Effect of fluvoxamine therapy on the activities of CYP1A2, CYP2D6, and CYP3A as determined by phenotyping

Objective: To determine the effect of 150 mg/day fluvoxamine on the activities of CYP1A2, CYP2D6, CYP3A, *N*-acetyltransferase-2 (NAT2), and xanthine oxidase (XO) by phenotyping with caffeine, dextromethorphan, and midazolam.

Methods: Oral caffeine (2 mg/kg), oral dextromethorphan (30 mg), and intravenous midazolam (0.025 mg/kg) were administered to 10 white male volunteers every 14 days for 4 months and to 10 white premenopausal female volunteers during the midfollicular and midluteal phases of the menstrual cycle for 4 complete cycles (8 total phenotyping measures). The first 6 phenotyping measures were used to establish baseline activity. Subjects were given 150 mg/day fluvoxamine for the fourth month or cycle of the study. Enzyme activity for CYP1A2, CYP2D6, NAT2, and XO was expressed as urinary metabolite ratios. Midazolam plasma clearance was used to express CYP3A activity.

Results: No difference between baseline and weeks 2 and 4 of fluvoxamine therapy was observed for NAT2 or XO metabolite ratios. For CYP1A2, CYP2D6, and CYP3A phenotypes, significant differences existed between baseline and fluvoxamine therapy. For CYP1A2, the mean urinary metabolite ratio (±SD) was 7.53 ± 7.44 at baseline and 4.30 ± 2.82 with fluvoxamine (P = .012). Mean CYP2D6 molar urinary dextromethorphan ratios before and after fluvoxamine therapy were 0.00780 ± 0.00694 and 0.0153 ± 0.0127, respectively (P = .011). Midazolam clearance decreased from 0.0081 ± 0.0024 L/min/kg at baseline to 0.0054 ± 0.0021 L/min/kg with therapy (P = .0091). For CYP1A2, CYP2D6, and CYP3A, fluvoxamine therapy changed the phenotyping measures by a median of −44.4%, 123.5%, and −34.4%, respectively.

Conclusions: We concluded that fluvoxamine may cause significant inhibition of CYP1A2, CYP2D6, and CYP3A activity. This metabolic inhibition may have serious implications for a variety of medications.
(Clin Pharmacol Ther 1998;64:257-68.)

Angela D. M. Kashuba, PharmD, Anne N. Nafziger, MD, MHS, Gregory L. Kearns, PharmD, FCP, J. Steven Leeder, PharmD, PhD, Russell Gotschall, MS, Mario L. Rocci, Jr, PhD, Robert W. Kulawy, BS, Debra J. Beck, BS, and Joseph S. Bertino, Jr, PharmD

Cooperstown and Whitesboro, N.Y., and Kansas City, Mo.

Drug interactions are commonly encountered among medications used in psychiatric practice.[1] With the advances in molecular pharmacology, it is now possible to characterize the specific cytochrome P450 isoforms responsible for drug metabolism. This knowledge may assist clinicians in anticipating drug interactions, as well as in predicting response or nonresponse to medications. Selective serotonin reuptake inhibitor

From the Clinical Pharmacology Research Center, the Department of Medicine, and the Department of Pharmacy Services, Bassett Healthcare, Cooperstown; the Division of Pediatric Clinical Pharmacology and Experimental Therapeutics, Children's Mercy Hospital, and the Department of Pediatrics and the Department of Pharmacology, University of Missouri, Kansas City, Kansas City; and Oneida Research Services, Inc., Whitesboro.

Supported in part by the E. Donnall Thomas Resident Research Program in Internal Medicine (Cooperstown, N.Y.), the American College of Clinical Pharmacists' (Kansas City, Mo.) Wyeth-Ayerst Laboratories Women's Healthcare Research Award, and grant 1U1031314-04 from the Network of Pediatric Pharmacology Research Units (Bethesda, Md., Dr. Kearns), National Institute of Child Health and Human Development (Bethesda, Md.), and Pfizer United States Pharmaceutical Group (New York, N.Y.).

Received for publication July 21, 1997; accepted May 13, 1998.

Reprint requests: Joseph S. Bertino Jr, PharmD, Clinical Pharmacology Research Center, Bassett Healthcare, One Atwell Road, Cooperstown, NY 13326. E-mail: jbertino@usa.net

0009-9236/98/$5.00 + 0 **13/1/91712**

(SSRI) antidepressants inhibit cytochrome P450 enzyme activity.[2] However, as shown in vitro, any given SSRI does not inhibit all cytochromes P450 to the same degree, and any given isozyme is not inhibited to the same extent by all SSRIs.[1] It is therefore clinically important to determine the in vivo effects of these drugs on enzyme activity.

Fluvoxamine, an SSRI, is eliminated predominantly by oxidation through the cytochromes P450.[3] Although the specific isozymes responsible for the metabolism of fluvoxamine have not been fully elucidated, investigations of its pharmacokinetics between smokers and nonsmokers and its interaction with caffeine indicate a correlation with CYP1A2 activity.[4,5] It has been suggested that the disposition of fluvoxamine may be associated with CYP2D6[6,7] and CYP2C19[8,9] activity, although findings are conflicting.[7,8] Fluvoxamine displays competitive, reversible potent inhibition of CYP1A2[10-12] and may also inhibit CYP3A[13] and CYP2D6.[14] Among the SSRIs, in vitro fluvoxamine has the most potent inhibitory effect on CYP3A and has a modest effect on CYP2D6 compared with paroxetine, fluoxetine, and norfluoxetine.[1,15]

The conflicting results observed in these in vivo investigations of the effect of fluvoxamine on P450 activity may be a result of the varying doses (50 to 100 mg/day) and treatment durations (from a single dose to 2 weeks of therapy) used. Many trials have not duplicated the clinical situation of higher doses and longer treatment duration. Small sample sizes and single phenotyping measures may not discriminate between intraindividual variability in isozyme activity and the true pharmacologic effect of fluvoxamine. In addition, use of substrates with multiple pathways of metabolism to examine the activities of single enzymes (ie, metoprolol for CYP2D6 and alprazolam and imipramine for CYP3A) may confound the results.

As the functional status of the cytochromes P450 has great impact on drug efficacy and interactions, it is important to conclusively determine the degree of enzyme inhibition in vivo with agents determined to impact activity in vitro. The usual clinically effective dose of fluvoxamine is 150 mg/day,[16] which may take as long as 14 days to reach steady state.[17] We therefore conducted a study to examine the effects of 4 weeks of therapy with 150 mg/day fluvoxamine on the activities of CYP1A2, CYP2D6, CYP3A, *N*-acetyltransferase-2, and xanthine oxidase as determined by phenotyping. Our specific aims were to (1) determine whether fluvoxamine affects drug-metabolizing enzyme activity as measured by phenotyping, (2) determine whether this effect differs after 2 and 4 weeks of fluvoxamine therapy, and (3) if an interaction exists, to determine whether there is a correlation between the magnitude of fluvoxamine effect and enzyme phenotype or *CYP2D6* genotype.

