Published in final edited form as:

J Clin Pharmacol. 2013 March ; 53(3): 305–313. doi:10.1002/jcph.1.

Role of cytochrome P4502B6 in methadone metabolism and clearance

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Abstract

Methadone N-demethylation *in vitro* is catalyzed by hepatic cytochrome P4502B6 (CYP2B6) and CYP3A4, but clinical disposition is often attributed to CYP3A4. This investigation tested the hypothesis that CYP2B6 is a prominent CYP isoform responsible for clinical methadone N-demethylation and clearance, using the *in vivo* mechanism-based CYP2B6 inhibitor ticlopidine, given orally for 4 days. A preliminary clinical investigation with the CYP3A4/5 substrate probe alfentanil established that ticlopidine did not inhibit intestinal or hepatic CYP3A4/5. Subjects received intravenous plus oral (deuterium-labeled) racemic methadone before and after ticlopidine. Ticlopidine significantly and stereoselectively (S>R) inhibited methadone N-demethylation, decreasing plasma metabolite/methadone area under the curve ratios and metabolite formation clearances. Ticlopidine also significantly increased the dose-adjusted plasma area under the curve for R- and S-methadone by 20% and 60%, respectively, after both intravenous and oral dosing. CYP2B6 inhibition reduces methadone N-demethylation and clearance, and alters methadone concentrations, demonstrating a predominant role for CYP2B6 in clinical methadone disposition.

Keywords

cytochrome P450 3A; CYP3A; cytochrome P450 2B6; CYP2B6; methadone; alfentanil

Methadone is a remarkably effective yet clinically challenging and inadequately understood opioid. Methadone maintenance is the cornerstone of opiate addiction therapy, preventing withdrawal and reducing death and codependent drug use, and is a public health strategy for reducing risks of HIV/AIDS, hepatitis, and other infectious diseases.¹ Methadone is also efficacious and cost-effective for treating acute, chronic, cancer, and neuropathic pain.^{2–4} Methadone is a frequent morphine alternative, used in opioid-rotation, and growing rapidly for first-line pain treatment.

The clinical utility of methadone is attenuated by considerable inter- and intra-individual variability in pharmacokinetics and pharmacodynamics, including metabolism, clearance, susceptibility to drug interactions, and autoinduction.⁵ Disposition of racemic methadone is

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No author has any conflict of interest.

Kristi Stubbert is deceased

This investigation was conducted before the requirement for clinical trials registration

stereoselective. The primary route of clearance is hepatic cytochrome P450 (CYP)-catalyzed metabolism, primarily N-demethylation to inactive 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), with some urinary excretion of unchanged drug. Despite decades of research, identification of the CYP isoform responsible for clinical methadone N-demethylation, clearance, and drug interactions remains controversial, if not elusive. This has been attributed variously to a plethora of CYPs, including 1A2, 2B6, 2C9, 2C19, 2D6, CYP3A4/5, and 19, often based on extrapolation of *in vitro* drug metabolism studies.^{5–7} Initial *in vitro* reports of CYP3A-catalyzed methadone metabolism^{8,9} engendered the methadone label, numerous articles, and therapeutic guidelines, to ascribe clinical methadone disposition to CYP3A4, warn of CYP3A4-mediated drug interactions, and recommend concomitant dose adjustments.^{5,10–12} For example, methadone interactions with antiretroviral therapies are widely attributed to CYP3A4.^{10–12}

Nevertheless, several clinical investigations are inconsistent with a predominant role for CYP3A4 in clinical methadone N-demethylation and clearance. For example, strong or profound inhibition of intestinal and hepatic CYP3A4/5 by troleandomycin, ritonavir, nelfinavir, indinavir, atazanavir or ritonavir/indinavir failed to increase methadone plasma concentrations, or to diminish systemic or apparent oral clearance, hepatic extraction or clearance, or bioavailability.^{6,13–17}

