of 3-HC in healthy smokers, including metabolism to (3′R,5′S)-*trans*-3′-hydroxycotinine glucuronide (3-HC-Gluc). We also studied pharmacologic responses to 3-HC and plasma levels of 3-HC in a group of smokers.

Methods Eight cigarette smokers were studied on a clinical research ward. After 5 days of supervised nonsmoking, each subject received an intravenous infusion of 3-HC, 4 $\mu g\ kg^{-1}\ min^{-1}$ for 60 min. Plasma and urine levels of 3-HC and 3-HC-Gluc and cardiovascular and subjective responses were examined. Plasma levels of 3-HC, nicotine, and cotinine were measured in 62 smokers on up to three occasions.

Results The total plasma clearance of 3-HC averaged 1.3 ml $min^{-1}\ kg^{-1}$, of which 63% was renal excretion of unchanged drug. An average of 29% of the dose was excreted as 3-HC-Gluc. 3-HC did not have nicotine-like cardiovascular effects.

Conclusions These findings extend our understanding of the quantitative nature of nicotine metabolism. Such data may be of use in quantitating human exposure to nicotine from tobacco and in studying individual variability in nicotine metabolism.

Keywords: 3′-hydroxycotinine, cotinine, metabolism, nicotine, pharmacodynamics, pharmacokinetics, smoking, tobacco

Introduction

Nicotine is metabolized to a number of metabolites, some of which have pharmacologic actions that could contribute to the effects of nicotine in people [1]. Individual differences in the rate and/or pattern of metabolism of nicotine might be a determinant of individual susceptibility to effects or toxicity of tobacco. The major proximate metabolite of nicotine is cotinine, which accounts for 70–80% of nicotine metabolism (Figure 1) [2]. Nicotine in tobacco and in medications, and metabolically formed cotinine, are largely the levorotatory (S)-isomers [3–6]. (S)-Cotinine is metabolized in turn, primarily to (3′R,5′S)-*trans*-3′-hydroxycotinine (3-HC) (unless otherwise noted, 3-HC refers to the natural (3′R,5′S) stereoisomer) [2, 6–11]. In people, very little cotinine is metabolized to the stereoisomer (3′R,5′S)-*cis*-3′-hydroxycotinine, although the percentage appears to be higher in some animal species [11, 12]. 3-HC is on average the most abundant metabolite of nicotine found in the urine, accounting for on average 40% of the nicotine dose [8, 10]. 3-HC is also conjugated to what is believed to be an *O*-glucuronide, the latter of which is also found in the urine [10, 13].

Only one study of the fate of 3′-hydroxycotinine in humans has been published [14]. In that study, the racemate, a mixture of (3′R,5′S)- and (3′R,5′S)-*trans*-3′-hydroxycotinine, was administered to six smokers who had abstained from smoking, and the disposition kinetics were determined. Infusion of the racemic mixture was reported to have no effect on the electrocardiogram and to produce no subjective effects.

We have developed a method to synthesise (3′R,5′S)-*trans*-3′-hydroxycotinine (3-HC) which, as noted above, is the isomer found in people [11]. Different stereoisomers can have different pharmacokinetics and different

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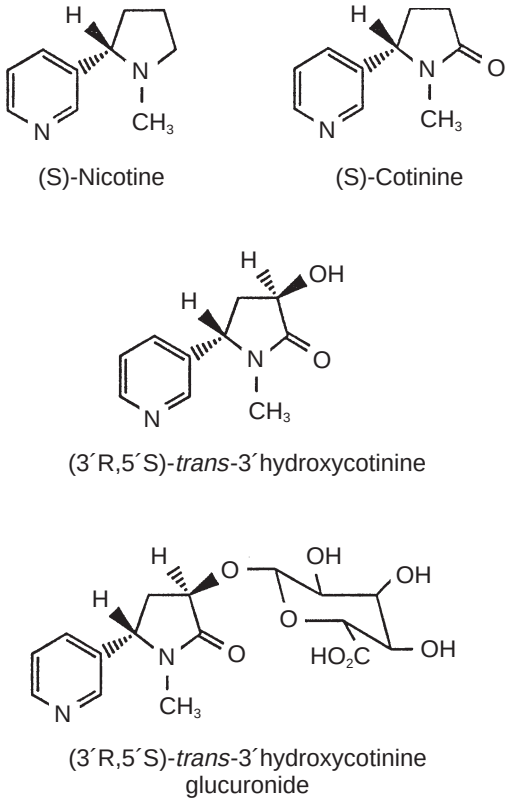