METHODS

This study was approved by the Institutional Review Board of Bassett Healthcare (Cooperstown, N.Y.). Written informed consent was obtained from all subjects.

Study subjects

Twenty white subjects were recruited for this investigation. A complete history was obtained from all subjects, and all subjects underwent a physical examination, an ECG, and blood chemistry and urinalysis screening before study. Subjects were excluded if they were receiving any medications on a long-term basis or if they were receiving concomitant therapy with drugs known to induce or inhibit the cytochromes P450. Smokers and binge drinkers were also excluded. Moderate alcohol intake was allowed (1 drink equivalent to 1 12-oz beer daily). Subjects were excluded if hepatic transaminases (AST and ALT) were greater than 1.5 times the upper limit of normal (men, AST from 0 to 50 U/L; ALT from 0 to 60 U/L; women, AST from 0 to 40 U/L; ALT from 0 to 50 U/L), bilirubin was greater than 1.5 mg/dL, or serum creatinine was not within the normal range (Men, 0.6 to 1.2 mg/dL, women, 0.5 to 1.0 mg/dL). Women were required to have regular menstrual cycles, defined as a predictable cycle length (ie, ±3 days) for a 3-month history. Women who were not surgically sterile underwent urine pregnancy testing (qualitative β-human chorionic gonadotropin) during screening and before each study phase (One-Step Clearblue Easy, Whitehall Laboratories, Madison, N.J.).

Phenotyping procedure

To obtain baseline enzyme activity data, men were phenotyped once every 14 days for 12 weeks. Women were phenotyped during the midfollicular and midluteal phases of the menstrual cycle for 3 complete cycles. To establish menstrual cycle patterns, women were instructed to keep a diary for 3 months before the study began and to use a home diagnostic ovulation kit for qualitative luteinizing hormone (One-Step Clearplan Easy, Whitehall Laboratories) during the menstrual cycle before the beginning of study. In addition, during each month of study, women were instructed to test first morning urine for qualitative luteinizing hormone 3 days before predicted midcycle and to continuing until a positive result was noted to accurately determine ovulation. Women were phenotyped during the midfollicular (days

5 to 8 of the cycle) and midluteal (days 17 to 20 of the cycle) menstrual cycle phases. All subjects refrained from ingesting ethanol, chocolate, caffeine-containing beverages, grapefruit or grapefruit juice, charbroiled foods, watercress, and cruciferous vegetables (eg, broccoli, cauliflower, cabbage, brussels sprouts, and kale) for 3 days before and the day of each phenotyping study.

On the morning of each study day (9 AM), subjects received a single dose of 0.025 mg/kg intravenous midazolam (Versed, provided by Hoffmann–La Roche, Inc., Nutley, N.J.) administered over 60 seconds into an antecubital vein. Blood samples were collected through an intravenous catheter (Angio-Set, Becton Dickinson Vascular Access, Sandy, Utah) placed in the opposite arm of each subject. Patency was maintained with 3 mL flushes of 10 U/mL heparin solution, and 3 mL blood was withdrawn from the catheter dead space and discarded immediately before each blood sample. Fifteen milliliter blood samples were collected at 0, 5, 30, 60, 120, 240, 300, and 360 minutes after midazolam administration. Respiratory status of all subjects was monitored by pulse oximetry for the first hour after midazolam administration. Samples were collected in ethylenediaminetetraacetic acid tubes, kept on ice, and centrifuged within 2 hours of collection at 2800 rpm at 4°C for 15 minutes. Plasma was separated and stored at –80°C until analysis.

At 4 PM, subjects emptied their bladders and were given an oral dose of caffeine, 2 mg/kg total body weight, rounded to the nearest 50 mg (NoDoze, Bristol Myers Inc., Princeton, N.J.), and 30 mg dextromethorphan (Robitussin Pediatric Solution, AH Robbins, Madison, N.J.). All urine was collected overnight in a single container with 2 g ascorbic acid, up to and including the first morning void. Total time of urine collection, total urine volume, and urine pH were recorded after mixing of the specimen. Fifteen milliliter aliquots of urine were combined with 20 mg/mL ascorbic acid to maintain a pH of <4 and were frozen at –80°C until analysis.

Fluvoxamine phase

After the sixth baseline phenotyping visit, all subjects were given 50 mg fluvoxamine capsules (Luvox, lot number 87145, Solvay, Marietta, Ga.), and instructed to titrate their dosage to 150 mg/day over 7 days: 50 mg in the evening (8 PM) for 3 days, 50 mg in the morning (6 AM) and evening (8 PM) for the next 3 days, 50 mg in the morning (6 AM) and 100 mg in the evening (8 PM) for the remainder of the study. Men were phenotyped 14 days and 28 days after the start of fluvoxamine therapy, and women were phenotyped in the subsequent midluteal and midfollicular phases of their menstrual cycle, as outlined above. Subject report and capsule counts assessed compliance at each visit. Adverse drug reactions were monitored by the use of a 1-page standardized self-report questionnaire modified from Corso et al.[18] Any events were described in greater detail on the comprehensive questionnaire by a clinician.[18]

Analytical procedure

Caffeine metabolites. Determination of 1-methylurate (1U), 1-methylxanthine (1X), 1,7-dimethylurate (17U), and 5-acetylamino-6-formylamino-3-methyluracil (AFMU) in urine was accomplished with useof a modification of HPLC methods of Evans et al.[19] In brief, internal standard (sulfamethoxazole) was added to 400 μL urine saturated with approximately 240 mg ammonium sulfate. The caffeine metabolites were then extracted with 3.5 mL of a 90:10 solution of chloroform/2-propanol. The aqueous layer was removed by aspiration and discarded. The organic layer was dried in a Savant SpeedVac (Holbrook, N.Y.) at 50°C. Samples were dissolved in 100 μL of 0.05% acetic acid and 75 μL was injected into the HPLC system (HP model 1100 chromatographic system, Hewlett-Packard, San Fernando, Calif.). Chromatography was performed at 50°C on a Nova-Pak C_{18} column (Waters Corp., Milford, Mass.) equilibrated with 95% solvent A (0.05% acetic acid) and 5% solvent B (100% methanol) at a flow rate of 1.2 mL/min, after which the chromatogram was developed with a multistep gradient produced by applying the following linear change in solvent: 0 minutes, 5%; 3.0 minutes, 5%; 5.0 minutes, 7%; 6.0 minutes, 15%; 7.0 minutes, 20%; 10.01 minutes, 30%; 14.0 minutes, 35%; 14.01 minutes, 100%; 17 minutes, 100%; and 17.01 minutes, 5%. Ultraviolet detection at 290 nm was used to monitor the separation of analytes. Data output was normalized to the internal standard, and the molar amount determined with use of standard curves prepared daily in drug free urine specimens.

