The Role of Cytochrome P450 3A4 in Alfentanil Clearance

Implications for Interindividual Variability in Disposition and Perioperative Drug Interactions

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Background: There is considerable unexplained variability in alfentanil pharmacokinetics, particularly systemic clearance. Alfentanil is extensively metabolized in vivo, and thus systemic clearance depends on hepatic biotransformation. Cytochrome P450 3A4 was previously shown to be the predominant P450 isoenzyme responsible for human liver microsomal alfentanil metabolism in vivo. This investigation tested the hypothesis that P450 3A4 is responsible for human alfentanil metabolism and clearance in vivo.

Methods: Nine healthy male volunteers who provided institutional approval written informed consent were studied in a three-way randomized crossover design. Each subject received alfentanil (20 μg/kg given intravenously) 30 min after midazolam (1 mg injected intravenously) on three occasions: control; high P450 3A4 activity (rifampin induction); and low P450 3A4 activity (selective inhibition by troleandomycin). Midazolam is a validated selective in vivo probe for P450 3A4 activity. Venous blood was sampled for 24 h and plasma concentrations of midazolam and alfentanil and their primary metabolites 1'-hydroxymidazolam and noralfentanil were measured by gas chromatography-mass spectrometry. Pharmacokinetic parameters were determined by two-stage analysis using both noncompartmental and three-compartment models.

Results: Plasma alfentanil concentration-time profiles depended significantly on P450 3A4 activity. Alfentanil noncompartmental clearance was 5.3 ± 2.3, 14.6 ± 3.8, and 1.1 ± 0.5 mL·kg⁻¹·min⁻¹, and elimination half-life was 58 ± 13, 35 ± 7, and 630 ± 374 min, respectively, in participants with normal (controls), high (rifampin), and low (troleandomycin) P450 3A4 activity (means ± SD; p < 0.05 compared with controls). Multicompartmental modeling suggested a time-dependent inhibition-resynthesis model for troleandomycin effects on P450 3A4 activity, characterized as k_{id}(t) = k_{i0}[1 − \phi e^{-\sigma t}]

Conclusions: Modulation of P450 3A4 activity by rifampin and troleandomycin significantly altered alfentanil clearance and disposition. These results strongly suggest that P450 3A4 is the major isoenzyme of P450 responsible for clinical alfentanil metabolism and clearance. This observation, combined with the known population variability in P450 3A4 activity, provides a mechanistic explanation for the interindividual variability in alfentanil disposition. Furthermore, known susceptibility of human P450 3A4 activity to induction and inhibition provides a conceptual framework for understanding and predicting clinical alfentanil drug interactions. Finally, human liver microsomal alfentanil metabolism in vivo is confirmed as an excellent model for human alfentanil metabolism in vivo.

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Anesthesiology. V 87, No 1, Jul 1997

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CONFOUNDING the considerable efforts to design reliable infusion schemes to attain and maintain specific plasma concentrations of alfentanil1-4 is the wide interpersonal variability in alfentanil pharmacokinetics.5,6 The most significant factor responsible for unpredictable alfentanil disposition is the tenfold interindividual variability in alfentanil systemic clearance,7-9 from 1.5-17.6 ml·kg\(^{-1}\)·min\(^{-1}\). Interindividual differences in alfentanil plasma concentrations after fixed-dose administration derive primarily from this variability in clearance.5 Potential consequences of variable alfentanil clearance, due either to inherent individual differences or to drug interactions, are delayed anesthetic emergence and prolonged respiratory depression;9,12 Nevertheless, the mechanisms for variable alfentanil clearance remain unknown.

There also appear to be age- and sex-dependent contributors to the variability in alfentanil clearance. Alfentanil clearance diminishes linearly after age 40 yr, and one half of the total population variance in alfentanil clearance is accounted for by age.5 Mean alfentanil clearance decreased from 6.5 ml·kg\(^{-1}\)·min\(^{-1}\) in young adults to 4.4 ml·kg\(^{-1}\)·min\(^{-1}\) in those 68 yr and older, and the coefficient of variation increased from 32% to 60%.8,13 Another possible factor is the influence of sex. A significant negative correlation between alfentanil clearance and age was found in women but not in men, suggesting that aging effects on alfentanil kinetics are sex dependent.13 Possibly related to menopausal status,14 Another investigation only of men also found no effect of age on alfentanil clearance.15 Despite these observational investigations, the mechanism of potential age and sex dependence of alfentanil kinetics are unexplained.

