Effects of nicotine on cytochrome P450 2A6 and 2E1 activities

Janne Hukkanen, Peyton Jacob III,^{1,2,3} Margaret Peng,^{1,2,3}
Delia Dempsey^{1,2,3} & Neal L. Benowitz^{1,2,3}

Department of Internal Medicine, University of Oulu, Oulu, Finland, ¹Division of Clinical Pharmacology and Experimental Therapeutics, Medical Service, San Francisco General Hospital Medical Center, Departments of ²Medicine and ³Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA, USA

Correspondence

Dr Neal L. Benowitz, MD, Division of Clinical Pharmacology and Experimental Therapeutics, University of California, San Francisco, Box 1220, San Francisco, CA 94143-1220, USA.

Tel: + 1 415 206 8324

Fax: + 1 415 206 4956

E-mail: nbenowitz@medsfgh.ucsf.edu

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Smoking slows the metabolism of nicotine and accelerates the metabolism of chlorzoxazone.
- Nicotine is a useful probe for phenotyping cytochrome P450 2A6 activity and chlorzoxazone is a frequently used probe for CYP2E1 activity.
- The tobacco smoke constituents responsible for the reduced CYP2A6 and increased CYP2E1 activities are unknown.

WHAT THIS PAPER ADDS

- This study demonstrates that CYP2A6 and CYP2E1 activities are not affected by nicotine dosing.
- High-dose nicotine treatment has a low potential of interaction with CYP2A6 and CYP2E1 substrates.
- The mechanisms of tobacco smoke-elicited changes in CYP2A6 and CYP2E1 activities are yet to be determined.

AIMS

Smoking slows the metabolism of nicotine and accelerates the metabolism of chlorzoxazone, which are probe reactions for cytochrome P450 2A6 (CYP2A6) and CYP2E1 activities, respectively. We aimed to determine the role of nicotine in these metabolic effects of cigarette smoking.

METHODS

The study had a single-blind, randomized, crossover two-arm design. Twelve healthy smokers were given two transdermal patches with 42-mg nicotine a day or placebo patches, each for 10 days. The subjects abstained from smoking during the study arms. Oral chlorzoxazone was given on day 7 and deuterium-labelled nicotine-d₂ and cotinine-d₄ infusion on day 8.

RESULTS

There was no significant influence of transdermal nicotine administration on pharmacokinetic parameters of nicotine-d₂ or on the formation of cotinine-d₂. Nicotine decreased the volume of distribution (62.6 vs. 67.7 l, 95% confidence interval of the difference -9.7, -0.6, *P* = 0.047) of infused cotinine-d₄. There were no significant differences in disposition kinetics of chlorzoxazone between the treatments.

CONCLUSIONS

CYP2A6 and CYP2E1 activities are not affected by nicotine. The tobacco smoke constituents responsible for the reduced CYP2A6 and increased CYP2E1 activities remain unknown.

Introduction

Cigarette smoking accelerates the metabolism of certain drugs, particularly those primarily metabolized by cytochrome P450 1A2 (CYP1A2) and, to a lesser extent, CYP2E1 and some UDP-glucuronosyltransferases [1, 2]. The induc-

tion of CYP1A2 is mediated via binding of polycyclic aromatic hydrocarbons of the tobacco smoke to the aryl hydrocarbon receptor with consequent transcriptional activation of *CYP1A2* gene, whereas the mechanism of CYP2E1 induction is usually the stabilization of CYP2E1 mRNA and protein [3]. In contrast to its well-known effects

to induce drug metabolism, cigarette smoking slows the metabolism of nicotine. In previous studies, we found that the clearance of nicotine is significantly slower in cigarette smokers compared with nonsmokers [4], and in the same subjects when smoking compared with not smoking [5, 6].

