Cytochrome P4503A Does Not Mediate the Interaction between Methadone and Ritonavir-Lopinavir

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Received July 31, 2013; accepted September 25, 2013

ABSTRACT

Plasma concentrations of orally administered methadone are reduced by the human immunodeficiency virus protease inhibitor combination ritonavir and lopinavir, but the mechanism is unknown. Methadone metabolism, clearance, and drug interactions have been attributed to CYP3A4, but this remains controversial. This investigation assessed the effects of acute (2 days) and steady-state (2 weeks) ritonavir-lopinavir on intravenous and oral methadone metabolism and clearance, hepatic and intestinal CYP3A4/5 activity (using the probe substrate intravenous and oral alfentanil), and intestinal transporter activity (using oral fexofenadine) in healthy volunteers. Plasma and urine concentrations of methadone and metabolite enantiomers, and other analytes, were determined by mass spectrometry. Acute and chronic ritonavir-lopinavir reduced plasma methadone enantiomer concentrations in half, with an average 2.6- and 1.5-fold induction of systemic and apparent oral methadone clearances. Induction was attributable to stereoselectively increased hepatic methadone

N-demethylation, hepatic extraction, and hepatic clearance, and there was a strong correlation between methadone *N*-demethylation and clearance. Methadone renal clearance was unchanged. Alfentanil's systemic clearance and hepatic extraction, apparent oral clearance, and intestinal extraction were reduced to 25%, 16%, and 35% of control, indicating strong inhibition of hepatic and intestinal CYP3A activities. Ritonavir-lopinavir (acute > chronic) increased fexofenadine exposure, suggesting intestinal P-glycoprotein inhibition. No correlation was found between methadone clearance and CYP3A activity. Acute and steady-state ritonavir-lopinavir stereoselectively induced methadone *N*-demethylation and clearance, despite significant inhibition of hepatic and intestinal CYP3A activity. Ritonavir-lopinavir inhibited intestinal transporters activity but had no effect on methadone bioavailability. These results do not support a significant role for CYP3A or ritonavir-lopinavir-inhibitable intestinal transporters in single-dose methadone disposition.

Introduction

Methadone is a clinically efficacious and cost-effective analgesic that is used to treat acute and chronic pain in a variety of settings (Nicholson, 2007; Chou et al., 2009; Kharasch, 2011). Methadone is also used to treat opiate addiction and effectively prevents opiate withdrawal, diminishes illicit drug use, and reduces human immunodeficiency virus (HIV)/AIDS and other infectious diseases (Lobmaier et al., 2010). Methadone is used in various countries as a racemate, or as the single R-enantiomer, which has approximately 50 times greater μ opioid receptor activity than its antipode. Methadone pharmacokinetics and pharmacodynamics, including metabolism and clearance, are considered to have considerable and unpredictable interindividual and intraindividual variability (Ferrari et al., 2004; McCance-Katz et al., 2010). Methadone drug interactions are a specific concern (McCance-Katz et al., 2010; Saber-Tehrani et al., 2011). As a result of increased use, primarily for pain treatment, methadone prescribing has increased significantly in the past decades but has also been accompanied by a commensurate increase in toxicity and adverse events (Webster, 2005; Sims et al., 2007).

This work was supported by the National Institutes of Health [Grants R01-GM63674, R01-DA14211, K24-DA0041, and UL1-TR000448].
dx.doi.org/10.1124/dmd.113.053991.

Highly active antiretroviral drugs used for the therapy of HIV and AIDS are notorious perpetrators of drug interactions. Influence on the activity of hepatic and extrahepatic enzymes such as cytochromes P450 and glucuronosyltransferases, as well as on influx and efflux transporters in several organs, have been well described, along with clinically significant consequences for pharmacokinetics, pharmacodynamics, and clinical therapeutics (Dickinson et al., 2010; Griffin et al., 2011; Pal et al., 2011).

Ritonavir-boosted lopinavir is one of several fixed-dose protease inhibitor combinations, in which ritonavir is used primarily to inhibit CYP3A-mediated elimination of the coadministered protease inhibitor and thus enhance bioavailability and exposure. Ritonavir-lopinavir is known to inhibit the activity of CYP3A isoforms and clinically may inhibit CYP3A activity but also induce CYP3A expression, potentially resulting in net induction or net inhibition of CYP3A activity (Dickinson et al., 2010; Griffin et al., 2011; Pal et al., 2011).

Ritonavir-lopinavir has been shown to decrease significantly methadone exposure [area under the curve (AUC)] and increase methadone clearance (Clarke et al., 2002; McCance-Katz et al., 2003) and to variably cause symptoms of opioid withdrawal in methadone-maintained patients (Clarke et al., 2002; Stevens et al., 2003). Cessation of ritonavir-lopinavir was associated with an arrhythmia, attributed to an acute increase in methadone exposure (Lüthi et al., 2007). Induction of methadone clearance was attributed to either or both CYP3A4 and

ABBREVIATIONS: AUC, area under the plasma concentration-time curve; CL/F, apparent oral clearance; CL_{IV}, systemic clearance; EDDP, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine; E_G, intestinal extraction; E_H, hepatic extraction; F_{oral}, bioavailability; HIV, human immunodeficiency virus; P-gp, P-glycoprotein; V_{ss}, steady-state volume of distribution; Vz/F, apparent volume of distribution.

CYP2D6 (McCance-Katz et al., 2003). Nevertheless, the actual mechanism by which ritonavir-lopinavir alters methadone disposition and clinical effects is unknown.

The purpose of this investigation was to determine the mechanism by which ritonavir-lopinavir alters methadone disposition and clinical effect, assessing CYP3A and/or P-glycoprotein (P-gp)-mediated methadone intestinal absorption, first-pass elimination, systemic clearance, intestinal or hepatic metabolism, and bioavailability and to compare the effects of single-dose and steady-state ritonavir-lopinavir. A comprehensive crossover investigation in healthy volunteers evaluated the metabolism and clearance of both intravenous and oral methadone. Clearances of intravenous and oral alfentanil, a nonselective CYP3A4/5 (henceforth referred to as CYP3A) substrate, phenotyped hepatic and first-pass CYP3A activities and oral fexofenadine probed P-gp (Kharasch et al., 2004b, 2005, 2007, 2008a,b, 2009a,b, 2011a,b, 2012a,b).

