Stereoselective Metabolism of Methadone N-Demethylation by Cytochrome P4502B6 and 2C19

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ABSTRACT Methadone is a clinically used opioid agonist that is oxidatively metabolized by cytochrome P450 (CYP) isoforms to a stable metabolite, EDDP. Methadone is a chiral drug administered as the racemic mixture of (R)-(–)- and (S)-(+) -methadone, but (R)-methadone is the active isomer. The cytochrome P450 (CYP) isoform involved in methadone’s metabolism is thought to be CYP3A4, but human drug–drug interaction studies are not consistent with this. The ability of the common human drug-metabolizing CYPs (obtained from baculovirus-infected insect cell supernatants) to generate 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrilidine (EDDP) from racemic methadone was examined and then determined if the CYP isoforms metabolized methadone stereoselectively. Only CYP2B6, 2C19, and 3A4 generated measurable EDDP from 1 mg/ml of racemic methadone. The hierarchy of EDDP generation was CYP2B6 > CYP2C19 ≥ CYP3A4. At 10 μg/ml of methadone, CYP2C9 and CYP2D6 also generated EDDP, but in at least 10-fold lower quantities than CYP2B6. Michaelis-Menten kinetic data demonstrated that CYP2B6 had the highest Vmax (44 ng/min/10pmol) and the lowest Km (12.6 μg/ml) for EDDP formation of all the CYP isoforms. In human liver microsomes with high and low CYP2B6 expression but equivalent CYP3A4 expression, high CYP2B6 expression microsomes generated twice the amount of EDDP from 10 μg/ml of methadone than low CYP2B6 expression microsomes. When stereoselective metabolism of racemic methadone by CYP2B6, 2C19, and 3A4 was examined using an enantiospecific methadone assay, CYP2B6 preferentially metabolized (S)-methadone, CYP2C19 preferentially metabolized (R)-methadone, and CYP3A4 showed no preference. These data suggest that multiple CYPs metabolized methadone but CYP2B6 had the highest Vmax/Km. In addition, only CYP2B6 and 2C19 showed stereoselective metabolism. Our data could explain why the plasma concentration ratio of R/S methadone is variable and why drugs that induce CYP2B6 such as nevirapine and efavirenz also induce methadone metabolism, while the CYP3A4 inducer rifabutin has no effect on methadone pharmacokinetics. Chirality 16:36–44, 2004. © 2003 Wiley-Liss, Inc.

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Methadone is a narcotic agonist in clinical use for the treatment of narcotic addiction and severe pain syndrome.1–3 Methadone contains a single chiral carbon atom and thus exists as a pair of enantiomers. The drug is clinically administered as an equal (racemic) mixture of (R)-(–)- and (S)-(+) -methadone in the USA (Fig. 1). (R)-Methadone has significantly higher affinity towards the μ-opioid receptor and numerous studies have demonstrated that it is (R)-methadone that is responsible for the drug’s activity when administered as the racemic mixture.4,5 The major mode of metabolism of methadone is through hepatic N-demethylation to the stable metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrilidine (EDDP)6 (Fig. 1). After steady-state administration of methadone there is a large variability in the plasma (R)-methadone/(S)-methadone ratio across a population, suggesting that methadone is metabolized stereo-
selectively in vivo. The cytochrome P450 (CYP) isofrom responsible for methadone N-demethylation is thought to be CYP3A4 based on in vitro liver-microsomal studies using high substrate concentrations of methadone. However, Foster et al. demonstrated that methadone N-demethylation through CYP3A4 was not stereoselective. In addition, drug–drug interaction studies using the CYP3A4 inhibitors ritonavir and indinavir do not suggest that this isofrom is the main CYP involved in methadone metabolism. The present study had two major goals. One was to screen the major human CYPs involved in drug metabolism for their ability to perform methadone N-demethylation at low substrate concentrations. The other was to examine if the CYP isofroms involved in methadone metabolism do so stereoselectively. It was hoped that these studies would explain both the variability in ($R$)/($S$) ratio of methadone plasma concentrations in large populations as well as the presently available human drug–drug interaction data. Understanding the metabolism of methadone is important for the safe use of this drug across all population groups. For example, methadone use can coincide with antiretroviral drug use because intravenous drug use is a major risk factor for HIV-infection. Thus, knowledge of the CYP isofroms involved in methadone’s metabolism to EDDP, the major pathway of inactivation, takes on importance for the prevention of unwanted drug–drug interactions.

