DOI:10.1111/j.1365-2125.2005.02406.x

Letter to the Editors

Effect of pregnancy on a measure of FMO3 activity

Janne Hukkanen, Delia Dempsey, Peyton Jacob III & Neal L. Benowitz
Division of Clinical Pharmacology and Experimental Therapeutics, Medical Service, San Francisco General Hospital Medical Center, and the Departments of Medicine, Psychiatry and Biopharmaceutical Sciences, University of California, San Francisco, USA

Flavin-containing monooxygenases (FMO) are a family of microsomal enzymes that catalyse the oxygenation of xenobiotics [1]. Six *FMO* genes exist in humans, but only FMO3 enzyme is abundantly expressed in human liver. FMO3 contributes to the metabolism of several drugs including methimazole, nicotine, cimetidine, ranitidine, tamoxifen, and clozapine [1, 2]. Dysfunctional FMO3 enzymes with deficient N-oxygenation of dietary trimethylamine cause trimethylaminuria (fish-odour syndrome).

FMO3 is the main enzyme responsible for nicotine N′-oxide formation, which is a minor pathway of nicotine metabolism. The FMO content of human liver microsomes is correlated with nicotine N′-oxide formation, and N′-oxygenase activity is abolished by FMO inhibitors *in vitro* [3]. Also, cDNA-expressed FMO3 is active in nicotine N′-oxide formation [4]. A preliminary report on two siblings with fish-odour syndrome showed impaired urinary excretion of nicotine N′-oxide compared with normal subjects [5]. Methimazole, an antithyroid agent and FMO3 inhibitor, has been reported to reduce nicotine N′-oxide excretion [6]. Thus, formation of nicotine N′-oxide can be used as a probe of human FMO3 activity [1].

We recently published a study on pregnant smokers investigating the effect of pregnancy on nicotine and cotinine metabolism [7]. Ten subjects were given deuterium-labelled nicotine-d_2 and cotinine-d_4 infusion (30 or 45 $\mu g/kg^{-1}$ of each compound) during pregnancy and postpartum. The systemic clearance was increased by 60% and 140% for nicotine and cotinine, respectively, in pregnancy compared with postpartum. This increase is most likely mediated by the induction of cytochrome P450 2A6, the main enzyme metabolizing nicotine and cotinine [8].

Urine samples were collected and nicotine and metabolite concentrations were analysed by gas chromatography – mass spectrometry [7]. We have now reanalysed the data to examine the effects of pregnancy on the urinary ratio of nicotine N′-oxide/nicotine, as an index of FMO3 activity. Ten subjects completed the study; a urine sample from one subject was lost. We measured both nicotine N′-oxide-d_2 and nicotine-d_2 concentrations in urine samples collected over 8 h following the infusion in nine subjects during pregnancy and postpartum. In two subjects the calculation of the urine ratio during pregnancy was not possible because the concentration of nicotine (one subject) or concentration of nicotine N′-oxide (one subject) was below the limit of quantification. In the remaining subjects the ratio of nicotine N′-oxide/nicotine was substantially increased during pregnancy compared with postpartum (2.03 *vs.* 0.78; paired t-test, $P = 0.017$; 95% CI of the difference 0.5–2.0) (Table 1).

A possible confounding factor is that renal clearance of nicotine is pH-dependent, whereas nicotine N′-oxide excretion is not. On average, pregnancy increases urine pH by 0.3–0.4 units [9] and might increase the nicotine N′-oxide/nicotine ratio by decreasing nicotine excretion. Urine pH values were not recorded in our study. To answer the question of whether a change in the renal clearance of nicotine related to pH might explain our observations, we calculated the ratio of nicotine N′-oxide excreted in urine/area under the plasma nicotine concentration–time curve over the 8 h of urine collection (AUC). As the plasma concentration of nicotine is the driving force behind nicotine N′-oxide formation in liver, this ratio can be used as an another indicator of FMO3 activity. The ratio of urine nicotine N′-oxide/plasma $AUC_{nicotine}$ was increased significantly in pregnancy compared with postpartum (45.3 *vs.* 28.6; paired t-test, $P = 0.047$; 95% CI of the difference 3.1–30.3) (Table 1).

Table 1
Effect of pregnancy on the nicotine N′-oxide metabolic ratios

Subject	Urine nicotine N′-oxide/ Urine nicotine		Urine nicotine N′-oxide/ Plasma $AUC_{nicotine}$ (ml min^{-1})	
	Pregnant	Post-Partum	Pregnant	Post-Partum
1	2.57	1.34	44.7	35.5
2	1.37	0.20	22.6	37.2
3		–*	36.5	27.8
4	2.03	0.27	51.9	41.4
5	1.17	1.58	55.5	20.5
6	2.01	1.57	52.9	46.0
7	2.22	0.36	59.8	12.1
8	2.83	0.12	38.2	8.2
Mean	2.03	0.78	45.3	28.6
SD	0.60	0.68	12.3	13.8

*Difference between pregnant and postpartum is statistically significant (*P = *0.017, paired* t*-test for urine nicotine N′-oxide/urine nicotine ratio;* P = *0.047, paired* t*-test for urine nicotine N′-oxide/plasma* $AUC_{nicotine}$ *ratio). *The ratio not calculated due to the concentration of urine nicotine below the limit of quantification.*

Our observation provides evidence that FMO3 activity may be induced during pregnancy. We are aware of only one previous article describing the effect of pregnancy on human FMO3-related activity. In this work, the apparent clearance of methimazole was found to be higher (164 ml min^{-1} *vs.* 110 ml min^{-1}) in pregnant hyperthyroid patients compared with nonpregnant patients after administration of 10 mg oral carbimazole, which is efficiently hydrolysed to methimazole [10]. Also of note is that hepatic FMO enzymes in rabbits and sheep are induced during pregnancy [11, 12]. Although gender does not affect FMO3-related activities and hepatic enzyme expression in humans [13–15], menstruation increases the symptoms of the fish-odour syndrome and reduces the excretion of trimethylamine N-oxide in some women with this syndrome [16, 17]. This would further suggest that sex hormones play a role in the regulation of FMO3.

