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Mechanism of ritonavir changes in methadone pharmacokinetics and pharmacodynamics I. Evidence against CYP3A mediation of methadone clearance

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Abstract

Ritonavir diminishes methadone plasma concentrations, attributed to CYP3A induction, but actual mechanisms are unknown. We determined ritonavir effects on stereoselective methadone pharmacokinetics and clinical effects (pupillary miosis) in healthy HIV-negative volunteers. Subjects received intravenous plus oral (deuterium-labeled) racemic methadone after nothing, short-term (3 day) and steady-state ritonavir. Acute and steady-state ritonavir, respectively, caused 1.5- and 2-fold induction of systemic and apparent oral R- and S-methadone clearances. Ritonavir increased renal clearance 40-50%, and stereoselectively (S>R) increased methadone hepatic N-demethylation 50-80%, extraction 2-fold, and clearance 2-fold. Bioavailability was unchanged, despite significant inhibition of intestinal P-glycoprotein. Intestinal and hepatic CYP3A was inhibited >70%. Ritonavir shifted methadone plasma concentration-miosis curves leftward and upward. Rapid ritonavir induction of methadone clearance results from increased renal clearance and induced hepatic metabolism. Induction of methadone metabolism occurred despite profound CYP3A inhibition, suggesting no role for CYP3A in clinical methadone metabolism and clearance. Ritonavir may alter methadone pharmacodynamics.

Methadone is a cornerstone therapy of opiate dependence.¹ Methadone maintenance prevents withdrawal, craving, concomitant use of opiates and illicit drugs, and is a vital public health strategy for HIV/AIDS risk reduction. Methadone is an efficacious and cost-effective first- or second-line treatment for acute, chronic, neuropathic, and cancer pain, in adults and children, and can be administered via multiple routes.^{2,3} Methadone use increased more than 700% between 1997-2004.⁴ Attainment and maintenance of predictable and reproducible methadone concentrations within therapeutic ranges is confounded, however, by considerable and unpredictable interindividual variability in methadone pharmacokinetics, susceptibility to drug interactions, and a long elimination half-life.^{5,6} Potential consequences include inadequate treatment or adverse events such as withdrawal, respiratory depression, or death. Methadone-related adverse events increased nearly 1800% between 1997-2004, and it is the sixth most frequent suspected drug in death and serious nonfatal outcomes.⁴

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Methadone is mainly cleared by hepatic metabolism, and also by urinary excretion of unchanged drug. The primary metabolic route is N-demethylation to pharmacologically inactive 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). There is large interindividual variability in methadone clearance.^{5,7} Methadone drug interactions occur with numerous CYP inducers and inhibitors.^{5,6} For over a decade, methadone clearance has been attributed to CYP3A4. Numerous *in vitro* studies demonstrated CYP3A4 involvement in methadone N-demethylation by human liver or intestinal microsomes and expressed CYPs.⁸⁻¹² Methadone metabolism and clearance *in vivo* have been widely attributed to CYP3A4; numerous dosing guidelines warn about potential CYP3A4-mediated methadone drug interactions and the need to adjust dosing accordingly.^{5-7,10,13-16} Methadone is a substrate for the efflux transporter P-glycoprotein (P-gp) *in vitro* and in brain in animals *in vivo*, which influences methadone brain access, pharmacodynamics, and analgesia.¹⁷⁻¹⁹ The role of P-gp in methadone brain penetration, pharmacodynamics and clinical effects, is unknown.

The epidemics of opioid dependence and HIV/AIDS are inextricably intertwined and methadone-HIV/AIDS cotreatment increasingly common. Antiretrovirals are notorious perpetrators of drug interactions. The protease inhibitors ritonavir, indinavir, nelfinavir, saquinavir, lopinavir, and amprenavir can induce and/or inhibit the metabolism and clearance of numerous drugs *in vivo*.⁶ Ritonavir is the most potent and efficacious inhibitor of CYP3A isoforms. Duration of ritonavir administration may affect the degree of CYP3A inhibition, with short-term dosing causing inhibition, with longer administration causing apparent induction with concurrent inhibition.^{20,21} Ritonavir, both alone and in combination with other antiretrovirals, causes decreases in both S- and R-methadone (active enantiomer) plasma concentrations and area under the concentration-time curve, and variably causes methadone withdrawal.⁶ Ritonavir effects on methadone clearance have been attributed to CYP3A4. Nonetheless, the apparent paradox of ritonavir induction of methadone clearance, despite profound inhibition of CYP3A4 activity, has never been addressed. Indeed, mechanism(s) of ritonavir modulation of methadone pharmacokinetics and clinical effects still remain fundamentally unknown. Typically, methadone-ritonavir interaction studies have evaluated methadone but not EDDP plasma concentrations, and only some have evaluated stereoselectivity. Thus effects on methadone metabolism, and their stereoselectivity, are unknown.

The purpose of this clinical investigation was to determine: 1) mechanism(s) of acute and steady-state ritonavir alterations in methadone disposition and clinical effect; including the role of CYP3A4/5- and/or P-gp-mediated methadone bioavailability, first-pass metabolism, and systemic clearance; 2) the influence of ritonavir on methadone pharmacodynamics, 3) acute and steady-state ritonavir effects on hepatic CYP3A4/5, first-pass CYP3A4/5, and intestinal P-gp activities using validated *in vivo* probes; 4) the ability of a noninvasive *in vivo* CYP3A4/5 probe to detect ritonavir drug interactions.

