DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

DMD Fast Forward. Published on January 8, 2013 as doi:10.1124/dmd.112.050625

Title Page

Methadone N-demethylation by the common *CYP2B6* allelic variant CYP2B6.6

Sarah Gadel, Amanda Crafford, Karen Regina, Evan D. Kharasch

Department of Anesthesiology, Division of Clinical and Translational Research (SG, AC, KR, EDK), and the Department of Biochemistry and Molecular Biophysics (EDK), Washington University in St. Louis, St. Louis, MO

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

Copyright 2013 by the American Society for Pharmacology and Experimental Therapeutics.

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Running Title Page

Running title: Methadone metabolism by CYP2B6.6

Corresponding author: Evan D. Kharasch, Department of Anesthesiology, Washington University in St. Louis, 660 S Euclid Ave, Campus Box 8054, St. Louis, MO 63110-1093, Tel: 314-362-8796, Fax: 314-362-8571, email: kharasch@wustl.edu

Word count:
Text pages - 20
Tables – 1
Figures – 2
Abstract - 230
Introduction - 493
Discussion - 1168
Number of references: 40

Abbreviations: EDDP, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

The long-acting opioid methadone displays considerable unexplained interindividual pharmacokinetic variability. Methadone metabolism clinically occurs primarily by N-demethylation to 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), catalyzed predominantly by cytochrome P450 2B6 (CYP2B6). Retrospective studies suggest an influence of the common allele variant *CYP2B6*6* on methadone plasma concentrations. The catalytic activity of CYP2B6.6, encoded by *CYP2B6*6,* is highly substrate-dependent. This investigation evaluated methadone N-demethylation by CYP2B6.6, and in comparison to that by wild-type CYP2B6.1. Methadone enantiomer and racemate N-demethylation by recombinant expressed CYP2B6.6 and CYP2B6.1 was determined. At substrate concentrations (0.25-2 μM) approximating plasma concentrations occurring clinically, rates of methadone enantiomer N-demethylation by CYP2B6.6, incubated individually or as the racemate, were one-third to one-fourth those by CYP2B6.1. For methadone individual enantiomers metabolism by CYP2B6.6 compared with CYP2B6.1, V_{max} was diminished, K_s was greater, the *in vitro* intrinsic clearance was diminished 5- to 6-fold. The intrinsic clearance for R- and S-EDDP formation from racemic methadone was diminished approximately 6-fold and 3-fold for R- and S-methadone. Both CYP2B6.6 and CYP2B6.1 showed similar stereoselectivity (S>R-methadone). Human liver microsomes with diminished CYP2B6 content due to a *CYP2B6*6* allele had lower rates of methadone N-demethylation. Results show that methadone N-demethylation catalyzed by CYP2B6.6, the CYP2B6 variant encoded by the *CYP2B6*6* polymorphism, is catalytically deficient compared with wild-type CYP2B6.1. Diminished methadone N-demethylation by CYP2B6.6 may provide a mechanistic explanation for clinical observations of altered methadone disposition in individuals carrying the *CYP2B6*6* polymorphism.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

The long-acting opioid methadone is used to treat opiate addiction, as well as acute, chronic, and cancer pain. Clinical use of methadone is challenging, however, because of considerable and unpredictable inter- and intra-individual variability in pharmacokinetics, including metabolism, clearance, and susceptibility to drug interactions (Ferrari et al., 2004; McCance-Katz et al., 2010). This can result in opiate withdrawal, inadequate analgesia, or drug accumulation and toxicity. Indeed, with increasing methadone use over the past decade there has been an epidemic of toxicity, including a nearly 1800% increase in adverse events and a 390% increase in fatalities, which persist today (2012).

Methadone in humans is cleared primarily by hepatic cytochrome P450 (CYP)-catalyzed metabolism, to the pharmacologically inactive metabolite 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), with some urinary excretion of unchanged drug. Methadone clearance and N-demethylation are stereoselective. After considerable research, a consensus has emerged that methadone N-demethylation *in vitro*, both by human liver microsomes and expressed CYPs, is catalyzed most efficiently by CYP2B6 and CYP3A4 (while CYP3A5 is comparatively inactive), and, CYP2B6 but not CYP3A4 N-demethylates methadone stereoselectively (Gerber et al., 2004; Kharasch et al., 2004; Totah et al., 2007; Totah et al., 2008; Chang et al., 2010). Clinical drug interaction studies indicate that CYP2B6, rather than CYP3A4, is a major or predominant CYP isoform responsible for clinical methadone disposition. CYP3A induction (Vourvahis et al., 2012), and very strong CYP3A inhibition (Kharasch et al., 2004; Kharasch et al., 2008; van Heeswijk et al., 2011; Kharasch et al., 2012), had no influence on (or in some studies even increased) methadone N-demethylation or clearance. However CYP2B6 induction or inhibition did correspondingly modulate methadone plasma concentrations, metabolism and clearance (Kharasch et al., 2004; Kharasch et al., 2008; Kharasch and Stubbert, 2013).