Materials and Methods

Clinical Protocol. The investigation was a three-part sequential crossover (control first, for logistical reasons), approved by the Washington University Institutional Review Board. All subjects provided written informed consent. Subjects were their own control. Subjects were normal, healthy volunteers (smokers or nonsmokers) aged 18–40 years, within 25% of ideal body weight (body mass index <30). Exclusion criteria were 1) major medical problems; 2) history of hepatic or renal disease; 3) use of drugs or herbals known to alter CYP3A activity, including female hormonal contraceptives; 4) known history of drug or alcohol addiction or routine handling of addicting drugs in the regular course of employment; 5) family history of type 2 diabetes; 6) fasting blood glucose >110 mg/dl (because protease inhibitors can cause glucose intolerance); and 7) HIV seropositivity (because monotherapy can cause HIV resistance). The final study cohort was four men and eight women, aged 29 ± 7 years, range 20–40; 772 ± 13 kg, range 57–995. Subjects were instructed to consume no grapefruit, apples, or oranges for 7 days before and on any study day, no alcohol or caffeine for 1 day before and on a study day, and no food or water after midnight before each study session.

First-pass CYP3A activity and intestinal P-gp (and other transporters) activity were evaluated on day 1 using oral alfentanil and fexofenadine as *in vivo* probes (Kharasch et al., 2004b, 2005, 2007, 2008b, 2009a,b, 2011a,b, 2012a,b). Subjects received ondansetron (4 mg *i.v.*) for antinausea prophylaxis followed 30 minutes later by oral alfentanil (45 and 5 μ g/kg with 100 ml water at control and ritonavir-lopinavir sessions, respectively), followed 1 hour later by fexofenadine (60 mg, 100 ml of water). Venous blood was sampled for 48 hours after alfentanil dosing and plasma stored at -20°C for later analysis. Hepatic CYP3A activity was evaluated on day 2 using *i.v.* alfentanil as an *in vivo* probe (Kharasch et al., 2004b, 2007, 2008b, 2009a,b, 2011a,b, 2012a,b). Subjects received ondansetron followed 30 minutes later by alfentanil bolus (15 and 5 μ g/kg at control and ritonavir-lopinavir sessions, respectively). Venous blood was sampled for 24 hours after alfentanil dosing. Subjects received a standard breakfast and lunch 3 and 6 hours, respectively, after alfentanil in both sessions.

Methadone metabolism and clearance were assessed on day 3 by simultaneously administering *i.v.* and oral methadone (Kharasch et al., 2004a, 2008a, 2009a,b, 2012a,b). Subjects received *i.v.* ondansetron followed 30 minutes later by *i.v.* racemic unlabeled (d0)-methadone HCl (6.0 mg, equivalent to 5.4 mg free base) and oral deuterated racemic (d5)-methadone HCl (11.0 mg, equivalent to 9.86 mg free base, with 100 ml of water, synthesized and used under Investigational New Drug approval (Kharasch et al., 2004a). Subjects received a standard breakfast and lunch 2 and 4 hours after methadone, respectively, and free access to food and water thereafter. Venous blood was sampled for 96 hours after methadone. Continuous urine samples were collected at 24, 48, 72, and 96 hours. Nausea and/or vomiting were treated with ondansetron (4 mg *i.v.* or 8 mg orally) as needed.

Hepatic and first-pass CYP3A, intestinal transporters activity, and methadone disposition were assessed at baseline. After 1 to 2 months, subjects began taking ritonavir-lopinavir 100 and 400 mg, respectively (Kaletra; Abbott Laboratories, Abbott Park, IL) twice daily. Dosing was adjusted on study days (morning dose at breakfast) to preclude an acute effect from the morning dose.

First-pass CYP3A and transporter activities were determined on day 2 of ritonavir-lopinavir and methadone disposition on day 3. At steady-state ritonavir-lopinavir, first-pass CYP3A and intestinal transporters activity were assessed on day 15, hepatic CYP3A on day 17, and methadone disposition on day 17. Because the duration of ritonavir administration may affect the degree of CYP3A alteration (Hsu et al., 1998; Greenblatt et al., 1999; Kharasch et al., 2008a,b), both acute and steady-state ritonavir-lopinavir were evaluated.

Sample size was determined using a simplified analysis (paired *t* test) for the outcome variable methadone systemic clearance. A previous study found 22% and 33% interday and intrasubject variability in *i.v.* and oral methadone clearances, respectively (Kharasch et al., 2004a). To detect a 30% change in clearance, using a paired *t* test with 33% variability, $1-\beta = 0.8$, $\alpha = 0.05$, would require 12 subjects.

Analytical Methods. Plasma alfentanil and fexofenadine concentrations were quantified simultaneously by solid-phase extraction and electrospray liquid chromatography-mass spectrometry as described previously (Kharasch et al., 2005). Plasma and urine methadone and EDDP enantiomer concentrations were quantified by chiral liquid chromatography-tandem electrospray mass spectrometry as described (Kharasch and Stubbett, 2013).

Data Analysis. Pharmacokinetic data were analyzed using noncompartmental methods (Phoenix, Pharsight Corp, Mountain View, CA), assuming complete absorption, as described previously (Kharasch et al., 2004a,b, 2007, 2008a,b, 2009a,b, 2012a,b). AUC was determined using the trapezoidal rule. Systemic clearance (CL_{IV}) was $\text{dose}_{IV}/AUC_{IV}$, apparent oral clearance (CL/F) was $\text{dose}_{oral}/AUC_{oral}$, bioavailability (F_{oral}) was $(AUC_{oral}/\text{dose}_{oral}) \times (\text{dose}_{IV}/AUC_{IV})$, steady-state volume of distribution (V_{ss}) was mean residence time $\times CL$, volume of distribution based on the terminal phase (V_z) was $\text{dose}/(AUC \times \lambda)$, where λ is the terminal elimination rate constant. Hepatic extraction ratio (E_H) was determined as (CL_{IV}/Q_p) , where hepatic plasma flow (Q_p) was estimated as the product of hepatic blood flow (25.3 and 25.5 ml/kg in males and female subjects) and hematocrit (estimated as 40 and 36 in male and female subjects, respectively), and negligible extrahepatic metabolism was assumed. Gastrointestinal extraction ratio (E_G) was $1 - F_{oral}/(F_H \times F_{abs})$, where the oral dose was assumed to be entirely absorbed and thus F_{abs} was considered to be unity, and $F_H = 1 - E_H$. Renal clearance (CL_r and CL_r/F) was determined as the amount excreted in urine/ $AUC_{0-\infty}$. EDDP formation clearance was determined from urine data as: $CL_f = \text{fraction of dose recovered in urine} \times CL_{IV}$ and $CL_f/F = \text{fraction of dose recovered in urine} \times CL/F$, for *i.v.* and oral dosing, respectively. Hepatic clearance (CL_H) was $CL_{IV} - CL_r$.