The main pathway of nicotine metabolism is C-oxidation to cotinine, which accounts for 70–80% of nicotine metabolism on average [7]. Cotinine is further metabolized to trans-3'-hydroxycotinine, and both of these reactions are catalysed primarily by hepatic CYP2A6 [8, 9]. Thus, nicotine has been widely used to phenotype CYP2A6 activity [10]. Also studies with another CYP2A6-specific probe, coumarin, support the proposition that smoking inhibits CYP2A6-mediated metabolism [11, 12]. In aggregate, these studies suggest that there are substance(s) in tobacco smoke, as yet unidentified, that inhibit the metabolism of nicotine. Another possibility is that reduced CYP2A6 activity is due to downregulation of CYP2A6 expression and not due to inhibition. The administration of nicotine for 21 days to monkeys decreased CYP2A6 activity by downregulating CYP2A6 mRNA and protein in liver [13]. Furthermore, expression of CYP2A6 mRNA is lower in human pulmonary bronchial epithelial cells in smokers compared with nonsmokers [14].

Both transcriptional and post-transcriptional mechanisms influence the induction of CYP2E1, with stabilization of mRNA and protein having major significance in contrast to many other CYP forms [15]. Smoking increased CYP2E1-mediated chlorzoxazone clearance by 23% when compared with the same individuals after 4-day tobacco abstinence [2]. The induction of CYP2E1 protein in the brain of smoking alcoholics in comparison with non-alcoholic nonsmokers has been proposed [16]. Several studies with experimental animals have shown the induction of CYP2E1 protein and activity in liver and brain by nicotine administration [17–20]. Furthermore, nicotine treatment increased CYP2E1 protein in a human neuroblastoma cell line [16].

No previous study has studied the effects of nicotine on human CYP2A6 and CYP2E1 activities *in vivo*. The aim of our study was to determine the effects of nicotine on the disposition kinetics of intravenously infused deuterium-labelled nicotine and cotinine and oral chlorzoxazone, and to test the hypotheses that nicotine reduces its own CYP2A6-mediated metabolism and induces CYP2E1 activity.

Methods

Healthy smokers (>10 cigarettes day⁻¹), including men and women between 18 and 65 years old, were recruited for the study. Exclusion criteria included major medical or psychiatric conditions, current use of smokeless tobacco, pipes or cigars, inability to abstain from nicotine use, obesity [body mass index (BMI) >30], history of significant caffeine

withdrawal, pregnancy, lactation, drug or alcohol abuse, inability to speak English, and history of fainting. Plasma cotinine levels were measured at screening to confirm smoking. The study was approved by the Committee on Human Research at the University of California, San Francisco. Written, informed consent was obtained from each subject. The subjects were financially compensated for participation.

The number of subjects ($n = 12$) was based on a power analysis to detect an effect size of 25% in nicotine clearance assuming a coefficient of variation of 25% with $\alpha = 0.05$ and $\beta = 0.2$.

Experimental protocol

This was a single-blind, randomized, crossover two-arm study. Subjects were admitted to the General Clinical Research Center at San Francisco General Hospital Medical Center (San Francisco, CA, USA) for two 10-day inpatient periods separated by an at least 1-week wash-out period. The sequence (i.e. nicotine or placebo period first) of the two admissions was randomized. Subjects were asked to abstain from the use of grapefruit juice, alcohol, and dietary and herbal supplements for 2 days before the inpatient stays. No grapefruit juice, charcoal-broiled meat, cruciferous vegetables or caffeine-containing beverages were served during the study. Subjects were not allowed to smoke or to use any tobacco products during hospitalization, and expired air carbon monoxide was measured two to three times a day to ensure that subjects were not smoking surreptitiously.

Patches releasing 21-mg nicotine (Nicoderm CQ; Glaxo-SmithKline, Philadelphia, PA, USA) or placebo patches were administered twice daily at 08.00 h and at 12.00 h (except on the first day only one patch was administered at 08.00 h), and each patch was worn for 24 h. The total daily dose of nicotine in the active patch condition was 42 mg. This is higher than the usual therapeutic dose (21 mg) so that we could be sure that nicotine levels were adequate to test its possible effects on drug metabolism. One subject experienced nausea and vomiting during the first days of the nicotine arm and the dose of nicotine was reduced to 21 mg day⁻¹ (one patch a day) starting on the day 3 of the inpatient stay. Placebo patches were different in appearance from active patches, so all patches were covered with a gauze bandage to preserve the subject's blind.

