Lack of Indinavir Effects on Methadone Disposition Despite Inhibition of Hepatic and Intestinal Cytochrome P4503A (CYP3A)

Evan D. Kharasch, M.D., Ph.D.,* Pamela Sheffels Bedynek, B.S.,† Christine Hoffer, B.A.,‡ Alysa Walker, B.S.,† Dale Whittington, B.S.†

ABSTRACT

Background: Methadone disposition and pharmacodynamics are highly susceptible to interactions with antiretroviral drugs. Methadone clearance and drug interactions have been attributed to cytochrome P4503A4 (CYP3A4), but actual mechanisms are unknown. Drug interactions can be clinically and mechanistically informative. This investigation assessed effects of the protease inhibitor indinavir on methadone pharmacokinetics and pharmacodynamics, hepatic and intestinal CYP3A4/5 activity (using alfentanil), and intestinal transporter activity (using fexofenadine).

Methods: Twelve healthy volunteers underwent a sequential crossover. On three consecutive days they received oral alfentanil plus fexofenadine, intravenous alfentanil, and intravenous plus oral (deuterium-labeled) methadone. This was repeated after 2 weeks of indinavir. Plasma and urine analytes were measured by mass spectrometry. Opioid effects were measured by miosis. Results: Indinavir significantly inhibited hepatic and first-pass CYP3A activity. Intravenous alfentanil systemic clearance and hepatic extraction were reduced to 40–50% of control. Methadone plasma concentrations, methadone N-demethylation and clearance, suggesting little or no effect on methadone plasma concentrations, methadone N-demethylation, systemic or apparent oral clearance, renal clearance, hepatic extraction or clearance, or bioavailability. Methadone plasma concentration-effect relationships were unaffected by indinavir.

Conclusions: Despite significant inhibition of hepatic and intestinal CYP3A activity, indinavir had no effect on methadone bioavailability after oral administration.

METHADONE is a utilitarian opioid because of its effectiveness in treating numerous pain conditions and opiate addiction, administration by numerous routes, and long duration of effect. Methadone is cost-effective for treating acute, chronic, neuropathic, and cancer pain, in adults and children, in first- or second-line therapy, and can be administered orally, intravenously, nasally, and rectally.1–4 It is particularly useful perioperatively.4 Methadone use grew substantially during the last decade, primarily if not...
exclusively for pain, becoming a “darling drug of the pain management community.”\textsuperscript{5} Methadone prescriptions increased 13-fold from 1997 to 2006\textsuperscript{6} and 7-fold (population-adjusted) from 1997 to 2004.\textsuperscript{6} Tragically, however, during the same period, population-adjusted accidental methadone-related deaths increased 1,800\%, and fatalities increased 390\%; methadone was the drug with the greatest increase in fatalities, and the sixth most frequently suspected drug in death and serious nonfatal outcomes.\textsuperscript{6} The decade-long increase in methadone toxicity persists.

Methadone use is confounded by considerable inter- and intraindividual variability in pharmacokinetics, including metabolism, clearance, and drug interaction susceptibility, which can cause toxicity, inadequate analgesia, or withdrawal.\textsuperscript{7–9} Toxicity may occur at seemingly therapeutic plasma concentrations, suggesting pharmacodynamic variability or drug interactions. Despite decades of investigation, cause(s) of variable methadone disposition remain enigmatic. Much attention focused on identifying cytochrome P450 (CYP) responsible for methadone clearance and metabolism to the inactive N-demethylated metabolite 2-ethyl-1,5-dimethyl-3,3-diphenylpyrroolidine (EDDP). N-demethylation is considered a determinant of methadone elimination because clearance and the plasma EDDP/methadone area under the curve ratio are correlated.\textsuperscript{10} For more than a decade, and based on extrapolation of initial in vitro metabolism studies, numerous publications and clinical guidelines attributed clinical methadone disposition to CYP3A4 and warned of CYP3A4-mediated drug interactions.\textsuperscript{8,11–17} Newer information, however, consistently shows both CYP2B6 and CYP3A4 having the highest activity toward methadone N-demethylation in vitro, and CYP2B6 but not CYP3A4 metabolizing methadone stereoselectively, mirroring stereoselective methadone clearance.\textsuperscript{18–22} Nevertheless, it remains disputed which isoform determines clinical methadone disposition. Another potential pharmacokinetic factor is the efflux transporter P-glycoprotein (P-gp), which influences methadone intestinal absorption, brain access, pharmacodynamics, and analgesia in animals.\textsuperscript{23–26} In humans, the nature of P-gp participation in clinical methadone disposition and drug effects remains unknown.

Antiretroviral drugs cause well-known drug interactions which may be untoward or therapeutically useful. Such interactions may also provide useful insights into mechanisms of drug disposition. Methadone-antiretroviral drug interactions are well-noted.\textsuperscript{8,17} Previous mechanistic investigations of methadone-antiretroviral interactions, including ritonavir, ritonavir-boosted indinavir, and nelfinavir, showed no reduction (or an increase) in methadone metabolism and clearance, despite profound inhibition of hepatic and intestinal CYP3A4,\textsuperscript{27–30} suggesting lack of major CYP3A4 involvement in clinical methadone disposition. However, alternative explanations have been offered, such as compensatory increases in methadone renal clearance.\textsuperscript{17}

This comprehensive crossover investigation in healthy volunteers determined (1) indinavir effects on methadone disposition and clinical effects; (2) potential mechanisms, including altered CYP3A and/or P-gp–mediated bioavailability, first-pass, and systemic clearance; (3) indinavir effects on methadone pharmacodynamics; and (4) ability of noninvasive miosis to detect indinavir-CYP3A drug interactions. Intravenous and oral (deuterium-labeled) methadone were administered simultaneously.\textsuperscript{19,27–30} Clearances of intravenous and oral alfentanil, a nonselective CYP3A4 inhibitor (henceforth referred to as CYP3A) substrate, phenytoin hepatic and first-pass CYP3A activities.\textsuperscript{31,32} Oral fexofenadine probed P-gp.\textsuperscript{27–30} Miosis was a surrogate for opioid plasma concentrations to estimate CYP3A activity non-invasively and assess methadone pharmacodynamics.

Materials and Methods

Clinical Protocol

The investigation was approved by the University of Washington Institutional Review Board (Seattle, Washington), and subjects provided written informed consent. Inclusion and exclusion criteria were the same as described previously.\textsuperscript{27–30} The final study population was 12 healthy subjects (six men, six women; 23 ± 4 yr, range 18–31; 76 ± 13 kg, range 62–97).

The protocol was a two-period sequential crossover (controls first, for logistical considerations) with each subject serving as his or her own control. Hepatic and first-pass CYP3A, intestinal transporter activity, and methadone disposition were assessed before and after 2 weeks of indinavir. Detailed aspects of the protocol were similar to those described previously\textsuperscript{19,27–32} and are summarized here: First-pass CYP3A activity and intestinal P-gp (and other transporters) activity were evaluated using oral alfentanil and fexofenadine as in vivo probes. Subjects received 4 mg intravenous ondansetron followed 30 min later by 43 μg/kg oral alfentanil, and fexofenadine (60 mg) was administered 1 h later. Subjects received a standard breakfast and lunch 3 and 6 h after alfentanil. Venous blood was sampled and plasma stored for later analysis. Coincident with blood sampling, dark-adapted pupil diameter was measured (Pupilscan 60, Keeler Instruments, Broomall, PA). The next day, hepatic CYP3A activity was evaluated using intravenous alfentanil (15 μg/kg alfentanil bolus) given 30 min after ondansetron. Subjects received a standard lunch 4 h after alfentanil and free access to food and water thereafter. Venous blood was sampled, and dark-adapted pupil diameter was measured coincident with blood sampling using the infrared pupillometer.