Statistical Analysis. Differences between treatment groups for pharmacokinetic and effect parameters were analyzed using one-way repeated measures analysis of variance followed by the Student-Newman-Keuls test for multiple comparisons, or paired *t* tests, as appropriate (Sigmaplot 12.5, Systat Software, Inc, San Jose, CA). Non-normal data were log transformed for analysis but reported as the nontransformed results. Statistical significance was assigned at $P < 0.05$. Results are reported as the arithmetic mean $\pm$ S.D. 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. Relationships between methadone metabolism and clearance were evaluated by linear regression analysis.

Results

Both acute and steady-state ritonavir-lopinavir diminished plasma R- and S-methadone concentrations and exposure (Fig. 1; Table 1). Plasma $AUC_{0-\infty}$ ratios (ritonavir-lopinavir/control) were significantly reduced to 0.5–0.7, with a slightly greater effect on S- than R-methadone and a slightly greater reduction by acute versus steady-state ritonavir-lopinavir. Intravenous R- and S-methadone systemic clearance, hepatic clearance, and hepatic extraction were significantly increased by both acute versus steady-state ritonavir-lopinavir. Methadone renal clearance was unaffected by ritonavir-lopinavir. Methadone *N*-demethylation was also increased by ritonavir-lopinavir, with the plasma R- and S-EDDP/methadone AUC_{∞} ratio (ritonavir-lopinavir/control) increased, with a greater effect on the S-enantiomer. S-EDDP formation clearance was significantly increased by acute but not by steady-state ritonavir-lopinavir.

Fig. 1. Effect of acute and steady-state ritonavir-lopinavir (RTV/LOP) on i.v. methadone disposition and metabolism. Shown are plasma R- and S-methadone and R- and S-EDDP concentrations. Subjects received 6.0 mg of i.v. methadone HCl (5.4 mg free base). Each data point is the mean $\pm$ S.D. ($n = 12$). Some S.D. values are omitted for clarity.

When control and both ritonavir-lopinavir sessions were analyzed together, there was a significant positive correlation ($r = 0.90$, $P < 0.001$) between methadone *N*-demethylation (plasma EDDP/methadone AUC ratio) and systemic clearance after i.v. methadone administration (Fig. 2A). Intravenous methadone disposition was stereoselective, with greater exposure to S-methadone and a time-dependent increase in the plasma methadone R/S concentration ratio, which was further amplified by acute ritonavir-lopinavir (Fig. 2B).

Acute and steady-state ritonavir-lopinavir also diminished oral methadone plasma concentrations, with a greater effect of acute versus steady-state ritonavir-lopinavir (Fig. 3; Table 2). Plasma $AUC_{0-\infty}$ ratios (ritonavir-lopinavir/control) were significantly reduced to 0.4–0.8, with a greater reduction by acute versus steady-state ritonavir-lopinavir and slightly greater effect on S- than R-methadone. The apparent oral clearance of both methadone enantiomers was significantly increased by acute and steady-state ritonavir-lopinavir. Methadone bioavailability was unaffected, as was renal clearance. Oral methadone *N*-demethylation was apparently induced, with the plasma EDDP/methadone AUC_{∞} ratio (ritonavir-lopinavir/control) increased by acute and steady-state ritonavir-lopinavir, with a greater effect on S- than R-methadone *N*-demethylation. Oral methadone disposition was stereoselective, and the time-dependent increase in the plasma methadone R/S concentration ratio augmented by acute, and less so, by steady-state ritonavir-lopinavir (Fig. 2C).

Both acute and steady-state ritonavir-lopinavir markedly inhibited hepatic and first-pass CYP3A activity. Alfentanil plasma concentrations

are shown in Fig. 4, and pharmacokinetic parameters are provided in Table 3. The $AUC_{0-\infty}/\text{dose}$ ratio (ritonavir-lopinavir/control) for oral alfentanil was increased 11- and 7-fold, respectively, by acute and steady-state ritonavir-lopinavir, and that of i.v. alfentanil was increased almost 5-fold by steady-state ritonavir-lopinavir. These results indicate a 75% inhibition of hepatic alfentanil clearance and CYP3A activity and 84%–90% inhibition of first-pass alfentanil clearance and CYP3A activity. Both hepatic and intestinal alfentanil extractions were substantially inhibited.

Disposition of oral fexofenadine was used to probe the activity of intestinal P-gp and potentially other intestinal transporters. Both acute and steady-state ritonavir-lopinavir, the former more than the latter, increased fexofenadine peak plasma concentrations and AUC, with no effects on apparent elimination half-life (Fig. 5; Table 3).

Discussion

This investigation determined the role of CYP3A and P-gp in the mechanism by which ritonavir-lopinavir alters methadone disposition and compared effects of single-dose and steady-state ritonavir-lopinavir. Steady-state (14 days) ritonavir-lopinavir (100/400 mg twice daily) had previously been found to reduce steady-state oral methadone AUC_{0-24} (36%) and $C_{\max}$ (44%) (Clarke et al., 2002). These results were considered surprising, given protease inhibitors' effects on CYP3A and the consideration at that time of methadone as a CYP3A substrate (Clarke et al., 2002). However, neither methadone

TABLE 1

Intravenous methadone pharmacokinetic parameters

Subjects received 6.0 mg i.v. methadone HCl at all sessions. Results are the arithmetic mean $\pm$ S.D., except area under the plasma concentration-time curve (AUC) ratios (ritonavir-lopinavir/control), which are the geometric mean (90% confidence interval); n = 12, except renal clearances and EDDP formation clearances (n = 10, due to incomplete urine collections in two subjects).