CYP2B6 comprises a small fraction of hepatic CYP content, but is responsible for metabolizing a much larger percentage of drugs and xenobiotics (Wang and Tompkins, 2008; Mo et al., 2009) *CYP2B6* is a highly polymorphic gene (Zanger et al., 2007), with numerous single nucleotide polymorphisms encoding thirty CYP2B6 protein variants identified to date. The *CYP2B6*6* allele (516G>T, Q172H;

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

785A>G, K262R) is of considerable interest, owing to its common occurrence (particularly in populations or descendants of Africans, Asians, and Hispanics) and therapeutic significance (Zanger et al., 2007). For example, *CYP2B6*6* influences the metabolism, pharmacokinetics, and clinical effects of efavirenz, nevirapine, cyclophosphamide, and bupropion (Mo et al., 2009) *CYP2B6*6* has been associated with diminished CYP2B protein expression in human liver microsomes (Xie et al., 2003; Desta et al., 2007; Hofmann et al., 2008) Expressed CYP2B6.6, compared with CYP2B6.1, has diminished catalytic activity towards bupropion and efavirenz (Zhang et al., 2011; Xu et al., 2012), modest-to-moderately increased activity towards cyclophosphamide (Xie et al., 2003; Ariyoshi et al., 2011; Raccor et al., 2012), and artemether (Honda et al., 2011), and indifferent metabolism of selegiline (Watanabe et al., 2010). Human liver microsomes from *CYP2B6*6* carriers had diminished metabolism of mephenytoin (Lang et al., 2001), bupropion (Hesse et al., 2004; Xu et al., 2012), and efavirenz (Desta et al., 2007; Xu et al., 2012), but not cyclophosphamide (Xie et al., 2003; Raccor et al., 2012). Thus, the influence of *CYP2B6*6* genotype on the direction (increased or decreased metabolism), magnitude, pharmacokinetic consequence, clinical implications and mechanism of CYP2B6-dependent biotransformation are complex, and, substrate-dependent.

Available clinical evidence suggests that *CYP2B6* polymorphisms can influence methadone disposition. Specifically, associations between *CYP2B6*6* genotype and higher dose-adjusted steady-state plasma methadone concentrations (Crettol et al., 2005; Crettol et al., 2006; Eap et al., 2007; Wang et al., 2011), or need for lower methadone doses (Hung et al., 2011; Levran et al., 2012), have been reported.

Despite these pharmacogenetic association studies evaluating single steady-state plasma methadone concentrations, the influence of *CYP2B6*6* alleles or *CYP2B6* polymorphism in general, on clinical methadone metabolism and clearance is unknown, as are their influence on human liver microsomal methadone metabolism. In addition, the activity of CYP2B6.6 towards methadone metabolism is also unknown. Therefore, the purpose of this investigation was to evaluate methadone N-demethylation by CYP2B6.6, and compare it to that by wild-type CYP2B6.1.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Materials and Methods

Chemicals and reagents. EDDP and EDDP-d3 were purchased from Cerilliant (Round Rock, TX). Insect cell microsomes (Supersomes®) containing CYP2B6.1 coexpressed with human cytochrome P450 reductase and human cytochrome b_5 were purchased from BD Gentest Corporation (Woburn, MA). Microsomes containing CYP2B6.6 coexpressed with P450 reductase and cytochrome b_5 were a generous gift from BD Gentest Corporation. All other reagents were from Sigma-Aldrich (St. Louis, MO).

Methadone metabolism. Incubations (200 µl, 10 pmol/ml CYP2B6) with RS-, R-, or S-methadone were performed as previously described (Totah et al., 2007; Totah et al., 2008), with minor modifications. Reactions (10 min) were quenched with 20 µl 20% trichloroacetic acid containing internal standard (d3-EDDP, final concentration 4.55 ng/ml), centrifuged, and supernatant (200µl) processed immediately by solid-phase extraction as described previously (Kharasch et al., 2004), except that Strata-X-C 33 µm plates (Phenomenex, Torrance, CA) were used. EDDP analysis was performed on an API 3200 triple-quadrupole mass spectrometer with Turbo Ion Spray source (Applied Biosystems/MDS Sciex, Foster City, CA), Shimadzu HPLC (Columbia, MD), autosampler (Gerstel, Germany), and chiral AGP column (3 x 50mm, 5µm) with chiral AGP guard cartridge (3 x 10mm) (Chrom Tech, Apple Valley, MN), as described (Sharma et al., 2011), except that the mobile phase was 20mM ammonium formate (pH 5.0) and methanol. Retention times were 10.4 and 11.2 min, respectively, for R- and S-EDDP. EDDP was quantified using peak area ratios and standard curves prepared using calibration standards in buffer. Control incubations lacking NADPH and protein were included for all reactions to determine the background EDDP, which was subtracted from all results.

Data and Statistical Analysis. Results are the mean ± SD (3-6 replicates) unless otherwise indicated. Differences between groups were determined by analysis of variance followed by the Student-Newman-Keuls test (SigmaPlot 12.3 Systat, San Jose, CA). EDDP formation vs substrate concentration data were analyzed by nonlinear regression analysis (SigmaPlot 12.3) using the Adair-Pauling model, based on the recognition that CYP2B6 contains at least two binding sites, and as described previously (Totah et al., 2007; 2008). Results are the parameter estimate ± standard error of the estimate.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Results

N-demethylation of racemic methadone and individual enantiomers was evaluated at concentrations (0.25-2 μM) approximating those in patients receiving low and high doses of methadone, respectively, for treatment of pain (typically 10-20 mg) or substance abuse (60-100 mg). Rates of methadone enantiomer N-demethylation by CYP2B6.6 were typically one-third those by CYP2B6.1, when enantiomers were incubated individually (Figure 1A). When racemic methadone N-demethylation was evaluated (Figure 1B), rates of R- and S-EDDP formation by CYP2B6.6 were even lower, approximately one-fourth those by CYP2B6.1. N-demethylation by CYP2B6.1 was stereoselective, with S-methadone metabolism exceeding that of R-methadone, both with individual enantiomers and the racemate. Although EDDP formation by CYP2B6.6 was much lower than that by CYP2B6.1, stereoselectivity (S>R-methadone) was preserved, with individual enantiomers and the racemate.