---


sampling. The next day, oral deuterated racemic (d5)-methadone HCl (11.0 mg, equivalent to 9.86 mg free base) and intravenous racemic unlabeled (d0)-methadone HCl (6.0 mg, equivalent to 5.4 mg free base, Roxane Laboratories, Columbus, OH) were simultaneously administered 30 min after ondansetron. Venous blood samples were obtained, dark-adapted pupil diameters were measured, and 24-h urine samples were collected for 96 h. After approximately 1 month, subjects began taking oral indinavir 800 mg three times daily for 21 days. The above drug administration and sampling protocols were repeated, beginning on day 16 of indinavir.

Sample size was determined using a paired t test for the outcome variable methadone systemic clearance. A previous study found 22 and 33% inter-day-intrasubject variability in intravenous and oral methadone clearances, respectively, and to detect a 30% change in clearance using a paired t test with 33% variability (1-β = 0.8, α = 0.05) would require 12 subjects.27–30

Plasma alfentanil and fexofenadine were quantified simultaneously using solid-phase extraction and liquid chromatography electrospray mass spectrometry, and plasma and urine methadone and EDDP enantiomer concentrations were quantified using online extraction and electrospray mass spectrometry as described previously.27,29,30 Pharmacokinetic and pharmacodynamic data were analyzed using noncompartmental methods, as described previously.19,27–32 Area under the plasma concentration-time curve (AUC) was determined using the trapezoidal rule. Systemic clearance was (CL/F) = Doseoral/AUCoral, apparent oral clearance was (CL/V) = Doseoral/AUCoral, bioavailability was (Foral) = (AUCoral/Doseoral) × (DoseIV/AUCIV), steady-state volume of distribution was (Vss) = mean residence time × CL, volume of distribution based on the terminal phase was (Vz) = Dose/(AUC × λ) where λ is the terminal elimination rate constant. Hepatic extraction ratio (EH) was determined as (CLIV/Qp), where hepatic plasma flow (Qp) was estimated as the product of hepatic blood flow (25.3 and 25.5 ml/kg in males and females)33 and hematocrit (estimated as 40% and 36%, respectively, in males and females), and negligible extrahepatic metabolism was assumed. Gastrointestinal extraction ratio was (EC) = 1 − Foral/(Fh × Fabs), where the oral dose was assumed to be entirely absorbed, so Fabs was considered to be unity, and Fh = 1 − EH. Alfentanil effect (miosis) disposition curves were analyzed using noncompartmental analysis, analogously to conventional plasma concentration curves, to yield effect parameters (area under the effect curve, AUEC) similar to conventional pharmacokinetic parameters, as described previously.19,27–30

**Statistical Analysis**

Two-tailed paired t tests assessed differences between groups for pharmacokinetic and effect parameters. Non-normal data were log transformed for analysis but reported as nontransformed results (arithmetic mean ± SD). Statistical significance was assigned at P < 0.05. Area under the curve ratios (indinavir/control) were evaluated using the geometric mean and 90% confidence intervals, as recommended.** Confidence intervals excluding 1.0 were considered statistically significant. Relationships between methadone clearance and CYP3A activity were evaluated by Spearman correlation analysis. Data were analyzed using SigmaPlot 11.2 (Systat Software Inc., San Jose, CA).

**Modeling and Prediction**

**Mathematical Model.** *In vitro-in vivo* modeling using drug metabolism data were conducted to understand and predict inhibitor effects on alfentanil and methadone disposition. Reversible (competitive or noncompetitive) inhibition of a intravenously administered “victim” drug by a “perpetrator” drug is described by34,35:

\[
\frac{AUC^{IV}_{\text{victim}}}{AUC^{IV}_{\text{control}}} = \frac{Cl^{IV}}{Cl^{IV}_{\text{control}}} = \frac{1}{1 + \frac{1}{I_{H,u}}K_{I,u}} \cdot f_m + (1 - f_m)
\]

(1)

where \(AUC^{IV}_{\text{victim}}/AUC^{IV}_{\text{control}}\) is the plasma AUC ratio of the victim drug in the absence (AUC) and presence (AUC') of perpetrator, \(Cl^{IV}_{\text{control}}\) and \(Cl^{IV}_{\text{control}}\) are the uninhibited and inhibited clearances, \(f_m\) is the fraction of drug cleared by the altered metabolic pathway of interest, \(I_{H,u}\) is the unbound inhibition of the perpetrators in the liver (typically estimated using the unbound plasma concentration), and the unbound inhibitor constant \(K_{I,u}\) is the free drug concentration that produces half-maximum enzyme inhibition. \(K_{I,u}\) is determined directly by measuring the unbound inhibitor concentrations in *in vitro* microsomal incubations or by correcting the nominal (added) inhibitor concentrations for nonspecific binding to microsomal lipid and protein, using the unbound fraction (\(f_{u,mic}\)) calculated as:

\[
K_{I,u} = K_i \cdot f_{u,mic} = K_i \cdot \frac{1}{C \cdot 10^{6.56OgP/D–1.41} + 1}
\]

(2)

where \(f_{u,mic}\) is the unbound drug fraction in a microsomal incubation, \(C\) is the microsomal protein concentration (mg/ml), and log P/D is the octanol/buffer partition coefficient (for a weak base, \(pK_a > 7.4\)) or distribution coefficient (neutral or weak acid, \(pK_a < 7.4\)).36
For hepatic clearance of an intravenous drug that is catalyzed exclusively by one process, enzyme or isoform(s) (e.g., CYP3A4/5), and incorporating the influence of the hepatic extraction ratio ($E_H$),\textsuperscript{37,38} equation 1 becomes:

\[
\frac{AUC_{IV}}{AUC_{IV}} = \frac{CL_{IV}}{CL_{IV}} = \frac{1}{1 - f_{hep}} \left( \frac{1}{E_H} + \sum_{n=1}^{\infty} \frac{1}{1 + I_{H,d} K_{u,n}} \right) + (1 - f_{hep})
\]

(3)

where $f_{hep}$ is the fraction of systemic clearance that results from hepatic clearance.

For hepatic clearance of an intravenous drug eliminated by multiple enzymatic pathways (p) and/or where each pathway is catalyzed by one or more enzymes or isoforms,\textsuperscript{37,38} equation 3 becomes:

\[
\frac{AUC_{IV}}{AUC_{IV}} = \frac{f_{hep}}{1 - f_{hep}} \left( \frac{1}{E_H} + \sum_{n=1}^{\infty} \frac{1}{1 + I_{H,d} K_{u,n}} \right) + (1 - f_{hep})
\]

(4)

where $f_{m,n}$ is the fraction of hepatic clearance that is the result of the $n$th enzyme.