On the morning of the seventh hospital day after an overnight fast, an intravenous catheter was inserted in forearm vein for blood drawings. At 08.00 h a tablet of bupropion 75 mg was given (data on bupropion kinetics will be presented elsewhere). At 12.00 h a tablet of chlorzoxazone 250 mg was given. Urine was collected for 24 h to measure chlorzoxazone and metabolites. Blood samples were taken at 0, 15, 30, 45, 60 and 90 min after, and then 2, 3, 4, 5, 6, 7 and 8 h after ingestion of chlorzoxazone. The breakfast was served at 10.00 h and the lunch at 14.00 h.

On the morning of day 8 of the inpatient stay after an overnight fast, a second intravenous catheter was inserted in forearm vein for intravenous infusion. Deuterium-labelled nicotine-d₂ (3',3'-dideuteronicotine) and cotinine-d₄ (2,4,5,6-tetradeutercotinine) infusion was given (1.5 µg kg⁻¹ min⁻¹ for each compound for 30 min). The mean dose of nicotine-d₂ (and cotinine-d₄) administered by intravenous infusion was 3.1 mg (range 2.5–4.1 mg). Urine was collected for 48 h for the measurement of nicotine and metabolites. Blood was sampled for measurements at 0, 15, 30, 45, 60 and 90 min after, and then 2, 3, 4, 6, 8, 12, 16, 24, 36, 48 and 56 h after infusion. Four hours after the start of the infusion a tablet of 200 mg caffeine was given. Urine was collected for 24 h to measure caffeine and metabolites. Data on caffeine kinetics will be presented elsewhere. The breakfast was served 2 h and lunch 6 h after the infusion. Subjects were discharged on the afternoon of the tenth inpatient day.

Analytical methods

Nicotine and cotinine concentrations in plasma were determined by gas chromatography–mass spectrometry (MS) [21]. Concentrations of chlorzoxazone and 6-hydroxychlorzoxazone in plasma and 6-hydroxychlorzoxazone in urine were measured by liquid chromatography (LC)–MS/MS. Plasma and urine samples, standards, and quality control specimens with deuterium-labelled chlorzoxazone (chlorzoxazone-d₃) as an internal standard were incubated overnight at 37°C with Type IX-A β-glucuronidase (Sigma-Aldrich Corp., St Louis, MO, USA) in a pH 7 phosphate buffer to deconjugate glucuronides of chlorzoxazone and 6-hydroxychlorzoxazone. Protein was precipitated with acetonitrile, and the samples were centrifuged for 4 min at about 12 000 *g* to ensure a particle-free supernatant. The acetonitrile supernatant was evaporated to dryness and samples were reconstituted for LC–MS/MS with 85:15 water/acetonitrile containing 0.1% formic acid. LC–MS used a Synergi 4-µm Polar-RP 80Å 150 × 2.0 mm column (Phenomenex Inc., Torrance, CA, USA) with a flow rate of 0.4 ml min⁻¹. A gradient was used, from 15% to 60% acetonitrile in water with 0.1% formic acid between 0 and 6 min, changing to 100% acetonitrile with 0.1% formic acid between 6 and 7 min. Retention times were 6.7 min for chlorzoxazone and chlorzoxazone-d₃ and 4.6 min for 6-hydroxychlorzoxazone. MS/MS detection used negative ion atmospheric pressure chemical ionization and selected reaction monitoring. The transitions monitored were 168–132 for chlorzoxazone, 173–134 for chlorzoxazone-d₃ and 184–120 for 6-hydroxychlorzoxazone. As the glucuronides, if present, eluted before the analytes and exhibited the same ion transitions as the aglycones, it was possible to monitor for completion of the deconjugation reactions. For chlorzoxazone in plasma, precision (per cent coefficient of variation) was 2.9–10.3% and accuracy (per cent of expected values) 101–108% for concentration range of 0.025–15 µg ml⁻¹. For hydroxychlorzoxazone in plasma,