Intraday variability values for 17U at concentrations of 500, 100, 10, and 1 ng/mL were 4.9%, 5.5%, 3.3%, and 10.5%, respectively. Interday variability values for 17U at concentrations of 500, 100, 10, and 1 ng/mL were 4.3%, 8.4%, 8.0%, and 14.2%, respectively. Intraday variability values for 500, 100, 10, and 1 nm/mL concentrations of 1X were 5.5%, 2.0%, 5.9%, and 4.6%, respectively. Interday variability values for 500, 100, 10, and 1 nm/mL concentrations of 1X were 10.5%, 9.3%, 6.2%, and 21.0%, respectively. For 1U, the intraday and interday variability values were consistently <10%. Intraday variability for AFMU at concentrations of 100, 10, and 1 nm/mL were 5.2%, 4.1%, and 4.0%. Interday variability for AFMU at concentra-

Table I. Demographic data for enrolled subjects

Variable	*Men (n = 10)*	*Women (n = 10)*
Age (y)	34.8 ± 7.9	38.2 ± 9.3)
Total body weight (kg)	76.3 ± 13.3	79.4 ± 20.1
Ideal body weight (kg)*	74.0 ± 6.8	58.5 ± 6.3†
AST (U/L)	24.2 ± 4.8	24.0 ± 7.4
ALT (U/L)	30.1 ± 8.3	24.6 ± 9.2
Serum creatinine (mg/dL)	1.0 ± 0.1	0.8 ± 0.1†
Calculated creatinine clearance (mL/min/1.73 m^2)‡	99.4 ± 14.7	81.3 ± 19.7
Caffeine dose (mg)	155 ± 43.8	150 ± 33.3
Length of overnight urine collection (h)	15.0 ± 1.0	14.3 ± 0.6
Midazolam dose (mg)	2.0 ± 0.5	1.9 ± 0.3
Length of menstrual cycle (days)	—	27.2 ± 2.3
Follicular phase study day (days after menstruation)	—	5.6 ± 1.4
Menstrual cycle day of ovulation	—	14.1 ± 2.8
Luteal phase study day (days after menstruation)	—	18.3 ± 3.3
Luteal phase study day (days after ovulation)	—	3.9 ± 1.3
Length of fluvoxamine therapy (days)		
Visit 1	13.8 ± 0.63	15.3 ± 3.5
Visit 2	28.1 ± 0.74	27.5 ± 3.8
Fluvoxamine compliance		
Week 1-2	98.6%	98.0%
Week 3-4	97.2%	95.9%

Data are mean values ± SD.
*See Devine.[41]
†$P < .05$ compared to males; fluvoxamine compliance calculated as follows: (Number of doses taken) ÷ (number of doses scheduled to be taken) × 100%.
‡See Cockcroft and Gault.[42]

tions of 100, 10, and 1 nm/mL were 8.3%, 5.2%, and 4.0%, respectively.

Dextromethorphan and metabolites Determination of dextromethorphan, dextrorphan, and 3-methoxymorphinan in urine was accomplished with use of a modification of the HPLC methods of Park et al[20] and Lam and Rodriguez.[21] In brief, 100 standard mU of β-glucuronidase/arylsulfatase was added to each 3 mL aliquot of urine, which was placed in a shaking incubator for 18 hours at 37°C. Internal standard (levallorphan tartrate) was then added and the pH adjusted to 12 before solid-phase extraction with use of Chem Elute (Varian, Harbor City, Calif.) columns. Eluants were back extracted with 0.01 mol/L hydrochloric acid and the aqueous layer dried in a Savant SpeedVac at 50°C. Samples were then redissolved in 100 μL of 0.01N hydrochloric acid, and 75 μL was injected into the HPLC system (HP model 1100 chromatographic system with an HP model 1046A fluorescence detector, Hewlett-Packard). Chromatography was performed at 25°C on a Novapak (Waters Corp.) phenyl column with use of mobile phase that consisted of 20 mmol/L potassium phosphate–hexane sulfonic acid, (60%, pH 4.0) and acetonitrile (40%) pumped at 1.2 mL/min, with monitoring at excitation and emission wavelengths of 235 and 310 nm, respectively. Data output was normalized to the internal standard, and the molar amount determined with standard curves prepared daily in drug-free urine specimens.

Intraday variability values for dextromethorphan at concentrations of 10, 1, and 0.1 nm/mL were 3.1%, 1.7%, and 4.7%. Interday variability values for dextromethorphan at concentrations of 10, 1, and 0.1 nm/mL were 3.4%, 3.5%, and 7.8%, respectively. Intraday variability values for 100, 10, and 1 nm/mL concentrations of dextrorphan were 2%, 4.8%, and 1.9%. Interday variability for 100, 10, and 1 nm/mL concentrations of dextrorphan were 2.8%, 6.9%, and 10.1%, respectively. For 3-methoxymorphinan, the intraday and interday variability values were consistently <5% and <10%, respectively.

Midazolam and metabolites Plasma midazolam, 1-hydroxymidazolam, and 4-hydroxymidazolam concentrations were determined at Oneida Research Services, Inc. with use of an LC/MS/MS method and with alprazolam as the internal standard.† In brief, 1 mL plasma samples were deprotonated and extracted with methanol and C_{18} solid-phase columns. Samples were evaporated to dryness and reconstituted in 50 μL of methanol/5 mmol/L ammonium acetate (80/20). Chro-

†Rocci ML, Kulawy RW, Beck DJ. Analysis of midazolam, 1-hydroxymidazolam and 4-hydroxymidazolam in human plasma by LC/MS/MS. Submitted for publication, J Chromatogr.

Table II. Comparison of phenotyping measures before and after therapy with 150 mg/day fluvoxamine, and percent change of those measures, in 10 men and 10 women for CYP1A2, NAT2, XO, CYP2D6, and CYP3A

Enzyme	*Baseline pheontype**	*Fluvoxamine phenotype therapy†*	*Change in phenotyping measure (%)‡*
CYP1A2	7.53 ± 7.44	4.30 ± 2.82	–44.4 (–25.6, 49.0)
NAT2	0.462 ± 0.168	0.543 ± 0.149	–8.89 (–2.3, –50.4)
XO	0.667 ± 0.0630	0.637 ± 0.0415	–6.03 (–11.2, 1.3)
CYP2D6	0.00780 ± 0.00694	0.0153 ± 0.0127	123.5 (–10.2, 180.6)
CYP3A	0.0081 ± 0.0024	0.0054 ± 0.0021	–34.4 (–26.0, –41.5)

NAT2, *N*-Acetyltransferase 2; XO, xanthine oxidase.
*Mean ± SD of six measures.
†Mean ± SD of two measures.
‡Data reported as median; 25th and 75th percentiles are given in parentheses. $P < .0167$ for baseline versus fluvoxamine therapy for CYP1A2, CYP2D6, and CYP3A; see text for phenotype measure calculations.

matographic separation of the compounds was accomplished with use of a Waters symmetry C_{18} column and a methanol/5 mmol/L ammonium acetate (80/20) mobile phase. A PE Sciex API III+ LC/MS/MS system (Perkin-Elmer Sciex Instruments, Rochester, N.H.) equipped with a Waters 616 pump and 600S controller (Waters Corporation) and a Waters 717+ autosampler was used in this analysis. Approximate retention times for alprazolam, 4-hydroxymidazolam, 1-hydroxymidazolam, and midazolam were 4.0, 4.5, 5.5, and 7.5 minutes, respectively. The method used a standard curve that ranged in concentration from 0.25 to 100 ng/mL for each analyte of interest. Interassay precision of the method was 9.89% or less at quality control sample concentrations of 0.75, 7.5, and 75.0 ng/mL. Interassay accuracy for the same quality control samples ranged from –5.73% to 9.20% of nominal values. (A more detailed method description can be obtained by writing to the corresponding author, J.S.B.)