When a victim drug is administered orally, reversible inhibition of intestinal enterocyte and hepatocyte metabolism is described by\textsuperscript{34,35,39}:

\[
\frac{AUC_{PO}}{AUC_{PO}} = \frac{1}{1 + I_{H,d} K_{u,m}} \cdot f_m + (1 - f_m) \left( \frac{1}{1 + I_{G,d} K_{u,m}} \right) \cdot (1 - F_G) + F_G
\]

(5)

When the perpetrator and substrate are not coadministered, the concentration of the perpetrator in enterocytes ($I_G$) can be estimated using the unbound concentration of perpetrator in the systemic circulation.\textsuperscript{46} When the perpetrator and substrate are administered together, the predicted maximum concentration of perpetrator in the enterocyte ($I_G$) is given by equation 2, where dose, $k_s$, $f_s$, and $Q_a$ are the perpetrator dose given orally, the first-order absorption rate constant, the fraction of perpetrator dose that is absorbed into the gut wall, and enterocyte blood flow, respectively.\textsuperscript{34} Values for $k_s$, $f_s$, and $Q_a$ are 0.03 min$^{-1}$, 1.0, and 248 ml/min, respectively.\textsuperscript{34}

\[
I_G = \frac{\text{dose} \cdot k_s \cdot f_s}{Q_a}
\]

(6)

**Model Application.** Indinavir is a reversible (variably described as noncompetitive, competitive, or mixed) human CYP3A inhibitor at clinically relevant concentrations (unlike other protease inhibitors, which cause irreversible mechanism-based inactivation).\textsuperscript{40–44} The $K_i$, for indinavir in human liver microsomes has been reported (or calculated from a reported IC$_{50}$) as 3 $\mu$M,\textsuperscript{41} 0.17–0.86 $\mu$M in six studies,\textsuperscript{42} and 0.5 $\mu$M,\textsuperscript{43} with an overall average of 0.49 $\mu$M in the latter studies. The $K_i$ values reported or calculated for expressed CYP3A4 are 0.24 $\mu$M\textsuperscript{44} and 0.75 $\mu$M.\textsuperscript{45} Based on protein concentrations provided in these reports, an indinavir octanol-water partition coefficient of 2.7 at pH 7, and equation 2, the average $f_{m,n,mic}$ is 0.53, and the average calculated indinavir $K_{i,u}$ is 0.25 and 0.42 $\mu$M in human liver microsomes and CYP3A, respectively. Therefore, subsequent calculations used 0.34 $\mu$M as the indinavir $K_{i,u}$.

Alfentanil is a low- to moderate-extraction drug cleared exclusively by hepatic metabolism, with systemic clearance equivalent to hepatic intrinsic clearance, and metabolism is catalyzed exclusively by CYPs 3A4 and 3A5.\textsuperscript{31,45,46} Based on previous studies,\textsuperscript{31} $E_H$ and $F_G$ are 0.3 and 0.6, respectively, $f_m$ (which is due entirely to CYP3A, and thus equal to $f_{m,CYP3A}$) is 0.98, and $f_{hep}$ is 0.99.\textsuperscript{38} Therefore, equation (4) for intravenous dosing reduces to:

\[
\frac{AUC_{IV}}{AUC_{IV}} = \frac{1}{1 - f_{hep}} \left( \frac{1}{E_H} + \sum_{n=1}^{\infty} \frac{1}{1 + I_{H,d} K_{u,n}} \right)
\]

(7)

For oral alfentanil, equation 5 becomes:

\[
\frac{AUC_{PO}}{AUC_{PO}} = \frac{1}{1 + I_{H,d} K_{u,m}} \cdot f_m + (1 - f_m) \left( \frac{1}{1 + I_{G,d} K_{u,m}} \right) \cdot (1 - F_G) + F_G
\]

(8)

Indinavir plasma concentrations were not measured. To estimate $I_{H,u}$, it is necessary to note that indinavir is rapidly eliminated (1–2 h half-life), so even at steady state, predose plasma concentrations are extremely low if not negligible.\textsuperscript{47,48} Absorption of oral indinavir is slow, with maximum plasma concentrations occurring after 2 h.\textsuperscript{47,48} Average steady-state total indinavir concentrations over an 8-h dosing interval are 3.6 $\mu$M (based on total AUC divided by the dosing interval),\textsuperscript{42,47,48} so based on 61% protein binding,\textsuperscript{49} the average unbound indinavir concentrations calculated as 1.4 $\mu$M. Subjects received their
morning indinavir dose 3 h after intravenous alfentanil administration. Therefore, plasma indinavir concentrations were likely low or insignificant during the first 4 h of alfentanil elimination and higher than average during the second 4 h. That supposition was confirmed by the observation that over the 8 h intravenous alfentanil AUC determinations, indinavir inhibition of alfentanil elimination was much greater in the second 4 h compared with the first 4 h (vide infra). Therefore, over the total 8 h period of alfentanil measurement, the average effective unbound indinavir concentrations (K_{in,w}) were estimated as 0.7 μM. Subjects received their morning indinavir dose 2 h after oral alfentanil and took their evening dose after the last blood sample (12 h). Analogous to the above calculations, for oral alfentanil dosing, unbound indinavir plasma concentrations based on 8 h AUC should be divided over the 12 h, giving an average unbound concentration of 1.0 μM. The calculated maximal enterocyte indinavir concentration after oral indinavir (equation 8) is 158 μM, equivalent to 84 μM when considering f_{u,mic}.

Methadone is a low-extraction drug cleared by a combination of hepatic and renal clearance. For intravenous methadone, hepatic clearance (CL_H) of R- and S-methadone averaged 73 and 79% of total clearance (CL_T), respectively, so f_{hep} is 0.76, and E_{14} averaged 0.09. The fraction of total hepatic methadone clearance catalyzed by CYP3A (f_{m,CYP3A}) is unknown. However, it can be approximated using urine excretion of methadone and EDDP, because the latter reflects CYP3A metabolism. For intravenous methadone, urine R-EDDP averaged 19% of the dose and 41% of the total R-enantiomer recovered, and urine S-EDDP averaged 33% of the dose and 61% of the total S-enantiomer recovered. If all of the nonrecovered portion of the dose was non-CYP3A4–mediated metabolism, then f_{m,CYP3A} would be 0.19 and 0.33 for R- and S-methadone, respectively. Conversely, if the nonrecovered portion of the dose was metabolized proportionally by CYP3A4, f_{m,CYP3A} would be 0.41 and 0.61 for R- and S-methadone, respectively. These represent the lower and upper estimates of f_{m,CYP3A} for R-methadone (0.19–0.41) and S-methadone (0.33–0.61). For the plasma methadone AUC ratio after intravenous dosing, equation 4 becomes:

\[
\frac{AUC_{IV}}{AUC_{po}} = \frac{1}{f_{hep}} \left( \frac{1}{E_{Hi}} - 1 \right) \left( \frac{1}{f_{m,CYP3A} + 1 + F_G} \right) + (1 - f_{hep})
\]

(9)

Methadone undergoes little apparent intestinal metabolism, with F_G predicted as 0.97, and averaging 0.96 in several investigations. Therefore, for the plasma methadone AUC ratio after oral dosing, equations (5) and (9) give:

\[
\frac{AUC_{po}}{AUC_{IV}} = \frac{1}{f_{hep}} \left( \frac{1}{E_{Hi}} - 1 \right) \left( \frac{1}{f_{m,CYP3A} + 1 + F_G} \right) + (1 - f_{hep})
\]

(10)

However, if only the CYP3A-catalyzed pathways for methadone are considered, and CYP3-dependent parameters such as the plasma EDDP/methadone AUC ratio or urine EDDP formation clearance (CL_E) are the outcome, f_{m,CYP3A} is by definition 1.0, and the relevant equation is 11 for intravenous dosing (equivalent to f_{m,CYP3A} = 1 in equation 9), and equation 12 for oral dosing:

\[
\frac{AUC_{EDDP/methadone,IV}}{AUC_{EDDP/methadone,IV}} = \frac{1}{f_{hep}} \left( \frac{1}{E_{Hi}} - 1 \right) \left( \frac{1}{1 + F_G} \right) + (1 - f_{hep})
\]

(11)

\[
\frac{AUC_{EDDP/methadone,PO}}{AUC_{EDDP/methadone,PO}} = \frac{CL_{f,PO}}{CL_{f,IV}}
\]

(12)

Because indinavir is administered three times daily, and methadone concentrations are measured for 4 days, the average unbound indinavir plasma concentration of 1.4 μM is used in the above calculations. Indinavir was taken 3 h after oral methadone, and because methadone absorption peaks after approximately 4 h, this would coincide with peak indinavir concentrations. Therefore, the inhibitor and substrate are effectively coadministered, and equation 6 is used to calculate the predicted maximum enterocyte indinavir concentration (84 μM) after oral administration for application in the above models.
Indinavir increased the AUC ratio for oral alfentanil more than 3-fold, reduced apparent oral clearance to 30% of control, and increased oral bioavailability, indicating 70% inhibition of first-pass CYP3A activity. The intestinal extraction ratio were both reduced to 40–50% of control, beginning 3–4 h after alfentanil administration (fig. 2). As consistent with subjects receiving their morning indinavir dose 3 h after intravenous alfentanil and 2 h after oral alfentanil administration. Because predose plasma indinavir concentration AUC.