	R-methadone			S-methadone		
	Control	Acute Ritonavir-Lopinavir	Steady-State Ritonavir-Lopinavir	Control	Acute Ritonavir-Lopinavir	Steady-State Ritonavir-Lopinavir
C_{max} (ng/ml)	56 $\pm$ 28	36 $\pm$ 13*	36 $\pm$ 17*	78 $\pm$ 35	44 $\pm$ 17*	45 $\pm$ 22*
AUC_{0-96} (ng $\cdot$ h $\cdot$ ml $^{-1}$)	304 $\pm$ 81	213 $\pm$ 102*	234 $\pm$ 111*	441 $\pm$ 142	244 $\pm$ 161*	312 $\pm$ 201*
$AUC_{0-\infty}$ (ng $\cdot$ h $\cdot$ ml $^{-1}$)	427 $\pm$ 152	304 $\pm$ 160*	354 $\pm$ 213	547 $\pm$ 215	313 $\pm$ 238*	410 $\pm$ 284*
$AUC_{0-\infty}$ ratio (ritonavir-lopinavir/control)		0.65 (0.54, 0.79)	0.74 (0.59, 0.93)		0.47 (0.34, 0.63)	0.64 (0.50, 0.83)
CL_{IV} (ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$)	1.66 $\pm$ 0.57	2.80 $\pm$ 1.71*	2.49 $\pm$ 1.41*	1.37 $\pm$ 0.65	3.62 $\pm$ 3.17*	2.49 $\pm$ 1.99*
CL_H (ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$)	1.54 $\pm$ 0.52	2.63 $\pm$ 1.61*	2.33 $\pm$ 1.31*	1.29 $\pm$ 0.63	3.47 $\pm$ 3.06*	2.38 $\pm$ 1.92*
Renal clearance (ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$)	0.13 $\pm$ 0.09	0.16 $\pm$ 0.13	0.14 $\pm$ 0.13	0.08 $\pm$ 0.05	0.13 $\pm$ 0.10	0.10 $\pm$ 0.08
Elimination $t_{1/2}$ (h)	48 $\pm$ 10	52 $\pm$ 21	54 $\pm$ 24	35 $\pm$ 10	37 $\pm$ 18	41 $\pm$ 15
V_{ss} (l/kg)	6.2 $\pm$ 1.6	10.4 $\pm$ 4.9*	9.3 $\pm$ 2.8*	3.6 $\pm$ 1.0	8.3 $\pm$ 4.7*	6.8 $\pm$ 3.3
E_H	0.10 $\pm$ 0.04	0.17 $\pm$ 0.11*	0.15 $\pm$ 0.09*	0.08 $\pm$ 0.04	0.22 $\pm$ 0.20*	0.15 $\pm$ 0.13*

	R-EDDP			S-EDDP		
	Control	Acute Ritonavir-Lopinavir	Steady-State Ritonavir-Lopinavir	Control	Acute Ritonavir-Lopinavir	Steady-State Ritonavir-Lopinavir
C_{max} (ng/ml)	0.39 $\pm$ 0.08	0.34 $\pm$ 0.12	0.43 $\pm$ 0.34	0.74 $\pm$ 0.22	0.68 $\pm$ 0.23	0.74 $\pm$ 0.36
AUC_{0-96} (ng $\cdot$ h $\cdot$ ml $^{-1}$)	20 $\pm$ 5	17 $\pm$ 5*	17 $\pm$ 5*	37 $\pm$ 10	32 $\pm$ 9	32 $\pm$ 8
AUC_{∞} (ng $\cdot$ h $\cdot$ ml $^{-1}$)	28 $\pm$ 7	26 $\pm$ 11	23 $\pm$ 8	45 $\pm$ 12	41 $\pm$ 12	40 $\pm$ 12
Elimination $t_{1/2}$ (h)	48 $\pm$ 14	59 $\pm$ 35	44 $\pm$ 13	37 $\pm$ 12	40 $\pm$ 15	36 $\pm$ 10
AUC_{0-96} (EDDP/methadone)	0.08 $\pm$ 0.03	0.10 $\pm$ 0.05	0.08 $\pm$ 0.03	0.10 $\pm$ 0.04	0.21 $\pm$ 0.17*	0.15 $\pm$ 0.10
AUC_{∞} (EDDP/methadone)	0.09 $\pm$ 0.05	0.11 $\pm$ 0.06	0.08 $\pm$ 0.03	0.11 $\pm$ 0.04	0.22 $\pm$ 0.18*	0.15 $\pm$ 0.11
AUC_{0-96} (EDDP/methadone) ratio (ritonavir-lopinavir/control)		1.15 (0.98, 1.36)	1.08 (0.99, 1.17)		1.73 (1.35, 2.22)	1.32 (1.13, 1.55)
EDDP formation clearance (ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$)	0.15 $\pm$ 0.08	0.17 $\pm$ 0.09	0.16 $\pm$ 0.08	0.21 $\pm$ 0.09	0.41 $\pm$ 0.26*	0.27 $\pm$ 0.13

CL_H , hepatic clearance; CL_{IV} , systemic clearance; E_H , hepatic extraction; V_{ss} , steady-state volume of distribution.

*Significantly different from control ($P < 0.05$).

pharmacokinetics nor metabolism was assessed, and mechanism(s) of altered methadone concentrations were considered unclear. Another study found that 7 days of ritonavir-lopinavir twice daily caused a 26%–28% reduction in AUC_{0-24} and C_{max} and a 42% increase in racemic methadone clearance, and some patients suffered opiate withdrawal (McCance-Katz et al., 2003). Reduced methadone exposure was attributed to net induction of metabolic clearance, involving CYP3A and/or CYP2D6 and possibly also intestinal P-gp. Nevertheless, it subsequently became clear that protease inhibitors do not cause net CYP3A induction (Dickinson et al., 2010), and CYP2D6 does not contribute

materially to methadone clearance (Shiran et al., 2009), hence driving the need to understand the mechanism of the methadone interaction (Khalsa and Elkashef, 2010). This is the first investigation to evaluate ritonavir-lopinavir effects on i.v. methadone disposition, oral and i.v. methadone concurrently, methadone metabolism, renal excretion, the stereoselectivity of these effects, and the mechanisms by which they occur.