Several investigations have now established that CYP2B6 is a major isoform catalyzing methadone N-demethylation *in vitro*, and many clinical studies are consistent with or provide indirect evidence for prominent CYP2B6 involvement in clinical methadone disposition. Methadone N-demethylation was catalyzed as effectively by expressed CYP2B6 as CYP3A4, and CYP2B6 content in human liver microsomes strongly influenced methadone metabolism.^{6,18–21} CYP2B6 but not CYP3A4 metabolized methadone stereoselectively (S>R), liver microsomes expressing higher CYP2B6 vs CYP3A4 showed greater stereoselective metabolism, CYP2B6 inhibition reduced methadone metabolism, and only CYP2B6 inhibition was stereoselective.^{6,18–21} Clinically, CYP2B6 induction by ritonavir doubled methadone clearance, despite profound CYP3A4 inhibition.^{14,22} Methadone disposition was influenced by CYP2B6 genotype, with CYP2B6*6/*6 carriers having higher plasma concentrations, and presumably, lower clearance.^{23,24} *In vitro-in vivo* modeling predicted CYP3A modulation would minimally affect plasma R/S-methadone ratios, whereas CYP2B6 modulation would alter stereoselective disposition.^{19,20} This was confirmed experimentally.^{6,14–17,20,22,25}

Despite *in vitro* and indirect clinical evidence, direct evidence establishing or refuting a predominant role for CYP2B6 in clinical methadone N-demethylation and clearance does not exist. Isoform-selective CYP inhibitors are a standard approach to determining CYP isoform involvement in clinical drug metabolism. This investigation tested the hypothesis that CYP2B6 is a prominent CYP isoform responsible for clinical methadone N-demethylation and clearance, using the *in vivo* CYP2B6 inhibitor probe ticlopidine.^{26,27} Although ticlopidine is an effective CYP2B6 inhibitor, its clinical selectivity is unknown. Given the overlap in substrate specificity in general between CYP2B6 and CYP3A, an additional clinical investigation determined if ticlopidine might inhibit CYP3A and thereby confound interpretation of the methadone-ticlopidine interaction.

Methods

Clinical Protocol

Protocols were approved by the Institutional Review Board of Washington University in St. Louis. All subjects provided written informed consent. Eligible were normal healthy smoking or nonsmoking 18–40 yr male and female volunteers within 30% of ideal body

weight (body mass index <33). Exclusion criteria were major medical problems (including hematopoietic disorders), history of hepatic or renal disease, use of drugs or nonprescription preparations known to alter CYP3A or CYP2B6 activity, known history of drug or alcohol addiction, or access to and routine handling of addicting drugs in the regular course of employment.

Protocols were 2-period sequential crossovers (controls first, for logistical considerations). Each subject was their own control. Subjects were instructed to consume no food or beverages containing grapefruit throughout the study period, none containing apples or oranges for 5d before any study day, no alcohol for 1d before each study session and on the study day, and no food or water after midnight before each study session. Venous catheters were placed in opposite arms for blood sampling and drug administration (if needed). Subjects were monitored with a pulse oximeter and blood pressure cuff, and received supplemental oxygen for saturations <94%.

Ticlopidine effects on methadone metabolism and clearance were assessed by simultaneously administering intravenous and oral methadone.^{6,14–17,25} The final study population was twelve subjects (4 men, 8 women; 33 ± 12 yr, range 23–45; 78 ± 16 kg, range 66–116). Subjects received intravenous ondansetron followed by oral deuterated racemic d5-methadone HCl (11.0 mg, equivalent to 9.86 mg free base), and intravenous racemic unlabelled d0-methadone HCl (6.0 mg, equivalent to 5.4 mg free base, Roxane Laboratories, Columbus, OH). Deuterated methadone was synthesized and used under Investigational New Drug approval.⁶ Blood was sampled for 96 hr after methadone dosing and plasma stored (-20°C) for later analysis. Subjects received a standard breakfast 2 hr after methadone and had free access to food and water thereafter. Continuous urine samples were collected at 24, 48, 72, and 96 hr. Nausea and/or vomiting were treated with ondansetron (4 mg intravenous or 8 mg orally) as needed. At least 2–3 weeks later, subjects began taking oral ticlopidine 250 mg twice daily, with food or just after eating. On the fourth day of ticlopidine, the above methadone protocol was repeated, except that methadone doses were decreased to 8 mg oral d5-methadone HCl and 4 mg intravenous d0-methadone HCl, and blood and urine sampling continued for 144 hr, anticipating potentially decreased methadone clearance. Subjects continued to take ticlopidine 250 mg twice daily for the five days after taking methadone.