Subjects received 6.0 mg intravenous and 11.0 mg oral methadone HCl, 15 μg/kg intravenous alfentanil, and 43 μg/kg oral alfentanil at all sessions. Results (n = 12) are the arithmetic mean ± SD, except area under the curve ratios (indinavir/control) which are the geometric mean (90% CI).

<table>
<thead>
<tr>
<th>Table 1. Alfentanil and Methadone Effect Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Intravenous alfentanil</td>
</tr>
<tr>
<td>Maximum miosis (mm)</td>
</tr>
<tr>
<td>AUEC0–∞ (mm/h)</td>
</tr>
<tr>
<td>AUEC0–∞ ratio (indinavir/control)</td>
</tr>
<tr>
<td>Oral alfentanil</td>
</tr>
<tr>
<td>Maximum miosis (mm)</td>
</tr>
<tr>
<td>AUEC0–∞ (mm/h)</td>
</tr>
<tr>
<td>AUEC0–∞ ratio (indinavir/control)</td>
</tr>
<tr>
<td>Methadone</td>
</tr>
<tr>
<td>Maximum miosis (mm)</td>
</tr>
<tr>
<td>AUEC0–∞ (mm/h)</td>
</tr>
<tr>
<td>AUEC0–∞ ratio (indinavir/control)</td>
</tr>
<tr>
<td>AUEC0–∞/AUEC∞ (mm · ml · ng⁻¹)</td>
</tr>
<tr>
<td>AUEC0–∞/AUEC∞ ratio (indinavir/control)</td>
</tr>
</tbody>
</table>

Indinavir increased and prolonged alfentanil miosis and significantly increased the AUEC0–∞ ratio for both intravenous and oral alfentanil. These early results predicted significant inhibition of CYP3A activity.

Indinavir effects on alfentanil plasma concentrations are shown in figure 2, and pharmacokinetic parameters are provided in table 2. Indinavir significantly inhibited hepatic and first-pass CYP3A activity. The AUC ratio (indinavir/control) for intravenous alfentanil was significantly increased 2-fold by indinavir, and systemic clearance and the hepatic extraction ratio were both reduced to 40–50% of control, indicating 50% inhibition of hepatic CYP3A activity. Indinavir increased the AUC ratio for oral alfentanil more than 3-fold, reduced apparent oral clearance to 30% of control, and increased oral bioavailability, indicating 70% inhibition of first-pass CYP3A activity. The intestinal extraction ratio was decreased by half, indicating significant inhibition by indinavir of intestinal CYP3A activity. It is notable that indinavir inhibition of alfentanil elimination was much greater than that of liver CYP3A.

**Fig. 1.** Indinavir effects on first-pass and hepatic cytochrome P4503A (CYP3A) activity assessed using alfentanil as a CYP3A probe. Pupil diameter change from baseline (miosis) was used as a surrogate for alfentanil plasma concentrations. Shown is miosis after 43 μg/kg oral alfentanil (A) and 15 μg/kg intravenous alfentanil (B). Each data point is the mean ± SD (n = 12).

**Fig. 2.** Indinavir effects on first-pass and hepatic cytochrome P4503A (CYP3A) activity assessed using alfentanil as a CYP3A probe. Shown are alfentanil concentrations after oral (43 μg/kg) (A) and intravenous (15 μg/kg) (B) administration. Subjects received their morning indinavir dose 3 h after intravenous alfentanil and 2 h after oral alfentanil administration. Each data point is the mean ± SD (n = 12).

**Results**

Miosis has been used as a surrogate for alfentanil plasma concentrations and clearance and a noninvasive probe for CYP3A activity, and pupil data were available before plasma alfentanil concentrations. Thus, alfentanil miosis was used for early assessment of indinavir effects on CYP3A (fig. 1 and table 1). Indinavir increased and prolonged alfentanil miosis and significantly increased the AUEC0–∞ ratio for both intravenous and oral alfentanil. These early results predicted significant inhibition of CYP3A activity.
rate (fig. 3, table 3).

3-fold, with only a small decrease in the systemic elimination fenadine peak plasma concentrations and overall AUC intestinal transporters. Indinavir significantly increased fexo-

activity of the intestinal efflux pump P-gp and other gastro-

alfentanil elimination and much higher in the 4 – 8 h

tations were likely low or insignificant during the first4ho f

/\%

AUC

* Significantly different from control (90% CI).

Each data point is the mean

subject received 60 mg oral fexofenadine on all occasions.

assessed using fexofenadine as a transporter probe. Each

Fig. 3.


given below.

Table 2. Intravenous and Oral Alfentanil Pharmacokinetic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Indinavir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous alfentanil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>103 ± 21</td>
<td>113 ± 32</td>
</tr>
<tr>
<td>AUC0-∞ (ng · h · ml⁻¹)</td>
<td>72 ± 27</td>
<td>136 ± 35*</td>
</tr>
<tr>
<td>AUC0-∞ ratio (indinavir/ control)</td>
<td>—</td>
<td>2.0 (1.7, 2.3)</td>
</tr>
<tr>
<td>CLV (ml · kg⁻¹ · min⁻¹)</td>
<td>4.0 ± 1.5</td>
<td>2.0 ± 0.8*</td>
</tr>
<tr>
<td>Elimination t1/2 (h)</td>
<td>1.3 ± 0.2</td>
<td>3.7 ± 1.2*</td>
</tr>
<tr>
<td>Ei</td>
<td>0.25 ± 0.09</td>
<td>0.12 ± 0.02*</td>
</tr>
<tr>
<td>Oral alfentanil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>46 ± 19</td>
<td>68 ± 23*</td>
</tr>
<tr>
<td>AUC0-∞ (ng · h · ml⁻¹)</td>
<td>107 ± 59</td>
<td>333 ± 157*</td>
</tr>
<tr>
<td>AUC0-∞ ratio (indinavir/ control)</td>
<td>—</td>
<td>3.3 (2.6, 4.1)</td>
</tr>
<tr>
<td>CL/F (ml · kg⁻¹ · min⁻¹)</td>
<td>9.4 ± 5.9</td>
<td>2.8 ± 1.7*</td>
</tr>
<tr>
<td>Elimination t1/2 (h)</td>
<td>1.3 ± 0.3</td>
<td>2.2 ± 0.8*</td>
</tr>
<tr>
<td>Foral</td>
<td>0.51 ± 0.19</td>
<td>0.78 ± 0.23*</td>
</tr>
<tr>
<td>Ei</td>
<td>0.35 ± 0.17</td>
<td>0.16 ± 0.21*</td>
</tr>
</tbody>
</table>

Subjects received 15 μg/kg intravenous alfentanil and 43 μg/kg oral alfentanil. Results are the arithmetic mean ± SD (n = 12), except the AUC0-∞ ratio (indinavir/control), which is the geometric mean (90% CI).