A comprehensive crossover investigation was conducted in healthy volunteers (Figure 1). An accompanying manuscript describes ritonavir effects on hepatic and intestinal CYP3A4/5 and P-gp activities.²² This manuscript reports ritonavir effects on methadone pharmacokinetics and pharmacodynamics. Intravenous and oral (deuterium-labeled) methadone were simultaneously administered to concurrently assess drug kinetics, hepatic extraction and bioavailability, and, by avoiding a crossover design (for different routes of administration on different days) thereby diminish interday variability and double the protocol efficiency.¹² Miosis was used to assess one of the methadone clinical effects and pharmacodynamics.

Results

Plasma R- and S-methadone concentrations after IV unlabelled (d0) racemic methadone were diminished by short- and longer-duration ritonavir, with reductions greater after steady-state ritonavir (Fig 2). Acute and steady-state ritonavir caused statistically significant 1.5- and 2-fold increases in the systemic clearance, hepatic clearance, and hepatic extraction of both R- and S-methadone (Table 1). The plasma $AUC_{0-\infty}$ ratio (ritonavir/control) for both enantiomers was reduced to approximately 0.6 and 0.5, respectively, by acute and steady-state ritonavir. Ritonavir acutely increased V_{ss} of both methadone enantiomers, and these effects were not different at steady-state. Methadone N-demethylation was induced by ritonavir. The plasma AUC_{∞} ratio (ritonavir/control) was increased for both R- and S-EDDP/methadone by both short- and longer-term ritonavir. Formation clearance of R-EDDP was increased 26% and 51% by acute and steady-state ritonavir, while that for S-EDDP was increased 59% and 77% (Table 2). Renal clearance accounted for 25% and 21% of R- and S-methadone systemic clearance, respectively. Renal clearance of R- and S-methadone was increased approximately 40 and 50%, respectively, by ritonavir, however there was no difference between acute and steady-state ritonavir effects. The fraction of total methadone clearance attributable to renal clearance was not significantly affected by ritonavir. About 40-50% of the dose was recovered in urine for both enantiomers, and this was not affected by ritonavir.

The influence of ritonavir on plasma R- and S- d5-methadone and d5-EDDP concentrations after oral methadone is shown in Fig 3. Both short- and longer-duration ritonavir diminished plasma concentrations of both methadone enantiomers. The apparent oral clearance of R-methadone was significantly increased 1.5- and 1.9-fold, and that for S-methadone was increased 1.8- and 2-fold, by acute and steady-state ritonavir (Table 1). The plasma $AUC_{0-\infty}$ ratio (ritonavir/control) for both enantiomers was reduced to approximately 0.6 and 0.5, respectively, by acute and steady-state ritonavir. Ritonavir had no effect on methadone bioavailability, which exceeded 80%. Oral methadone N-demethylation was induced by ritonavir. The plasma AUC_{∞} ratio (ritonavir/control) for both R- and S-EDDP/methadone was significantly increased 1.5-fold and 1.8-fold by short- and longer-term ritonavir. This ritonavir regimen increased the apparent formation clearance of R-EDDP by 33% and 58%, and that of S-EDDP by 67% and 92% (Table 2). Renal clearance accounted for approximately 21% and 16% of R- and S-methadone systemic clearance, respectively. Renal clearance of R- and S-methadone was increased approximately 40 and 50%, respectively, by ritonavir, however there was no difference between acute and steady-state ritonavir effects. The fraction of apparent oral methadone clearance attributable to renal clearance was not significantly affected by ritonavir. About 40% of the oral methadone dose was recovered in urine for both enantiomers, and this was not affected by ritonavir.

Methadone disposition was stereoselective. R-methadone had a greater clearance and volume of distribution, and hence longer elimination half-life than S-methadone. As a result, there was a time-dependent increase in the plasma methadone R/S ratio (Fig 4), as reported previously.^{7,23} This increase was augmented by ritonavir, with similar effects after short- and longer-term ritonavir. Augmentation of the R/S ratio by acute ritonavir cannot be attributed to increasing induction 2-4 d after methadone administration, because similar effects were observed at steady-state ritonavir induction.

The relationship between methadone clearance and CYP3A4/5 activity is shown in Fig 5. Intravenous and oral alfentanil were used to probe hepatic and first-pass CYP3A4/5 activity, measured as alfentanil clearance.²² For IV R- and S-methadone, there was no significant correlation between systemic methadone clearance and hepatic CYP3A4/5 activity ($r^2 = 0.15$ and 0.10 , both $p > 0.05$). For oral R- and S-methadone, there was similarly no significant

correlation between apparent methadone oral clearance and first-pass CYP3A4/5 activity ($r^2 = 0.08$ and 0.06 , both $p > 0.05$).