Methadone and EDDP in plasma and urine were quantified by solid phase extraction and stereoselective HPLC-mass spectrometry using a modification of a previous method.^{16,28} Briefly, to 0.5 ml plasma were added 25 μl of internal standard stock (racemic 20ng/ml d3-EDDP and 40ng/ml d9-methadone) and 0.75ml 4% phosphoric acid. Samples were loaded onto Oasis MCX 96-well plates (Waters, Milford, MA) previously conditioned with 100% methanol then 0.1N HCl. Plates were washed with 0.1N HCl then 100% methanol, and eluted with ethyl acetate:isopropanol:ammonium hydroxide (70:25:5). To 100 μl urine were added internal standards (17.4 ng d3-EDDP and 87 ng d9-methadone) and water. Samples were loaded onto previously conditioned Oasis MCX plates, washed with 0.1N HCl, 100% methanol, and methanol:water:ammonium hydroxide:methanol (55:40:5), and eluted with 5% ammonium hydroxide in methanol. Samples were evaporated to dryness under nitrogen and resuspended in 250 μl (plasma) or 100 μl (urine) mobile phase buffer A.

Analysis was performed on an API 3200 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA)-Shimadzu Prominence LC-20AC HPLC (Shimadzu, Columbia, MD) equipped with a Turbo Ion Spray ionization source and a chiral AGP analytical column (100 $\times$ 2.0 mm, 5 μm) with chiral AGP guard column (10 $\times$ 2.0 mm) (ChromTech, Apple Valley, MN). Injections were 75 μl (plasma) or 30 μl (urine) and the oven temperature was 30 $^{\circ}\text{C}$. The mobile phase (0.22 ml/min) was (A) 20mM ammonium

acetate (pH 5.7) and (B) methanol. The gradient was 10% B for 1 min, linear to 25% B over 0.5 min, held for 3.5 min, linear to 50% over 1 min, held for 9.5 min, immediately decreased back to 10% and re-equilibrated for 5 min. Retention times were 10.0 and 10.6 for R- and S-methadone, and 9.7 and 10.7 for R- and S-EDDP. Mass spectrometer parameters were: positive mode, source temperature 600°C, ion spray 5500V, curtain gas 35, ion source gas one 30, ion source gas two 30 and collision gas 5, exit potential 5, unit mass resolution. Multiple reaction monitoring transitions were m/z 278.2→234.2, 281.2→234.2, and 283.2→239.2 for d0-, d3- and d5-EDDP; m/z 310.2→265.2, 315.2→270.2, and 319.2→268.2 for d0-, d5- and d9-methadone. Analytes were quantified using peak area ratios and standard curves prepared using calibration standards in blank plasma or urine. Interday coefficients of variation in plasma averaged 4, 4, and 6% for EDDP enantiomers (0.025, 0.25, 2.5 ng/ml) and 7, 6, 6, and 5% for methadone enantiomers (0.05, 0.5, 5, 50 ng/ml).

Ticlopidine effects on CYP3A activity were determined using alfentanil clearance as the *in vivo* probe.²⁹ The final study population was thirteen subjects (5 men, 8 women; 30 ± 7 yr, range 18–40; 76 ± 14 kg, range 59–105). Subjects received 50 µg/kg oral alfentanil. Blood was sampled for 10 hr after dosing and plasma stored (–20°C) for later analysis. Nausea and/or vomiting were treated with ondansetron as needed. Subjects began taking oral ticlopidine 250 mg twice daily, and on the fourth day of ticlopidine, the alfentanil protocol was repeated. Alfentanil plasma concentrations were determined by HPLC-mass spectrometry as described.²⁹ Interday coefficients of variation were 7, 4 and 4% at 1, 10 and 100 ng/ml, respectively.