Concentration-dependence of methadone N-demethylation was determined for racemic methadone and the individual enantiomers (Figure 2). As observed previously (Totah et al., 2008), Eadie-Hofstee plots for racemic methadone N-demethylation by CYP2B6.1 were not strictly linear, nor were those for R- and S-methadone, indicating that the nonlinearity of the racemate did not represent the two enantiomers interacting differently with a single enzyme site, but rather, each enantiomer interacting with two apparent enzyme sites. Nonlinear Eadie-Hofstee plots were also observed for CYP2B6.6-catalyzed racemic methadone and methadone enantiomers N-demethylation. The Adair-Pauling equation, which allows for two binding sites, was used to model EDDP formation from individual methadone enantiomers, and kinetic parameters are provided in Table 1. For CYP2B6.6, V_{max} was diminished to approximately one-third to one-fifth that for CYP2B6.1, K_s greater than for CYP2B6.1, and the *in vitro* intrinsic clearance (Cl_{int}, V_{max}/K_s), was diminished 5- to 6-fold. The Adair-Pauling equation was also used to model EDDP enantiomer formation from racemic methadone. For CYP2B6.6, the apparent K_s for both enantiomers was approximately 50% greater than for CYP2B6.1, V_{max} was diminished more for R-methadone (to one fourth) than S-methadone (to approximately half), and the *in vitro* intrinsic clearance was diminished approximately 6-fold and 3-fold for R- and S-methadone, respectively.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

The major finding of this investigation is that the N-demethylation of methadone catalyzed by CYP2B6.6, the CYP2B6 variant encoded by the *CYP2B6*6* polymorphism, is catalytically deficient compared with wild-type CYP2B6.1 With CYP2B6.6 compared with CYP2B6.1, EDDP formation from both individual methadone enantiomers was diminished, K_s was increased, V_{max} was reduced, and the *in vitro* intrinsic clearance was diminished to approximately one-fifth that for the wild-type enzyme. With racemic methadone, EDDP formation from both methadone enantiomers was also lower with CYP2B6.6, V_{max} was reduced, and the *in vitro* intrinsic clearance was diminished to approximately one-fifth to one-half that for wild-type CYP2B6.1. At substrate concentrations approximating total plasma methadone concentrations (0.25-0.5 μM each enantiomer) occurring clinically, for both individual enantiomers and racemic methadone, rates of N-demethylation by CYP2B6.6 were generally only one-third those for CYP2B6.1. Although rates of methadone metabolism by CYP2B6.6 were diminished compared with CYP2B6.1, the stereoselectivity of metabolism (S-methadone > R-methadone) seen previously with CYP2B6.1 (Gerber et al., 2004; Totah et al., 2007; Totah et al., 2008; Chang et al., 2010), was preserved with CYP2B6.6.

Modeling of methadone N-demethylation by CYP2B6 is complex (Totah et al., 2007). Methadone enantiomers N-demethylation by CYP2B6.1 showed apparent multiple-site or multiple-affinity binding with complex allosteric kinetics or homotropic cooperativity, which was best described using the Adair-Pauling equation (Totah et al., 2007). This approach was used to model methadone enantiomers N-demethylation by CYP2B6.1 and CYP2B6.6 in the present investigation. With CYP2B6.6, at the highest substrate concentrations, the possibility of substrate or product inhibition cannot be eliminated, however there are insufficient data with which to evaluate such models in an identifiable fashion, and hence it most appropriate to use the simplest model for which there is precedent. With racemic methadone, a previous investigation with CYP2B6.1 found a competitive inhibitory interaction, with each enantiomer in the racemate (R- or S-) inhibiting the metabolism of its antipode (S- or R-methadone). The Adair-Pauling model was found to be mis-specified for racemic methadone, and a

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

more complex model was required to describe methadone metabolism, however that required evaluating metabolism of a complex matrix of individual antipode concentrations at each enantiomer concentration. In the present investigation, only racemic methadone metabolism by CYP2B6.6 was evaluated, precluding application of the complex CYP2B6 model, and the simpler Adair-Pauling equation used to model the data, accepting some misspecification in the parameter estimates. Thus for racemic methadone the reported K_s and V_{max} are best considered as apparent parameters. Use of a Michaelis-Menten model did not result in improved fits to the data (not shown).