Figure 1 Structures of nicotine, cotinine, *trans*-3′-hydroxy-cotinine, and *trans*-3′-hydroxycotinine glucuronide.

pharmacological activities. Therefore, we studied the disposition kinetics and effects of the natural stereoisomer. We also studied plasma levels of 3-HC in comparison with levels of nicotine and cotinine in a group of cigarette smokers during *ad libitum* smoking.

Methods

Subjects

Eight healthy cigarette smokers were recruited by newspaper advertisements and were paid for their participation. The subjects included five men and three women. They ranged in age from 25–55 years (average, 41 years) and smoked an average of 24 cigarettes per day (range, 18–35). The plasma cotinine concentration during *ad libitum* smoking averaged 252 ng ml^{-1} (range, 29–534 ng ml^{-1}). Data were lost from one subject due to technical problems, so the data presented are for seven subjects.

Another group of 62 healthy cigarette smokers provided a blood sample as part of a screening examination for studies involving intravenous infusion of nicotine and cotinine (not reported here). Fifty-one of these subjects also provided samples during *ad libitum* smoking 1 and 2 weeks later, allowing us to examine within-subject reproducibility of the measurements. These 62 subjects included 24 men and 38 women, with an average age of 30 years (range, 20–57) and an average cigarette consumption of 13.1 cigarettes per day (range, 2–40). The average cigarette consumption is lower than that of the typical U.S. smoker.

Written informed consent was obtained from each subject. The study was approved by the Committee on Human Research at the University of California, San Francisco.

Study protocol

Subjects were hospitalized on the General Clinical Research Center at San Francisco General Hospital Medical Center for 8 days, during which time they were not permitted to use tobacco. The first 5 days were washout days during which cotinine and 3-HC levels were allowed to fall to low levels prior to the administration of 3-HC.

The 3-HC infusion was administered on the morning of the sixth day. 3-HC was infused at 4 $\mu g\ kg^{-1}\ min^{-1}$ for 60 min in the forearm vein of one arm. The dose was selected as similar to the dose of cotinine (its precursor) in a previous study that produced blood levels consistent with those of smokers [15]. Venous blood samples were withdrawn from a forearm vein in the other arm at frequent intervals for 48 h after the infusion. Blood pressure was measured at frequent intervals using an automatic blood pressure recorder (Dynamap, Critikon, Tampa, FL, USA), and heart rate was measured by electrocardiogram. Cardiovascular measurements were made for 30 min before and for 60 min after the 3-HC infusion.

A seven item visual analogue questionnaire was administered every 15 min during and for 60 min after the infusion. The questions included:

1 How strong was the treatment effect?
2 Did the drug have good effects?
3 Did the drug have bad effects?
4 How much did you like the drug?
5 How high do you feel?
6 How much would you like to receive this treatment again?
7 How energetic did the drug make you feel?

The Profile of Mood Scale [16] was administered before and immediately after the end of the infusion.

Urine samples were collected at intervals of 0–2, 2–4, 4–8, 8–16, 16–24, and 24–48 h after the beginning of the infusion.

Blood samples from smokers were taken in the clinic as a routine procedure in screening for participation in various research studies. Subjects were asked how many cigarettes they smoked per day. Subjects were asked to

return to the clinic in 1 and 2 weeks for additional blood samples during *ad libitum* smoking. Subjects were not attempting smoking cessation.