Material

AFMU was obtained from Dr. B.K. Tang at the Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada. All other standards and reagents used for the quantitation of caffeine metabolites were of the highest purity available commercially (Sigma Chemical Co., St. Louis, Mo.). Dextromethorphan was purchased commercially (Sigma Chemical Co.) and both levallorphan tartrate and dextrorphan were provided as gifts from Roche Laboratories. Standards of 3-methoxymorphinan were obtained from Gentest (Woburn, Mass.). All other reagents for CYP2D6 phenotyping and genotyping were of the highest purity available commercially (Sigma Chemical Co.). Midazolam, 1′-hydroxymidazolam, and 4′-hydroxymidazolam standards were provided by Mr. Thomas Mulligan and Dr. Jerry Sepinwall (Roche Laboratories).

Genotyping procedure

CYP2D6 genotyping was performed for 17 subjects according to methods established in our laboratory. For this method, highly pure genomic DNA suitable for extra-long polymerase chain reaction (XL-PCR) is prepared from peripheral blood mononuclear cells with the Super Quik-Gene kit (AGTC Inc., Denver, Colo.). To distinguish between the *CYP2D6* gene and the *CYP2D8* pseudogene and nonfunctional *CYP2D7* gene (both located immediately upstream from CYP2D6), a primer pair was designed to amplify the entire coding region of CYP2D6 by XL-PCR, generating an approximately 5.1 kb product. By including a second set of primers in this XL-PCR reaction, a 3.5 kb product is generated if the entire *CYP2D6* gene is deleted (*CYP2D6*5* allele; nomenclature according to Daly et al[22]). XL-PCR reactions also form the basis for detecting the **16* allele and the **2x2* gene duplication. The 5.1 kb CYP2D6 specific PCR product subsequently serves as template for a series of reamplification reactions designed to detect single nucleotide deletions or insertions compared to the wild-type allele. For example, mutation of the wild-type C to T at position 2938 abolishes a naturally occurring *Fsp*I restriction site. When a 240 base pair product flanking position 2938 is amplified and digested with *Fsp*I, the wild-type allele is cut into fragments 119 and 121 base pairs in length, while the **2A, *2B, *8, *11, *12, *14,* and **17* alleles remain uncut. The digestion products are analyzed by TWINgel electrophoresis, strained with ethidium bromide and visualized with a FluorImager scanning fluorimeter (Molecular Dynamics, Sunnydale, Calif.). To positively identify **2B, *8, *11, *12, *14,* or **17* alleles, additional assays are performed. In several cases, mutations do not introduce or abolish naturally occurring restriction sites. For example, the **4* allele has a G to A mutation at position 1934, leading to a splicing defect and absence of functional protein. To detect the presence of the A nucleotide at this position, a partial *Pst*I

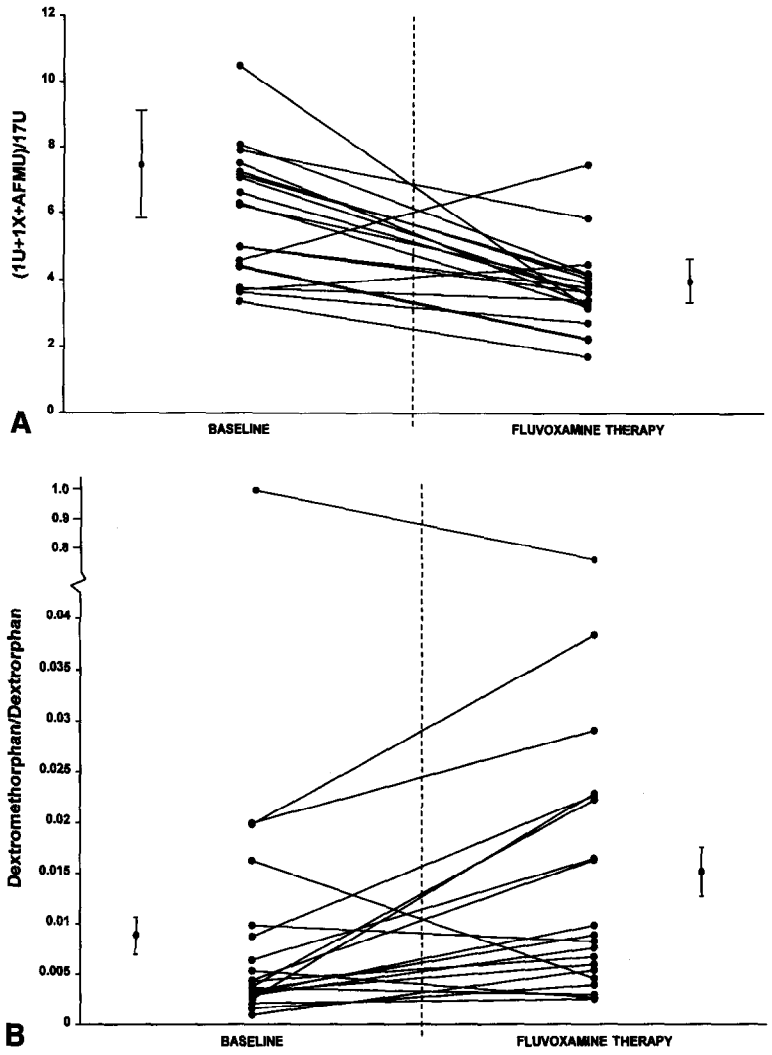

Fig. 1. **A,** Comparison of CYP1A2 phenotype as measured by caffeine metabolite ratios at baseline and after fluvoxamine therapy (150 mg/day) in 10 male and 10 female white volunteers. The baseline measure was calculated as the mean of 6 phenotyping measures, and the fluvoxamine therapy measure was calculated as the mean of 2 phenotyping measures. *Offset points* and *error bars* represent the mean and SE for baseline and fluvoxamine measures of CYP1A2 activity. **B,** Comparison of CYP2D6 phenotype as measured by dextromethorphan molar ratios at baseline and after fluvoxamine therapy (150 mg/day) in 10 male and 10 female white volunteers. The baseline measure was calculated as the mean of 6 phenotyping measures, and the fluvoxamine therapy measure calculated as the mean of 2 phenotyping measures. *Offset points* and *error bars* represent the mean and SE for baseline and fluvoxamine measures of CYP2D6 activity in extensive metabolizers. *Cont'd on page 263.*

restriction site was incorporated into one of the oligonucleotide primers. This engineered *Pst*I site is only complemented in the presence of the wild-type G such that a subsequent restriction digest will yield a cut PCR fragment only in the wild-type allele. A PCR product that remains uncut will occur in the presence of an A splice defect, identifying the **4* allele. Oligonucleotide primers incorporating partial restriction sites have been designed in a similar manner to assign genotypes for the **3, *4, *6, *7, *8, *9, *10, *13, *14, *15,* and **17* alleles. In cases where multiple mutations occur for a given allele (eg, **4A, *4B, *4C,* and **4D*) assays were conducted only for the defining mutation (position 1934 for **4* alleles.[23,24] Allele frequency data from published studies were incorporated