precision was 4.6–14.9% and accuracy was 96–108% for concentration range of 0.025–15 µg ml⁻¹. The lower limit of quantification for both chlorzoxazone and its metabolite was 0.025 µg ml⁻¹.

Data analysis

Pharmacokinetic parameters were estimated from the plasma concentrations using model-independent methods described previously [7] with WinNonlin pharmacokinetic analyses package (Pharsight Corp., Mountain View, CA, USA). Total clearance of nicotine-d₂ and cotinine-d₄ was computed as follows:

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

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

where CL is clearance (ml min⁻¹) and AUC is area under the plasma concentration–time curve extrapolated to infinity, nic is nicotine, and cot is cotinine.

Fractional conversion of nicotine to cotinine (*f*) was estimated using the plasma concentrations of cotinine-d₂ formed as a metabolite during the nicotine-d₂ infusion and the clearance of cotinine-d₄ estimated from its plasma concentrations following the cotinine-d₄ infusion.

$$f = \frac{\text{AUC}_{\text{cot-d}_2}}{\text{Dose}_{\text{nic-d}_2}} \times CL_{\text{cot-d}_4}$$

The metabolic clearance of nicotine via formation of cotinine (CL_{nic→cot}) was computed as CL_{nic} × *f*.

Oral clearance of chlorzoxazone was computed as follows: CL = Dose/AUC. The clearance of chlorzoxazone via the 6-hydroxychlorzoxazone pathway was estimated as oral clearance multiplied by the percentage of chlorzoxazone dose recovered as 6-hydroxychlorzoxazone in the urine. The pharmacokinetic parameters were compared across treatments by paired Student's *t*-test. The results of bupropion and caffeine pharmacokinetics will be reported in subsequent papers.

Results

Subjects

Twelve smokers (five women, seven men), aged 24–65 years, completed the study. The mean age was 39 years (SD ± 13), the mean weight 67 kg (SD ± 11, range 55–93 kg) and the BMI 24 (SD ± 4, range 19–30 kg m⁻²). There were 11 non-Hispanics and one Hispanic. The racial/ethnic profile of the subjects was seven non-Hispanic Whites, one Hispanic White, three Blacks and one Asian. Five subjects consumed 44 g (SD ± 79) of alcohol weekly on average; the others did not drink alcohol. The subjects ingested 518 mg (SD ± 355) caffeine and smoked 19 cigarettes (SD ± 5) a day on

Table 1Effect of nicotine administration on disposition kinetics of nicotine-d₂

	Nicotine	Placebo	P-value	95% CI of difference
C _{max} (ng ml ⁻¹)	23.3 ± 7.8	20.2 ± 6.7	0.11	-0.42, 6.51
t _{1/2} (min)	137 ± 62	153 ± 88	0.42	-53, 21
Clearance (ml min ⁻¹)	1416 ± 463	1353 ± 567	0.48	-106, 233
Mean residence time (min)	178 ± 79	201 ± 118	0.35	-70, 23
V _{ss} (l)	229 ± 54	226 ± 56	0.82	-22, 28
AUC (min ng ml ⁻¹)	2393 ± 785	2749 ± 1346	0.27	-953, 241
Fractional conversion of nicotine to cotinine	0.73 ± 0.24	0.72 ± 0.18	0.80	-0.07, 0.09
Metabolic clearance by the cotinine pathway (ml min ⁻¹)	1076 ± 520	1001 ± 510	0.27	-52, 202

Data are presented as mean ± SD. CI, confidence interval; C_{max}, peak plasma concentration; t_{1/2}, half-life; AUC, area under plasma concentration–time curve extrapolated to infinity; V_{ss}, steady-state volume of distribution.