Synthetic and analytical chemistry

(3′R,5′S)-*trans*-3′-hydroxycotinine (3-HC) perchlorate was synthesized as previously described [11]. The salt was converted to the free base and purified by column chromatography using ethyl acetate/methanol/concentrated ammonia (80 : 20 : 5) as the eluting solvent. The fractions containing 3-HC were combined and evaporated under reduced pressure to give an oil that crystallized on standing. This was recrystallized from acetone and dried under vacuum to give a white crystalline powder, mp 110–111.5°C (lit mp 110–112°C) [17]. Identity and purity were ascertained by t.l.c. and GC-MS [18]. The purified 3-HC base was dissolved in 0.9% sodium chloride USP, sterilized by millipore filtration, and stored refrigerated in nitrogen-flushed vials.

Plasma concentrations of 3-HC were measured by gas chromatography-mass spectrometry [18]. Glucuronide-conjugated 3-HC (3-HC-Gluc) in the urine was measured as the difference in the total concentration of 3-HC before and after incubation with beta glucuronidase, as described previously [10]. The extent of deconjugation of 3-HC during incubation with β-glucuronidase could not be determined definitively because no pure 3-HC glucuronide is available as a test standard. However, it is likely that deconjugation is complete based on observations that increasing the incubation time or increasing the concentration of the enzyme resulted in no further increase in the concentration of free 3-HC.

Data analysis

The pharmacokinetics of plasma 3-HC were estimated using model-independent methods [19]. Renal clearances were computed using plasma level data and urine excretion data collected from the onset of the infusion for 8 h. The elimination half-life of 3-HC in the plasma and half-lives of 3-HC and 3-HC glucuronide in the urine were computed by linear regression of the log plasma concentration or urine excretion rate *vs* time in the terminal log linear phase.

Pharmacologic response data were analysed by comparing baseline values to values at different times after 3-HC dosing by repeated measures analysis of variance, or paired *t*-tests, as appropriate.

Plasma concentrations of 3-HC, nicotine, and cotinine during ad libitum cigarette smoking were analysed in two ways. Correlations between plasma concentrations at the initial screening were examined by determining Pearson correlation coefficients. The consistency of plasma concentration measurements on successive weeks was examined by computing within-subject standard deviations and intraclass correlation coefficients (Cronbach alpha statistic) for each analyte [20].

Results

Infusion study

The plasma 3-HC concentrations prior to infusion averaged 3 ng ml^{-1}. Plasma levels of 3-HC during and after the infusion are shown in Figure 2. The peak concentration at the end of the infusion averaged 497 ng ml^{-1} (range, 361–1050 ng ml^{-1}). The plasma concentration time curve appears to be biexponential. Plasma pharmacokinetic parameters are presented in Table 1. Plasma clearance averaged 1.34 ml min^{-1} kg^{-1} (95% CI, 1.15–1.53), renal clearance averaged 0.83 ml min^{-1} kg^{-1} (0.63–1.02), and steady state volume of distribution (V_{ss}) averaged 0.66 l kg^{-1} (0.56–0.77). The plasma elimination half-life averaged 6.6 h (range, 4.6–8.3).

Average urine excretion rate curves for 3-HC and 3-HC-Gluc are shown in Figure 3. The average elimination half-life for 3-HC in urine was 6.4 h (range, 3.9–8.7 h), while the average half-life for 3-HC-Gluc excretion was 7.2 h (range, 4.6–9.4 h). These half-lives were not significantly different from one another.

On average, 87% of the dose of 3-HC was recovered in the urine as 3-HC (range, 38–100). An average of 29% of the dose of 3-HC was recovered as 3-HC-Gluc (range, 11–52). For most subjects, nearly all of the infused dose of 3-HC was recovered in the urine as the sum of 3-HC and 3-HC-Gluc. In one case, recovery was low, which