Methadone effects were quantified using changes in dark-adapted pupil diameter (miosis).¹² Plasma concentrations of total R-methadone (representing both IV d0- and oral d5- R-methadone), the pharmacologically active enantiomer, and miosis, are shown in Figure 6. Methadone caused immediate miosis, with effects detectable at the first time point (2 min), and peak effects occurring within 5-10 min. Due to the slow absorption of oral methadone, and a second plasma concentration peak at 3-4 hr (Fig 6A), there was a second peak in pupil diameter change (Fig 6B). Despite lower plasma total R-methadone concentrations, miosis was greater when methadone was administered after 2d of ritonavir compared with controls, and miosis was essentially unchanged from controls when methadone was administered after 2 wk of ritonavir. Due to considerable interindividual variability in the combined effects of pharmacokinetic and pharmacodynamics, however, the AUEC_{0-∞} ratio (ritonavir/control) for acute ritonavir was not significantly different from control (Table 3). The apparent paradox of greater miosis despite diminished plasma methadone concentrations is resolved by examination of R-methadone pharmacodynamics, assessed by plasma concentration-effect curves (Fig 6C). It appears that the curve is shifted upwards and to the left by ritonavir, with a greater effect of acute compared with steady-state methadone. A sigmoid $E_{\max}$ model was used to analyze all individual concentration-effect data (not shown). Acute and steady-state ritonavir significantly decreased EC_{50} , suggestive of increased potency. $E_{\max}$ was increased by acute but not steady-state ritonavir, suggestive of increased apparent efficacy after acute ritonavir, which abated after steady-state ritonavir. Thus, induction of methadone metabolism and clearance, and diminished plasma concentrations, were offset by apparently greater R-methadone potency and effect.

Discussion

One major purpose of this investigation was to determine ritonavir effects on methadone disposition. It is the first to evaluate ritonavir effects on IV methadone disposition, concurrent effects on both IV and oral methadone, effects on methadone metabolism and on renal excretion, and to compare short-term and steady-state ritonavir influence on methadone disposition. Acute and steady-state ritonavir, respectively, caused 1.5- and 2-fold induction of systemic and apparent oral methadone clearances. Clearance induction was not stereoselective, with changes in methadone AUC_{0-∞} ratios (ritonavir/control), and systemic and hepatic clearances not significantly different between enantiomers. Induction of systemic and apparent oral clearances was attributable to an increase in renal clearance, and more so to induction of hepatic N-demethylation, extraction, and clearance. Renal clearance was increased similarly by both acute and steady-state ritonavir, while systemic, oral and hepatic clearances were increased more so by steady-state ritonavir. Induction of IV and oral methadone metabolism was evidenced by increases in both plasma EDDP/methadone ratios and EDDP formation clearances. Induction of methadone N-demethylation was stereoselective, with a greater effect on S than R-EDDP formation clearance, and time/dose-dependent, with a greater induction after 2 wk. A notable aspect of induction was the speed, with increased methadone clearance apparent after only 3d of ritonavir. Ritonavir had no effect on oral methadone bioavailability, which averaged about 85% throughout. Together, these results suggest that ritonavir causes time- and/or dose-dependent decreases in methadone plasma concentrations, owing to induction of systemic clearance, in turn attributable to stereoselective induction of hepatic N-demethylation and, to a lesser extent, non-stereoselective and time- and/or dose-independent induction of methadone renal clearance. Similar effects of ritonavir on IV and oral methadone disposition, together with an absent effect on bioavailability, suggests that intestinal contributions to ritonavir alterations of methadone metabolism, if any, appear relatively small.

Ritonavir effects on methadone disposition resembled those reported in previous, albeit different and more limited investigations. Oral methadone racemic plasma $C_{\max}$ and AUC were decreased 36-38% by ritonavir.²⁰ Plasma racemic methadone $C_{\max}$ and AUC₀₋₂₄ were decreased 28% and 36% by 100mg ritonavir (with 400mg lopinavir) twice daily for 7d,²⁴ and 44% and 36%, respectively, after 14d.²⁵ In contrast, 100mg ritonavir alone for 7d was reported to have no effect.²⁴ Ritonavir 400mg (with 400mg saquinavir) twice daily for 15d caused 32% and 40% reductions, respectively, in the oral R- and S-methadone AUC.²⁶ 0-24 Thus, reduction of methadone plasma concentrations by ritonavir (at least at doses exceeding 100mg) is a consistent observation.

Although ritonavir reduction of methadone plasma concentrations was reported over a decade ago, the mechanism remained unknown. Therefore, the second, and indeed preeminent objective of this investigation, was to determine the role of CYP3A4/5 and/or P-gp-mediated methadone bioavailability, first-pass metabolism, and systemic clearance, in methadone disposition and its alteration by ritonavir. Results provide strong and unambiguous evidence against a role for CYP3A4/5 in ritonavir induction of methadone clearance. Specifically, ritonavir caused 1.5- to 2-fold *increases* in IV and oral methadone enantiomers N-demethylation and clearance, despite more than 70% *inhibition* of hepatic CYP3A4/5 and more than 90% *inhibition* of first-pass CYP3A4/5.²² There was no correlation between methadone clearance (IV and oral) and CYP3A4/5 activity (hepatic and first-pass). The lack of correlation between methadone clearance and CYP3A4/5 resembles that reported previously.^{12,15} For over a decade, methadone metabolism and clearance *in vivo* have been attributed to CYP3A4. Numerous *in vitro* studies demonstrated methadone N-demethylation by recombinant CYP3A4, and CYP3A4 involvement in human hepatic or intestinal microsomal methadone metabolism.⁸⁻¹² Based on these *in vitro* studies, and well-intended extrapolation, methadone metabolism and clearance *in vivo* have been widely attributed to CYP3A4.^{5,7,13,15} Ritonavir and other protease inhibitor effects on methadone disposition and clearance have been attributed to CYP3A4.^{6,24,27} Numerous dosing guidelines warn about the potential for CYP3A4-mediated methadone drug interactions and the need to adjust dosing as a result.^{5-7,10,13,15,16} According to even the most recent methadone label, revised October, 2007, and approved by the FDA November 2007, "Since the metabolism of methadone is mediated primarily by CYP3A4 isozyme, coadministration of drugs that inhibit CYP3A4 activity may cause decreased clearance of methadone. The expected clinical results would be increased or prolonged opioid effects."^{*} The present results do not support a role for CYP3A4/5 in clinical methadone N-demethylation and clearance. Clinical guidelines identifying methadone as a CYP3A4 substrate, and warnings about clinical effects of CYP3A4 induction and/or inhibition on methadone disposition, require thoughtful reevaluation. Such apparently incorrect extrapolations of *in vitro* data reinforce the need to confirm such observations with clinical investigations.