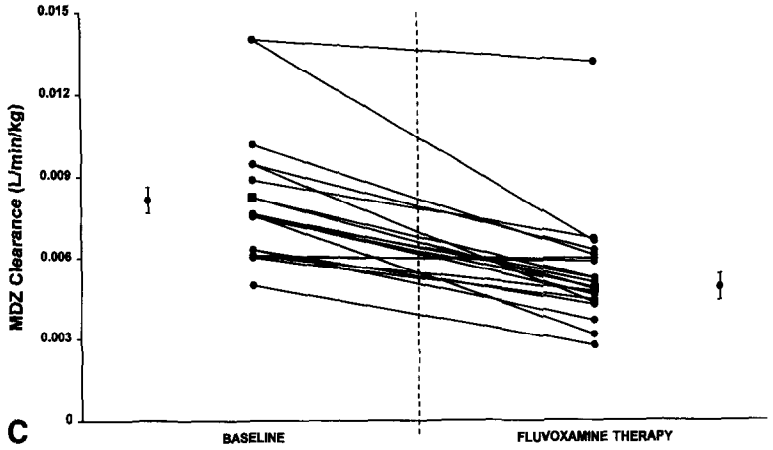

Fig. 1. *Cont'd.* **C,** Comparison of CYP3A phenotype as measured by midazolam (MDZ) plasma clearance at baseline and after fluvoxamine therapy (150 mg/day) in 10 male and 10 female white volunteers. The baseline measure was calculated as the mean of 6 phenotyping measures, and the fluvoxamine therapy measure calculated as the mean of 2 phenotyping measures. *Offset points* and *error bars* represent the mean and SE for baseline and fluvoxamine measures of CYP3A activity. 1U, 1-Methylurate; 1X, 1-methylxanthine; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; 17U, 1,7-dimethylurate.

into a genotyping algorithm that essentially targets the highest frequency alleles early in the genotyping process.[23,24]

Phenotype assignment

Demethylation ratios were used to express CYP1A2 activity.[25,26] The CYP1A2 index was defined as the molar ratio of (1U+1X+AFMU)/17U. Hydroxylation molar ratios were used to express xanthine oxidase (XO) activity: 1U/1X and 1U/(1U+1X).[27] Three molar ratios for *N*-acetyltransferase-2 activity were examined: AFMU/1X, AFMU/(1X+1U), and AFMU/(1X+1U+AFMU).[28] CYP2D6 activity was expressed as the molar ratio of dextromethorphan to dextrorphan.[29] Midazolam clearance was used as an indicator of CYP3A activity.[30] Noncompartmental analysis of midazolam plasma concentration–time data was performed with the pharmacokinetic software TOPFIT 2.0 (Gustav Fischer Verlag, Stuttgart, Germany).[31] Percentage change in phenotype was calculated by the following equation:

$$[(\text{Phenotype}_{\text{fluvoxamine}} - \text{phenotype}_{\text{baseline}})/\text{phenotype}_{\text{baseline}}] \times 100$$

Statistical analysis

Statistics were performed with use of SYSTAT V5.02 software.[32] Normal distribution was determined by tests of skewness and kurtosis. The Mann-Whitney rank sum test was used to evaluate data between men and women. ANOVA with Scheffe's testing was used to determine statistical significance of log-transformed enzyme activity measures of fluvoxamine therapy at baseline, week 2, and week 4. The significance limit accepted for the Mann-Whitney rank sum test was $\alpha = 0.05$, and the significance limit accepted for ANOVA with Scheffe's was $\alpha = 0.05 \div 3 = 0.0167$. Spearman's rank correlation coefficient was calculated to determine the relationship between baseline phenotype and fluvoxamine-induced percentage change in phenotype measure. Data are reported as mean ± SD unless otherwise noted.

RESULTS

All subjects completed all 8 phenotyping visits. Within the fluvoxamine cycle, one woman missed a midfollicular phenotyping visit and had to continue into a second month to complete her data set. Subject demographic data are listed in Table I. Statistically significant differences existed between men and women with respect to ideal body weight and serum creatinine. There were no other significant differences between these two groups. All female subjects and 9 male subjects had dextromethorphan metabolic ratios <0.3 and were classified as extensive metabolizer phenotype for CYP2D6. One male subject had a dextromethorphan metabolic ratio >0.3 and was classified as a poor metabolizer phenotype. Results for CYP2D6 genotyping were consistent with

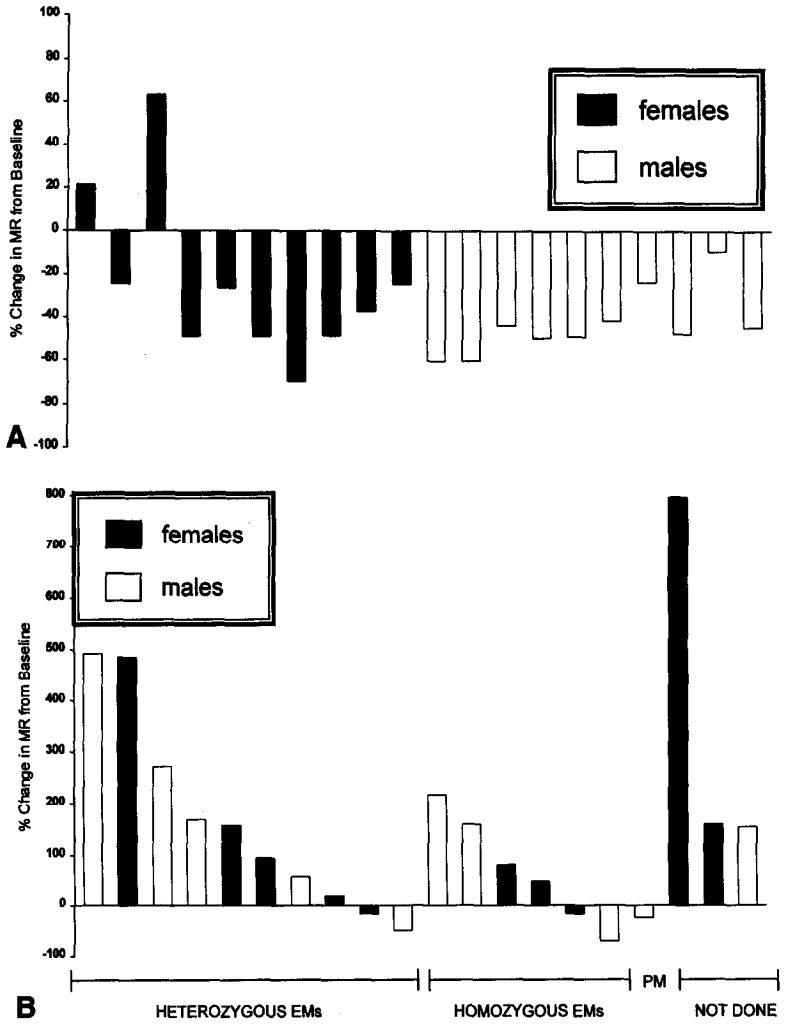

Fig. 2. A, Percentage change in CYP1A2 phenotype as measured by caffeine metabolite ratios after fluvoxamine therapy (150 mg/day) in 10 male and 10 female white volunteers. **B,** Percentage change in CYP2D6 phenotype, compared with genotype, as measured by dextromethorphan molar ratios after fluvoxamine therapy (150 mg/day) in 10 male and 10 female white volunteers. *Cont'd on page 265.*

