Letter to the Editors

Effect of nicotine on cytochrome P450 1A2 activity

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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 induction of CYP1A2 is mediated by binding of polycyclic aromatic hydrocarbons of the tobacco smoke to the aryl hydrocarbon receptor (AHR) with consequent transcriptional activation of the *CYP1A2* gene. Furthermore, CYP1A1 and CYP1B1 enzymes are induced by tobacco smoking via AHR in various human tissues such as lung and placenta [3]. As CYP1A1 and CYP1B1 are mostly expressed in extra-hepatic tissues, their induction by smoking is not known to affect the pharmacokinetics of any medication. There is evidence of the role of nicotine in the induction of CYP1A1 and CYP1A2 enzymes *in vitro* in rat lung [4], and *in vivo* in rat lung, kidney and liver [5–7], liver and placenta of pregnant rats [8] and in brains of mice and rats [9, 10], probably through mechanisms not involving AHR. Some evidence exists for the induction of CYP1A1 by nicotine in human pulmonary explant culture [11].

We recently published a study on the effects of 10 day dosing of nicotine on human CYP2A6 and CYP2E1 activities [12]. An additional aim of that study was to determine the effects of high dose nicotine on the pharmacokinetics of oral caffeine and to test the hypothesis that nicotine induces CYP1A2-mediated metabolism of caffeine to paraxanthine, a well-established probe reaction of CYP1A2 activity [13]. No previous study has studied the effects of nicotine on CYP1A2 activity in humans *in vivo*.

The details of the experimental protocol and the subject characteristics are described in a prior report [12]. Briefly, 12 healthy smokers were given two 21 mg transdermal patches delivering a total of 42 mg nicotine day⁻¹ or placebo patches, each for 10 days in a randomized and crossover design. Subjects were not allowed to smoke or to use any tobacco products during hospitalization. At noon on the eighth hospital day, 200 mg of oral caffeine was given. Blood samples were collected for measurement of

caffeine and metabolites at 0, 30 and 60 min, and then 2, 3, 4, 6, 8, 12, 20, 32, 44 and 52 h after ingestion of caffeine. In addition, deuterium-labelled nicotine-D₂ and cotinine-D₄ phenotyping for CYP2A6, bupropion phenotyping for CYP2B6 and chlorzoxazone phenotyping for CYP2E1 were performed on the seventh and eighth hospital days as previously described [12].

Concentrations of caffeine, paraxanthine, theobromine and theophylline in plasma were determined using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Stable isotope-labelled analogues of paraxanthine and caffeine were used as internal standards. Following protein precipitation, samples (0.2 ml) were treated with phosphate buffer and extracted with a mixture of methylene chloride, ethyl acetate and isopropyl alcohol. The extracts were evaporated, reconstituted in the LC mobile phase, and injected into the LC-MS/MS system. The mass spectrometer was operated using atmospheric pressure chemical ionization, and selected reaction monitoring was used for quantitation. Calibration curves were constructed using the peak area ratio of analyte : internal standard and linear regression. Limits of quantitation for all analytes were 10 ng ml⁻¹. Precision (within-run, % coefficient of variation) ranged from 1.7 to 10.3%, and accuracy (% of expected value) ranged from 88 to 118% for concentrations from 10 ng ml⁻¹ to 5000 ng ml⁻¹. Oral clearance of caffeine was computed as follows: CL = Dose/AUC. The oral CL of caffeine and the paraxanthine : caffeine AUC(0,52 h) ratio were used as measures of CYP1A2 activity. The pharmacokinetic parameters were compared across treatments by paired Student's *t*-test.

The effects of nicotine on the disposition kinetics of caffeine are presented in Table 1. There was no significant influence of nicotine administration on the pharmacokinetic parameters of caffeine or the formation pharmacokinetics of paraxanthine, theophylline and theobromine. Since caffeine metabolism to paraxanthine is a specific probe reaction for CYP1A2 [13], it can be concluded that

Table 1

Effect of nicotine on caffeine disposition kinetics and formation and elimination pharmacokinetics of paraxanthine, theobromine and theophylline