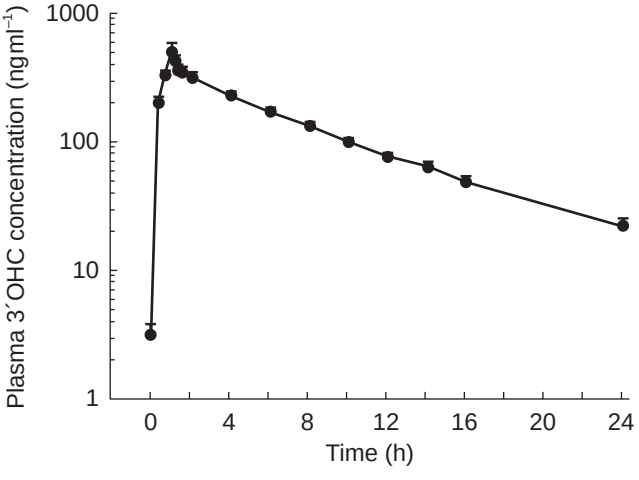

Figure 2 Plasma concentrations of 3′-hydroxycotinine during and after intravenous infusion of 3′-hydroxycotinine, 4 μg kg^{-1} for 30 min. Data represent average values for seven subjects.

Table 1 Pharmacokinetics of *trans*-3′-hydroxycotinine

Subject	*Gender*	*Body weight (kg)*	*Total plasma clearance (ml min^{-1})*	*Renal clearance (ml min^{-1})*	*% Renal clearance*	*% Recovery as 3HC-G*	*Plasma elimination half-life (min)*	V_{ss} *(l)*
1	M	61.6	82	62	76	25	499	52.3
2	F	61.0	91	50	55	23	311	39.4
3	M	65.2	95	–	–	52	472	50.6
4	M	60.3	58	34	59	37	487	34.3
5	M	68.5	102	50	49	11	278	38.1
6	F	46.3	67	49	73	27	327	30.2
7	F	65.0	78	53	68	29	395	40.9
Mean		61.1	82	50	63	29	396	40.8
s.d.		7.2	16	9	10	12	92	8.0
95% CI		54.5–67.7	67–96	41–59	52–74	18–41	311–481	33.3–48.3

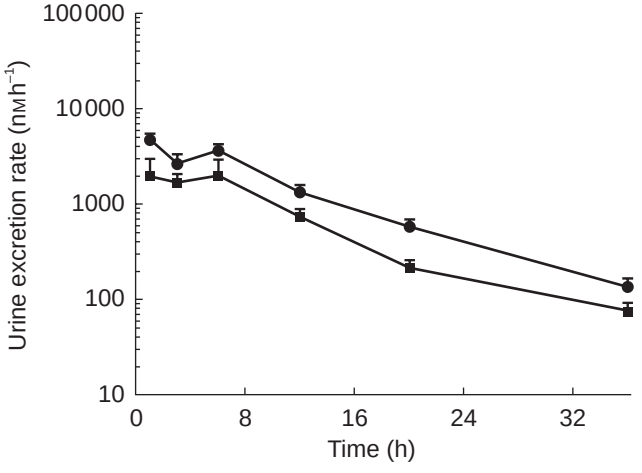

Figure 3 Urinary excretion rate curves for 3′-hydroxycotinine (●) and 3′-hydroxycotinine glucuronide (■) after intravenous infusion of 3′-hydroxycotinine. Data represent mean values for seven subjects. Concentrations are expressed as μM so that the relative molar excretion of the metabolites can be appreciated.

is suspected to be due to noncompliance with the urine collection protocol.

There was no significant change in blood pressure or heart rate during or after the infusion of 3-HC. The visual analogue scores showed no significant treatment effects. The POMS analysis revealed significant decreases in tension ($P=0.004$) and fatigue ($P=0.04$) after compared with prior to the infusion.

Ad libitum *cigarette smoking study*

The plasma level data during *ad libitum* cigarette smoking are shown in Table 2. The average plasma cotinine concentration in these subjects, 146 ng ml^{-1}, is lower than that in the experimental study discussed above, because the smokers in the *ad libitum* smoking group smoked fewer cigarettes per day on average than in the experimental study. Plasma levels of 3-HC were significantly correlated with the plasma cotinine levels ($r=0.62$, $P<0.001$), but not significantly correlated with plasma nicotine concentrations (Table 3). The ratio of 3-HC/cotinine during *ad libitum* cigarette smoking averaged 0.32 but was widely variable among individuals (range, 0.02–0.90). The ratio of plasma 3-HC/cotinine was significantly inversely correlated with plasma nicotine concentration ($r=-0.38$, $P<0.005$) (Figure 4).