Data and Statistical Analysis

Plasma and urine methadone and EDDP data and plasma and alfentanil data were analyzed using noncompartmental methods, assuming complete absorption, as described previously (WinNonlin 5.2, Pharsight Corp, Mountain View, CA).^{14–16} Differences between treatment groups were analyzed using paired t-tests (SigmaPlot 11.2, Systat Corp, Point Richmond, CA). Non-normal data were log transformed for analysis, but reported as the non-transformed results. Statistical significance was assigned at $p < 0.05$. Results are reported as the arithmetic mean $\pm$ standard deviation (SD). Plasma AUC and urine data were also assessed as ratios (treated/control) and the geometric mean and 90% confidence interval of the geometric mean. Confidence intervals excluding 1.0 were considered statistically significant.

Results

Methadone plasma concentrations were increased by ticlopidine, after both intravenous (Figure 1) and oral (Figure 2) methadone administration, and effects were stereoselective (Table 1). Dose-adjusted area under the concentration-time curve ($AUC_{0-\infty}/D$) was increased approximately 20% and 60%, respectively, for R- and S-methadone, after both intravenous and oral dosing. There was a similar increase in methadone elimination half-life, which was greater for S- than R-methadone. Elimination clearance of S-methadone was significantly diminished, but the decrease was not statistically significant for R-methadone. Methadone elimination is known to be stereoselective, with faster S-methadone elimination, which was reflected by a time-dependent increase in the plasma methadone R/S concentration ratio. Ticlopidine abolished the time-dependent increase in the plasma methadone R/S ratio (Figures 1E and 2E).

Ticlopidine significantly inhibited N-demethylation of both intravenous and oral methadone, and the effect was stereoselective (Figure 3). The metabolite/parent (EDDP/methadone) plasma AUC ratio was significantly decreased by ticlopidine, and that ratio (compared to

controls) was decreased more for S-methadone than for R-methadone. Ticlopidine effects on EDDP/methadone plasma AUC ratios were comparable for intravenous and oral methadone. Formation clearances of R- and S-EDDP were also significantly decreased by ticlopidine. Methadone enantiomers renal clearance was unchanged by ticlopidine (not shown).

To assess the specificity of ticlopidine as a CYP2B6 inhibitor, and determine whether ticlopidine effects on methadone metabolism and clearance could have been due to ticlopidine inhibition of CYP3A, the effect of ticlopidine on hepatic and intestinal CYP3A activity was assessed using alfentanil as an *in vivo* probe. Ticlopidine, in the same 4 day regimen as used in the methadone protocol, failed to increase plasma alfentanil concentrations, establishing that ticlopidine did not inhibit either intestinal or hepatic CYP3A activity (Figure 4). Indeed, there was some evidence for CYP3A induction, based on a decrease in the alfentanil plasma AUC ratio (ticlopidine/control) (Table 2).