Table 2Effect of nicotine administration on formation pharmacokinetics of cotinine-d₂

	Nicotine	Placebo	P-value	95% CI of difference
C _{max} (ng ml ⁻¹)	30.0 ± 10.3	28.4 ± 8.3	0.09	-0.11, 3.23
T _{max} (min)	264 ± 94	310 ± 117	0.16	-106, 14
t _{1/2} (min)	869 ± 203	891 ± 282	0.82	-208, 163
AUC (min ng ml ⁻¹)	43 361 ± 17 605	45 876 ± 17 746	0.48	-9226, 4195

average. One subject had a chronic hepatitis C infection with normal liver function tests, and low-grade inflammation and low-stage fibrosis in a liver biopsy taken shortly before the screening. The mean plasma cotinine concentration in screening was 259 ng ml⁻¹ (SD ± 153). Fifteen subjects were enrolled, and there were three drop-outs. The reasons for dropping out were high blood pressure on admission, tachycardia due to undisclosed use of high-dose cold medication, and abnormal ECG on admission.

Four and two subjects experienced headache during placebo and nicotine arms, respectively. One subject experienced nausea and vomiting in the beginning of the nicotine arm and the dose of nicotine was reduced to 21 mg day⁻¹ (one patch a day) starting on day 3 of the inpatient stay. The mean plasma nicotine and cotinine concentrations achieved prior to the infusion on the eighth inpatient day were 35 ng ml⁻¹ (SD ± 16) and 714 ng ml⁻¹ (SD ± 314), respectively, during the nicotine treatment. These concentrations are at the higher end of the concentration range detected in heavy smokers [10]. The corresponding concentrations during the placebo treatment were 0.8 ng ml⁻¹ (SD ± 0.2) and 17 ng ml⁻¹ (SD ± 9).

Disposition kinetics of nicotine, cotinine and chlorzoxazone

The effects of nicotine on the disposition kinetics of nicotine-d₂ and cotinine-d₄ are presented in Tables 1–3 and Figure 1. There was no significant influence of nicotine administration on pharmacokinetic parameters of

nicotine-d₂ or on the formation of cotinine-d₂. Nicotine decreased the volume of distribution ($P = 0.047$) and the mean residence time ($P = 0.042$), and increased the peak plasma concentration ($P = 0.017$), of infused cotinine-d₄. There were no significant differences in disposition kinetics of chlorzoxazone between the treatments (Tables 4 and 5).

Discussion

This study has demonstrated that high-dose nicotine administration has no significant effects on nicotine and chlorzoxazone pharmacokinetics. Since nicotine and chlorzoxazone are specific substrates for CYP2A6 and CYP2E1 [10, 22], respectively, it can be concluded that CYP2A6 and CYP2E1 activities are not affected by nicotine dosing. Thus, we disprove the hypotheses that nicotine inhibits its own CYP2A6-mediated metabolism and induces CYP2E1 activity.

In previous studies, we found that the clearance of nicotine is significantly slower in cigarette smokers compared with nonsmokers [4], and in the same subjects when smoking compared with not smoking. After 4 days' smoking abstinence, nicotine clearance was increased by 14% [5], and after 7 days' abstinence, nicotine clearance was 36% higher [6] when compared with overnight abstinence from cigarettes. Also the studies with another CYP2A6-specific probe, coumarin, support the reducing effect of smoking on CYP2A6-mediated metabolism [11,

Table 3Effect of nicotine administration on disposition kinetics of cotinine- d_4