The catalytic behavior of CYP2B6.6 is substrate-dependent, and the mechanism of altered CYP2B6.6-catalyzed biotransformation, when it has been observed, is not well understood. Some information is available from studies using expressed CYP2B6.6, those using liver microsomes of individuals carrying the *CYP2B6*6* allele, and from clinical pharmacogenetic studies. In COS-1 cells expressing CYP2B6.6, Cl_{int} for 7-ethoxy-4-trifluoromethylcoumarin O-deethylation was double that for CYP2B6.1 (Jinno et al., 2003). In COS-7 cells expressing CYP2B6.6, Cl_{int} for 7-ethoxy-4-trifluoromethylcoumarin O-deethylation, selegiline N-demethylation, and selegiline N-depropagylation was not different from that for CYP2B6.1 (Watanabe et al., 2010), and the activities towards bupropion and artemether were significantly less (Hofmann et al., 2008), and greater (Honda et al., 2011), respectively. In an insect cell CYP2B6 expression system co-expressing P450 reductase but not cytochrome b_5, Cl_{int} for efavirenz 8-hydroxylation by CYP2B6.6 was half that compared with CYP2B6.1, while the Cl_{int} for cyclophosphamide 4-hydroxylation was 60% greater (Ariyoshi et al., 2011). In a CYP2B6 reconstitution system with P450 reductase but not cytochrome b_5, the catalytic efficiency (k_{cat}/K_m) of CYP2B6.6 for 7-ethoxy-4-trifluoromethylcoumarin, bupropion 4-hydroxylation, and efavirenz 8-hydroxylation was decreased to two-thirds, one-half, and one-fifth that compared with CYP2B6.1 (Zhang et al., 2011). In a CYP2B6 expression system co-expressing P450 reductase and cytochrome b_5, CYP2B6.6-catalyzed efavirenz 8-hydroxylation was not significantly different and the Cl_{int} for bupropion 4-hydroxylation was reduced by approximately one-third compared with CYP2B6.1 (Xu et al., 2012). In the absence of b_5, efavirenz 8-hydroxylation Cl_{int} was approximately half for

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

CYP2B6.6 compared with CYP2B6.1, and bupropion 4-hydroxylation Cl_{int} approximately 50% greater (Xu et al., 2012). In the present insect cell CYP2B6 expression system, containing both co-expressed P450 reductase and cytochrome b_5, the catalytic difference between CYP2B6.6 and CYP2B6.1 for methadone enantiomer metabolism was generally greater than that observed previously for other substrates, with a 5-fold lower Cl_{int} for N-demethylation. Thus methadone appears to be one of the most susceptible substrates to the diminished catalytic efficiency of CYP2B6.6.

In human liver, the *CYP2B6*6* allele causes aberrant splicing (Hofmann et al., 2008), resulting in reduced functional mRNA and low hepatic CYP2B expression (Lang et al., 2001; Desta et al., 2007; Hofmann et al., 2008). Thus both diminished CYP content and deficient catalytic efficiency may combine to cause the phenotype of decreased CYP2B6-catalyzed biotransformation in CYP2B6*6 carriers. Human liver microsomes may therefore show greater catalytic differences between CYP2B6.1 and CYP2B6.6 and the effect of the *CYP2B6*6* polymorphism, compared with expressed enzyme systems. Indeed, in human liver microsomes from individuals with *6 genotypes, there was markedly diminished CYP2B6 protein expression, and cyclophosphamide 4-hydroxylation (Xie et al., 2003), bupropion hydroxylation (Hesse et al., 2004; Xu et al., 2012), and efavirenz 8-hydroxylation (Desta et al., 2007; Xu et al., 2012). Clinically, numerous investigations have shown an association between *CYP2B6*6* genotypes and increased efavirenz plasma concentrations, diminished metabolism and clearance, and greater neurotoxicity and hepatotoxicity (Haas et al., 2004; Holzinger et al., 2012; Turpeinen and Zanger, 2012). Bupropion metabolism was similarly diminished in *CYP2B6*6* carriers, based on lower plasma hydroxybupropion/bupropion AUC ratios (Chung et al., 2011).

We previously evaluated the influence of CYP2B6 content and genetic polymorphisms on human liver microsomal methadone metabolism (Totah et al., 2008). There was no apparent consistent relationship between methadone N-demethylation and CYP2B6 content (or genotype), and no definitive conclusions could be drawn regarding *CYP2B6* genotype and methadone metabolism. Since then, microsomal CYP3A content was re-quantified (Raccor et al., 2012), and, when pairs of livers were re-matched for CYP3A content but different CYP2B6 content (or genotype) and methadone enantiomers

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

metabolism again compared, a clear influence of *CYP2B6* genotype became apparent (Supplemental Table 1). For example, microsomes from human livers 141 and 144 both had high CYP3A content, but high (*CYP2B6*1/*1*) and moderate CYP2B6 (*CYP2B6*1/*6*) content, respectively, and lower N-demethylation of both methadone enantiomers was observed in HLM 144. Livers 124 and 148 both had high CYP3A content, but high (*CYP2B6*1/*6*) and low CYP2B6 (*CYP2B6*6/*6*) content, respectively, and lower methadone metabolism was observed in HLM 148. HLM 142 and 164 both had low CYP3A, but moderate (*CYP2B6*1/*4*) and low CYP2B6 (*CYP2B6*1/*6*) content, and lower methadone N-demethylation was observed with HLM 164. These data suggest that *CYP2B6* genotype, specifically the **6* allele, can influence human liver microsomal methadone N-demethylation.