Discussion

One (preliminary) purpose of this investigation was to determine the inhibitory selectivity of ticlopidine for CYP2B6 versus CYP3A4/5. Ticlopidine is a mechanism-based inhibitor of CYP2B6 *in vitro*,²⁶ and effectively inhibits *in vivo* hydroxylation of bupropion, the accepted CYP2B6 probe, reducing bupropion clearance, plasma hydroxybupropion AUC, and hydroxybupropion:bupropion AUC ratio, and increasing plasma bupropion AUC.²⁷ However ticlopidine is not purely isoform-specific, as it is also inhibits CYP2C19 *in vitro*.^{26,30} Although ticlopidine did not inhibit CYP3A4 *in vitro*,²⁶ effects of ticlopidine metabolites on CYP3A4 *in vitro* are unreported. More importantly, ticlopidine effects on clinical CYP3A4/5 activity were unknown. Owing to the importance of ticlopidine CYP2B6 inhibitory selectivity for interpreting the ticlopidine-methadone interaction, it was prudent to assess ticlopidine effects on CYP3A4/5. Alfentanil, the most sensitive probe for detecting intestinal or hepatic CYP3A4/5 inhibition,³¹ was used as the selective CYP3A4/5 probe.^{14-17,25} Ticlopidine did not increase oral alfentanil plasma concentrations, demonstrating a lack of intestinal or hepatic CYP3A4/5 inhibition, consistent with the lack of *in vitro* inhibition.²⁶ While ticlopidine actually appeared to weakly induce clinical CYP3A4/5, this was not explored further. Thus ticlopidine, an effective mechanism-based clinical CYP2B6 inhibitor, does not inhibit CYP3A4/5.

The primary purpose of this investigation was to test the hypothesis that CYP2B6 is a prominent CYP isoform responsible for clinical methadone N-demethylation and clearance. Ticlopidine decreased the plasma EDDP/methadone AUC ratio and EDDP formation clearance, indicating inhibition of methadone N-demethylation, and increased methadone exposure, evidenced by an increase in the plasma AUC_{0-∞}/D. Together these results demonstrate that clinical N-demethylation and clearance of single-dose methadone are mediated significantly by CYP2B6. This is the first investigation, using a CYP2B6 inhibitor probe, to establish the clinical significance of CYP2B6 in human methadone disposition.

The stereoselectivity of ticlopidine effects further supports the notion that CYP2B6 is a prominent determinant of methadone disposition. Ticlopidine had a greater effect on S- vs R-methadone N-demethylation and plasma concentrations, and abolished the characteristic time-dependent increase in plasma methadone R/S ratio. Several investigations showed that *in vitro* methadone N-demethylation by expressed and microsomal CYP2B6 but not CYP3A4 was stereoselective, and only CYP2B6 inhibition diminished metabolism stereoselectively.^{6,18-21} Stereoselective methadone disposition *in vivo* is reflected in a time-dependent increase in the plasma R/S-methadone ratio,³² because CYP2B6 preferentially metabolizes S-methadone and because S-methadone inhibits R-methadone metabolism.¹⁹ *In vitro-in vivo* modeling predicted that CYP2B6 inhibition or induction would diminish or

augment, respectively, time-dependent increases in R/S-methadone.^{19,20} The present and previous investigations confirm that CYP2B6 inhibition did indeed abrogate, and induction did indeed enhance, the time-dependent increase in plasma R/S-methadone ratio.^{6,14,20,22,25}

Ticlopidine effects on methadone N-demethylation and clearance are consistent with effects on other CYP2B6 substrates, including the standard substrate probe bupropion. Ticlopidine reduced the plasma hydroxybupropion AUC, the hydroxybupropion:bupropion AUC ratio, and increased bupropion AUC.²⁷ Ticlopidine doubled oral ketamine AUC and halved the metabolite:parent plasma AUC ratio.³³ Clopidogrel, another mechanism-based CYP2B6 inhibitor, also doubled the sibutramine AUC and halved the metabolite:parent plasma ratio.³⁴