the phenotyping results (data not shown). The CYP2D6 poor metabolizer, as defined by dextromethorphan phenotype, was homozygous for the *CYP2D6*4* allele. Of the two XO and three *N*-acetyltransferase-2 metabolic indices, the 1U/(1U+1X) ratio for XO and the AFMU/(1U+1X+AFMU) ratio for *N*-acetyltransferase-2 resulted in the least amount of intrasubject variability. Although only these ratios are reported, statistical analyses were performed on all NAT2 and XO indices, producing similar results to those for the aforementioned ratios (data not shown).

Analyses of baseline phenotyping measures for these enzymes established no significant differences in activity either during the menstrual cycle or between men and women (data not shown). Mean ± SD values for CYP1A2, CYP2D6 extensive metabolizer, and CYP3A phenotype were 7.53 ± 8.21, 0.00642 ± 0.00592, and 0.0078 ± 0.0021, respectively. The mean of the six baseline phenotyping measures were used for each subject. Mean coefficients of variation calculated for the six baseline measures for each subject for CYP1A2, CYP2D6, and CYP3A were 19.6% (range, 4.5% to 49.3%), 50.4% (range, 12.1% to 136%), and 10.8% (range, 5.3% to 16.6%), respectively.

By ANOVA with Scheffe's testing, no significant differences occurred between week 2 and week 4 of flu-

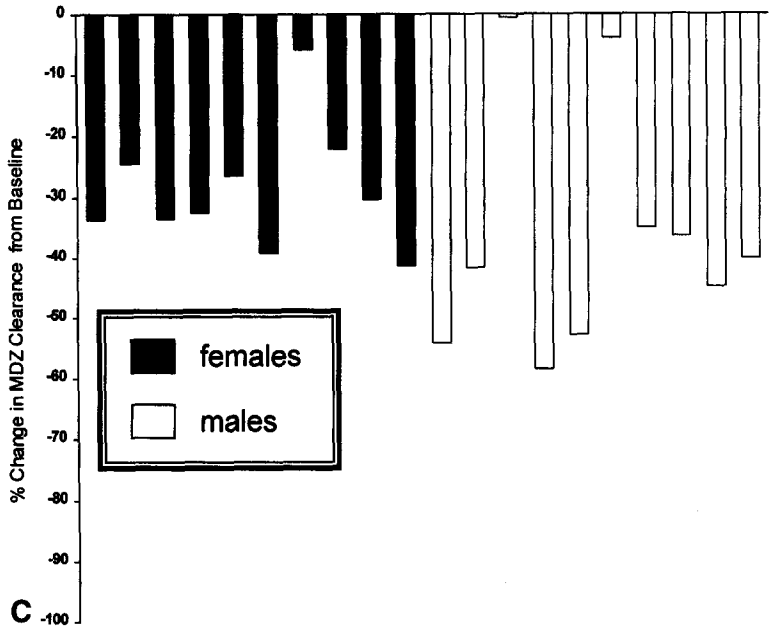

Fig. 2. *Cont'd* **C,** Percentage change in CYP3A phenotype as measured by midazolam (MDZ) plasma clearance after fluvoxamine therapy (150 mg/day) in 10 male and 10 female white volunteers. MR, Molar ratio; EMs, extensive metabolizers; PM, poor metabolizer.

voxamine therapy for any phenotyping measure ($P > .05$), suggesting that maximal effect on the isozymes was achieved within the first 14 days of therapy. For NAT2 and XO metabolite ratios, no significant difference was found between the mean baseline measures and fluvoxamine therapy ($P > .5$). Significant differences were observed between mean baseline phenotyping measures and weeks 2 and 4 of fluvoxamine therapy for CYP1A2, CYP2D6, and CYP3A. Because week 2 and 4 measures were not significantly different, the 2 were combined for further comparisons.

Table II lists mean phenotyping measures at baseline and with fluvoxamine therapy, and percentage change in phenotype for each enzyme. Mean ± SD CYP1A2 caffeine metabolite ratios at baseline and after fluvoxamine therapy were 7.53 ± 7.44 and 4.30 ± 2.82, respectively ($P = .012$). CYP2D6 molar dextromethorphan ratios before and after fluvoxamine therapy were 0.0078 ± 0.00694 and 0.0153 ± 0.0127 respectively ($P = .011$). Midazolam clearance, used as a measure of CYP3A activity decreased from 0.0081 ± 0.0024 L/min/kg before fluvoxamine therapy, to 0.0054 ± 0.0021 L/min/kg after therapy ($P = .0091$). Fig 1 illustrates the change in phenotype for CYP1A2, CYP2D6, and CYP3A for each subject.

The percentage change in phenotype is shown in Fig 2. For CYP1A2 activity, fluvoxamine decreased the caffeine metabolite ratios by a median of 44.4% from baseline. For NAT2 and XO activity, fluvoxamine changed the caffeine metabolite ratios by a median of 8.9% and 6.0%, respectively ($P > .5$). Although CYP2D6 activity was significantly decreased and dextromethorphan molar ratios increased a median of 123.5%, no subject's molar ratio was increased to a poor metabolizer phenotype. Fluvoxamine decreased midazolam clearance by a median of 34.4%. Fig 2, *B,* illustrates the relationship between CYP2D6 genotype and the percentage change in phenotype with fluvoxamine therapy.

A recent investigation suggested a relationship between baseline CYP2D6 activity and degree of enzyme inhibition in 6 patients prescribed varying doses of sertraline.[33] To examine this relationship in our study subjects, Spearman's rank correlation coefficients were calculated for baseline CYP1A2, CYP2D6, and CYP3A activity and fluvoxamine-induced percentage change in phenotype measure. Spearman's rank correlation coefficient measures for CYP1A2, CYP2D6, and CYP3A were 0.36, 0.25, and 0.28, respectively ($P > .5$). No significant relationship could be determined between baseline activity and degree of change in phenotype indices.