This investigation does not identify CYP isoform(s) responsible for clinical methadone metabolism and clearance, and their induction by ritonavir. Nonetheless, the results are consistent with previous studies which identified a prominent role for CYP2B6 in methadone N-demethylation *in vitro*, and suggested a role for CYP2B6 *in vivo*. Recombinant CYP2B6 catalyzed methadone N-demethylation as effectively as CYP3A4.^{11,12,23,28} Unlike CYP3A4, metabolism by CYP2B6 was stereoselective.^{11,28} In human liver microsomes, selective inhibitors of CYPs 2B6, 3A4 and 2C19, respectively, inhibited methadone metabolism by 50-60%, 20-30%, and <10%, and only inhibition by clopidogrel was stereoselective.²³ Greater stereoselective metabolism (S>R) occurred in livers expressing high levels of CYP2B6 compared to CYP3A4.²³ Clinically, CYP3A

* www.fda.gov/CDER/Drug/infopage/methadone/default.htm, last accessed Dec 1, 2007

inhibition had no effect on methadone clearance, while the CYP2B6 inducer rifampin increased clearance.¹² Other clinical evidence supporting a role for CYP2B6 in clinical methadone metabolism and clearance, including the role of CYP2B6 polymorphisms, has recently been published.^{14,15} Methadone disposition clinically is stereoselective. *In vitro* modeling predicted that hepatic CYP3A inhibition would minimally alter plasma R/S methadone ratios, while CYP2B6 induction would augment a time-dependent increase.²⁸ This was confirmed in a clinical investigation, which showed that CYP3A inhibition had no significant effect on plasma R/S methadone ratios, while rifampin caused a time-dependent increase.²³ In the present study, ritonavir induction of methadone clearance also resulted in time-dependent increases in the plasma R/S methadone ratios. Ritonavir induces CYP2B6 in human hepatocytes.^{29,30} Ritonavir, in the same dosing paradigm as used herein, was recently shown to cause 2-fold induction of clinical CYP2B6 activity.³¹ Thus, CYP2B6 may mediate ritonavir induction of IV and oral methadone clearance, and indeed, be responsible in general for clinical methadone N-demethylation and clearance.

This investigation does not identify mechanism(s) for ritonavir induction of methadone renal clearance, although it is apparently the first to report such a renal contribution to this interaction, indeed any renally-mediated methadone interaction with antiretroviral, or other drugs. Little in general is known about mechanisms of methadone renal clearance, or involvement of any renal transporters. Ritonavir inhibition of clinical renal P-gp activity was observed in some³² but not other³³ studies, but induction has not been reported. Ritonavir did induce P-gp (and MRP2) in human hepatocytes,³⁰ but effects on renal cells are unknown. Ritonavir interacts with renal transporters other than P-gp, including multidrug resistance transporters and organic cation transporters.³⁴ Mechanisms of ritonavir alteration in renal methadone clearance require further evaluation.

Results of the present investigation do not support the hypothesis that intestinal P-gp mediates methadone absorption and first-pass extraction,³⁵ or ritonavir-methadone interactions. Acute and steady-state ritonavir increased fexofenadine plasma AUC 2.8- and 1.4-fold, respectively,²² suggesting P-gp inhibition, yet methadone bioavailability was unchanged. Previously, the P-gp inhibitor quinidine increased oral methadone plasma concentrations during the absorptive phase and decreased t_{max} , but C_{max} and AUC were unchanged,³⁶ and P-gp polymorphisms had no effect on oral methadone C_{max} .¹⁵

Clinical studies of ritonavir initiation in patients on stable methadone doses reported an absence of withdrawal symptoms, despite 30-50% decreases in methadone plasma concentrations.^{26,37} This has never been explained. Therefore the third major purpose of this investigation was to evaluate ritonavir effects on methadone pharmacodynamics, which were assessed using pupil miosis. Short-term ritonavir caused an apparent left- and up-ward shift of the R-methadone concentration-miosis curve, suggesting increased apparent potency (decreased EC_{50}) and efficacy (apparent E_{max}). Actual E_{max} could not be ascertained, since safety considerations precluded giving volunteers maximally effective doses. Apparent E_{max} , but not EC_{50} , returned to control values with steady-state ritonavir. This is the first report suggesting that ritonavir, or any protease inhibitor, affects apparent methadone pharmacodynamics. Apparent concentration-effect shifts offset the reduced plasma concentrations, such that miosis after acute and steady-state ritonavir was increased and unchanged, respectively, compared with controls. If other clinical methadone effects are similarly altered by ritonavir, this may explain the lack of clinical withdrawal despite induced methadone clearance by ritonavir.