Steady-state ritonavir-lopinavir reduced total (R- and S-methadone) plasma concentrations by half after a single methadone dose, consistent with previous reports with steady-state methadone dosing (Clarke

Fig. 2. Effect of ritonavir-lopinavir (RTV/LOP) on methadone disposition. (A) Relationship between methadone *N*-demethylation (plasma EDDP AUC/methadone AUC ratio) and systemic methadone clearance after i.v. methadone administration. Data for each methadone enantiomer include control, acute, and steady-state ritonavir-lopinavir pretreatments. Each data point is the result from an individual subject. There was a significant correlation between methadone metabolism and clearance (Pearson product moment correlation, $r = 0.90$, $P < 0.001$). Plasma R/S-methadone concentration ratios in controls and after acute and steady-state ritonavir-lopinavir for intravenous (B) and oral (C) methadone. Each data point is the mean $\pm$ SD ($n = 12$). Some S.D. values are omitted for clarity.

Fig. 3. Effect of acute and steady-state ritonavir-lopinavir (RTV/LOP) on oral methadone disposition and metabolism. Shown are plasma R- and S-methadone and R- and S-EDDP concentrations. Subjects received 11.0 mg of oral methadone HCl (9.9 mg free base). Each data point is the mean $\pm$ S.D. ($n = 12$). Some S.D. values are omitted for clarity.

et al., 2002; McCance-Katz et al., 2003). Overall, there was an average 1.5-fold induction of systemic and apparent oral methadone clearances. Effects of acute (2 days) ritonavir-lopinavir on clearances were greater, averaging 2.6-fold. Clearance induction was attributable to increased hepatic methadone *N*-demethylation, hepatic extraction, and hepatic clearance, and there was a strong correlation between methadone *N*-demethylation and clearance. Induction of i.v. and oral methadone *N*-demethylation was evidenced by increases in plasma EDDP/methadone ratios and EDDP formation clearances. Neither acute nor steady-state ritonavir-lopinavir altered methadone renal clearance. Effects of ritonavir-lopinavir on methadone plasma concentrations, metabolism, and clearance were stereoselective. There was a time-dependent increase in plasma R/S-methadone concentration ratios, which were augmented by ritonavir-lopinavir. S-methadone systemic and oral clearances, hepatic clearance, hepatic extraction ratios, and EDDP/methadone ratios were induced approximately 2-fold, whereas those of R-methadone were induced less. Together, the major finding of this investigation suggests that ritonavir-lopinavir decreases methadone plasma concentrations via a stereoselective metabolic drug interaction, resulting from preferential induction of hepatic S-methadone clearance, in turn attributable to stereoselective (S- > R-methadone) induction of hepatic methadone *N*-demethylation and clearance.

The second major result of this investigation was that ritonavir-lopinavir increased fexofenadine AUC and C_{max} . Effects after short-term exposure (3.7- and 2.5-fold increases in AUC and C_{max}) were

greater than at steady-state (2.0- and 1.8-fold, respectively) and were not due to reduced elimination. These results are similar to the 4- and 2.9-fold increases in fexofenadine AUC caused by single-dose and steady-state ritonavir-lopinavir reported previously (van Heeswijk et al., 2006). When this investigation was designed, fexofenadine was considered selective for P-gp and was used to probe intestinal P-gp-mediated interactions based on existing recommendations. However, fexofenadine is now known as a substrate for multiple transporters, with absorption influenced by intestinal P-gp-mediated efflux and organic anion transporting polypeptide 1A2-mediated uptake, and hepatic organic anion transporting polypeptide 1A2-mediated hepatic (Bailey et al., 2007; Matsushima et al., 2008). Changes in fexofenadine AUC caused by ritonavir-lopinavir are consistent with decreased fexofenadine intestinal efflux and acute inhibition of P-gp, with partial P-gp induction (yet continued net P-gp inhibition) on continuing exposure. Similar conclusions were articulated previously (van Heeswijk et al., 2006) and also were reached using oral digoxin, considered to quantify primarily intestinal P-gp activity (Wyen et al., 2008). Ritonavir-lopinavir increased digoxin AUC and C_{max} 1.8- and 1.6-fold, respectively, interpreted as P-gp inhibition (Wyen et al., 2008). Ritonavir alone at steady state (300 or 400 mg twice daily) increased digoxin AUC 1.4- to 1.9-fold (Ding et al., 2004; Kirby et al., 2012). In vitro, acute lopinavir exposure inhibited P-gp, whereas extended exposure induced P-gp (Vishnuvardhan et al., 2003). Together, these findings suggest that ritonavir-lopinavir inhibits intestinal P-gp.

TABLE 2

Oral methadone pharmacokinetic parameters

Subjects received 11.0 mg oral methadone HCl at all sessions. Results are the arithmetic mean $\pm$ S.D., except area under the plasma concentration-time curve (AUC) ratios (ritonavir-lopinavir/control), which are the geometric mean (90% confidence interval), $n = 12$, except renal clearances and EDDP formation clearances ($n = 10$, due to incomplete urine collections in two subjects).