The most likely explanation for unpredictable alfentanil clearance is variability in hepatic intrinsic clearance. Because alfentanil undergoes extensive biotransformation, hepatic intrinsic clearance is equivalent to hepatic intrinsic metabolic clearance. Therefore, variability in alfentanil clearance may reflect individual differences in alfentanil metabolism. Alfentanil is metabolized predominantly by two independent pathways, piperidine N-dealkylation to noralfentanil and amide N-dealkylation to N-phenylpropionamide.16,17 Noralfentanil is the major metabolite recovered in human urine. Previous in vitro investigations have shown that total human liver microsomal alfentanil biotransformation,18 and specific microsomal alfentanil metabolism to noralfentanil17,19 and to N-phenylpropionamide,17 are catalyzed predominantly by cytochrome P450 3A4.† The identity of the P450 3A4 isoform responsible for alfentanil metabolism in vivo, however, is unknown.

This investigation tested the hypotheses that P450 3A4 is primarily responsible for human alfentanil metabolism in vivo, and that variability in alfentanil clearance is due to variability in P450 3A4-dependent alfentanil metabolism. These hypotheses were tested in healthy volunteers by selectively manipulating P450 3A4 activity and then assessing the effect on alfentanil metabolism and clearance. P450 3A4 activity was induced by pretreatment with rifampin for 5 days and inhibited by a single oral dose of troleandomycin before alfentanil administration. P450 3A4 activity and the adequacy of inducer and inhibitor effects were assessed by the clearance of midazolam, a validated selective probe for P450 3A4 activity.21

Methods

Participant Selection and Clinical Protocol
Nine nonsmoking male volunteers ages 20 to 43 yr (29 ± 7 yr; means ± SD) of normal weight (74 ± 5 kg; range, 67-80 kg) participated in the investigation after giving written informed consent. The protocol was ap-
proved by the University of Washington Human Subjects Committee. The men were in good health, weighed within 20% of ideal body weight, had no history of hepatic or renal disease, and took no prescription medications during the entire course of the investigation. They consumed no alcohol, caffeine, grapefruit, or grapefruit juice for 24 h before each study session and for 48 h thereafter. Grapefruit and grapefruit juice were avoided because of the possibility of a pharmacokinetic interaction.

The investigation was a three-way crossover design, with participants randomized by Latin squares balanced for potential carryover effects. They were studied on three occasions. On one occasion, participants received no pretreatment (control). On another occasion, they received trolenandomycin (500 mg given orally) 2 h before midazolam administration, and 12, 24, and 36 h after the initial dose. On another occasion, participants received rifampin (600 mg given orally) for five consecutive mornings before the study session. Rifampin was not administered on the day of midazolam and alfentanil administration. The order of the three sessions was randomized, and study sessions were separated by 3 weeks.

For each session, an intravenous catheter was placed in each arm, one for drug administration and the other for blood sampling. After a baseline venous blood sample was obtained, participants received midazolam (1 mg intravenous bolus dose), and samples were obtained 15 and 30 min later. Participants then received alfentanil (20 μg/kg given as an intravenous bolus dose) with blood samples obtained 0, 1, 3, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480, 600, and 720 min later. The men were fed a standard breakfast 2 h after alfentanil administration and had free access to food and water thereafter. The participants returned 24 h after alfentanil administration to give a single blood sample, which was obtained by venipuncture. Plasma was separated and stored at -20°C for later analysis. Spontaneously voided urine was collected before and for 48 h after drug administration, the volume was recorded, and an aliquot was stored at -20°C until analysis.

**Analytical Methods**

Plasma alfentanil and noralfentanil concentrations were measured by gas chromatography-mass spectrometry with selected ion monitoring, as described previously. Interday coefficients of variation were 6% and 14% for 50 and 1 ng/ml alfentanil, and 6% for 1 ng/ml noralfentanil, respectively. Urine noralfentanil concentration were determined by modification of previous assays. Briefly, 0.25 ml urine was incubated with 10 μl β-glucuronidase (Sigma Chemical Co., St. Louis, MO) in 1.5 m ammonium acetate buffer (pH 5.1) for 18 h at 37°C. The pH was adjusted to approximately 11, the internal standard (d4-noralfentanil) was added, and noralfentanil was extracted with ethyl acetate/heptane (1:1). Noralfentanil was converted into its derivative with perfluoropropionyl anhydride and quantified by gas chromatography-mass spectrometry with selected-ion monitoring.