Mechanism(s) by which ritonavir appears to alter methadone concentration-miosis relationships are not evident. While ritonavir and saquinavir somewhat diminished methadone protein binding and increased free plasma concentrations,²⁶ a concomitant

increase would still not explain the present findings. One plausible explanation is that methadone brain access is an active process, influenced by one or more xenobiotic transporters. This is the first clinical observation suggestive of methadone blood-brain barrier transport in humans. One potential explanation is acute ritonavir inhibition of methadone efflux (or augmentation of influx) followed by mild efflux induction, for example, brain P-gp inhibition followed by induction. Methadone is a P-gp substrate *in vitro* and in animals *in vivo*, which influences methadone absorption, brain access, pharmacodynamics, and analgesia. In P-gp knockout mice, and those treated with a P-gp inhibitor, methadone brain penetration was increased,^{17-19,38} the dose-response curve was shifted to the left,¹⁹ and analgesia was enhanced.^{19,38} Ritonavir and other protease inhibitors are well-established substrates, inhibitors, and inducers of brain P-gp *in vitro* and in animals. Ritonavir acutely inhibited P-gp-mediated transport in animal brain endothelial cells,^{39,40} while longer exposure induced P-gp expression and activity in cells⁴¹ and *in vivo*.⁴² Mouse brain amprenavir concentrations were increased by acute ritonavir exposure and P-gp inhibition, but unchanged after 4d.⁴³ In humans, acute ritonavir enhanced indinavir brain penetration.⁴⁴ Despite these laboratory findings, the role of P-gp in human methadone brain transport remains unknown.^{16,45} At present, the identity of the human methadone transporter(s), and any ritonavir effects on these transporter(s), remain unknown.

Similarities in acute (inhibition of first-pass CYP3A4/5, gastrointestinal P-gp, and brain P-gp, or other transporter) and steady-state (mild induction of CYP3A4/5, CYP2B6, gastrointestinal P-gp, brain P-gp, or other transporter), ritonavir effects may suggest a common mechanism. The pregnane X receptor (PXR) and constitutive androstane receptor (CAR) both regulate CYP2B6, CYP3A and P-gp expression. Human hepatocyte PXR activation nonselectively upregulates CYP2B6 and CYP3A4, while CAR preferentially upregulates CYP2B6.⁴⁶ PXR and CAR activation increased intestinal cell P-gp expression.⁴⁷ PXR activation in mice upregulated brain capillary P-gp and decreased methadone brain access.¹⁹ In human hepatocytes, ritonavir activated PXR, induced CYP3A and CYP2B6 expression,^{29,30} but completely inhibited CYP3A activity.^{29,30} Thus, PXR-mediated coordinate ritonavir upregulation of CYP2B6 and CYP3A4 expression was offset by selective CYP3A4 inhibition. Ritonavir is a more potent mechanism-based inhibitor of CYP3A4 than CYP2B6. In human intestinal cells, ritonavir acutely inhibits but then induces P-gp.⁴⁸ A similar *in vivo* scenario could encompass acute ritonavir inhibition of CYP3A and P-gp, followed by ritonavir activation of PXR resulting in mild relative upregulation of CYP3A4, CYP2B6 and P-gp expression, yet net inhibition of CYP3A4 and P-gp activity. Alternatively, ritonavir may be a preferential human CAR activator, resulting in more selective CYP2B6 upregulation, and likely also CYP3A4 inhibition. Additional investigation is needed to determine the mechanism of ritonavir effects on human clinical CYP3A, CYP2B6, and transporter activities.

There are recognized potential limitations with this investigation. First, the ritonavir dose differs from typical contemporary “boosted” antiretroviral regimens (100-200mg). The protocol was designed, however, as a mechanistic investigation, specifically to provide insights into the ritonavir reduction of methadone plasma concentrations first reported when higher ritonavir doses were used. Nonetheless, clearance induction did occur after only 3d of twice daily 200-300mg ritonavir, as has been observed after longer periods at lower (100mg twice daily) doses.⁴⁹ Thus the present investigation is of both mechanistic and clinical relevance. Second, ritonavir effects were evaluated in healthy volunteers, rather than HIV-infected patients. This was deliberate, because standard antiretroviral therapy involves several drugs, thereby precluding a careful mechanistic evaluation and attribution of results to any one specific drug. Third, a single methadone dose was evaluated, and, at concentrations much lower than typically used in opioid-dependent patients. It is not possible to study healthy volunteers at “therapeutic” (80-100mg) methadone doses.

Specifically, to tolerate such a “therapeutic” dose, opiate-naïve normal volunteers (in whom approximately 15mg methadone is normally the maximal safe dose) would need dose-escalation for several days or weeks, which is unethical because of a substantial risk of causing methadone addiction. Nonetheless, since methadone kinetics are independent of dose,⁵ results with acute “low-dose” methadone may be sufficiently similar to those of higher, steady-state methadone doses, to allow therapeutic application of the present results. Fourth, ritonavir changes in methadone concentration-miosis relationships were small, and, it remains unknown if other methadone clinical effects are similarly altered.

In summary, acute and steady-state ritonavir, respectively, caused 1.5- and 2-fold induction of systemic and apparent oral methadone clearances, due to increased renal clearance, and increased hepatic methadone N-demethylation, extraction and clearance. Ritonavir stereoselectively increased (S>R) EDDP formation clearance. Ritonavir had no effect on oral methadone bioavailability, despite apparent inhibition of intestinal P-gp. Rapid ritonavir induction of methadone clearance results from increased renal clearance and stereoselective induction of hepatic methadone metabolism, despite profound (70-95%) inhibition of hepatic and first-pass CYP3A4/5 activity. This suggests little if any role for CYP3A4/5 in clinical methadone metabolism and clearance. Ritonavir appeared to alter methadone concentration-miosis relationships, potentially suggestive of increased apparent potency and efficacy, methadone blood-brain barrier transport, and a transport-mediated drug interaction.