**MATERIALS AND METHODS**

**Chemical**

Racemic methadone, ($R$)-methadone, ($S$)-methadone, and EDDP were obtained from the National Institute of Drug Abuse (Dr. Pushpa Thadani). All other chemicals for the enzyme incubations were of analytical grade and were obtained commercially.

**Cloned Human Cytochrome P450 Enzymes**

All of the cloned human cytochrome P450 isofroms were obtained from Gentest Corp. (Woburn, MA) as baculovirus-infected insect cell-expressed supersomes without coexpressed cytochrome $b_5$.

**Human Liver Microsomes**

Human liver microsomes with defined total cytochrome P450 content and specific cytochrome P450 isofrom content were obtained from Gentest Corp. Two microsomal lots with donor designations of HG3 and HG43 were utilized. Both had protein concentrations of 20 mg/ml. HG3 had a CYP2B6 content of 18 pmol of enzyme per mg of protein and CYP3A4 content of 95 pmol of enzyme per mg of protein as determined by quantitative Western blotting. HG43 had a CYP2B6 content of 4 pmol of enzyme per mg of protein and a CYP3A4 content of 94 pmol of enzyme per mg of protein. Both donor liver microsomes had wildtype CYP2C19 expression, but HG43 had ~10-fold greater expression of CYP2C19 than HG3 as determined by ($S$)-mephenytoin 4-hydroxylase activity.

**Enzyme Incubations**

Incubations were performed in duplicate at 37°C in a shaking water bath for 20 min. The incubates of 200 µl final volume contained 50 mM potassium phosphate buffer (pH 7.4), baculovirus-infected insect cell-expressed supersomes or human liver microsomes, varying concentrations of methadone, and 1 mM NADPH. The enzyme mixture was preincubated for 5 min and the enzymatic reaction was initiated by the addition of 1 mM NADPH. After incubation for 20 min, the reaction was stopped with the addition of 100 µl of ice-cold acetonitrile and vortexed for 30 sec. The incubate was centrifuged at 10,000g for 10 min and 100 µl of the aliquot was directly injected into the HPLC system to quantify EDDP and methadone.

**HPLC Assays**

Nonstereospecific assay. EDDP and methadone were separated and quantified using reverse phase HPLC with a Carbosorb ODS-3 analytical column (MetaChem Technologies, Torrance, CA) with a mobile phase of acetonitrile/water (35/65 v/v) containing 1% (v/v) triethylamine, pH 2.8, adjusted with orthophosphoric acid. The flow rate was 1 ml/min, UV detection was at 210 nm, and peak areas were determined with a Shimadzu (Kyoto, Japan) CR501 Chromatopac integrator. A five-point external standard curve was generated for each set of incubations, with these standard points being treated exactly as the incubation media. Assay linearity was demonstrated over the concentration range of 50 ng/ml to 400 µg/ml. For each incubation a standard curve was generated to include the concentration range of the substrate and the product. The lower limit of quantitation (LLQ) was 50 ng/ml for both EDDP and methadone. The retention time for EDDP was 3.7 min and methadone 5.1 min. The intrassay coefficients of variation for EDDP were 5.3%, 3.3%, and 7.9% at low, mid-range, and high concentrations, respectively, and interassay coefficients of variation were 5.9%, 7.4%, and 3.8% at low, mid-range, and high concentrations, respectively. The intrassay coefficients of variation for methadone were 3.0%, 2.9%, and 6.9% at low, mid-range, and high concentrations, respectively, and interassay coefficients of variation were 5.9%, 7.4%, and 3.8% at low, mid-range, and high concentrations, respectively.
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**Enantiospecific assay.** \((R)-\) and \((S)-\)methadone were separated and quantified using HPLC with a Cyclobond I 2000 RSP column (Astec, Whippany, NJ) with a mobile phase of 1%TEA/ACN (90:10, v/v) pH 3.2, adjusted with phosphoric acid. The flow rate was 0.8 ml/min and UV detection was 200 nm. Dibenzepin (250 ng) was used as internal standard and added with the ACN at the end of the timed incubation. A 5-point standard curve was generated for each set of incubations. The LLQ was 75 ng/ml for both the \((R)-\) and \((S)-\)methadone. Both the intraassay and interassay coefficients of variation were less than 5% at high and low methadone concentrations.