Plasma midazolam concentrations were determined by negative chemical ionization gas chromatography-mass spectrometry with selected ion monitoring, as described previously. Plasma 1'-hydroxymidazolam concentrations were determined before and after hydrolysis with β-glucuronidase, as described before. Subtraction yielded the concentration of 1'-hydroxymidazolam glucuronide.

Urinary cortisol and 6-β-hydroxycortisol concentration were determined by high-pressure liquid chromatography according to a published method with some modification. Briefly, 2 ml urine was extracted with 4 ml ethyl acetate after adding the internal standard dexamethasone (200 ng). The organic layer was transferred to a new tube, washed with 1 ml 0.25 m sodium hydroxide saturated with sodium sulfate, evaporated to dryness in a clean tube under nitrogen, and resuspended in 125 μl 25% acetonitrile. The high-pressure liquid chromatography system consisted of Waters (Milford, MA) M45 and 6000A pumps, Waters 720 gradient controller, Hewlett Packard (Wilmington, DE) 1050 autoinjector with 35900 A/D controller, Waters 441 ultraviolet detector (254 nm), and an Alltech (Deerfield, IL) EcoSpheric C18 reverse-phase column (150 mm × 4.6 mm, 5μ). The mobile phase was a linear gradient of 0-100% solvent B in A over 60 min, where solvent A was 0.015% trichloroacetic acid in deionized water and solvent B was acetonitrile:0.015% trichloroacetic acid (1:1) in deionized water at 1.5 ml/min. Retention times for 6-β-hydroxycortisol, cortisol, and dexamethasone were 30, 49, and 53 min, respectively.

**Data Analysis**

Alfentanil plasma concentration-time data for each participant at each of three times were analyzed by noncompartmental and compartmental methods using a microcomputer-based nonlinear extended least-squares regression analysis (MKMODEL; Biosoft, Cam-
ALFENTANIL DISPOSITION DEPENDS ON P450 3A4

bridge, UK) with subsequent two-stage calculations of means and standard deviations. For noncompartmental analysis, the trapezoidal rule was used to calculate area under the curve and area under the moment curve, elimination half-life was estimated by linear regression, systemic clearance was calculated as alfentanil dose/area under the curve, and steady-state volume of distribution (Vdss) was calculated as dose · area under the moment curve/area under the curve.

Alfentanil data were also fit to a three-compartment model. Data from treoleandomycin-treated men could not, however, be readily formulated as the sum of exponentials (vide infra). Therefore, the model parameters were identified in terms of the microconstants and central compartment volume to allow exploration of a time-dependent model of k10 for treoleandomycin-treated participants. First-order differential equations describing the plasma concentration versus time data are expressed as equation 1.

\[
\frac{dX_1}{dt} = -(k_{10} + k_{12} + k_{13}) X_1 + k_{21} X_2 + k_{31} X_3
\]

\[
\frac{dX_2}{dt} = k_{12} X_1 - k_{21} X_2
\]

\[
\frac{dX_3}{dt} = k_{13} X_1 - k_{31} X_3
\]

\[
C_i(t=0) = \frac{\text{dose}}{V_i}
\]

The parameter models are \( k_{10}, k_{12}, k_{13}, k_{21}, k_{31}, \) and \( V_i \). The compartmental volumes and clearances and \( V_{dss} \) are derived from

\[
CI_1 = V_1 k_{10}
\]

\[
V_1 k_{12} = V_2 k_{21}
\]

\[
V_1 k_{13} = V_3 k_{31}
\]

\[
V_{dss} = V_1 \left[ 1 + \frac{k_{12}}{k_{21}} + \frac{k_{13}}{k_{31}} \right]
\]

After parameter estimation for all participants, the means, standard deviations, and log-normal averages were calculated. The log-normal average represents a logarithmic smoothing of the fit parameters, minimizes the effect of outliers on the two-stage means, and more accurately models population data.