Methods

Additional Methods information is provided in the on-line Supplement and accompanying manuscript.²² The protocol was a 3-session sequential crossover approved by the University of Washington Institutional Review Board (Figure 1). Twelve normal HIV-negative volunteers (25 ± 5 yr, 74 ± 13 kg) were studied.

Methadone disposition was assessed by simultaneously administering IV racemic unlabelled (d0)-methadone HCl (6.0mg, 5.4mg free base) and oral deuterated racemic (d5)-methadone HCl (11.2mg, 10.0mg free base) after ondansetron for antinausea prophylaxis.¹² Venous blood and urine were sampled for 96 hr. Concurrently, dark-adapted pupil diameter was measured.^{12,22} Plasma and urine methadone and EDDP enantiomer concentrations were quantified by stereoselective liquid chromatography-mass spectrometry.⁵⁰ Hepatic and first-pass CYP3A4/5 activities were evaluated using intravenous and oral alfentanil, and P-gp activity using oral fexofenadine, on days preceding methadone.²²

Subjects then began ritonavir, 200mg three times daily for 1d, 300mg twice daily for 6d, then 400mg twice daily for 2 wk.^{20,21} First-pass CYP3A4/5, P-gp, and methadone disposition were determined on the second and third day of ritonavir. Hepatic and first-pass CYP3A4/5, P-gp, and methadone disposition were again assessed at steady-state ritonavir (2 weeks). Dosing was adjusted on study days to preclude an acute inhibitor effect from the ritonavir morning dose.

Pharmacokinetic data were analyzed using noncompartmental methods as described.¹² One-way repeated measures analysis of variance followed by Student-Newman-Keuls tests assessed differences between groups for pharmacokinetic and effect parameters. Non-normal data were log transformed for analysis, but reported as non-transformed results (arithmetic mean $\pm$ standard deviation). Plasma AUC and urine data were also assessed as ratios (ritonavir/control) and the geometric mean and 90% confidence interval. Concentration-effect data were analyzed by nonlinear regression using a sigmoid $E_{\max}$ model. Results are parameter estimate $\pm$ standard error of the estimate. Parameter

differences between treatment groups were analyzed by t-test, corrected for multiple comparisons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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		Control							ritonavir (200 mg tid x 1d, 300 mg bid x 7d, then 400 mg bid																							
Procedure	Objective	Day	1	2	3	4	5	6	7	1-2 mo																						
oral alfentanil (dose, µg/kg)	first-pass CYP3A activity	43									4.3																				4.3	
oral fexofenadine (60 mg)	intestinal P-gp activity																															
IV alfentanil (Dose, µg/kg)	hepatic CYP3A activity	15																													5	
methadone HCl (6 mg IV, 11 mg oral)	methadone PK/PD																															

Figure 1. Study protocol for ritonavir-methadone interaction. Shaded boxes show drug administration and/or blood and urine sampling.

Figure 2.

Effect of acute and steady-state ritonavir on intravenous methadone disposition and metabolism. Shown are plasma (A, B) R-methadone and R-EDDP concentrations and (C, D) S-methadone and S-EDDP concentrations. Subjects received 6.0 mg IV methadone HCl (5.4 mg free base). Each data point is the mean $\pm$ SD (n=12). Some SD are omitted for clarity.

Figure 3. Effect of acute and steady-state ritonavir on oral methadone disposition and metabolism. Shown are plasma (A, B) R-methadone and R-EDDP concentrations and (C, D) S-methadone and S-EDDP concentrations. Subjects received 11.2 mg oral methadone HCl (10.0 mg free base). Each data point is the mean $\pm$ SD (n=12). Some SD are omitted for clarity.

Figure 4. Effect of acute and steady-state ritonavir on stereoselective methadone elimination. Shown are plasma R/S-methadone concentration ratios for (A) intravenous and (B) oral methadone. Each data point is the mean $\pm$ SD (n=12).

Figure 5.

Relationship between methadone enantiomers clearance and CYP3A activity. (A) IV methadone clearance and hepatic CYP 3A activity (IV alfentanil clearance) (B) apparent oral methadone clearance and first-pass CYP 3A activity (oral alfentanil apparent clearance). Each data point is the result for a single subject. There were no significant correlations between methadone clearance and CYP3A activity.

Figure 6.

Effect of acute and steady-state ritonavir on methadone pharmacodynamics. Subjects simultaneously received 11.2 mg oral and 6.0 mg IV methadone HCl. Results are shown for (A) total plasma R-methadone concentrations, (B) dark-adapted pupil diameter change from baseline (miosis), and (C) plasma concentration-effect relationships (miosis vs R-methadone plasma concentration). Each data point is the mean $\pm$ SD (n=12). Some SD are omitted for clarity.