Reported adverse events during fluvoxamine therapy included gastrointestinal complaints (nausea, 75%; indigestion, 15%; diarrhea, 20%; and constipation, 10%),

cardiovascular complaints (pounding heart beat, 15%), sexual dysfunction (decreased desire, 15%; decreased ability to achieve orgasm, 20%; and impaired erection, 5%), and neurologic complaints (dizziness, 20%; drowsiness, 25%; difficulty sleeping, 25%; and anxiety, 20%). The gastrointestinal complaints were reported soon after the start of fluvoxamine therapy and attenuated within 2 weeks. Cardiovascular, sexual, and neurologic complaints continued throughout therapy. The presence or severity of adverse events did not appear to be related to the magnitude of change in phenotyping indices.

DISCUSSION

Because SSRI-mediated cytochrome P450 inhibition is competitive and reversible, the degree of enzyme inhibition is attributable to the affinity of the SSRI for the enzyme and its concentration at that enzyme. The degree of inhibition is also dependent upon the inhibited substrate's affinity for, and concentration at, that enzyme.[33] Therefore it is important to examine the effects of enzyme inhibition or induction in vivo, with clinically used doses of substrate. For example, the relative in vitro potencies for CYP2D6 inhibition is paroxetine > fluoxetine > sertraline.[14,34] However, at the minimum effective dose, fluoxetine attains the highest plasma drug concentration, producing an estimated 80% CYP2D6 inhibition at 20 mg/day, followed by paroxetine producing an estimated 25% inhibition at 20 mg/day, followed by sertraline producing an approximate 15% inhibition at 50 mg/day.[33] The lack of in vivo information on the impact of clinically used doses of SSRIs was the impetus for the current investigation.

Fluvoxamine steady-state plasma concentrations in subjects given 150 to 300 mg/day have been reported to range from 20 to 400 ng/mL, with a mean of approximately 140 ng/mL.[35] von Moltke et al[36] reported mean liver:water partition ratios for fluvoxamine of 26.6 (SE, 1.3). Thus a mean steady-state fluvoxamine plasma concentration of 140 ng/mL (0.44 nmol/L), yields a hypothetical hepatic fluvoxamine concentration of approximately 0.012 μmol/L. The following Ki (inhibition constant) values for inhibiting the functional integrity of CYP isozymes have been determined from in vitro studies with use of human hepatic microsomes: 0.2 to 0.24 mol/L for phenacetin and theophylline (CYP1A2 substrates), 3.9 to 16.6 mol/L for sparteine, imipramine, and desipramine (CYP2D6 substrates), and 10 40 mol/L for alprazolam and cortisol (CYP3A substrates).[16] Reported in vivo clearance reductions attributable to SSRIs range from 25% to 60%, producing an increase in AUC for the substrate of no more than twofold to threefold.[13,37] For most medications this may not be significant; however, it may be important for drugs with a narrow therapeutic range or for patients titrated on high doses of medications. In this trial, 150 mg/day fluvoxamine affected maximal enzyme inhibition after 2 weeks of therapy. No significant effect on NAT2 or XO activity was noted. CYP1A2, CYP2D6, and CYP3A phenotyping measures were altered by a median of –44.4%, 123.5%, and –34.4%, respectively.

In vitro and in vivo investigations have shown fluvoxamine to be a potent inhibitor of CYP1A2.[10-12] Our investigation confirms these findings, with fluvoxamine therapy causing a statistically significant decrease in the caffeine metabolic ratio from baseline.

Although fluvoxamine causes some degree of inhibition of CYP2D6 in vitro, previous in vivo investigations with ≤100 mg/day fluvoxamine have not found a significant degree of inhibition.[14,38] In the current investigation, 150 mg/day fluvoxamine significantly inhibited CYP2D6 activity. These findings illustrate the importance of accounting for the medication dose, as well as its affinity for the enzyme, when predicting significant medication interactions.

The interaction of fluvoxamine with CYP3A substrates has been well described.[36,39] von Moltke et al[40] modeled a theoretical relationship using in vitro data between the percentage decrease in metabolic clearance of terfenadine (a CYP3A substrate) with the coadministration of fluvoxamine at varying doses. The authors determined that at 150 mg/day fluvoxamine, an approximate 25% decrease in terfenadine clearance would be expected. Our investigation yielded similar results, with midazolam clearance decreasing by a median of 34.4% with the coadministration of 150 mg/day fluvoxamine.

With use of a standard dose and length of therapy for fluvoxamine, no significant relationships were noted between baseline CYP1A2, CYP2D6, and CYP3A activity as determined by phenotyping and the percent change in phenotype produced by fluvoxamine.

This is the first investigation to systematically investigate the effect of fluvoxamine on multiple drug-metabolizing enzyme activities at a clinically relevant dose and suitable length of therapy. Future investigations of the impact of other SSRIs on drug-metabolizing enzyme activity will result in useful data that may direct clinicians in their attempt to predict significant medication interactions.

We gratefully acknowledge Linda Stragand, BS, BSN, for her countless hours of excellent nursing assistance for this investigation, Troy Smith, BS, for his valuable technical assistance, and Andrea Gaedigk, PhD, for the performance of *CYP2D6* genotyping assays.