Table 2 Plasma concentrations of nicotine, cotinine, and trans-3′-hydroxycotinine during *ad libitum* cigarette smoking (62 subjects).

	Mean	*s.d.*	*Range*
Cigarettes per day	13.1	8.1	2–40
Plasma nicotine (ng ml^{-1})	7.2	5.7	0.5–22.8
Plasma cotinine (ng ml^{-1})	146	106	17–499
Plasma 3′-hydroxycotinine (ng ml^{-1})	44	34	0.5–138
Ratio 3′-hydroxycotinine/cotinine	0.32	0.21	0.02–0.90
Plasma cotinine/CPD	13.6	11.0	1.0–44.7

Fifty-one subjects had three measurements of plasma nicotine, cotinine, and 3-HC. The mean values of the measurements were similar across weeks. The within-subject standard deviations were 4.7 (CV = 54%) for nicotine, 43 (CV = 29%) for cotinine, and 21 (CV = 45%) for 3-HC. The intraclass correlation coefficients were 0.81, 0.95, and 0.85 for nicotine, cotinine, and 3-HC, respectively.

Discussion

The main findings of this study include the first human data on the pharmacology of the natural stereoisomer

Table 3 Pearson correlation matrix for plasma nicotine and metabolite concentrations during *ad libitum* cigarette smoking (62 subjects)

	Nicotine	*Cotinine*	*3′-Hydroxycotinine*	*3′-Hydroxycotinine/cotinine ratio*
Nicotine	1.00			
Cotinine	0.45**	1.00		
3′-hydroxycotinine	0.14	0.62**	1.00	
3′-hydroxycotinine/cotinine ratio	−0.38*	−0.12	0.52**	1.00

* $P<0.005$, ** $P<0.001$

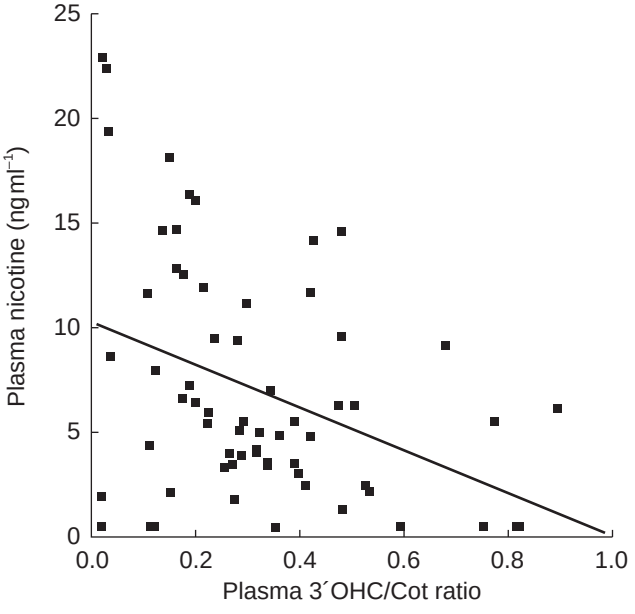

Figure 4 Correlation between plasma nicotine concentration and the ratio of plasma concentrations of *trans*-3′-hydroxycotinine/cotinine in 62 subjects during *ad libitum* cigarette smoking. The solid line represents the line of regression. The correlation coefficient was −0.38, $P<0.005$.