	R-methadone			S-methadone		
	Control	Acute Ritonavir-Lopinavir	Steady-State Ritonavir-Lopinavir	Control	Acute Ritonavir-Lopinavir	Steady-State Ritonavir-Lopinavir
C_{max} (ng/ml)	14 $\pm$ 6	8 $\pm$ 4*	10 $\pm$ 4*	23 $\pm$ 10	10 $\pm$ 7*	15 $\pm$ 8*
t_{max} (h)	5 $\pm$ 3	6 $\pm$ 6	5 $\pm$ 2	5 $\pm$ 3	6 $\pm$ 5	5 $\pm$ 3
AUC ₀₋₉₆ (ng $\cdot$ h $\cdot$ ml ⁻¹)	462 $\pm$ 154	288 $\pm$ 201*	399 $\pm$ 190	690 $\pm$ 289	324 $\pm$ 285*	525 $\pm$ 332*
AUC _{0-∞} (ng $\cdot$ h $\cdot$ ml ⁻¹)	672 $\pm$ 253	424 $\pm$ 292*	583 $\pm$ 301	862 $\pm$ 404	415 $\pm$ 397*	688 $\pm$ 435
AUC _{0-∞} ratio (ritonavir-lopinavir/control)		0.52 (0.38, 0.70)	0.79 (0.61, 1.02)		0.35 (0.24, 0.50)	0.70 (0.50, 0.97)
CL/F (ml $\cdot$ kg ⁻¹ $\cdot$ min ⁻¹)	1.98 $\pm$ 0.74	4.94 $\pm$ 4.70*	2.81 $\pm$ 1.78*	1.70 $\pm$ 0.90	6.48 $\pm$ 5.83*	3.04 $\pm$ 3.16
Renal clearance (ml $\cdot$ kg ⁻¹ $\cdot$ min ⁻¹)	0.15 $\pm$ 0.11	0.15 $\pm$ 0.15	0.16 $\pm$ 0.15	0.09 $\pm$ 0.06	0.11 $\pm$ 0.12	0.11 $\pm$ 0.09
Elimination $t_{1/2}$ (h)	50 $\pm$ 9	54 $\pm$ 29	49 $\pm$ 24	35 $\pm$ 8	36 $\pm$ 14	40 $\pm$ 16
Vz/F (l/kg)	8.4 $\pm$ 3.6	19.5 $\pm$ 15.4*	10.0 $\pm$ 4.2	4.9 $\pm$ 2.6	17.5 $\pm$ 14.3*	8.2 $\pm$ 5.4
F _{oral}	0.88 $\pm$ 0.20	0.79 $\pm$ 0.35	0.92 $\pm$ 0.24	0.87 $\pm$ 0.20	0.72 $\pm$ 0.31	0.92 $\pm$ 0.21

	R-EDDP			S-EDDP		
	Control	Acute Ritonavir-Lopinavir	Steady-State Ritonavir-Lopinavir	Control	Acute Ritonavir-Lopinavir	Steady-State Ritonavir-Lopinavir
C_{max} (ng/ml)	1.4 $\pm$ 0.9	1.0 $\pm$ 0.5	1.4 $\pm$ 1.1	2.0 $\pm$ 1.5	1.8 $\pm$ 1.2	1.9 $\pm$ 1.1
AUC ₀₋₉₆ (ng $\cdot$ h $\cdot$ ml ⁻¹)	46 $\pm$ 22	30 $\pm$ 14	39 $\pm$ 13	70 $\pm$ 36	54 $\pm$ 27 ^a	63 $\pm$ 17 ^a
AUC _∞ (ng $\cdot$ h $\cdot$ ml ⁻¹)	64 $\pm$ 40	40 $\pm$ 22	48 $\pm$ 18	96 $\pm$ 61	63 $\pm$ 30 ^a	74 $\pm$ 21
Elimination $t_{1/2}$ (h)	43 $\pm$ 12	36 $\pm$ 14	36 $\pm$ 9	40 $\pm$ 17	35 $\pm$ 25	31 $\pm$ 6
AUC ₀₋₉₆ (EDDP/methadone)	0.10 $\pm$ 0.03	0.12 $\pm$ 0.07	0.11 $\pm$ 0.05	0.11 $\pm$ 0.05	0.20 $\pm$ 0.14	0.19 $\pm$ 0.17
AUC _∞ (EDDP/methadone)	0.10 $\pm$ 0.04	0.12 $\pm$ 0.07	0.10 $\pm$ 0.05	0.12 $\pm$ 0.05	0.21 $\pm$ 0.15*	0.18 $\pm$ 0.17*
AUC ₀₋₉₆ (EDDP/methadone) ratio (ritonavir-lopinavir/control)		1.16 (0.92, 1.45)	1.11 (0.95, 1.29)		1.74 (1.32, 2.31)	1.46 (1.19, 1.78)
EDDP formation clearance (ml $\cdot$ kg ⁻¹ $\cdot$ min ⁻¹)	0.16 $\pm$ 0.10	0.17 $\pm$ 0.13	0.18 $\pm$ 0.10	0.20 $\pm$ 0.12	0.36 $\pm$ 0.30	0.28 $\pm$ 0.13

CLF, apparent oral clearance; F_{oral}, bioavailability; $t_{1/2}$, half-time.

*Significantly different from control ($P < 0.05$).

Another potential explanation for ritonavir-lopinavir reduction of oral methadone concentrations, in addition to increased clearance, was diminished bioavailability. Methadone has been considered a P-gp substrate, and rodent studies suggested a role for intestinal P-gp in methadone absorption (Ortega et al., 2007). Nevertheless, despite apparent P-gp inhibition by ritonavir-lopinavir, no change in methadone bioavailability was observed. Previous clinical studies also showed methadone bioavailability apparently unrelated to P-gp (or fexofenadine transporter) activity. Specifically, ritonavir, indinavir, ritonavir-indinavir, nelfinavir, and efavirenz, respectively, inhibited, inhibited, inhibited,

induced, and induced fexofenadine efflux (suggesting altered P-gp activity), whereas methadone bioavailability was unchanged, unchanged, unchanged, decreased, and unchanged (Kharasch et al., 2008a, 2009a, b, 2012a,b). Together, these results do not suggest that intestinal P-gp significantly mediates methadone absorption and first-pass extraction, and thus transporter mechanisms of methadone bioavailability and drug interactions remain unclear.

The third major finding of this investigation was that acute and steady-state ritonavir-lopinavir strongly inhibited both hepatic and intestinal CYP3A. Steady-state ritonavir-lopinavir inhibited combined

Fig. 4. Effect of acute and steady-state ritonavir-lopinavir (RTV/LOP) first-pass and hepatic CYP3A activity, assessed with alfentanil as a CYP3A probe. Shown are dose-adjusted plasma alfentanil concentrations after (A) oral and (B) intravenous administration. Subjects received 45 and 53 μ g/kg oral alfentanil at the control and ritonavir-lopinavir sessions, respectively, and 15 and 5 μ g/kg intravenous alfentanil at the control and ritonavir-lopinavir sessions, respectively. Each data point is the mean $\pm$ S.D. ($n = 12$). Some S.D. values are omitted for clarity.