* Significantly different from control (P < 0.05).

AUC = area under the plasma concentration-time curve; Cmax = peak plasma concentration; CLV = systemic clearance of intravenous alfentanil; CL/F = apparent oral clearance of alfentanil; Ei = hepatic extraction ratio; Ei = hepatic extraction ratio; Foral = bioavailability.

concentrations are very low, even at steady state, and they peak 2 h after dosing. Plasma indinavir concentrations were likely low or insignificant during the first 4 h of alfentanil elimination and much higher in the 4 – 8 h thereafter.

Disposition of oral fexofenadine was used to evaluate the activity of the intestinal efflux pump P-gp and other gastrointestinal transporters. Indinavir significantly increased fexofenadine peak plasma concentrations and overall AUC 3-fold, with only a small decrease in the systemic elimination rate (fig. 3, table 3).

Fig. 3. Indinavir effects on gastrointestinal 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 ± SD (n = 12).

Table 3. Fexofenadine Pharmacokinetic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Indinavir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)</td>
<td>134 ± 61</td>
<td>453 ± 298*</td>
</tr>
<tr>
<td>AUC0-∞ (ng · h · ml⁻¹)</td>
<td>729 ± 185</td>
<td>2,413 ± 1,344*</td>
</tr>
<tr>
<td>AUC0-∞ ratio (indinavir/ control)</td>
<td>—</td>
<td>2.9 (2.1, 4.0)</td>
</tr>
<tr>
<td>CL/F (ml · kg⁻¹ · min⁻¹)</td>
<td>19.6 ± 5.6</td>
<td>7.3 ± 3.5*</td>
</tr>
<tr>
<td>Elimination t1/2 (h)</td>
<td>13.6 ± 2.7</td>
<td>10.3 ± 3.6*</td>
</tr>
</tbody>
</table>

Results are the arithmetic mean ± SD (n = 12), except the AUC0-∞ ratio (indinavir/control), which is the geometric mean (90% CI).

* Significantly different from control (P < 0.05).

AUC = area under the plasma concentration-time curve; CL/F = apparent oral clearance of fexofenadine; Cmax = peak plasma concentration.

Disposition of both methadone enantiomers after intravenous administration was essentially unaffected by indinavir. Plasma concentrations are provided in figure 4, and pharmacokinetic parameters in tables 4 and 5. Methadone and EDDP plasma concentrations with and without indinavir pretreatment were essentially superimposable. The plasma AUCs and AUC ratios (indinavir/control) for both R- and S-methadone were unchanged, as were the systemic clearance, hepatic clearance, and hepatic extraction. R-methadone half-life was longer after indinavir, although clearance and volume of distribution were not changed. Methadone N-demethylation was similarly essentially unaffected by indinavir, which had no significant effect on the plasma AUC ratio (indinavir/control) for R- and S-EDDP/methadone or on the formation clearances of R- and S-EDDP. A small difference in R-EDDP/Cmax was observed. Renal clearance, which accounted for approximately one third of both methadone enantiomers’ systemic clearance, was not altered by indinavir. Renal clearance was not significantly affected by indinavir.

Indinavir also had no significant influence on the disposition of oral methadone. Plasma concentrations are shown in figure 5, and pharmacokinetic parameters provided in tables 4 and 5. Methadone and EDDP plasma concentrations with and without indinavir pretreatment were essentially superimposable. Methadone enantiomers plasma AUCs and AUC ratios (indinavir/control) were negligibly or not changed. Methadone Cmax was observed. Renal clearance, which accounted for approximately one third of both methadone enantiomers’ systemic clearance, was not altered by indinavir. Renal clearance was not significantly affected by indinavir.

Disposition is stereoselective, as is apparent from figures 4 and 5, and tables 3 and 4. Consequently, there is a well-described time-dependent increase in the plasma methadone R/S ratio (fig. 6). However, indinavir had no influence on the plasma R/S ratio of intravenous or oral methadone.

The relationship between methadone clearance and CYP3A activity, measured using alfentanil clearance, was
evaluated by linear correlation analysis (fig. 7). For intravenous R- and S-methadone, there was no significant correlation between systemic methadone clearance and hepatic CYP3A activity. Similarly for oral R- and S-methadone, there was no significant correlation between methadone oral clearance and first-pass CYP3A activity.

Methadone effects were evaluated using changes in dark-adapted pupil diameter (miosis). Plasma concentrations of total (sum of intravenous d0- and oral d5) R-methadone (the pharmacologically active enantiomer) are shown in figure 8A. Because of the slow absorption of oral methadone, there was a second plasma concentration peak at 3 h, after the initial intravenous peak. Total R-methadone plasma concentrations were not different between groups. Miosis in the indinavir-treated subjects was not significantly different from that in controls (fig. 8B), nor was the AUEC (table 1). The R-methadone plasma concentration-effect relationship (pharmacodynamics) was not affected significantly by indinavir (fig. 8C), nor was the effect/concentration AUEC0–H11009/AUC0–H11009 ratio (table 1).

In vitro drug metabolism and inhibition data can be extrapolated to predict the in vivo effects of a “perpetrator” drug on the disposition of a “victim” drug.34-39 Models incorporate hepatic interactions for intravenous drugs and intestinal as well as hepatic drug interactions for an oral drug. Models often predict the parent drug AUC ratio (perpetrator/control) in the absence and presence of perpetrator, but other relative metrics, such as those specifically reflecting metabolism, can also be used. For intravenous and oral alfentanil, assuming CYP3A-dependent clearance and based on in vitro indinavir inhibition of CYP3A, the predicted plasma alfentanil AUC ratios (indinavir/control) were 2.1 and 3.7, respectively. For methadone (where the fraction metabolized by CYP3A was assumed to be 0.19–0.41 and 0.33–0.61 for R- and S-enantiomers, respectively), assuming indinavir inhibition of CYP3A-dependent clearance, the predicted plasma R- and S-methadone AUC ratios were 1.1–1.3 and 1.2–1.6, respectively, for intravenous dosing, and 1.2–1.4 and 1.3–1.6 for oral dosing. For the specific CYP3A-catalyzed N-demethylation of methadone, the predicted (indinavir/control) ratios for plasma EDDP/methadone AUC and EDDP formation clearance were both 2.5 for both enantiomers after intravenous dosing, and both 2.6 for both enantiomers after oral dosing.