Diminished methadone metabolism by expressed CYP2B6.6 and livers from individuals with the *CYP2B6**6 allele is consistent with previous reports of a genetic influence of CYP2B6 on methadone plasma concentrations. Dose-adjusted steady-state trough and peak plasma S- methadone concentrations were greater in homozygous carriers of *CYP2B6*6*, compared with heterozygotes and non-carriers (Crettol et al., 2005; Crettol et al., 2006; Eap et al., 2007). Dose-adjusted steady-state trough S-methadone concentrations were 2-fold higher in **6/*6* genotypes than non-carriers (Crettol et al., 2005). Another investigation found that *CYP2B6*6* homozygotes similarly needed lower methadone doses (Hung et al., 2011). *CYP2B6*6* carriers had higher plasma S-methadone concentrations and a higher concentration-to-dose ratio for both enantiomers (Wang et al., 2011). Mean methadone doses required by methadone maintenance patients were significantly lower in *CYP2B6*6/*6* genotypes than in heterozygotes or non-carriers (Levran et al., 2012). In a series of methadone-related deaths, whole blood RS-methadone concentrations were significantly (approximately 2-fold) higher in *CYP2B6*6* carriers than non-carriers (Bunten et al., 2010). Together these reports suggest that the *CYP2B6*6* allele influences methadone disposition, although there have been no published studies investigating the influence of *CYP2B6*6* or other polymorphisms on clinical methadone metabolism or clearance. Diminished methadone N-demethylation by CYP2B6.6 further supports these clinical observations, and may provide a mechanistic explanation.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Acknowledgements:

The authors gratefully appreciate the generous gift of CYP2B6.6 Supersomes provided by Chris Patten, BD Gentest Corporation.

Authorship Contributions:

Participated in research design: Gadel, Kharasch

Conducted experiments: Gadel, Crafford, Regina

Performed data analysis: Gadel, Crafford, Regina, Kharasch

Wrote or contributed to the writing of the manuscript: Gadel, Crafford, Regina, Kharasch

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

References

(2012) Vital signs: risk for overdose from methadone used for pain relief - United States, 1999-2010. *MMWR Morb Mortal Wkly Rep* **61:**493-497.

Ariyoshi N, Ohara M, Kaneko M, Afuso S, Kumamoto T, Nakamura H, Ishii I, Ishikawa T and Kitada M (2011) Q172H replacement overcomes effects on the metabolism of cyclophosphamide and efavirenz caused by CYP2B6 variant with Arg262. *Drug Metab Dispos* **39:**2045-2048.

Bunten H, Liang WJ, Pounder D, Seneviratne C and Osselton MD (2010) CYP2B6 and OPRM1 gene variations predict methadone-related deaths. *Addict Biol* **16:**142-144.

Chang Y, Fang WB, Lin SN and Moody DE (2010) Stereo-selective metabolism of methadone by human liver microsomes and cDNA-expressed cytochrome P450s: a reconciliation. *Basic Clin Pharmacol Toxicol* **108:**55-62.

Chung JY, Cho JY, Lim HS, Kim JR, Yu KS, Lim KS, Shin SG and Jang IJ (2011) Effects of pregnane X receptor (NR1I2) and CYP2B6 genetic polymorphisms on the induction of bupropion hydroxylation by rifampin. *Drug Metab Dispos* **39:**92-97.

Crettol S, Deglon JJ, Besson J, Croquette-Krokar M, Hammig R, Gothuey I, Monnat M and Eap CB (2006) ABCB1 and cytochrome P450 genotypes and phenotypes: Influence on methadone plasma levels and response to treatment. *Clin Pharmacol Ther* **80:**668-681.

Crettol S, Deglon JJ, Besson J, Croquette-Krokkar M, Gothuey I, Hammig R, Monnat M, Huttemann H, Baumann P and Eap CB (2005) Methadone enantiomer plasma levels, CYP2B6, CYP2C19, and CYP2C9 genotypes, and response to treatment. *Clin Pharmacol Ther* **78:**593-604.

Desta Z, Saussele T, Ward B, Blievernicht J, Li L, Klein K, Flockhart DA and Zanger UM (2007) Impact of CYP2B6 polymorphism on hepatic efavirenz metabolism in vitro. *Pharmacogenomics* **8:**547-558.

Eap CB, Crettol S, Rougier JS, Schlapfer J, Sintra Grilo L, Deglon JJ, Besson J, Croquette-Krokar M, Carrupt PA and Abriel H (2007) Stereoselective block of hERG channel by (S)-methadone and QT interval prolongation in CYP2B6 slow metabolizers. *Clin Pharmacol Ther* **81:**719-728.

Ferrari A, Coccia CP, Bertolini A and Sternieri E (2004) Methadone--metabolism, pharmacokinetics and interactions. *Pharmacol Res* **50:**551-559.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Gerber JG, Rhodes RJ and Gal J (2004) Stereoselective metabolism of methadone N-demethylation by cytochrome P4502B6 and 2C19. *Chirality* **16:**36-44.

Haas DW, Ribaudo HJ, Kim RB, Tierney C, Wilkinson GR, Gulick RM, Clifford DB, Hulgan T, Marzolini C and Acosta EP (2004) Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS clinical trials group study. *AIDS* **18:**2391-2400.

Hesse LM, He P, Krishnaswamy S, Hao Q, Hogan K, von Moltke LL, Greenblatt DJ and Court MH (2004) Pharmacogenetic determinants of interindividual variability in bupropion hydroxylation by cytochrome P450 2B6 in human liver microsomes. *Pharmacogenetics* **14:**225-238.

Hofmann MH, Blievernicht JK, Klein K, Saussele T, Schaeffeler E, Schwab M and Zanger UM (2008) Aberrant splicing caused by single nucleotide polymorphism c.516G>T [Q172H], a marker of CYP2B6*6, is responsible for decreased expression and activity of CYP2B6 in liver. *J Pharmacol Exp Ther* **325:**284-292.