	Nicotine	Placebo	P-value	95% CI of difference
$C_{\max}$ (ng ml $^{-1}$)	80.6 $\pm$ 16.3	67.4 $\pm$ 17.8	0.017	4.0, 22.3
$t_{1/2}$ (min)	867 $\pm$ 286	926 $\pm$ 226	0.14	-133, 14
Clearance (ml min $^{-1}$)	56.3 $\pm$ 23.3	55.0 $\pm$ 25.3	0.51	-2.5, 5.2
Mean residence time (min)	1205 $\pm$ 423	1315 $\pm$ 359	0.042	-205, -16
V_{ss} (l)	62.6 $\pm$ 18.5	67.7 $\pm$ 23.7	0.047	-9.7, -0.6
AUC (min ng ml $^{-1}$)	61 667 $\pm$ 20 609	63 876 $\pm$ 19 371	0.37	-6832, 2414

12]. In earlier studies with crossover design, we demonstrated that cotinine and carbon monoxide are not responsible for this reduction in CYP2A6 activity [5, 23].

Tyndale and coworkers have shown that the administration of nicotine for 21 days to African green monkeys decreases CYP2A6 activity by downregulating significantly CYP2A6 mRNA and protein in liver [13]. Also, an early study reported a decrease in nicotine metabolism in mice after 3 days' nicotine pretreatment [24]. The results of the current study indicate that this phenomenon is not occurring in humans as the activity of CYP2A6 was not affected by 7-day nicotine dosing compared with placebo, and in previous studies 7-day tobacco abstinence (tobacco abstinence corresponds to placebo treatment in the current study) has been a sufficiently long time period to increase CYP2A6 activity [6]. β -Nicotyrine, a minor tobacco alkaloid, inhibits effectively human CYP2A6 *in vitro* and metabolism of nicotine in mice *in vivo* [25, 26]. Thus, β -nicotyrine is one candidate for the search for the inhibiting compound of nicotine metabolism in tobacco smoke.

It was recently proposed that nicotine is an activator of pregnane X receptor (PXR) [27], which is an important nuclear receptor regulating the induction of several CYP and other drug-metabolizing enzymes [3]. CYP2A6 enzyme is transcriptionally regulated by PXR *in vitro* [28], and this is reflected in the increase of nicotine metabolism by inducers such as artemisinin and phenobarbital *in vivo* [29, 30]. Our current finding that nicotine does not induce CYP2A6 activity and our previous results on the reducing effect of smoking on CYP2A6 activity speak against the role of nicotine as a PXR activator. Furthermore, the high molarity of nicotine (1 mM) required for the activation of PXR *in vitro* [27] suggests that nicotine activation of PXR probably does not occur *in vivo* in humans.

We found that nicotine administration decreases the volume of distribution of cotinine, reflected in a significant increase in peak plasma concentration and decrease in mean residence time of cotinine. This finding is in agreement with our previous result that cigarette smoking reduces the volume of distribution of cotinine in comparison with 4-day cigarette abstinence [5]. Possibly the high cotinine concentration resulting from the high-dose nicotine may interfere with the transport into tissues of infused labelled cotinine.

Numerous studies in rats have shown the induction of CYP2E1 protein and activity in liver and brain by chronic nicotine administration [16–20, 31, 32]. Also, a study with 21-day nicotine dosing in African green monkeys demonstrated the induction of hepatic CYP2E1 protein and activity, including increased chlorzoxazone metabolism *in vivo* [33]. Moreover, nicotine treatment increased markedly CYP2E1 protein as studied with immunocytochemistry in a human neuroblastoma cell line IMR-32 [16]. Thus, our finding that chlorzoxazone metabolism is not induced by nicotine administration was unexpected. Possibly, 6-day nicotine abstinence in this study was an inadequate time period for CYP2E1 to return to basal expression levels. However, this is an unlikely explanation as smoking-induced CYP2E1 activity decreased measurably after only 4 days' tobacco abstinence [2]. Another explanation may be a differing response to nicotine treatment between experimental animals and humans. To our knowledge, the only human *in vitro* study reporting increased CYP2E1 expression after incubation with nicotine is the above-mentioned study in IMR-32 neuroblastoma cell line [16]. Thus, confirmatory human *in vitro* studies are needed. Tissue-selective response to nicotine between human liver and brain could also explain divergent results. Nicotine and cotinine in high concentrations inhibit CYP2E1 activity *in vitro*, but estimated K_i values are as high as 2 and 54 mg ml $^{-1}$, respectively [34]. Thus, nicotine and cotinine inhibition of CYP2E1 that could have masked the induction of CYP2E1 is unlikely. This kind of masking was also not evident when smoking was shown to induce CYP2E1 [2].