**Discussion**

The first major finding of this investigation was that indinavir significantly inhibited clinical CYP3A activity. Indinavir in vitro is a reversible inhibitor of expressed and human liver microsomal CYP3A isoforms, and unlike other antiretroviral drugs (ritonavir, saquinavir, amprenavir, lopinavir, and nel-

---

**Fig. 4.** Effect of indinavir on intravenous methadone disposition. Shown are plasma R-methadone (A), R-EDDP (2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine) (B), S-methadone (C), and S-EDDP (D) concentrations. Subjects received 6.0 mg intravenous methadone HCl (5.4 mg free base). Each data point is the mean ± SD (n = 12). Some SD are omitted for clarity.
### Methadone Disposition and CYP3A Activity

**Table 4. Intravenous and Oral Methadone Pharmacokinetic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>R-methadone</th>
<th></th>
<th>S-methadone</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Indinavir</td>
<td>Control</td>
<td>Indinavir</td>
</tr>
<tr>
<td>Intravenous methadone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>59 ± 28</td>
<td>54 ± 29</td>
<td>83 ± 39</td>
<td>76 ± 40</td>
</tr>
<tr>
<td>AUC0–96 (ng · h · ml⁻¹)</td>
<td>304 ± 54</td>
<td>304 ± 34</td>
<td>430 ± 93</td>
<td>418 ± 63</td>
</tr>
<tr>
<td>AUC0–∞ (ng · h · ml⁻¹)</td>
<td>387 ± 106</td>
<td>410 ± 77</td>
<td>481 ± 110</td>
<td>493 ± 113</td>
</tr>
<tr>
<td>AUC0–∞ ratio (indinavir/control)</td>
<td>—</td>
<td>1.07 (0.99, 1.16)</td>
<td>—</td>
<td>1.03 (0.93, 1.13)</td>
</tr>
<tr>
<td>CLv (ml · kg⁻¹ · min⁻¹)</td>
<td>1.64 ± 0.42</td>
<td>1.53 ± 0.43</td>
<td>1.33 ± 0.43</td>
<td>1.29 ± 0.37</td>
</tr>
<tr>
<td>CLu (ml · kg⁻¹ · min⁻¹)</td>
<td>1.16 ± 0.36</td>
<td>1.02 ± 0.27</td>
<td>1.03 ± 0.38</td>
<td>0.96 ± 0.29</td>
</tr>
<tr>
<td>Elimination t1/2 (h)</td>
<td>44 ± 10</td>
<td>54 ± 17*</td>
<td>30 ± 6</td>
<td>37 ± 15</td>
</tr>
<tr>
<td>Vss (L/kg)</td>
<td>5.6 ± 1.1</td>
<td>5.9 ± 1.0</td>
<td>3.1 ± 1.0</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>Eₜₜ</td>
<td>0.07 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>R-EDDP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.38 ± 0.12</td>
<td>0.40 ± 0.12</td>
<td>0.73 ± 0.24</td>
<td>0.90 ± 0.35*</td>
</tr>
<tr>
<td>AUC0–96 (ng · h · ml⁻¹)</td>
<td>16 ± 7</td>
<td>15 ± 6</td>
<td>33 ± 10</td>
<td>35 ± 8</td>
</tr>
<tr>
<td>AUC0–∞ (ng · h · ml⁻¹)</td>
<td>22 ± 10</td>
<td>24 ± 6</td>
<td>38 ± 11</td>
<td>42 ± 8</td>
</tr>
<tr>
<td>Elimination t1/2 (h)</td>
<td>35 ± 12</td>
<td>40 ± 11</td>
<td>29 ± 6</td>
<td>33 ± 13</td>
</tr>
<tr>
<td>AUC0–96 (EDDP/methadone)</td>
<td>0.05 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.08 ± 0.03</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>AUC0–∞ (EDDP/methadone)</td>
<td>0.06 ± 0.03</td>
<td>0.06 ± 0.02</td>
<td>0.08 ± 0.03</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>AUC0–∞ (EDDP/methadone) ratio (indinavir/control)</td>
<td>—</td>
<td>1.03 (0.84, 1.27)</td>
<td>—</td>
<td>1.10 (0.98, 1.23)</td>
</tr>
<tr>
<td>Oral methadone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>15 ± 4</td>
<td>17 ± 4*</td>
<td>24 ± 7</td>
<td>30 ± 10*</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>4 ± 1</td>
<td>3 ± 2</td>
<td>4 ± 2</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>AUC0–96 (ng · h · ml⁻¹)</td>
<td>489 ± 104</td>
<td>509 ± 66</td>
<td>688 ± 169</td>
<td>685 ± 131</td>
</tr>
<tr>
<td>AUC0–∞ (ng · h · ml⁻¹)</td>
<td>641 ± 204</td>
<td>711 ± 154</td>
<td>780 ± 200</td>
<td>805 ± 194</td>
</tr>
<tr>
<td>AUC0–∞ ratio (indinavir/control)</td>
<td>—</td>
<td>1.12 (1.02, 1.23)</td>
<td>—</td>
<td>1.04 (0.94, 1.16)</td>
</tr>
<tr>
<td>CL/F (ml · kg⁻¹ · min⁻¹)</td>
<td>1.86 ± 0.57</td>
<td>1.67 ± 0.61</td>
<td>1.57 ± 0.69</td>
<td>1.46 ± 0.46</td>
</tr>
<tr>
<td>Elimination t1/2 (h)</td>
<td>45 ± 12</td>
<td>55 ± 20*</td>
<td>30 ± 6</td>
<td>35 ± 11*</td>
</tr>
<tr>
<td>Vz/F</td>
<td>6.8 ± 1.6</td>
<td>7.2 ± 1.2</td>
<td>4.0 ± 1.4</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>Foral</td>
<td>0.91 ± 0.10</td>
<td>0.92 ± 0.10</td>
<td>0.87 ± 0.10</td>
<td>0.90 ± 0.11</td>
</tr>
<tr>
<td>R-EDDP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-methadone</td>
<td>S-methadone</td>
<td>R-methadone</td>
<td>S-methadone</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Indinavir</td>
<td>Control</td>
<td>Indinavir</td>
</tr>
<tr>
<td></td>
<td>1.5 ± 0.6</td>
<td>1.7 ± 0.7</td>
<td>1.9 ± 0.7</td>
<td>2.5 ± 1.0*</td>
</tr>
<tr>
<td></td>
<td>54 ± 17</td>
<td>52 ± 12</td>
<td>73 ± 20</td>
<td>76 ± 16</td>
</tr>
<tr>
<td></td>
<td>83 ± 33</td>
<td>77 ± 20</td>
<td>101 ± 21</td>
<td>100 ± 22</td>
</tr>
<tr>
<td></td>
<td>56 ± 27</td>
<td>55 ± 29</td>
<td>47 ± 15</td>
<td>42 ± 10</td>
</tr>
<tr>
<td></td>
<td>0.11 ± 0.04</td>
<td>0.10 ± 0.03</td>
<td>0.11 ± 0.04</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>0.14 ± 0.06</td>
<td>0.12 ± 0.05</td>
<td>0.14 ± 0.06</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>0.86 (0.72, 1.03)</td>
<td>—</td>
<td>0.94 (0.84, 1.05)</td>
</tr>
</tbody>
</table>

Subjects received 6.0 mg intravenous and 11.0 mg oral methadone HCl at all sessions. Results are the arithmetic mean ± SD (n = 12), except area under the concentration-time curve (AUC) ratios (indinavir/controls), which are the geometric mean (90% CI).

* Significantly different from control (P < 0.05).

**AUC** = area under the plasma concentration-time curve; **CL** = hepatic clearance; **CLs** = systemic clearance; **CL/F** = apparent oral clearance; **Cmax** = peak plasma concentration; **EDDP** = 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine; **Ei** = hepatic extraction ratio; **Foral** = bioavailability; **Tmax** = time to maximum concentration; **Vss** = steady-state volume of distribution; **Vz/F** = apparent volume of distribution based on the terminal phase.

Rinfinavir, is not a mechanism-based inhibitor.43,44,52 Less information is available regarding indinavir effects on clinical drug disposition and on CYP3A activity specifically because indinavir usually is coadministered with ritonavir. Indinavir increased rifampicin and rifabutin AUCs 1.7- and 2-fold, respectively, increased nelfinavir and clarithromycin AUCs, and halved amniprovir clearance.53 Although attributed to CYP3A inhibition, actual mechanisms were never identified.