Holzinger ER, Grady B, Ritchie MD, Ribaudo HJ, Acosta EP, Morse GD, Gulick RM, Robbins GK, Clifford DB, Daar ES, McLaren P and Haas DW (2012) Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols implicates several CYP2B6 variants. *Pharmacogenet Genomics* **22:**858-867.

Honda M, Muroi Y, Tamaki Y, Saigusa D, Suzuki N, Tomioka Y, Matsubara Y, Oda A, Hirasawa N and Hiratsuka M (2011) Functional characterization of CYP2B6 allelic variants in demethylation of antimalarial artemether. *Drug Metab Dispos* **39:**1860-1865.

Hung CC, Chiou MH, Huang BH, Hsieh YW, Hsieh TJ, Huang CL and Lane HY (2011) Impact of genetic polymorphisms in ABCB1, CYP2B6, OPRM1, ANKK1 and DRD2 genes on methadone therapy in Han Chinese patients. *Pharmacogenomics* **12:**1525-1533.

Jinno H, Tanaka-Kagawa T, Ohno A, Makino Y, Matsushima E, Hanioka N and Ando M (2003) Functional characterization of cytochrome P450 2B6 allelic variants. *Drug Metab Dispos* **31:**398-403.

Kharasch ED, Bedynek PS, Hoffer C, Walker A and Whittington D (2012) Lack of indinavir effects on methadone disposition despite inhibition of hepatic and intestinal cytochrome P4503A (CYP3A). *Anesthesiology* **116:**432-447.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Kharasch ED, Bedynek PS, Park S, Whittington D, Walker A and Hoffer C (2008) Mechanism of ritonavir changes in methadone pharmacokinetics and pharmacodynamics. I. Evidence against CYP3A mediation of methadone clearance. *Clin Pharmacol Ther* **84:**497-505.

Kharasch ED, Hoffer C, Whittington D and Sheffels P (2004) Role of hepatic and intestinal cytochrome P450 3A and 2B6 in the metabolism, disposition and miotic effects of methadone. *Clin Pharmacol Ther* **76:**250-269.

Kharasch ED and Stubbert K (2013) Role of cytochrome P4502B6 in methadone metabolism and clearance. *J Clin Pharmacol, in press*.

Lang T, Klein K, Fischer J, Nussler AK, Neuhaus P, Hofmann U, Eichelbaum M, Schwab M and Zanger UM (2001) Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. *Pharmacogenetics* **11:**399-415.

Levran O, Peles E, Hamon S, Randesi M, Adelson M and Kreek MJ (2012) CYP2B6 SNPs are associated with methadone dose required for effective treatment of opioid addiction. *Addict Biol***:***in press*.

McCance-Katz EF, Sullivan LE and Nallani S (2010) Drug interactions of clinical importance among the opioids, methadone and buprenorphine, and other frequently prescribed medications: a review. *Am J Addict* **19:**4-16.

Mo SL, Liu YH, Duan W, Wei MQ, Kanwar JR and Zhou SF (2009) Substrate specificity, regulation, and polymorphism of human cytochrome P450 2B6. *Curr Drug Metab* **10:**730-753.

Raccor BS, Claessens AJ, Dinh JC, Park JR, Hawkins DS, Thomas SS, Makar KW, McCune JS and Totah RA (2012) Potential contribution of cytochrome P450 2B6 to hepatic 4-hydroxycyclophosphamide formation in vitro and in vivo. *Drug Metab Dispos* **40:**54-63.

Sharma A, Tallchief D, Blood J, Kim T, London A and Kharasch ED (2011) Perioperative pharmacokinetics of methadone in adolescents. *Anesthesiology* **115:**1153-1161.

Totah RA, Allen KE, Sheffels P, Whittington D and Kharasch ED (2007) Enantiomeric metabolic interactions and stereoselective human methadone metabolism. *J Pharmacol Exp Ther* **321:**389-399.

Totah RA, Sheffels P, Roberts T, Whittington D, Thummel K and Kharasch ED (2008) Role of CYP2B6 in stereoselective human methadone metabolism. *Anesthesiology* **108:**363-374.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Turpeinen M and Zanger UM (2012) Cytochrome P450 2B6: function, genetics, and clinical relevance. *Drug Metabol Drug Interact* **27:**185-197.

van Heeswijk R, Vandevoorde A, Verboven P, Boogaerts G, De Paepe E, van Solingen-Ristea R, Garg V and Beumont M (2011) Pharmacokinetic interaction between methadone and the investigational HCV protease inhibitor telaprevir. *J. Hepatology* **54:**S491-492.

Vourvahis M, Wang R, Gruener DM, Bruce RD, Haider S and Tawadrous M (2012) Effect of lersivirine co-administration on pharmacokinetics of methadone in healthy volunteers. *Drug Alcohol Depend* **126:**183-188.

Wang H and Tompkins LM (2008) CYP2B6: new insights into a historically overlooked cytochrome P450 isozyme. *Curr Drug Metab* **9:**598-610.

Wang SC, Ho IK, Tsou HH, Tian JN, Hsiao CF, Chen CH, Tan HK, Lin L, Wu CS, Su LW, Huang CL, Yang YH, Liu ML, Lin KM, Chen CY, Liu SC, Wu HY, Chan HW, Tsai MH, Lin PS and Liu YL (2011) CYP2B6 polymorphisms influence the plasma concentration and clearance of the methadone S-enantiomer. *J Clin Psychopharmacol* **31:**463-469.