In addition to nicotine and chlorzoxazone, caffeine and bupropion were also administered during the study to phenotype CYP1A2 and CYP2B6 activities, respectively (data to be provided in subsequent reports). We are not aware of any clinical studies demonstrating that bupropion, caffeine or chlorzoxazone inhibit CYP2A6 activity. In human liver microsomes *in vitro*, bupropion, caffeine and chlorzoxazone incubated with coumarin (a specific CYP2A6 substrate) show no inhibitory effects on coumarin 7-hydroxylation [35–37]. To the best of our knowledge, there are no human *in vivo* studies on the inhibitory properties of bupropion on CYP2E1 activity, and bupropion does not inhibit chlorzoxazone metabolism *in vitro* in human liver microsomes [37, 38]. As caffeine was dosed

Figure 1
Plasma clearance of nicotine-d₂ (a), cotinine-d₄ (b) and chlorzoxazone (c) for individual subjects across treatments

Table 4
Effect of nicotine on disposition kinetics of chlorzoxazone

	Nicotine	Placebo	P-value	95% CI of difference
C _{max} (µg ml ⁻¹)	6.0 ± 2.1	5.2 ± 3.2	0.50	-1.4, 2.8
T _{max} (min)	120 ± 48	123 ± 49	0.92	-47, 42
t _{1/2} (min)	55.5 ± 12.9	60.9 ± 21.8	0.19	-13.0, 2.2
Clearance/F (ml min ⁻¹)	350 ± 187	449 ± 290	0.23	-253, 54
AUC (min µg ml ⁻¹)	852 ± 326	740 ± 394	0.23	-61, 283

Clearance/F, systemic clearance/bioavailability.

Table 5

Effect of nicotine on formation pharmacokinetics of 6-hydroxychlorzoxazone

	Nicotine	Placebo	P-value	95% CI of difference
$C_{\max}$ ($\mu\text{g ml}^{-1}$)	1.9 ± 0.9	1.7 ± 0.6	0.50	-0.24, 0.51
$T_{\max}$ (min)	155 ± 48	165 ± 56	0.70	-59, 39
$t_{1/2}$ (min)	64.5 ± 12.8	75.7 ± 27.8	0.20	-27.4, 5.0
AUC (min $\mu\text{g ml}^{-1}$)	306 ± 121	332 ± 146	0.33	-75, 24
Hydroxychlorzoxazone/chlorzoxazone AUC ratio	0.39 ± 0.16	0.50 ± 0.21	0.078	-0.22, 0.001
Urinary recovery of hydroxychlorzoxazone (%)	53.9 ± 8.6	51.1 ± 9.0	0.29	-2.2, 7.9
Formation clearance of hydroxychlorzoxazone (ml min^{-1})	191 ± 110	213 ± 94	0.44	-77, 33

24 h after chlorzoxazone administration, it could not have affected the analysis of chlorzoxazone pharmacokinetics based on the 8-h plasma and 24-h urine collections. Thus, there is no known interaction between the study medications affecting the phenotyping of CYP2A6 and CYP2E1.

In conclusion, human CYP2A6 and CYP2E1 activities are not affected by nicotine. Hence, this study provides evidence that high-dose nicotine treatment has a low potential for interaction with concurrently administered CYP2A6 and CYP2E1 substrates. The tobacco smoke compounds responsible for the reduced CYP2A6 and increased CYP2E1 activities are still unknown. Further studies are warranted.

Competing interests

None to declare.

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