The current investigation revealed that indinavir reduced hepatic and first-pass CYP3A activities to 50% and 70% of control, respectively. Alfentanil hepatic and intestinal extraction ratios were both reduced by half, and bioavailability increased. Greater effects of indinavir on oral versus intravenous alfentanil are consistent with intestinal contributions to first-pass metabolism and inhibition.39 Indinavir increased both alfentanil miosis AUGC and plasma AUC ratios, demonstrating the value of alfentanil miosis as a noninvasive approach to assessing CYP3A and drug interactions.28–32
Although indinavir was reported not to inhibit CYP3A, using dapsone hydroxylation and cortisol 6β-hydroxylation as phenotyping probes, it was later recognized that neither were valid CYP3A probes. This first demonstration of indinavir inhibiting both hepatic and intestinal CYP3A shows that both liver and intestine can be sites of indinavir drug interactions. Indinavir effects on CYP3A were quantitatively comparable with those of nelfinavir but less than those of other antiretroviral agents. For example, intravenous and oral al-

<table>
<thead>
<tr>
<th>% Dose recovered 0–96 h</th>
<th>Control</th>
<th>Indinavir</th>
<th>Control</th>
<th>Indinavir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous d0-methadone</td>
<td>29 ± 10</td>
<td>33 ± 5</td>
<td>23 ± 9</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Oral d5-methadone</td>
<td>24 ± 8</td>
<td>29 ± 3</td>
<td>20 ± 7</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>d0-EDDP</td>
<td>17 ± 5</td>
<td>15 ± 3</td>
<td>30 ± 10</td>
<td>31 ± 7</td>
</tr>
<tr>
<td>d5-EDDP</td>
<td>18 ± 5</td>
<td>18 ± 2</td>
<td>30 ± 9</td>
<td>32 ± 6</td>
</tr>
</tbody>
</table>

Results are the mean ± SD (n = 12).
* Significantly different from control (P < 0.05).

CLf = formation clearance; CLr = renal clearance; EDDP = 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine.

![Fig. 5. Effect of indinavir on oral methadone disposition. Shown are plasma R-methadone (A), R-EDDP (2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine) (B), S-methadone (C), and S-EDDP (D) concentrations. Subjects received 11.0 mg oral methadone HCl (9.9 mg free base). Each data point is the mean ± SD (n = 12). Some SD are omitted for clarity.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931115/ on 11/04/2018)
Fentanyl AUCs were increased, respectively, 4- and 10-fold by ritonavir, and 13- and 30-fold by ritonavir-indinavir. This is consistent with the lower CYP3A inhibitory potency of indinavir. Nonetheless, the aim of this investigation was not profound CYP3A inhibition but rather CYP- and organ-specific effects. Indinavir did not appreciably inhibit CYP1A2, CYP2B6, CYP2C9, or CYP2E1 in human liver microsomes or clinically.52,55,56

The second major finding of this investigation was that indinavir altered intestinal transporter activity. Indinavir increased fexofenadine Cmax and AUC, with comparatively minimal effect on systemic elimination, suggesting increased intestinal efflux or increased absorption. Fexofenadine was used as an intestinal P-gp probe based on published recommendations and previous application.28–30,57 Conceived originally as a selective P-gp probe, fexofenadine is now known to be a multiple transporter substrate, with intestinal absorption influenced by P-gp–mediated efflux and organic anion transporting polypeptide (OATP) 1A2-mediated uptake.58,59 Therefore, increased fexofenadine concentrations could reflect inhibited P-gp–mediated efflux or enhanced OATP1A2-mediated uptake. In vitro, indinavir is a poor P-gp inhibitor compared with other protease inhibitors.60,61 No information is available on indinavir and the potential for OATP1A2 induction. An alternative explanation for the results is that indinavir altered hepatic fexofenadine uptake, which determines fexofenadine clearance.62 Nevertheless, this hypothesis is inconsistent with the minimal effect of indinavir on fexofenadine elimination rates.

Indinavir effects on fexofenadine can be compared with those of other antiretroviral agents. Nelfinavir decreased fexofenadine Cmax, suggesting increased intestinal efflux and...
P-gp activity, although AUC was not altered. Combination ritonavir and indinavir was similar to indinavir, causing a 4-fold increase in AUC, even despite enhanced fexofenadine elimination, suggesting impaired intestinal efflux and inhibition of P-gp. In contrast, ritonavir alone increased fexofenadine AUC only 1.4-fold. This suggests that ritonavir-indinavir effects were attributable primarily to indinavir and that ritonavir transport effects may differ from those of indinavir.

The primary purpose of this investigation was to determine indinavir effects on methadone disposition and attendant mechanism(s). It is the first to evaluate indinavir effects on intravenous methadone disposition, oral and intravenous methadone concurrently, metabolism, and renal excretion. Thus, the third major finding was that indinavir had no effect on intravenous or oral methadone plasma concentrations, systemic or apparent oral clearance, hepatic clearance, hepatic extraction, bioavailability, or renal clearance, and negligibly increased plasma AUC ratios. Indinavir had no effect on methadone metabolism because EDDP/methadone plasma AUC ratios, EDDP enantiomer formation clearances, and plasma R/S-methadone ratios were essentially unchanged. A previous investigation of oral methadone also found no indinavir effects on plasma methadone and EDDP concentrations or AUCs.

A fourth purpose of this investigation was to evaluate relationships between methadone bioavailability and indinavir effects on gastrointestinal drug transporters. Bioavailability was unchanged despite inhibition of intestinal P-gp efflux and/or OATP1A2 uptake activity. Methadone is a P-gp substrate in vitro, and the P-gp inhibitor valspodar in rats increased oral methadone bioavailability. The current investigation does not support the hypothesis that intestinal P-gp significantly mediates methadone absorption and first-pass extraction, possibly because of high intestinal passive permeability or involvement of transporters other than those affected by indinavir.

A fifth purpose was to evaluate the role of CYP3A in methadone disposition. Methadone metabolism and clearance were not decreased, despite reduction of hepatic and first-pass CYP3A activities. Additional insights may be gained from modeling and prediction of indinavir effects on clinical alfentanil and methadone disposition. For intravenous and oral alfentanil, the predicted plasma alfentanil AUC ratios based on in vitro indinavir inhibition of CYP3A (2.1 and 3.7, respectively) compare favorably with observed ratios (2.0 and 3.3), overestimating them by a negligible 5–12%. Indeed, predictions within 2-fold of measured values are considered highly accurate. Therefore, indinavir effects on alfentanil disposition are consistent with predicted inhibition of hepatic and intestinal CYP3A, validating the clinical prediction models. Predicated on a hypothesis that CYP3A4 also mediates methadone metabolism and clearance, the models similarly predicted that indinavir would also impair methadone elimination. Nevertheless, whereas predicted plasma methadone AUC ratios were 1.1–1.6 and 1.2–1.6 for intravenous and oral methadone, indinavir caused no change in AUC ratios. More importantly, for N-demethylation, whereas predicted EDDP-related ratios were 2.5 and 2.6 for intravenous and oral methadone, respectively, indinavir had no effect on these AUC ratios. Although the predicted 10–60% increase in plasma methadone AUC was less than the 2-fold increase in plasma alfentanil AUC, due to nonhepatic routes of methadone elimination and potential non-CYP3A–mediated hepatic metabolism, such changes should be detectable in clinical studies, particularly for S-methadone. In addition, 2-fold changes in EDDP-related...
lated ratios were detectable in other methadone studies.\(^{27}\) A post hoc power analysis, using a paired \(t\) test, 19% coefficient of variation for intravenous methadone AUC (indinavir/control) ratios, \(α = 0.05\), and 80% power, suggested that 12 subjects would be sufficient to detect a 17% change in the AUC ratio and that 31 subjects would be needed to detect a 10% difference. Therefore, indinavir effects are inconsistent with those predicted by CYP3A-dependent methadone metabolism and clearance, whereas indinavir effects on alfentanil disposition were exactly those predicted by CYP3A mediating alfentanil metabolism and clearance. These results do not support a role for CYP3A in clinical N-demethylation and clearance of single-dose methadone.