Watanabe T, Sakuyama K, Sasaki T, Ishii Y, Ishikawa M, Hirasawa N and Hiratsuka M (2010) Functional characterization of 26 CYP2B6 allelic variants (CYP2B6.2-CYP2B6.28, except CYP2B6.22). *Pharmacogenet Genomics* **20:**459-462.

Xie HJ, Yasar U, Lundgren S, Griskevicius L, Terelius Y, Hassan M and Rane A (2003) Role of polymorphic human CYP2B6 in cyclophosphamide bioactivation. *Pharmacogenomics J.* **3:**53-61.

Xu C, Ogburn ET, Guo Y and Desta Z (2012) Effects of the CYP2B6*6 allele on catalytic properties and inhibition of CYP2B6 in vitro: Implication for the mechanism of reduced efavirenz metabolism and other CYP2B6 substrates in vivo. *Drug Metab Dispos* **40:**717-725.

Zanger UM, Klein K, Saussele T, Blievernicht J, Hofmann MH and Schwab M (2007) Polymorphic CYP2B6: molecular mechanisms and emerging clinical significance. *Pharmacogenomics* **8:**743-759.

Zhang H, Sridar C, Kenaan C, Amunugama H, Ballou DP and Hollenberg PF (2011) Polymorphic variants of cytochrome P450 2B6 (CYP2B6.4-CYP2B6.9) exhibit altered rates of metabolism for

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

bupropion and efavirenz: A charge-reversal mutation in the K139E variant (CYP2B6.8) impairs formation of a functional cytochrome P450-reductase complex. *J Pharmacol Exp Ther* **338:**803-809.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Footnote

This work was supported by the National Institutes of Health [grants R01-DA14211 and K24-DA0041]

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Legends for Figures

Figure 1

Recombinant CYP2B6-catalyzed methadone N-demethylation at therapeutic concentrations. Results are shown for (A) metabolism of individual methadone enantiomers (0.25-1 μM each) and (B) racemic methadone (0.5-2 μM, corresponding to 0.25-1 μM of each enantiomer) by CYP2B6.1 and CYP2B6.6. Results are the mean ± SD of 3-6 determinations. *Significantly different vs CYP2B6.1 ($p<0.05$).

Figure 2

Concentration-dependence and kinetics of recombinant CYP2B6-catalyzed N-demethylation of methadone to EDDP. Results are shown for metabolism of (A and B) individual methadone enantiomers (0.25-500 μM each) and (C and D) racemic methadone (0.5-1000 μM, corresponding to 0.25-500 μM of each enantiomer) by CYP2B6.1 and CYP2B6.6. Corresponding Eadie-Hofstee plots are shown in B and D. For ease of comparison to enantiomers metabolism (A and B), racemic methadone is shown as the concentration of the individual enantiomers (C and D). Symbols represent CYP2B6.1-catalyzed EDDP formation from R-methadone (△) and S-methadone (▽) (single enantiomers or the racemate) and CYP2B6.6-catalyzed EDDP formation from R-methadone (▲) and S-methadone (▼) (single enantiomers or the racemate). Each data point is the mean ± SD of 3-6 determinations. Lines represent rates predicted from nonlinear regression analysis of data using the Adair-Pauling equation.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

Table 1 Kinetic Parameters for methadone *N*-demethylation

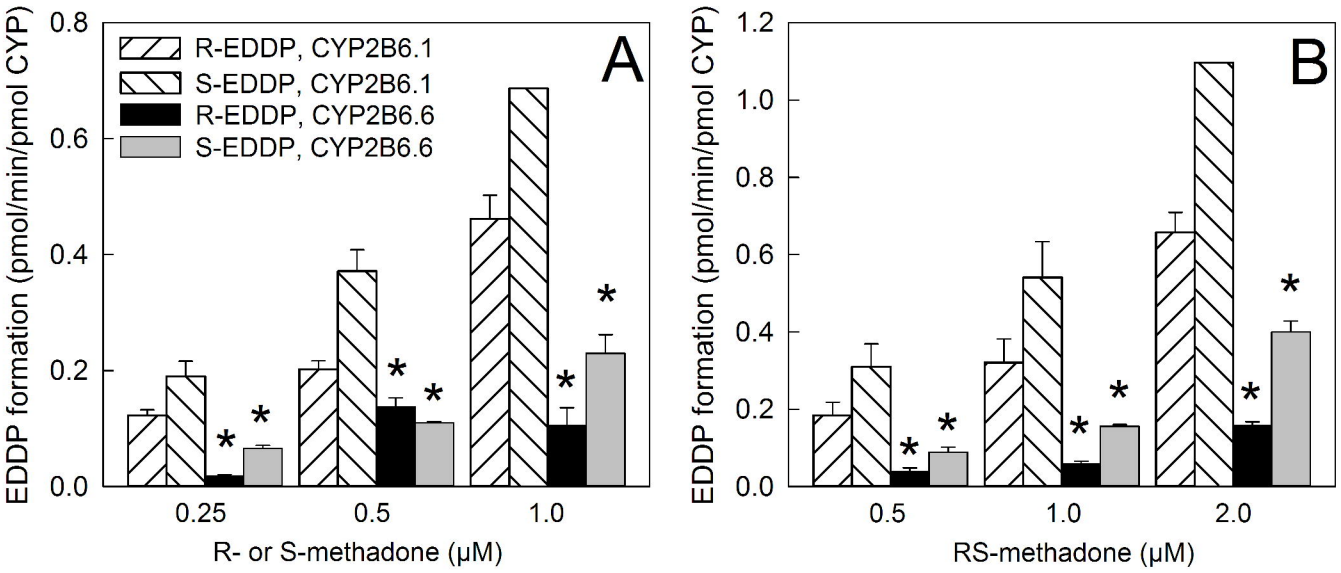
Parameter	Single Enantiomer Metabolism				Racemate Metabolism			
	CYP2B6.1		CYP2B6.6		CYP2B6.1		CYP2B6.6	
	R-EDDP formation	*S*-EDDP formation	*R*-EDDP formation	*S*-EDDP formation	*R*-EDDP formation	*S*-EDDP formation	*R*-EDDP formation	*S*-EDDP formation
K_s (μM)	75 ± 18	25 ± 5	97 ± 7	40 ± 7	40 ± 20	13 ± 3	57± 6	20 ± 3
V_{max} (nmol/nmol P450/min)	20.8 ± 0.6	14.6 ± 1.0	5.7 ± 0.3	3.5 ± 0.3	18.9± 7.4	8.7 ± 0.2	4.7 ± 0.6	5.1 ± 0.2
Cl_{int} (ml/min/nmol)	0.3 ± 0.1	0.6 ± 0.1	0.06 ± 0.01	0.09± 0.02	0.5 ± 0.3	0.7 ± 0.2	0.08 ± 0.01	0.3 ± 0.1