**Experiments**

Preliminary experiments demonstrated linearity in EDDP formation from 3 \(\mu\)g/ml of methadone with CYP additions of 3–40 pmol to the incubation media. In addition, product formation was linear to at least 30 min with 20 pmol of CYP2B6 and CYP2C19 at 10 \(\mu\)g/ml methadone concentration.

**Experiment 1. Examination of the ability of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 to generate EDDP at methadone substrate concentrations of 1 \(\mu\)g/ml and 10 \(\mu\)g/ml.** These experiments were designed to examine comparatively the generation of EDDP from methadone over 20 min at two concentrations of methadone via the various human CYP isoforms at equivalent enzyme content. Both concentrations of methadone are higher than what is measured in the plasma during usual dosing of methadone. However, in order to generate enough EDDP for assay accuracy, a methadone concentration of 1 \(\mu\)g/ml was the lowest concentration studied. For the CYPs that avidly metabolized methadone, these results did not simulate steady-state conditions because the rate of metabolism was rapid enough to change the substrate concentration over the 20 min of incubation. Nonetheless, this provided a preliminary look at the relative EDDP generation via CYP.

![Fig. 2](image_url)

**Fig. 2.** The concentration of EDDP generated after 20 min of incubation with various human CYP isoforms using racemic methadone at 1 \(\mu\)g/ml (A) and 10 \(\mu\)g/ml (B) as the substrate. The EDDP concentration is shown on the ordinate in ng/ml. For each CYP isoform, 20 pmol of baculovirus insect cell-expressed supersomes were added to the incubation mixture. These data are from a single experiment performed in duplicate. A repetition of this experiment showed similar results (data not shown).
oxidation from the most common drug-metabolizing CYP isoforms. For the most active CYP isoforms, enzyme kinetic curves to estimate the \( V_{\text{max}} \) and \( K_m \) of EDDP formation were generated.

**Experiment 2. Calculation of \( V_{\text{max}} \) and \( K_m \) of EDDP formation from CYP2B6, CYP2C19, and CYP3A4.** After determining that CYP2B6, CYP2C19, and CYP3A4 were the most active at metabolizing methadone to EDDP, the following experiments were performed. The various CYPs were incubated with increasing concentrations of methadone from 1–300 \( \mu \)g/ml. At 300 \( \mu \)g/ml of methadone metabolism via CYP3A4 was not saturated and 1,000 \( \mu \)g/ml point was added to the CYP3A4 incubations. Concentrations higher than 1,000 \( \mu \)g/ml were not possible because of methadone's limited aqueous solubility and increasing difficulty in separating methadone peaks from EDDP peaks by HPLC. The rates of EDDP formation for these studies were expressed as ng/min/10 pmol of CYP. \( V_{\text{max}} \) and \( K_m \) were calculated using the Michaelis-Menten kinetics equation with PRISM software (San Diego, CA). \( V_{\text{max}}/K_m \) as a measure of intrinsic clearance was calculated for each CYP isoform.

**Experiment 3. EDDP generation from human liver microsomes with defined CYP2B6 and CYP3A4 expression.** In using human liver microsome lots designated HG3 and HG43, the incubation conditions were as described for the CYP expressed experiments except that 500 \( \mu \)g of microsomal protein per incubation was utilized. The methadone concentration was 10 \( \mu \)g/ml and incubation time was 20 min. In preliminary experiments it was

![Fig. 3](image-url). The relationship between racemic methadone concentration and rates of formation of EDDP by baculovirus insect cell-expressed supersomes expressing human CYP 2B6 (A), CYP2C19 (B), and CYP3A4 (C). The abscissa has concentrations in \( \mu \)g/ml and the ordinate is the velocity in ng/min/10 pmol. The curves were generated using Michaelis-Menten kinetic parameters derived from nonlinear regression analysis of the data. The insert shows the raw data. The calculated \( V_{\text{max}} \) and \( K_m \) are indicated for CYP2B6 and CYP2C19. For CYP3A4, accurate \( V_{\text{max}} \) and \( K_m \) could not be calculated because the reaction was never saturated. These data represent one experiment each. A repetition gave identical results (see Results).
found that EDDP generation was linear with increasing concentration of microsomal protein from 100–500 µg at a methadone concentration of 10 µg/ml. The EDDP formation was linear up to 30 min of incubation. These experiments were performed three times in duplicate.