TABLE 3

Pharmacokinetic parameters for intravenous and oral alfentanil and oral fexofenadine

Subjects received 15 and 5 $\mu\text{g/kg}$ i.v. alfentanil and 45 and 5 $\mu\text{g/kg}$ oral alfentanil, at control and ritonavir-lopinavir sessions, respectively. All oral fexofenadine doses were 60 mg. Alfentanil C_{max} and area under the concentration-time curve (AUC) are shown normalized to dose. No other variables are dose adjusted. Results are the arithmetic mean $\pm$ S.D. ($n = 12$), except the $\text{AUC}_{0-\infty}/\text{dose}$ ratio (ritonavir-indinavir/lopinavir), which is the geometric mean (90% confidence interval).

	Control	Acute Ritonavir-Lopinavir	Steady-State Ritonavir-Lopinavir
Intravenous alfentanil			
$C_{\text{max}}/\text{dose}$ (ng/ml/mg)	100 $\pm$ 37		109 $\pm$ 42
$\text{AUC}_{0-\infty}/\text{dose}$ (ng $\cdot$ h $\cdot$ ml $^{-1}$ /mg)	68 $\pm$ 26		383 $\pm$ 287*
$\text{AUC}_{0-\infty}/\text{dose}$ ratio (ritonavir-lopinavir/control)			4.6 (3.2, 6.8)
CL_{IV} (ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$)	3.89 $\pm$ 1.33		0.97 $\pm$ 0.61*
Elimination $t_{1/2}$ (h)	1.5 $\pm$ 0.4		8.2 $\pm$ 3.1*
E_{H}	0.28 $\pm$ 0.08		0.06 $\pm$ 0.04*
Oral alfentanil			
$C_{\text{max}}/\text{dose}$ (ng/ml/mg)	12 $\pm$ 4	30 $\pm$ 9*	28 $\pm$ 11*
$\text{AUC}_{0-\infty}/\text{dose}$ (ng $\cdot$ h $\cdot$ ml $^{-1}$ /mg)	32 $\pm$ 17	367 $\pm$ 193*	246 $\pm$ 165*
$\text{AUC}_{0-\infty}/\text{dose}$ ratio (ritonavir-lopinavir/control)		11.4 (7.4, 17.7)	6.9 (4.3, 11.2)
CL/F (ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$)	9.2 $\pm$ 4.6	0.8 $\pm$ 0.3*	1.5 $\pm$ 1.2*
Elimination $t_{1/2}$ (h)	1.4 $\pm$ 0.3	8.8 $\pm$ 3.0*	7.7 $\pm$ 3.0*
F_{oral}	0.45 $\pm$ 0.09		0.84 $\pm$ 0.15*
E_{G}	0.40 $\pm$ 0.10		0.14 $\pm$ 0.13*
Oral fexofenadine			
C_{max} (ng/ml)	172 $\pm$ 90	440 $\pm$ 192*	303 $\pm$ 175*
$\text{AUC}_{0-\infty}$ (ng $\cdot$ h $\cdot$ ml $^{-1}$)	1180 $\pm$ 620	4420 $\pm$ 2180*	2470 $\pm$ 1360*
$\text{AUC}_{0-\infty}$ ratio (ritonavir-lopinavir/control)		3.7 (2.8, 4.9)	2.0 (1.6, 2.6)
CL/F (ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$)	14.4 $\pm$ 6.1	4.0 $\pm$ 1.9*	7.4 $\pm$ 3.8*
Elimination $t_{1/2}$ (h)	9.6 $\pm$ 4.8	7.8 $\pm$ 2.7	10.1 $\pm$ 3.0

CLF, apparent oral clearance; CL_{H} , hepatic clearance; CL_{IV} , systemic clearance; F_{oral} , bioavailability; $t_{1/2}$, half-time.

*Significantly different from control ($P < 0.05$).

hepatic and intestinal CYP3A and hepatic CYP3A activities by 85% and 78%, respectively, as assessed by apparent oral and intravenous alfentanil clearances. Hepatic and intestinal alfentanil extractions were inhibited 78% and 65%, respectively. Effects of shorter-duration (2 days) ritonavir-lopinavir on CYP3A were even greater, causing 91% inhibition of oral alfentanil clearance. Previously, combined hepatic and intestinal CYP3A and hepatic CYP3A activities were inhibited by ritonavir-lopinavir by 92% and 77%, respectively (Yeh et al., 2006), and 82% and 76%, respectively (Wyen et al., 2008), as assessed by apparent oral and i.v. midazolam clearances. The present results recapitulate the ability of ritonavir to cause both acute CYP3A inhibition or inactivation and mild induction of new CYP3A protein synthesis with continuing exposure, albeit with strong net CYP3A inhibition always predominant (Kirby et al., 2011b).

Fig. 5. Effect of acute and steady-state ritonavir-lopinavir (RTV/LOP) on intestinal transporter activity, assessed using fexofenadine as a transporter probe. Each subject received 60 mg oral fexofenadine on all occasions. Each data point is the mean $\pm$ S.D. ($n = 12$).

The ability of ritonavir-lopinavir to inhibit CYP3A while simultaneously inducing metabolism mediated by other CYP isoforms is consistent with previous observations, whether ritonavir is used either alone at therapeutic doses or in combination with other protease inhibitors at boosting doses (Foisy et al., 2008). For example, steady-state ritonavir alone (400 mg twice daily) caused 90% inhibition of hepatic and first-pass CYP3A activity while inducing CYP1A2, CYP2B6, and CYP2C9 3-, 2-, and 2-fold, respectively (Kharasch et al., 2008b,c; Kirby et al., 2011a,b). Steady-state ritonavir-lopinavir decreased hepatic CYP3A activity by 77%, whereas hepatic CYP1A2, CYP2C9, and CYP2C19 activities increased by 43%, 29%, and 100% (Yeh et al., 2006). Ritonavir-lopinavir inhibited CYP3A-dependent elimination of lomefentanyl while concurrently inducing metabolism of artemether, attributed to induction of CYP2B6, CYP2C9, or CYP2C19 (Byakika-Kibwika et al., 2012). More specifically, and of particular pertinence to this investigation, is that ritonavir-lopinavir inhibits CYP3A while significantly inducing hepatic CYP2B6 activity (Piscitelli et al., 2000; Hogeland et al., 2007).