Table 1A

Intravenous methadone pharmacokinetic parameters

	Control	Acute ritonavir	Steady-state Ritonavir	Control	Acute ritonavir	Steady-state Ritonavir
	R-methadone			S-methadone		
$C_{\max}$ (ng/ml)	41 ± 13	35 ± 15	44 ± 20	60 ± 19	47 ± 21	57 ± 29
AUC_{0-96} (ng •hr •ml ⁻¹)	252 ± 95	166 ± 44 ^a	127 ± 46 ^{a b}	379 ± 164	215 ± 93 ^a	187 ± 107 ^a
$AUC_{0-\infty}$ (ng •hr •ml ⁻¹)	311 ± 139	195 ± 62 ^a	143 ± 54 ^{a b}	430 ± 211	241 ± 120 ^a	211 ± 129 ^a
$AUC_{0-\infty}$ ratio (ritonavir/control)		0.66 (0.57, 0.76)	0.47 (0.40,0.57)		0.57 (0.60,0.65)	0.47 (0.40,0.56)
CL_{IV} (ml•kg ⁻¹ •min ⁻¹)	2.37 ± 1.01	3.44 ± 0.93 ^a	4.84 ± 1.74 ^{a b}	1.74 ± 0.73	2.96 ± 0.92 ^a	3.83 ± 1.96 ^a
CL_H (ml•kg ⁻¹ •min ⁻¹)	1.80 ± 0.73	2.64 ± 0.78 ^a	3.95 ± 1.62 ^{a b}	1.44 ± 0.61	2.46 ± 0.88 ^a	3.32 ± 1.88 ^{a b}
Elimination $t_{1/2}$ (hr)	37 ± 13	31 ± 12	25 ± 12 ^{a b}	27 ± 11	24 ± 10	22 ± 10 ^a
V_{ss}	6.2 ± 1.6	8.2 ± 2.5 ^a	8.6 ± 3.2 ^a	3.3 ± 0.8	5.0 ± 1.6 ^a	5.6 ± 2.1 ^a
E_H	0.12 ± 0.05	0.17 ± 0.05 ^a	0.26 ± 0.11 ^{a b}	0.10 ± 0.04	0.16 ± 0.06 ^a	0.22 ± 0.12 ^{a b}
	R-EDDP			S-EDDP		
$C_{\max}$ (ng/ml)	0.40 ± 0.20	0.28 ± 0.04	0.31 ± 0.07	0.71 ± 0.23	0.61 ± 0.12	0.61 ± 0.16
$t_{\max}$ (hr)	3 (2, 10)	4 (2, 12)	3 (1.5, 8)	8 (3, 12)	6 (3, 12)	6 (2, 10)
AUC_{0-96} (ng •hr •ml ⁻¹)	22 ± 12	16 ± 3 ^a	14 ± 4 ^a	33 ± 13	30 ± 7	24 ± 8 ^{a b}
AUC_{∞} (ng •hr •ml ⁻¹)	31 ± 18	21 ± 5 ^a	18 ± 6 ^a	41 ± 18	35 ± 8	29 ± 9 ^{a b}
Elimination $t_{1/2}$ (hr)	46 ± 12	44 ± 12	42 ± 14	34 ± 12	31 ± 11	29 ± 9
AUC_{0-96} (EDDP/methadone)	0.08 ± 0.02	0.10 ± 0.02 ^a	0.11 ± 0.03 ^a	0.09 ± 0.03	0.16 ± 0.05 ^a	0.16 ± 0.06 ^a
AUC_{∞} (EDDP/methadone)	0.09 ± 0.03	0.11 ± 0.02 ^a	0.14 ± 0.03 ^a	0.10 ± 0.04	0.16 ± 0.05 ^a	0.16 ± 0.06 ^a
AUC_{∞} (EDDP/methadone) ratio (ritonavir/control)		1.13 (0.91,1.41)	1.33 (1.05,1.68)		1.55 (1.30,1.85)	1.51 (1.22,1.86)

Subjects received 6.0 mg IV and 11.2 mg oral methadone HCl at all sessions. Results are the arithmetic mean ± SD (n=12), except $t_{\max}$ which is the median (range), and AUC ratios (ritonavir/control) which are the geometric mean (90% CI).

^aSignificantly different from control (p<0.05)

^bSignificantly different from acute ritonavir (p<0.05)