The identity of the CYP isoform responsible for clinical methadone N-demethylation, clearance, and drug interactions has remained ambiguous, if not controversial, notwithstanding decades of research. *In vitro* studies have more recently converged to the recognition that CYP2B6 and CYP3A4 are the major isoforms involved in methadone N-demethylation, and that the former but not the latter metabolizes methadone stereoselectively.^{6,18–21,23,35} This investigation helps clarify which isoform is a predominant determinant of clinical methadone disposition. CYP2B6 inhibition diminished methadone N-demethylation and increased plasma methadone concentrations, and abolished the increase in the methadone R/S ratio. In contrast, mechanism-based CYP3A-selective inhibition has consistently failed to impair methadone metabolism and clearance. Troleandomycin (which does not affect CYP2B6 activity³⁶) had no effect on plasma methadone or EDDP enantiomer concentrations, methadone clearance, or plasma R/S-methadone ratios.^{6,20} Ritonavir, despite profound inhibition of hepatic (>70%) and first-pass (>95%) CYP3A, actually induced intravenous and oral methadone N-demethylation and clearances.¹⁴ Nelfinavir, despite 50% and >75% inhibition of hepatic and first-pass CYP3A, increased methadone N-demethylation, and systemic, hepatic and apparent oral clearances.¹⁵ Indinavir alone inhibited 50% and 70%,¹⁷ and ritonavir/indinavir inhibited 90% and 97%,¹⁶ of hepatic and first-pass CYP3A, yet had no significant effects on methadone plasma concentrations, N-demethylation, or clearances. The CYP3A inhibitor atazanavir also had no effect on methadone plasma concentrations.¹³ Thus CYP inhibition studies support a predominant role for CYP2B6, but not CYP3A4, in single-dose disposition. CYP induction studies also support a prominent role for CYP2B6 in methadone disposition. CYP2B6 induction, either alone (by ritonavir) or co-induction with CYP3A (by rifampin or efavirenz) increased methadone N-demethylation and clearance.^{6,14,22,25} Finally, CYP2B6 induction by rifampin, ritonavir, or efavirenz amplified time-dependent increases in the plasma R/S methadone ratio,^{6,14,20,22,25} while CYP3A inhibition by troleandomycin, ritonavir, nelfinavir, indinavir, or ritonavir/indinavir had no significant effect on the ratio.^{6,14–17,25} Together, CYP induction and inhibition studies support a predominant role for CYP2B6 rather than CYP3A4 in single-dose methadone N-demethylation, clearance, and plasma concentrations.

Genetic investigations also provide evidence for CYP2B6 influencing methadone disposition. In methadone maintenance patients, *CYP2B6**6/*6 homozygotes had significantly higher dose-adjusted trough and peak S-methadone plasma concentrations compared with heterozygotes or noncarriers, and genotype effects were greater for S- than R-methadone.²³ Similarly, *CYP2B6**6 carriers had higher plasma S-methadone concentrations, and methadone concentration/dose ratios for both enantiomers, compared with non-carriers.²⁴ Methadone maintenance doses required by *CYP2B6**6 homozygotes were lower than those of heterozygotes and noncarriers.³⁷ No such associations were found for CYP3A variants.³⁷ Some other studies found lesser associations (numerically present but non-significant³⁸) or none³⁹ between *CYP2B6**6 alleles and methadone concentrations³⁸ or

effect.³⁹ Nonetheless, most available genetic evidence suggesting an influence, and its stereoselectivity, of *CYP2B6* polymorphisms on methadone disposition, is consonant with drug-interactions data also suggesting a prominent role for *CYP2B6*.

The results of this investigation have significant clinical implications. Methadone can be difficult to use, in part because slow elimination and variable clearance challenge predictable dosing and clinical effect.⁵ Methadone use has grown substantially, primarily if not exclusively for pain, with prescriptions increasing 7-fold (population-adjusted) from 1997 to 2004,⁴⁰ and 13-fold between 1997 and 2006.¹⁷ Increased methadone use has tragically resulted in an exponential increase in adverse events, including an 1800% increase in population-adjusted accidental methadone-related deaths and 390% increase in fatalities.^{40*} Might incorrect identification of the CYP responsible for methadone disposition be contributory? For more than a decade, based on extrapolation of initial *in vitro* drug metabolism studies,^{8,9} the methadone label, innumerable publications, and practitioner guidelines have ascribed methadone disposition to *CYP3A4*, warned of *CYP3A4*-mediated drug interactions, and suggested dosing adjustments.^{5,10–12} Results of the present investigation, together with accumulating drug interactions and pharmacogenetic evidence for *CYP2B6* predominance in methadone N-demethylation and disposition, argue for a reformation of thought and guidance regarding methadone use.