	Nicotine	Placebo	P value	95% CI of difference
Caffeine				
$C_{\max}$ (ng ml ⁻¹)	4 361 ± 1 150	4 286 ± 1 237	0.87	-936, 1085
$t_{\max}$ (min)	129 ± 65	110 ± 66	0.44	-32, 70
$t_{1/2}$ (min)	312 ± 136	326 ± 113	0.51	-60, 32
AUC (ng ml ⁻¹ h)	36 783 ± 18 326	33 797 ± 14 346	0.32	-3378, 9352
CL/F (ml min ⁻¹)	113 ± 54	119 ± 61	0.57	-31, 18
V_z/F (l)	43 ± 15	52 ± 26	0.062	-18, 0.53
MRT (min)	484 ± 185	480 ± 138	0.87	-51, 59
Paraxanthine				
$C_{\max}$ (ng ml ⁻¹)	1 393 ± 296	1 434 ± 314	0.31	-125, 43
$t_{\max}$ (min)	398 ± 156	350 ± 108	0.26	-40, 135
$t_{1/2}$ (min)	378 ± 168	375 ± 99	0.92	-66, 72
AUC(0,52 h) (ng ml ⁻¹ h)	22 897 ± 8 225	22 604 ± 6 993	0.80	-2168, 2754
Paraxanthine : caffeine AUC(0,52 h) ratio	0.68 ± 0.17	0.73 ± 0.19	0.29	-0.14, 0.045
Theobromine				
$C_{\max}$ (ng ml ⁻¹)	239 ± 48	322 ± 229	0.21	-66, 9.1
$t_{\max}$ (min)	594 ± 498	730 ± 798	0.59	-203, 257
$t_{1/2}$ (min)	633 ± 366	692 ± 307	0.75	-142, 189
AUC(0,52 h) (ng ml ⁻¹ h)	5 570 ± 2 654	7 486 ± 6 278	0.27	-1549, 76
Theobromine : caffeine AUC(0,52 h) ratio	0.16 ± 0.038	0.25 ± 0.26	0.23	-0.066, 0.018
Theophylline				
$C_{\max}$ (ng ml ⁻¹)	85 ± 15	85 ± 17	0.95	-9.2, 8.7
$t_{\max}$ (min)	514 ± 292	570 ± 281	0.48	-227, 115
$t_{1/2}$ (min)	738 ± 405	626 ± 172	0.29	-108, 332
AUC(0,52 h) (ng ml ⁻¹ h)	1 823 ± 977	1 724 ± 702	0.51	-222, 419
Theophylline : caffeine AUC(0,52 h) ratio	0.050 ± 0.010	0.053 ± 0.012	0.44	-0.012, 0.0049

Data are presented as mean ± SD. CI, confidence interval; $C_{\max}$, peak plasma concentration; $t_{\max}$, time to peak plasma concentration; $t_{1/2}$, elimination half-life; AUC, area under plasma concentration–time curve extrapolated to infinity; CL/F, systemic clearance/bioavailability; V_z/F , apparent volume of distribution during terminal phase.

CYP1A2 activity is not affected by 8 days of nicotine dosing. Although previous studies in experimental animals have provided evidence for the role of nicotine in the induction of CYP1A1 and CYP1A2 enzymes [4–10], our study disproves the hypothesis that nicotine induces CYP1A2 activity in humans *in vivo*. The discrepancy between human and animal data may be explained by tissue and species specific expression patterns. The human caffeine phenotyping probes the hepatic CYP1A2 activity, whereas the animal studies are mainly on extrahepatic CYP1A1 induction or based on methods not capable of differentiating between CYP1A1 and CYP1A2 enzymes.

In addition to nicotine and caffeine, bupropion and chlorzoxazone were administered during the study to phenotype CYP2B6 and CYP2E1 activities, respectively. We are not aware of any clinical studies demonstrating that bupropion inhibits CYP1A2 activity. In human liver microsomes *in vitro*, bupropion incubated with melatonin or phenacetin (probes for CYP1A2) shows no inhibitory effects on CYP1A2-mediated pathways [14–16]. Chlorzoxazone and caffeine have been dosed together in several metabolic cocktail approaches to phenotype CYP enzymes without any detected metabolic interactions [17–19]. Thus, there is no known interaction between the study medications affecting the phenotyping of CYP1A2.

In conclusion, this study shows that human CYP1A2 activity is not affected by nicotine and provides evidence that high dose nicotine treatment has a low potential for interaction with concurrently administered CYP1A2 substrates. Nicotine has no role in the induction of CYP1A2 that is known to occur in smokers.

Competing Interests

Dr Benowitz has been a paid consultant to several pharmaceutical companies that are developing or market smoking cessation medications.

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