References

1. Ketter TA, Flockhart DA, Post RM, Denicoff K, Pazzaglia PJ, Marangell LB, et al. The emerging role of cytochrome P450 3A in psychopharmacology. J Clin Pharmacol 1995;15:387-98.
2. van Harten J. Clinical pharmacokinetics of selective serotonin reuptake inhibitors. Clin Pharmacokinet 1993;24: 203-20.
3. Perucca E, Gatti G, Spina E. Clinical pharmacokinetics of fluvoxamine. Clin Pharmacokinet 1994;27:175-90.
4. Spigset O, Carleborg L, Hedenmalm K, Dahlqvist R. Effect of cigarette smoking on fluvoxamine pharmacokinetics in humans. Clin Pharmacol Ther 1995;58:399-403.
5. Jeppesen U, Loft S, Poulsen HE, Brosen K. A fluvoxamine-caffeine interaction study. Pharmacogenetics 1996;6:213-22.
6. Carrillo JA, Dahl ML, Svensson JO, Alm C, Rodriguez I, Bertilsson L. Disposition of fluvoxamine in humans is determined by the polymorphic CYP2D6 and also by the CYP1A2 activity. Clin Pharmacol Ther 1996;60:183-90.
7. Spigset O, Granberg K, Haag S, Norstrom A, Dahlqvist R. Relationship between fluvoxamine pharmacokinetics and CYP2D6/CYP2C19 phenotype polymorphisms. Eur J Clin Pharmacol 1997;52:129-33.
8. Xu ZH, Xie HG, Z HH. In vivo inhibition of CYP2C19 but not CYP2D6 by fluvoxamine. Br J Clin Pharmacol 1996;42:518-21.
9. Vandel S, Bertschy G, Baumann P, Bouquet S, Bonin B, Francois T, et al. Fluvoxamine and fluoxetine: interaction studies with amitriptyline, clomipramine and neuroleptics in phenotyped patients. Pharmacol Res 1995;31:347-53.
10. Brosen K, Skjelbo E, Rasmussen BB, Poulsen HE, Loft S. Fluvoxamine is a potent inhibitor of cytochrome P4501A2. Biochem Pharmacol 1993;45:1211-4.
11. Rasmussen BB, Maenpaa J, Pelkonen O, Loft S, Pousen HE, Lykkesfeldt J, et al. Selective serotonin reuptake inhibitors and theophylline metabolism in human liver microsomes: potent inhibition by fluvoxamine. Br J Clin Pharmacol 1995;39:151-9.
12. Pastrakuljic A, Tang BK, Roberts EA, Kalow W. Distinction of CYP1A1 and CYP1A2 activity by selective inhibition using fluvoxamine and isosafrole. Biochem Pharmacol 1997;53:531-8.
13. Fleishaker JC, Hulst LK. A pharmacokinetic and pharmacodynamic evaluation of the combined administration of alprazolam and fluvoxamine. Eur J Clin Pharmacol 1994;46:35-9.
14. Crewe HK, Lennard MS, Tucker GT, Woods FR, Haddock RE. The effect of selective serotonin re-uptake inhibitors of cytochrome P4502D6 (CYP2D6) activity in human liver microsomes. Br J Clin Pharmacol 1992;34:262-5.
15. von Moltke LL, Greenblatt DJ, Cotreau-Bibbo MM, Duan SX, Harmatz JS, Shader RI. Inhibition of desipramine hydroxylation in vitro by serotonin-reuptake-inhibitor antidepressants, and by quinidine and ketoconazole: a model system to predict drug interactions in vivo. J Pharmacol Exp Ther 1994;268:1278-83.
16. Preskorn SH. Clinically relevant pharmacology of selective serotonin reuptake inhibitors: an overview with emphasis on pharmacokinetics and effects on oxidative drug metabolism. Clin Pharmacokinet 1997;32(suppl 1):1-21.
17. DeVane CL. Pharmacokinetics of the selective serotonin reuptake inhibitors. J Clin Psychiatry 1992;53(suppl 2):13-20.
18. Corso DM, Pucino F, DeLeo JM, Calis KA, Gallelli JF. Development of a questionnaire for detecting potential adverse drug reactions. Ann Pharmacother 1992;26:890-6.
19. Evans WE, Relling MV, Petros WP, Meyer WH, Mirro J Jr, Crom WR. Dextromethorphan and caffeine as probes for simultaneous determination of debrisoquin oxidation and N-acetylation phenotypes in children. Clin Pharmacol Ther 1989;45:568-73.
20. Park YH, Kullberk MD, Hinsvark ON. Quantitative determination of dextromethorphan and three metabolites in urine by reverse-phase high performance liquid chromatography. J Pharm Sci 1984;73:24-9.
21. Lam YW, Rodriguez SY. High performance liquid chromatography determination of dextromethorphan and dextrorphan for oxidation phenotyping by fluorescence and ultraviolet detection. Ther Drug Monit 1993;15:300-4.
22. Daly AK, Brockmoeller J, Broly F, Eichelbaum M, Evans WE, Gonzalez FJ, et al. Nomenclature for human CYP2D6 alleles. Pharmacogenetics 1996;6:193-201.
23. Marez D, Legrand M, Sabbagh N, Guidice JM, Spire C, Lafitte JJ, et al. Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution. Pharmacogenetics 1997;7:193-202.
24. Sachse C, Brockmoller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 1997;60:284-95.
25. Grant DM, Tang BK, Kalow W. Variability in caffeine metabolism. Clin Pharmacol Ther 1983;33:591-602.
26. Campbell ME, Spielberg SP, Kalow W. A urinary metabolite ratio that reflects systemic caffeine clearance. Clin Pharmacol Ther 1987;42:157-65.
27. Grant DM, Tang BK, Campbell ME, Kalow W. Effect of allopurinol on caffeine disposition in man. Br J Clin Pharmacol 1986;21:454-8.
28. Tang BK, Kadar D, Qian L, Iriah J, Yip J, Kalow W. Caffeine as a metabolic probe: validation of its use for acetylator phenotyping. Clin Pharmacol Ther 1991;49: 648-57.
29. Schmid B, Birder J, Presig R, Kupfer A. Polymorphic dextromethorphan metabolism: cosegregation of oxidative O-demethylation with debrisoquin hydroxylation. Clin Pharmacol Ther 1985;38:618-24.
30. Thummel KE, Shen DD, Podoll TD, Kunze KL, Trager WF, Hartwell PS, et al. Use of midazolam as a human cytochrome P4503A probe; I: in vitro–in vivo correla-

tions in liver transplant patients. J Pharmacol Exp Ther 1994;271:549-56.
31. Tanswell P, Koup J. Topfit: a PC based pharmacokinetic/pharmacodynamic data analysis program. Int J Clin Pharmacol Ther Toxicol 1993;31:514-20.
32. Systat user's guide. Evanston (IL): SYSTAT Intelligent Software; 1990.
33. Preskorn SH, Magnus RD. Inhibition of hepatic P-450 isoenzymes by serotonin selective reuptake inhibitors: in vitro and in vivo findings and their implications for patient care. Psychopharmacol Bull 1994;30:251-9.
34. Otton SV, Ball SE, Cheung SW, Inaba T, Sellers EM. Comparative inhibition of the polymorphic enzyme CYP2D6 by venlafaxine and other 5HT uptake inhibitors. Clin Pharmacol Ther 1994;55:141.
35. Kasper S, Dotsch M, Kick H, Vieira A, Moller HJ. Plasma concentrations of fluvoxamine and maprotiline in major depression: implications on therapeutic efficacy and side effects. Eur Neurophychopharmacol 1993;3:13-21.
36. von Moltke LL, Greenblatt DJ, Court MH, Duan SX, Harmatz JS, Shader RI. Inhibition of alprazolam and desipramine hydroxylation in vitro by paroxetine and fluvoxamine: comparison with other serotonin-reuptake-inhibitor antidepressants. J Clin Psychopharmacol 1995; 15:125-31.
37. Perruca E, Gatti G, Cipolla G, Spina E, Barel S, Soback S, et al. Inhibition of diazepam metabolism by fluvoxamine: a pharmacokinetic study in normal volunteers. Clin Pharmacol Ther 1994;56:471-6.
38. Ozdemir V, Naranjo CA, Shulman RW, Herrmann N, Sellers EM, Kalow W. An in vivo comparison of paroxetine and fluvoxamine: effects on CYP1A2 and CYP2D6 activities [abstract]. Clin Pharmacol Ther 1997;61:177.
39. Fleishaker JC, Hulst LK. A pharmacokinetic and pharmacodynamic evaluation of the combined administration of alprazolam and fluvoxamine. Eur J Clin Pharmacol 1994;46:35-9.
40. von Moltke LL, Greenblatt DJ, Duan SX, Harmatz JS, Wright CE, Shader RI. Inhibition of terfenadine metabolism in vitro by azole antifungal agents and by selective serotonin reuptake inhibitor antidepressants: relation to pharmacokinetic interactions in vivo. J Clin Psychopharmacol 1996;16:104-12.
41. Devine BJ. Gentamicin pharmacokinetics. Drug Intell Clin Pharm 1974;8:650-5.
42. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41.