Effects of acute ritonavir-lopinavir to inhibit CYP3A and apparent P-gp activity, and to induce methadone clearances and apparent CYP2B6 activity, were greater after short-term compared with steady-state exposure. This finding is consistent with acute inhibition of P-gp and CYP3A, with partial P-gp and CYP3A induction (yet continued net inhibition). This may relate to effects of ritonavir or lopinavir on nuclear receptors and their regulation of CYPs 2B6 and 3A, and P-gp (Svård et al., 2010). Relationships of these clinical observations to mechanism-based inactivation of CYPs by ritonavir are presently unknown (Lin et al., 2013).

This investigation provides mechanistic insights regarding methadone metabolism and clearance and drug interactions. Although methadone plasma concentrations were known to be reduced by ritonavir-lopinavir, the mechanism was never explained (Clarke et al., 2002; McCance-Katz et al., 2003). For many years, methadone clearance was attributed to CYP3A4. Nevertheless, the present results provide strong evidence against CYP3A mediating ritonavir-lopinavir induction

Fig. 6. Relationship between methadone apparent oral clearance and first-pass CYP3A activity. The latter was measured as the apparent oral clearance of alfentanil. Data for each methadone enantiomer include control, acute, and steady-state ritonavir-lopinavir pretreatments. Each data point is the result from an individual subject. No significant correlation was found between the clearance of either methadone enantiomer and CYP3A activity.

of methadone metabolism and clearance. Specifically, ritonavir-lopinavir caused 1.3- to 3-fold increases in intravenous and oral methadone enantiomers *N*-demethylation, and clearance, despite 78% and 85% inhibition of hepatic and combined intestinal and hepatic CYP3A activity. Moreover, no correlation was found between apparent oral methadone clearance and combined intestinal and hepatic CYP3A activity (Fig. 6) or between i.v. methadone clearance and hepatic CYP3A activity. CYP3A appears not to mediate ritonavir-lopinavir effects on methadone disposition, and, more generally, these results do not support a predominant role for CYP3A in clinical *N*-demethylation and clearance of single-dose methadone.

The preponderance of other clinical evidence also vitiates the long-held notions that CYP3A4 activity determines clinical methadone *N*-metabolism and clearance, CYP3A4 interindividual variability is a major factor in variable methadone bioavailability, and CYP3A4 mediates methadone interactions with antiretrovirals and other drugs (Ferrari et al., 2004; McCance-Katz et al., 2010), which were extrapolated from in vitro methadone *N*-demethylation by CYP3A4 (Iribarne et al., 1996; Foster et al., 1999; Gerber et al., 2004; Chang et al., 2011). Strong hepatic and first-pass CYP3A inhibition by troleandomycin, and by ritonavir-indinavir, had no significant effects on methadone plasma concentrations, systemic or apparent oral clearance, hepatic clearance, or bioavailability (Kharasch et al., 2004a, 2009a). Ritonavir alone and nelfinavir induced methadone *N*-demethylation and clearance, despite strong hepatic and first-pass CYP3A inhibition (Kharasch et al., 2008a,b; 2009b). Neither these nor the present investigation showed a significant correlation between CYP3A activity and methadone clearance or *N*-demethylation. Taken together, these investigations do not support a significant role for CYP3A in clinical single-dose methadone *N*-demethylation and clearance.

In contrast, accumulating evidence suggests CYP2B6 as a major determinant of clinical methadone *N*-demethylation and clearance. CYP2B6 catalyzes methadone *N*-demethylation in vitro as avidly as CYP3A4 and CYP2B6 but not CYP3A4 is stereoselective (Gerber et al., 2004; Kharasch et al., 2004a; Totah et al., 2007, 2008; Chang et al., 2011). Methadone clearance was doubled by efavirenz and rifampin, which induce CYP2B6, as well as CYP3A4, and by ritonavir, which induces CYP2B6 while inhibiting CYP3A (Kharasch et al., 2004a, 2008a,c). Increasing the activity of CYP2B6, which metabolizes methadone stereoselectively (Gerber et al., 2004; Kharasch et al., 2004a; Totah et al., 2007, 2008; Chang et al., 2011),

increased plasma R/S methadone ratios (Kharasch et al., 2004a, 2008a,c, 2012b; Totah et al., 2008), whereas inhibiting CYP3A, which metabolizes methadone nonstereoselectively, did not (Kharasch et al., 2009a,b, 2012a). CYP2B6 inhibition by ticlopidine increased methadone enantiomers AUC and reduced methadone *N*-demethylation and clearance (Kharasch and Stubbett, 2013). Pharmacogenetic studies also evidence CYP2B6 involvement in methadone disposition, with *CYP2B6**6/*6 homozygotes having higher dose-adjusted S-methadone plasma concentrations and/or lower dose requirements than heterozygotes or noncarriers (Crettol et al., 2006; Wang et al., 2011; Levran et al., 2013).

In summary, acute and steady-state ritonavir-lopinavir induced the clinical *N*-demethylation and clearance of single-dose methadone. This occurred despite significant inhibition of hepatic and intestinal CYP3A activity. Steady-state, and more so acute ritonavir-lopinavir, inhibited intestinal P-gp activity but had no effect on methadone bioavailability. These observations, together with previous results, do not support a significant role for CYP3A or ritonavir-lopinavir-inhibitable intestinal transporters in single-dose methadone disposition. In general, the results demonstrate the potential for CYP2B6- as well as CYP3A-mediated ritonavir-lopinavir drug interactions.

Acknowledgments

The authors thank Darain Mitchell and Jing Berry for technical assistance.

Authorship Contributions

Participated in research design: Kharasch.

Conducted experiments: Kharasch, Stubbett.

Contributed new reagents or analytic tools: Stubbett.

Performed data analysis: Kharasch, Stubbett.

Wrote or contributed to the writing of the manuscript: Kharasch.

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