Experiment 4. The metabolism of (R)- and (S)-methadone with CYP isoforms at racemic methadone of 3 µg/ml. The stereoselectivity of methadone metabolism was evaluated using incubations with human CYP2B6, 2C19, and 3A4 supersomes. The enzymatic reaction was stopped at 20, 40, and 60 min of incubation for each CYP isomorphs to quantify (R)- and (S)-methadone concentrations in order to determine the relative rate of metabolism of the methadone enantiomers. The substrate concentration was 3 µg/ml of racemic methadone (1.5 µg/ml of each enantiomer). These experiments were performed three times in duplicate for reproducibility.

Experiment 5. Enzyme kinetics of (R)- and (S)-methadone with CYP2B6 and 2C19. Enzyme kinetics of EDDP formation from (R)-methadone and (S)-methadone were evaluated with 20 pmol of CYP2B6 and 2C19 for 20 min as described in Experiment 2. Eight concentrations of substrate that ranged from 1–300 µg/ml were used for each experiment. Vmax and K<sub>m</sub> were estimated for each enantiomer of methadone using Michaelis-Menten kinetics equation with PRISM software. These experiments were repeated for reproducibility.

RESULTS

Experiment 1

At a methadone concentration of 1 µg/ml, only CYP2B6, 2C19, and 3A4 generated measurable concentrations of EDDP in 20 min. All the other CYP isoforms generated less than 50 ng/ml of EDDP, which was the LLQ of the assay. In comparative terms, CYP2B6 generated more EDDP than either CYP2C19 or CYP3A4 (Fig. 2A). This was observed despite the fact that the velocity of the enzymatic reaction with CYP2B6 was decelerating over time as the substrate concentration in the incubation media was rapidly depleted. CYP2C19 generated slightly more EDDP than CYP3A4 in 20 min, which was consistent in two experiments.

At 10 µg/ml of methadone, the hierarchy of EDDP production of CYP2B6 > CYP2C19 > CYP3A4 was again demonstrated; however, at this higher substrate concentration both CYP2C9 and CYP2D6 also generated small but measurable quantities of EDDP. However, incubation with CYP1A2 and CYP2E1 still showed no evidence of EDDP formation (Fig. 2B). These data suggest that the three main CYP isoforms involved in methadone metabolism to EDDP are CYP2B6, CYP2C19, and CYP3A4, with CYP2B6 performing this most rapidly.

Experiment 2

The calculation of V<sub>max</sub> and K<sub>m</sub> using Michaelis-Menten equation was straightforward for both CYP2B6 and CYP2C19 in that both enzymes were saturated at a substrate concentration approaching 100 µg/ml. The calculated V<sub>max</sub> and K<sub>m</sub> for either CYP2B6 (V<sub>max</sub> of 43 and 45 ng/min/10 pmol and K<sub>m</sub> of 10.4 and 14.8 µg/ml) or CYP2C19 (V<sub>max</sub> of 15 and 19 ng/min/10 pmol and K<sub>m</sub> of 11.3 and 16.6 µg/ml) were very similar in the two separate experiments (Fig. 3A,B). The V<sub>max</sub> was 2.6-fold higher, but the K<sub>m</sub> was only slightly lower with CYP2B6 as compared to CYP2C19. Overall, the calculated V<sub>max</sub>/K<sub>m</sub> as a measure of intrinsic clearance was 3-fold higher for CYP2B6 as compared to CYP2C19. CYP3A4 metabolism demonstrated a difference in that the enzymatic reaction was not saturated up to a methadone concentration of 1,000 µg/ml (Fig. 3C).

Because of solubility limitations and HPLC separation problems of EDDP from methadone at high methadone concentrations, a further increase in the methadone substrate concentration was not possible. Since the enzymatic reaction with CYP3A4 was not saturated, accurate determination of the V<sub>max</sub> and K<sub>m</sub> could not be performed for CYP3A4. Nonetheless, at lower substrate concentrations in these experiments, CYP2B6 demonstrated many-fold more rapid metabolism of methadone to EDDP than CYP3A4 (Fig. 2).