(3′R,5′S)-*trans*-3′-hydroxycotinine, including quantitative data on the conjugation of 3-HC. Our pharmacokinetic data for (3′R,5′S)-*trans*-3′-hydroxycotinine are somewhat different from those reported for racemic 3-HC by Scherer *et al.* [14]. In our study, the plasma clearances (both total and renal) were about 30% lower than those reported by Scherer and coworkers, although the elimination half-lives were similar. Possibly, the results differed because Scherer administered ammonium chloride to acidify the urine, which is expected to increase renal clearance, while in our own subjects urine pH was uncontrolled. It is also possible that the pharmacokinetics of (3′R,5′S)-*trans*-3′-hydroxycotinine differs from that of racemic mixture. In any case, both studies indicate that the route of elimination of 3-HC is primarily renal, with an average of 63–75% excreted unchanged in the urine. The steady state distribution volume of 3-HC averaged 0.66 l kg^{-1}, which is smaller than that of nicotine and cotinine, reflecting the relative greater polarity and water solubility of 3-HC. Scherer *et al.* found that 75% of 3-HC was excreted unchanged in the urine, but they did not account for the remainder of the 3-HC dose. Our data indicate that metabolism to 3-HC-Gluc accounts for most or all of the remaining route of elimination.

The elimination rate of 3-HC in urine (half-life, 6.4 h) was similar to that in plasma (half-life, 6.6 h), as expected. The elimination rate of 3-HC-Gluc paralleled that of 3-HC, suggesting that the elimination half-life of the conjugate is formation limited. There was considerable individual variability in the percent of 3-HC that was conjugated (11–52%), presumably reflecting genetic variability in the activity of the conjugating enzyme.

The plasma level data from a previous study of cigarette smoking, nasal spray, and transdermal nicotine assessed at steady state revealed an average ratio of 3-HC to cotinine of 0.24 [21]. Our data from ad libitum smoking in the present study revealed an average ratio of 0.32. These ratios are lower than the ratio of 0.45 reported by Neurath *et al.* [22], but similar to the ratio of 0.32 reported by Neurath & Pein [8] and Pichini *et al.* [23] based on measurements in regular smokers.

We found extremely large variability in the ratio of 3-HC/cotinine in plasma during *ad libitum* cigarette smoking (range, 0.02–0.90). The most likely explanation is individual variability in the rate and extent of metabolism of cotinine to 3-HC. The conversion of cotinine to 3-HC is determined substantially by CYP2A6 activity [24]. CYP2A6 activity is reported to be extremely variable from person to person based on *in vitro* liver microsomal activity [25]. Nicotine metabolism is also substantially mediated by CYP2A6 [25, 26]. The ratio of plasma 3-HC/cotinine was inversely correlated with the plasma nicotine concentration during *ad libitum* cigarette smoking, suggesting that the ratio might be a marker of CYP2A6 activity. Thus, the higher the ratio, the greater the CYP2A6 activity and, correspondingly, the higher the CYP2A6 activity, the greater the clearance of nicotine and the lower the plasma nicotine concentration.

The results of the pharmacologic response measures should be interpreted after consideration of the high 3-HC levels which were produced by this infusion compared with that seen with tobacco use or use of nicotine medications. Another limitation of our study is that the pharmacologic measurements were not controlled. There were some individuals who reported feeling good on the infusion or that the infusion made them feel energetic, and there was a significant reduction in tension and fatigue. However, it is impossible to say if these responses were different from placebo responses or had more to do with relief of anxiety after the completion of an experimental procedure. There were no nicotine-like effects to increase heart rate or blood pressure.

The present study furthers our quantitative understanding of the metabolism of nicotine. A quantitative understanding of nicotine metabolism is important when using biological measurements to assess daily exposure to nicotine from tobacco, as a benchmark to examine individual differences in the pattern of nicotine metabolism. The present study provides pharmacokinetic parameters by which plasma levels or urinary excretion data for 3-HC and 3-HC-Gluc can be interpreted in relation to daily exposure to nicotine. Pharmacologic activity of metabolites could contribute to the action of nicotine. We also report plasma level data and data on within- and across-subject variability in nicotine, cotinine, and 3-HC levels during ad libitum cigarette smoking. Data on within-subject variability will be useful in designing longitudinal studies of changes in nicotine exposure. The present study finds no evidence of nicotine-like effects for 3-HC. Some subjective effects were reported; however, these are difficult to assess because the study was not placebo-controlled.

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