Several previous investigations also contravene the notion, based on extrapolation of in vitro methadone N-demethylation by CYP3A\(_4\),\(^{11,18,22,65,66}\) that clinical methadone metabolism and clearance are mediated by CYP3A\(_4\),\(^{8,13–16,22,67,68}\) interindividual variability in CYP3A\(_4\) is a major factor in variable methadone bioavailability,\(^7\) and methadone interactions with antiretroviral and other drugs are attributable to CYP3A\(_4\).\(^{8,17}\) Despite profound inhibition of hepatic (more than 70%) and first-pass (more than 95%) CYP3A, ritonavir actually induced methadone N-demethylation and clearance.\(^{27,28}\) Nelfinavir increased methadone systemic clearance, hepatic clearance, hepatic extraction ratios, and apparent oral clearance 1.6- to 2-fold, despite 50% and more than 75% inhibition of hepatic and first-pass CYP3A.\(^{29}\) Ritonavir-indinavir had no significant effects on methadone plasma concentrations, systemic or apparent oral clearance, hepatic clearance, or bioavailability, despite 90% and 97% inhibition of hepatic and first-pass CYP3A.\(^{30}\) None of these investigations showed a significant correlation between CYP3A activity and methadone clearance or N-demethylation. In the current investigation, there was no increase in methadone renal clearance, which was suggested to offset and explain previous absent effects of CYP3A inhibition.\(^{17}\) In aggregate, these investigations do not support a significant role for CYP3A in clinical single-dose methadone metabolism and clearance.

An alternative explanation is that CYP2B6 (vide infra) predominates at low methadone concentrations after a single low dose, whereas CYP3A4 predominates at higher steady-state methadone concentrations. Evidence does not appear to support this. CYP2B6 but not CYP3A4 catalyzes methadone N-demethylation stereoselectively,\(^{18–22}\) whereas methadone clearance is stereoselective regardless of dose.\(^{27,29,30,60}\) At both “lower” (0.5–1 \(\mu\)M) and “higher” (\(≥\)2.5 \(\mu\)M) methadone concentrations, CYP2B6 had equivalent or greater activity than did CYP3A4.\(^{18–22}\) Finally, both methadone concentration ranges are so much lower than \(K_m\) values for CYP2B6 (60 and 16 \(\mu\)M for R- and S-methadone, respectively) and CYP3A4 (137 and 149 \(\mu\)M), and the difference between ranges small relative to the large difference between concentrations and \(K_m\), that plasma concentration differences would not drive isomof specificity.

Methadone disposition has been investigated repeatedly and yet remains persistently enigmatic, if not misunderstood. For more than a decade, methadone metabolism and clearance have been variably and variously attributed to CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, 3A5 and 19,\(^{11–16,18–22,66,70,71}\) although most frequently to CYP3A\(_4\).\(^{7,8,13–17,22}\) A now consistent finding is that CYP2B6 and CYP3A4 have the highest activity toward methadone metabolism in vitro, and that CYP2B6 is stereoselective while CYP3A4 is not.\(^{18–22}\) In addition, there is growing evidence that CYP2B6 is a major determinant of methadone metabolism and clearance. Rifampin and efavirenz, which induce CYP2B6 and CYP3A4, and ritonavir, which induces CYP2B6 while inhibiting CYP3A4, all doubled methadone clearance.\(^{19,27,72}\) Voriconazole increased \(R-\) and \(S\)-methadone AUCs 47% and 103%, respectively, which was attributed to CYP3A inhibition.\(^{73}\) Nonetheless, it was shown recently that voriconazole also inhibits CYP2B6.\(^{74}\) Modulating the activity of CYP2B6, which metabolizes methadone stereoselectively,\(^{18–22}\) altered clinical \(R/S\) methadone plasma ratios,\(^{19,27}\) whereas inhibiting CYP3A4, which metabolizes methadone nonstereoselectively, had no such effect.\(^{29,30}\) CYP3A inhibition did not affect methadone disposition, but CYP2B6 inhibition by ticlopidine\(^{29}\) decreased clinical methadone N-demethylation (Unpublished observation, Evan Kharasch, M.D., Ph.D., Department of Anesthesiology, Washington University, St. Louis, Missouri, 2010).

The last major purpose of this investigation was to evaluate indinavir effects on methadone pharmacodynamics. Previous investigations showed that drug interactions could alter significantly \(R\)-methadone concentration-effect relationships. Specifically, rifampin, ritonavir, nelfinavir, and efavirenz, but not ritonavir-indinavir, caused a leftward and upward shift of the concentration-response curves, consistent with an increase in apparent potency and efficacy.\(^{19,27,29,30}\) These effects were considered consistent with blood-brain barrier active methadone influx or efflux, and modulation by transporter-mediated interactions. One candidate transporter is P-gp, because altered blood-brain barrier P-gp activity changed brain methadone access.\(^{24}\) The current investigation shows that indinavir alone had no effect on methadone pharmacodynamics. If P-gp mediates methadone brain access in humans,\(^{76}\) poor activity of indinavir as a P-gp inhibitor\(^{60,61,77}\) would be consistent with the lack of indinavir effects on methadone pharmacodynamics. A preliminary experiment using a human blood-brain barrier model (cocultured endothelial cells, pericytes, and astrocytes) showed no effect of indinavir on methadone transport, whereas known P-gp inhibitors (cyclosporine and ritonavir) reduced methadone efflux (Unpublished observation, Scott Campbell, Ph.D., and Evan Kharasch, M.D., Ph.D., both Department of Anesthesiology, Washington University, St. Louis, Missouri, 2010).

Anesthesiology 2012; 116:432–47 Kharasch et al.
The current investigation has potential limitations. Methadone was studied after a single low dose because the risk of causing addiction of healthy volunteers with steady state dosing makes it neither possible nor ethical. Doses were small, more like those used for treating pain than for opiate addiction. Nonetheless, methadone kinetics are independent of dose.13 Results with a single methadone dose theoretically might differ from those at steady state, although indinavir also had no effect on steady-state methadone disposition.63 Indinavir effects were evaluated in healthy volunteers, not patients with human immunodeficiency virus. This was deliberate because antiretroviral therapy involves polypharmacy, thereby precluding a mechanistic evaluation and attribution of results to any one specific drug.

In summary, despite significant inhibition of hepatic and intestinal CYP3A activity and modulation of intestinal transporters, indinavir had no effect on clinical methadone N-demethylation or clearance. This, together with previous observations, does not support a significant role for CYP3A or certain intestinal transporters in single-dose methadone disposition.

References


42. Unadkat JD, Wang Y: Protease inhibitors, Metabolic Drug Ther 2005; 312:583–60


63. Maas B, Kerr T, Fairbairn N, Montaner J, Wood E: Pharma-
cokinetic interactions between HIV antiretroviral therapy and drugs used to treat opioid dependence. Expert Opin Drug Metab Toxicol 2006; 2:533–43
66. Wang JS, DeVane CL: Involvement of CYP3A4, CYP2C8, and CYP2D6 in the metabolism of (R)- and (S)-methadone in vitro. Drug Metab Dispos 2003; 31:742–7