This investigation has potential limitations. Ticlopidine only partially reduced methadone N-demethylation, and effects on methadone plasma concentrations were small. Nonetheless, ticlopidine is only a weak *CYP2B6* inhibitor (causing less than a 2-fold increase in AUC or 20–50% decrease in clearance, according to FDA classification).[†] Strong *CYP2B6* inhibitors, which might have elicited greater effects, are not available. In addition, only a single methadone dose was evaluated, because steady-state methadone cannot be studied in healthy volunteers. However, since methadone clearance undergoes autoinduction,^{41,42} ticlopidine effects on steady-state methadone might be even greater. A relatively small methadone dose was studied, and the role of *CYP2B6* and *CYP3A* in the disposition of higher doses is untested. Lastly, ticlopidine is a selective but not perfectly specific *CYP2B6* inhibitor. Ticlopidine also inhibits *CYP2C19* *in vitro*,^{26,30} and ticlopidine effects on clinical *CYP2C19* and 1A2, 2C9, 2D6 and 19 activities have not been reported. Nonetheless, methadone metabolism by CYPs 1A2, 2C9, 2C19 and 2D6 is not thought to be significant.^{18,20,21}

In summary, the mechanism-based *CYP2B6* inhibitor ticlopidine significantly and stereoselectively reduced hepatic N-demethylation of methadone, and increased methadone plasma concentrations. Inhibition of *CYP2B6*, but not *CYP3A*, impairs methadone metabolism and clearance, and alters plasma methadone concentrations. These results support a predominant role for *CYP2B6* in clinical methadone disposition.

Acknowledgments

This investigation was supported by National Institutes of Health grants R01-GM63674, R01-DA14211, and K24-DA00417 (to E.D.K.) and UL1 RR024992 from the National Center for Research Resources to Washington University in St. Louis.

*Methadone-associated overdose deaths: Factors contributing to increased deaths and efforts to prevent them. United States Government Accountability Office Report GAO-09-341 (2009) (<http://www.gao.gov/new.items/d09341.pdf>). (last accessed March 1, 2012)

† Guidance for industry: Drug interaction studies - Study design, data analysis, implications for dosing, and labeling recommendations. *US Food and Drug Administration, Center for Drug Evaluation and Research* (2012) (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf>) (last accessed March 1, 2012)

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Figure 1.

Ticlopidine effect on intravenous methadone disposition. Shown are plasma concentrations of (A) R-methadone, (B) S-methadone (C) R-EDDP, and (D) S-EDDP, and (E) the plasma R/S-methadone concentration ratio. Methadone HCl doses were 6.0 mg (5.4 mg free base) and 4.0 mg (3.6 mg free base), respectively, in the control session and after ticlopidine. Results are dose-adjusted. Each data point is the mean $\pm$ SD ($n = 12$). Some SD are omitted for clarity.

Figure 2.

Ticlopidine effect on oral methadone disposition. Shown are plasma concentrations of (A) R-methadone, (B) S-methadone (C) R-EDDP, and (D) S-EDDP, and (E) the plasma R/S-methadone concentration ratio. Methadone HCl doses were 11.0 mg (9.9 mg free base) and 8.0 mg (7.2 mg free base), respectively, in the control session and after ticlopidine. Results are dose-adjusted. Each data point is the mean $\pm$ SD ($n = 12$). Some SD are omitted for clarity.

Figure 3. Ticlopidine effect on methadone metabolism. Shown are (A) plasma AUC_{∞} (EDDP/methadone) ratios and (B) urine EDDP formation clearances. Results are the mean $\pm$ SD ($n = 12$). *Significantly different from control ($p < 0.05$)

Figure 4. Ticlopidine effect on oral alfentanil disposition. Each data point is the mean $\pm$ SD ($n = 12$). The inset shows alfentanil areas under the curve in individual subjects.