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

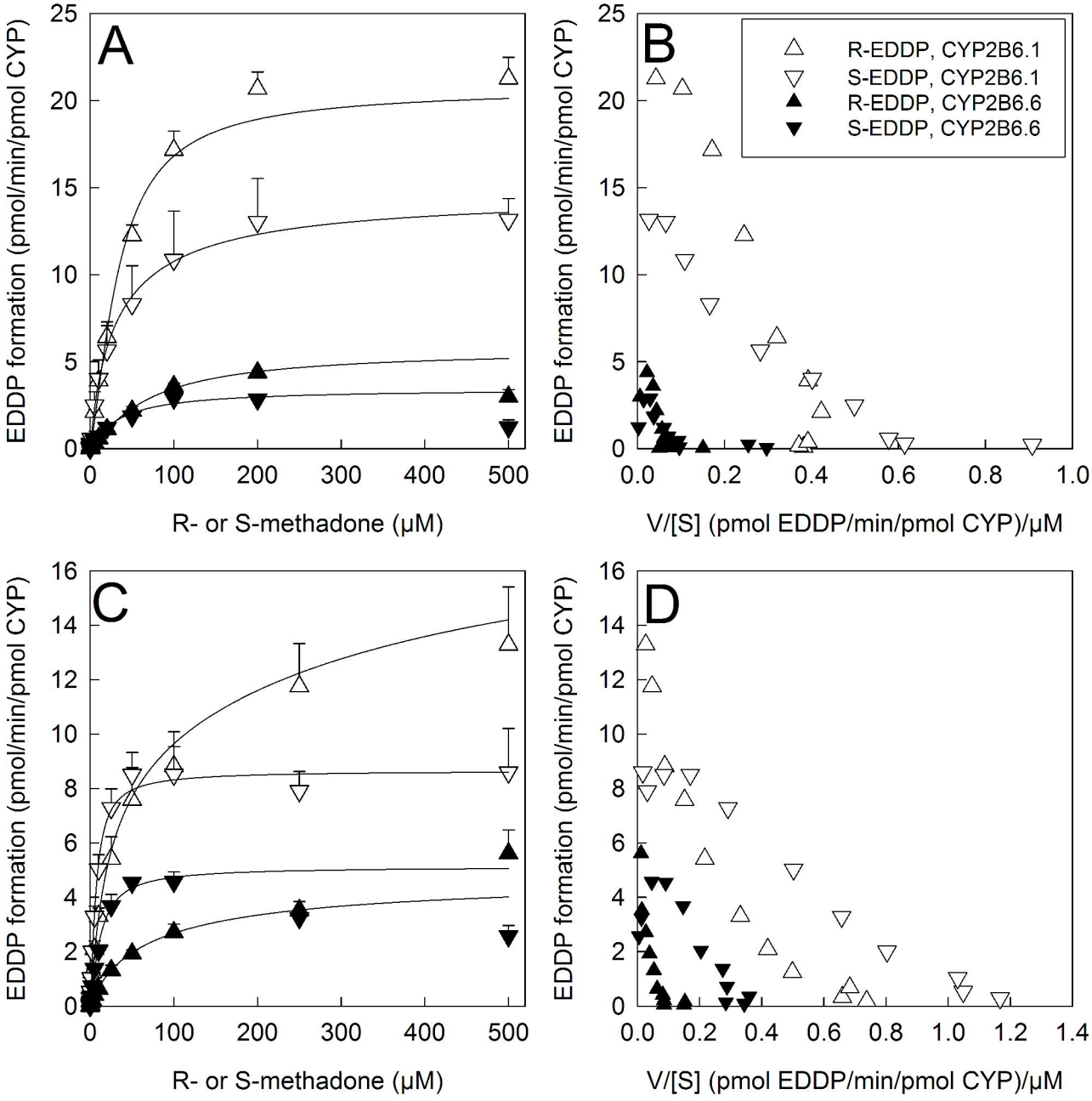
Figure 1



Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on January 8, 2013 as DOI: 10.1124/dmd.112.050625
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 2



Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024