Experiment 3

Liver microsomes from HG3, which has five times as much CYP2B6 expression as microsomes from HG43, generated almost 2-fold more EDDP from 10 µg/ml of racemic methadone (Fig. 4). The content of CYP3A4 was equal in both liver microsomes, and CYP2C19 expression was actually 10-fold greater in HG43. These data would strongly suggest that CYP2B6 expression in the liver is an important determinant of the rate of metabolism of methadone to EDDP.

Experiment 4

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Fig. 4. The concentration of EDDP in the incubation media after 20 min of incubation with a racemic methadone concentration of 10 µg/ml. HG3 and HG43 are two human liver microsomes with defined CYP isoform contents. HG3 has a CYP2B6 content of 18 pmol/mg of protein while HG43 has a CYP2B6 content of 4 pmol/mg of protein. Each incubation media contained 500 µg of microsomal protein. The graph is the mean ± SD of the three experiments. The ordinate shows the EDDP concentration at 20 min in ng/ml with a racemic methadone concentration of 10 µg/ml. The abscissa shows the two human liver microsome lots. Statistical comparison used unpaired Student’s t-test with a P value of <0.05 considered significantly different, as marked by an asterisk.
3 μg/ml generated the following observations. CYP2B6, at an equivalent CYP content, metabolized methadone more avidly than either CYP2C19 or CYP3A4 (Fig. 5), consistent with the observed data with racemic methadone (Fig. 2). In addition, CYP2B6 and CYP2C19 demonstrated stereoselective metabolism, while CYP3A4 did not. With CYP2B6, (S)-methadone was more rapidly metabolized than (R)-methadone and the (R)/(S) methadone ratio at 60 min was 1.77 ± 0.21. With CYP2C19 the opposite pattern emerged, in that (R)-methadone was more rapidly metabolized than (S)-methadone and the (R)/(S) methadone ratio at 60 min was 0.74 ± 0.09. CYP3A4 did not demonstrate any stereoselectivity in methadone metabolism and the (R)/(S) methadone ratio was 0.94 ± 0.04.

**Experiment 5**

In order to determine the potential mechanism of the stereoselective metabolism by CYP2B6 and CYP2C19, full enzyme kinetics for (R)- and (S)-methadone with CYP2B6 and CYP2C19 were performed. For CYP2B6 (Fig. 6A), (R)-methadone demonstrated a higher V\text{max} but also a higher K\text{m} than for (S)-methadone. Because the difference in K\text{m} was greater than the difference in V\text{max} at lower methadone concentrations the actual velocity of methadone N-demethylation was more rapid for (S)-methadone than for (R)-methadone. Since the plasma concentration of methadone with usual dosing is in the 500–750 ng/ml range, the enzyme kinetics at the lower concentrations of substrate more closely approached the stereoselective metabolism of methadone in vivo.

For CYP2C19 (Fig. 6B), (R)-methadone had a higher V\text{max} but also a higher K\text{m} than (S)-methadone, and at all substrate concentrations the velocity of N-demethylation of methadone was more rapid for (R)-methadone. This is consistent with the observed substrate disappearance data with racemic methadone, where (R)-methadone was metabo-
lized faster than (S)-methadone. These enzyme-kinetic data suggest that stereoselective metabolism of methadone is based on fundamental differences in the N-demethylation kinetics of the isomers of methadone with both CYP2B6 and CYP2C19 and not secondary to enantiomeric drug–drug interaction. The repeat experiment gave similar data as shown in Figure 5.

**DISCUSSION**

Our studies demonstrated that multiple CYP isoforms can metabolize methadone to EDDP, but CYP2B6, 2C19, and 3A4 appeared to be the most active in this regard. At low substrate concentrations CYP2B6 generated more EDDP than CYP2C19 or CYP3A4. Using Michaelis-Menten kinetics, only CYP2B6 and CYP2C19 generated an accurate V<sub>max</sub> and K<sub>m</sub>, and thus intrinsic clearance (V<sub>max</sub>/K<sub>m</sub>). Although CYP3A4 also metabolized methadone, an accurate V<sub>max</sub> and K<sub>m</sub> could not be calculated because saturation of the enzymatic reaction was not possible. This suggests that the K<sub>m</sub> for this reaction may be quite high. Both Iribarne et al.<sup>8</sup> and Foster et al.<sup>10</sup> described methadone’s K<sub>m</sub> for CYP3A4 in the high micromolar range. This would indicate that CYP3A4 is a low-affinity, high-capacity enzyme in the human liver microsomes. Both CYP2B6 and 2C19 have higher affinity towards methadone and, certainly at low methadone concentrations, as is observed in the clinical use of methadone, the preferred pathway of N-demethylation could be CYP2B6. Although in vitro data do not always correlate with what occurs in vivo, the overall rate of methadone N-demethylation is likely dependent on the level of expression of these CYPs in the liver.