Table 1

Intravenous and oral methadone pharmacokinetics

	Control	Ticlopidine	Control	Ticlopidine
<u>IV methadone</u>	<u>R-methadone</u>		<u>S-methadone</u>	
$C_{\max}/D$ ($\text{ng}\cdot\text{ml}^{-1}\cdot\text{mg}^{-1}$)	27 ± 11	25 ± 7	38 ± 16	33 ± 10
$AUC_{0-\infty}/D$ ($\text{ng}\cdot\text{hr}\cdot\text{ml}^{-1}\cdot\text{mg}^{-1}$)	243 ± 96	280 ± 87	284 ± 120	$438 \pm 177^*$
$AUC_{0-\infty}/D$ ratio (ticlopidine/control)	$1.19 (1.04, 1.35)$		$1.57 (1.33, 1.86)$	
CL_{IV} ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	1.1 ± 0.6	0.9 ± 0.4	1.0 ± 0.6	$0.6 \pm 0.3^*$
Elimination $t_{1/2}$ (hr)	61 ± 19	$79 \pm 23^*$	38 ± 10	$65 \pm 18^*$
V_{ss} (L/kg)	5.0 ± 2.0	5.2 ± 1.5	2.9 ± 1.6	2.9 ± 1.1
E_H	0.07 ± 0.04	0.06 ± 0.03	0.06 ± 0.04	$0.04 \pm 0.02^*$
<u>Oral methadone</u>	<u>R-methadone</u>		<u>S-methadone</u>	
$C_{\max}/D$ ($\text{ng}\cdot\text{ml}^{-1}\cdot\text{mg}^{-1}$)	2.1 ± 0.6	2.2 ± 0.5	3.5 ± 1.1	3.6 ± 1.2
$AUC_{0-\infty}/D$ ($\text{ng}\cdot\text{hr}\cdot\text{ml}^{-1}\cdot\text{mg}^{-1}$)	116 ± 47	139 ± 51	137 ± 52	$227 \pm 95^*$
$AUC_{0-\infty}/D$ ratio (ticlopidine/control)	$1.18 (1.02, 1.37)$		$1.62 (1.32, 1.97)$	
CL/F ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	2.2 ± 1.1	2.0 ± 1.3	1.9 ± 1.0	$1.3 \pm 1.0^*$
Elimination $t_{1/2}$ (hr)	58 ± 21	$75 \pm 20^*$	38 ± 10	$64 \pm 19^*$
V_z/F	10.1 ± 3.4	11.5 ± 5.0	5.9 ± 2.5	6.2 ± 3.3

Results are the arithmetic mean $\pm$ SD (n=12), except area under the concentration-time curve (AUC) ratios (ticlopidine/control), which are the geometric mean (90% CI). AUC/D , dose-normalized area under the plasma concentration-time curve; $C_{\max}/D$, dose-normalized peak plasma concentration; CL_{IV} , systemic clearance; CL/F , apparent oral clearance; E_H , hepatic extraction ratio.

* Significantly different from control ($p < 0.05$)

Table 2

Alfentanil pharmacokinetic parameters

	Control	Ticlopidine
$C_{\max}$ (ng/ml)	41 ± 15	39 ± 23
$AUC_{0-\infty}$ (ng •hr •ml ⁻¹)	111 ± 74	$81 \pm 68^*$
$AUC_{0-\infty}$ ratio (ticlopidine/control)		0.69 (0.58, 0.83)
CL/F (ml•kg ⁻¹ •min ⁻¹)	12 ± 10	$18 \pm 15^*$
Elimination $t_{1/2}$ (hr)	1.5 ± 0.7	1.3 ± 0.4

Subjects received 50 µg/kg oral alfentanil. Results are the arithmetic mean ± SD (n=12), except the $AUC_{0-\infty}$ ratio (ticlopidine/control), which is the geometric mean (90% CI). AUC, area under the plasma concentration-time curve; $C_{\max}$, peak plasma concentration; CL/F, apparent oral clearance of alfentanil.

* Significantly different from control (p<0.05)