Table 1B

Oral methadone pharmacokinetic parameters

	Control	Acute ritonavir	Steady-state Ritonavir	Control	Acute ritonavir	Steady-state Ritonavir
	R-methadone			S-methadone		
$C_{\max}$ (ng/ml)	12 ± 4	9 ± 4 ^a	8 ± 2 ^a	21 ± 8	13 ± 6 ^a	12 ± 4 ^a
$t_{\max}$ (hr)	3 (2, 10)	4 (2, 6)	4 (3, 6)	2 (1.5, 10)	4 (1.5, 6)	4 (2, 8)
AUC_{0-96} (ng •hr •ml ⁻¹)	399 ± 122	276 ± 79 ^a	229 ± 72 ^{a b}	580 ± 253	336 ± 170 ^a	308 ± 161 ^a
AUC_{∞} (ng •hr •ml ⁻¹)	513 ± 190	344 ± 116 ^a	261 ± 92 ^{a b}	672 ± 335	388 ± 233 ^a	340 ± 205 ^a
AUC_{∞} ratio (ritonavir/control)		0.68 (0.58, 0.79)	0.51 (0.44, 0.60)		0.57 (0.49, 0.65)	0.50 (0.42, 0.59)
CL/F (ml•kg ⁻¹ •min ⁻¹)	2.52 ± 0.90	3.70 ± 1.27 ^a	4.80 ± 1.37 ^{a b}	2.08 ± 0.88	3.72 ± 1.70 ^a	4.16 ± 1.65 ^a
Elimination $t_{1/2}$ (hr)	42 ± 10	38 ± 12	29 ± 11 ^{a b}	30 ± 8	28 ± 10	24 ± 7 ^{a b}
Vz/F	8.8 ± 2.4	11.7 ± 4.6 ^a	11.4 ± 3.1 ^a	5.0 ± 1.4	8.4 ± 3.7 ^a	8.0 ± 2.4 ^a
F_{oral}	0.86 ± 0.13	0.91 ± 0.17	0.92 ± 0.11	0.82 ± 0.10	0.84 ± 0.14	0.83 ± 0.12
	R-EDDP			S-EDDP		
$C_{\max}$ (ng/ml)	1.6 ± 0.5	1.3 ± 0.5	1.4 ± 0.5	2.0 ± 0.5	2.0 ± 0.6	2.0 ± 0.6
$t_{\max}$ (hr)	3 (1.5, 6)	4 (1.5, 6)	3 (1.5, 8)	3 (1.5, 6)	3 (1.5, 6)	4 (2, 6)
AUC_{0-96} (ng •hr •ml ⁻¹)	54 ± 13	53 ± 20	48 ± 17	72 ± 23	73 ± 27	68 ± 20
AUC_{∞} (ng •hr •ml ⁻¹)	83 ± 28	91 ± 53	90 ± 69	105 ± 35	94 ± 46	95 ± 39
Elimination $t_{1/2}$ (hr)	54 ± 26	70 ± 35	67 ± 46	49 ± 17	38 ± 16 ^a	42 ± 15
AUC_{0-96} (EDDP/methadone)	0.14 ± 0.06	0.20 ± 0.06 ^a	0.22 ± 0.06 ^a	0.14 ± 0.06	0.25 ± 0.10 ^a	0.26 ± 0.09 ^a
AUC_{∞} (EDDP/methadone)	0.17 ± 0.06	0.28 ± 0.17 ^a	0.33 ± 0.14 ^a	0.18 ± 0.06	0.28 ± 0.11 ^a	0.33 ± 0.13 ^a
AUC_{∞} (EDDP/methadone) ratio (ritonavir/control)		1.52 (1.26, 1.86)	1.86 (1.55, 2.24)		1.52 (1.33, 1.73)	1.79 (1.50, 2.14)

Subjects received 6.0 mg IV and 11.2 mg oral methadone HCl at all sessions. Results are the arithmetic mean ± SD (n=12), except $t_{\max}$ which is the median (range), and AUC ratios (ritonavir/control) which are the geometric mean (90% CI).

^aSignificantly different from control (p<0.05)

^bSignificantly different from acute ritonavir (p<0.05)

Table 2

Methadone and metabolite excretion

	Control	Acute ritonavir	Steady-state Ritonavir	Control	Acute ritonavir	Steady-state Ritonavir
	R-methadone			S-methadone		
% dose recovered 0-96 hr						
IV d0-methadone	25 ± 7	24 ± 8	20 ± 5	19 ± 6	18 ± 9	15 ± 6
Oral d5-methadone	21 ± 6	21 ± 7	16 ± 4	16 ± 6	15 ± 9	13 ± 5
d0-EDDP	19 ± 4	16 ± 4 ^a	15 ± 5 ^a	32 ± 6	30 ± 7	28 ± 8
d5-EDDP	19 ± 4	18 ± 4	16 ± 5	31 ± 6	30 ± 8	30 ± 7
clearance (ml•kg-1•min ⁻¹)						
IV d0-methadone Cl _f	0.61 ± 0.35	0.84 ± 0.37 ^a	0.89 ± 0.34 ^a	0.33 ± 0.17	0.50 ± 0.21 ^a	0.52 ± 0.20 ^a
Oral d5-methadone Cl _f /F	0.51 ± 0.23	0.72 ± 0.32 ^a	0.78 ± 0.32 ^a	0.31 ± 0.14	0.48 ± 0.22 ^a	0.48 ± 0.16 ^a
d0-EDDP Cl _f	0.43 ± 0.18	0.54 ± 0.21	0.65 ± 0.21 ^a	0.56 ± 0.29	0.89 ± 0.36 ^a	0.99 ± 0.49 ^a
d5-EDDP Cl _f /F	0.45 ± 0.20	0.60 ± 0.20 ^a	0.71 ± 0.22 ^{a b}	0.64 ± 0.30	1.07 ± 0.46 ^a	1.23 ± 0.60 ^a

Results are the mean ± SD (n=12)

^aSignificantly different from control (p<0.05)

^bSignificantly different from acute ritonavir (p<0.05)

Table 3**Ritonavir effect on methadone miosis**

	Control	Acute ritonavir	Sieauy-siaie Ritonavir
Maximum miosis (mm)	4.3 ± 1.2	5.1 ± 1.0	4.2 ± 0.6
AUEC _{0-∞} (mm•hr)	117 ± 61	135 ± 61	91 ± 46
AUEC _{0-∞} ratio (ritonavir/control)		1.27 (0.82, 1.95)	0.88 (0.64, 1.22)
R-methadone EC ₅₀ (ng/ml) ^a	6.1 ± 0.5	4.5 ± 0.2 ^b	4.4 ± 0.2 ^b
E _{max} (mm) ^c	3.0 ± 0.2	4.2 ± 0.2 ^b	3.3 ± 0.1 ^c

Subjects received 6.0 mg IV and 11.2 mg oral methadone HCl at all sessions. Results are the mean ± SD (n=11) except the AUC_{0-∞}/dose ratio (ritonavir/control), which is the geometric mean (90% CI).

^aMiosis vs total R-methadone concentration data were analyzed using a sigmoid E_{max} model. Results are the parameter estimate ± standard error of the estimate.

^bSignificantly different from control (p<0.05)

^cSignificantly different from acute ritonavir (p<0.05)