In conclusion, human FMO3 activity seems most likely to be induced in pregnancy, most likely by sex hormones. This observation should be taken into account when designing studies on FMO3-metabolized drugs in pregnant women. Further studies of the pharmacokinetics of drugs metabolized by FMO3 are warranted.

Supported by US Public Health Service grants DA09761, DA02277 and DA12393 from the National Institute on Drug Abuse, National Institutes of Health. Carried out in part at the General Clinical Research Center at San Francisco General Hospital Medical Center with the support of the Division of Research Resources, National Institutes of Health (5-MO1-RR-00083–41).

References

1 Cashman JR, Zhang J. Interindividual differences of human flavin-containing monooxygenase 3: genetic polymorphisms and functional variation. Drug Metab Dispos 2002; 30: 1043–52.
2 Overby LH, Carver GC, Philpot RM. Quantitation and kinetic properties of hepatic microsomal and recombinant flavin-containing monooxygenases 3 and 5 from humans. Chem Biol Interact 1997; 106: 29–45.
3 Cashman JR, Park SB, Yang ZC, Wrighton SA, Jacob 3rd P, Benowitz NL. Metabolism of nicotine by human liver microsomes. stereoselective formation of trans-nicotine N′-oxide. Chem Res Toxicol 1992; 5: 639–46.
4 Park SB, Jacob III P, Benowitz NL, Cashman JR. Stereoselective metabolism of (S)-(-)-nicotine in humans: formation of trans-(S)-(-)-nicotine N-1′-oxide. Chem Res Toxicol 1993; 6: 880–8.
5 Ayesh R, Al-Waiz M, Crothers J, Cholerton S, Mitchell SC, Idle JR, Smith RL. Deficient nicotine N-oxidation in two sisters with trimethylaminuria. Br J Clin Pharmacol 1988; 25: 664–5.
6 Cholerton S, Ayesh R, Idle JR, Smith RL. The pre-eminence of nicotine N-oxidation and its diminution after carbamizole administration. Br J Clin Pharmacol 1988; 26: 652–3.
7 Dempsey D, Jacob P. 3rd, Benowitz NL. Accelerated metabolism of nicotine and cotinine in pregnant smokers. J Pharmacol Exp Ther 2002; 301: 594–8.
8 Nakajima M, Kuroiwa Y, Yokoi T. Interindividual differences in nicotine metabolism and genetic polymorphisms of human CYP2A6. Drug Metab Rev 2002; 34: 865–77.
9 Maikranz P, Holley JL, Parks JH, Lindheimer MD, Nakagawa Y, Coe FL. Gestational hypercalciuria causes pathological urine calcium oxalate supersaturations. Kidney Int 1989; 36: 108–13.
10 Skellern GG, Knight BI, Otter M, Low CK, Alexander WD. The pharmacokinetics of methimazole in pregnant patients after oral administration of carbimazole. Br J Clin Pharmacol 1980; 9: 145–7.
11 Williams DE, Meyer HH, Dutchuk MS. Distinct pulmonary and hepatic forms of flavin-containing monooxygenase in sheep. Comp Biochem Physiol B 1989; 93: 465–70.
12 Lee MY, Smiley S, Kadkhodayan S, Hines RN. Williams DE. Developmental regulation of flavin-containing monooxygenase (FMO) isoforms 1 and 2 in pregnant rabbit. Chem Biol Interact 1995; 96: 75–85.
13 Chung WG, Kang JH, Park CS, Cho MH, Cha YN. Effect of age and smoking on in vivo CYP1A2, flavin-containing monooxygenase,

and xanthine oxidase activities in Koreans: determination by caffeine metabolism. Clin Pharmacol Ther 2000; 67: 258–66.

14 Kang JH, Chung WG, Lee KH, Park CS, Kang JS, Shin IC, Roh HK, Dong MS, Baek HM, Cha YN. Phenotypes of flavin-containing monooxygenase activity determined by ranitidine N-oxidation are positively correlated with genotypes of linked FM03 gene mutations in a Korean population. Pharmacogenetics 2000; 10: 67–78.

15 Koukouritaki SB, Simpson P, Yeung CK, Rettie AE, Hines RN. Human hepatic flavin-containing monooxygenases 1 (FMO1) and 3 (FMO3) developmental expression. Pediatr Res 2002; 51: 236–43.

16 Zhang AQ, Mitchell SC, Smith RL. Exacerbation of symptoms of fish-odour syndrome during menstruation. Lancet 1996; 348: 1740–1.

17 Yamazaki H, Fujieda M, Togashi M, Saito T, Preti G, Cashman JR, Kamataki T. Effects of the dietary supplements, activated charcoal and copper chlorophyllin, on urinary excretion of trimethylamine in Japanese trimethylaminuria patients. Life Sci 2004; 74: 2739–47.

Correspondence

Neal L. Benowitz, MD, Division of Clinical Pharmacology and Experimental Therapeutics, University of California, San Francisco, Box 1220, San Francisco, CA 94143–1220, California, USA. Tel: +1 415 206 8324; Fax: +1 415 206 4956; Email: nbeno@itsa.ucsf.edu