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**Fig. 6.** This figure demonstrates the Michaelis-Menten kinetics of pure enantiomers of R- and S-methadone in the presence of human CYP2B6 (A) and CYP2C19 (B). The abscissa has concentrations in µg/ml and the ordinate has the velocity in ng/min/10 pmol. The curves were generated using Michaelis-Menten kinetic parameters derived from the nonlinear regression analysis of the data. The insert shows the raw data. The calculated V<sub>max</sub> and K<sub>m</sub> are shown for R- and S-methadone with CYP2B6 and CYP2C19. These data represent one experiment; however, repetition gave identical results.
Additional data that CYP2B6 and CYP2C19 might be important in methadone metabolism were provided by the demonstration that these CYP isoforms metabolize methadone stereoselectively. Human CYP2B6 metabolized (S)-methadone more avidly than (R)-methadone, while CYP2C19 showed the reverse. CYP3A4 metabolized both methadone enantiomers equivalently. Both the substrate disappearance experiments with racemic methadone and the enzyme kinetic experiments of specific methadone enantiomers demonstrated this stereoselective metabolism by CYP2B6 and 2C19. Our data showed that the stereoselective metabolism of methadone by CYP2B6 and CYP2C19 is the result of enantioselective metabolism by these CYPs and not because of an enantiomeric interaction between (R)- and (S)-methadone. In addition, our data suggested that at steady state the (R)/(S)-methadone ratio in plasma is determined by the relative expression of the hepatic CYPs involved in methadone metabolism. Thus, induction of one of the isoforms of CYP could result in a greater effect on one of the enantiomers of methadone that would make the interpretation of total methadone concentrations difficult. The identification of CYP2B6 and CYP2C19 in methadone metabolism is interesting because CYP2B6 is not usually expressed at a high level in the liver, in vivo drug–drug interaction data with methadone cannot be explained solely by inhibition or induction of CYP3A4. Indinavir, an effective CYP3A4 inhibitor, and ritonavir, a very potent inhibitor of CYP3A4 but inducer of CYP2C1911,12 have no effect and induce methadone metabolism, respectively. In addition, rifabutin, which is a moderate inducer of CYP3A4, had no significant effect on methadone pharmacokinetics in vivo.17 Many of the other drugs that induce CYP3A4 and induce methadone metabolism can also induce other CYP isoforms. For example, rifampin can induce CYP2C9, CYP2C19 and probably CYP2B6, but not to the extent that it induces CYP3A4.19 Rifampin usually induces the metabolism of CYP3A4 substrates, such as HIV protease inhibitors, to such an extent that the concentration of these drugs is difficult to measure.20,21 Although rifampin has been shown to induce methadone metabolism, the extent of induction is not nearly as great as for HIV protease inhibitors.22 Nevirapine and efavirenz, HIV nonnucleoside reverse transcriptase inhibitors, are inducers of both CYP3A4 and CYP2B6, and they induced methadone metabolism by greater than 2-fold.23–25 Fluconazole is one drug that has been shown to increase methadone exposure in humans, but this drug is much more of an inhibitor of CYP2C isoforms than CYP3A4.26 In addition, in preliminary experiments we have demonstrated that fluconazole inhibits CYP2B6-mediated methadone metabolism in vitro (Gerber, unpubl. data).

In summary, the present experiments support the conclusion that the three main CYP isoforms are capable of methadone N-demethylation. CYP2B6 and CYP2C19 metabolized methadone enantioselectively, while CYP3A4 did not. Our present drug metabolism data in human CYP isoforms in vitro could explain the results of the in vivo drug–drug interaction studies as well as the variable plasma (R)/(S)-methadone concentration ratios in large population studies at steady state.

**LITERATURE CITED**