Although the three-compartmental model of equation 1 provided an excellent description for alfentanil data from control and rifampin-treated participants, it was an unsatisfactory model for the men treated with treoleandomycin. The results were poorly conditioned and there were systematic deviations of the residuals and predicted concentrations. Visual inspection of the raw data revealed a postdistributitional period (60–100 min) of relatively constant alfentanil concentrations followed by decreasing concentrations, consistent with an apparent increase in alfentanil clearance. The three-compartment model was thus expanded. A four-compartment model was tested but also poorly described the data. The apparent time-dependent increase in alfentanil clearance was modeled by postulating two enzyme populations, representing uninhibited and treoleandomycin-inhibited P450 and time-dependent regeneration of uninhibited enzyme activity. Here the uninhibited elimination rate constant is \( k_{10} \), the proportion of P450 inhibited is \( \phi \), and the reappearance of uninhibited enzyme is described by the rate constant \( \alpha \) after a lag time \( t_0 \). The apparent time-dependent rate constant is \( k_{10}(t) \):

for \( t \leq t_0: \quad k_{10}(t) = k_{10}(1 - \phi) \quad (4) \)

for \( t > t_0: \quad k_{10}(t) = k_{10}(1 - \phi) + k_{10}\phi(1 - e^{-\alpha(t-t_0)}) = k_{10}[1 - \phi e^{-\alpha(t-t_0)}] \]

Average clearances for the treoleandomycin-treated men were then calculated using the average value of \( k_{10}(t) \):

\[
CI = V_1 \frac{\int_0^t k_{10}(t) dt}{t}
\]

Improvements in model fitting to \( k_{10}(t) \) were assessed by F ratio testing and by plotting measured and predicted alfentanil concentrations and inspecting for accuracy and bias. Noralfentanil formation clearance was calculated as the product of systemic alfentanil clearance and the fraction of alfentanil dose recovered in urine as noralfentanil.

Midazolam data were analyzed by noncompartmental methods as described for alfentanil, and by a simple two-compartment model (WinNonlin; Scientific Consulting Inc., Apex, NC), with subsequent two-stage calculations of means and standard deviations. Insufficient

†† Calculated using the Excel macro Convert, obtained from Dr. Steve Shafer at http://pkpd.icon.palo-alto.med.va.gov

Anesthesiology, V 87, No 1, Jul 1997
sample numbers precluded using the time-dependent $k_{in}(t)$ model for midazolam.

Results are expressed as the means ± SD, unless indicated otherwise. Repeated measures analysis of variance was used to assess the significance of differences between values for control and treatment groups.

Results

Troleandomycin is the prototypic selective inhibitor of hepatic P450 3A4 activity in vitro but has not been established as an inhibitor in humans in vivo. Therefore rifampin induction and troleandomycin inhibition of P450 3A4 activity were confirmed using midazolam as a noninvasive probe of P450 3A4 activity. This probe was validated in previous investigations that showed an excellent correlation between the systemic clearance of intravenous midazolam and hepatic cytochrome P450 3A4 protein content as measured by liver biopsy. 21 As expected, midazolam elimination was substantially enhanced and inhibited, respectively, after rifampin and troleandomycin pretreatment (fig. 1). The threefold increase and fourfold decrease in midazolam clearance after rifampin and troleandomycin pretreatment conferred a fourfold decrease and a threefold increase in elimination half-life (table 1). Thus induction and inhibition of P450 3A4 by rifampin and troleandomycin was established unambiguously.

Alfentanil plasma concentrations in a typical participant on each of three occasions (control, rifampin induction, troleandomycin inhibition) are shown in figure 2, along with mean values from all participants. Pretreatment with rifampin or troleandomycin markedly altered alfentanil disposition. Alfentanil disappearance was extremely rapid after rifampin, and plasma concentrations were less than the limits of detection after 4 h. In contrast, alfentanil elimination was markedly diminished after troleandomycin, with alfentanil detectable for as long as 24 h.

Changes in alfentanil disposition were due primarily to alterations in systemic clearance. Noncompartmental analysis showed a threefold increase in alfentanil clearance in rifampin-induced participants and a fivefold decrease in troleandomycin-inhibited participants (table 2). Log-normal averages also showed a 14-fold range in alfentanil clearance between the rifampin and troleandomycin-treated men, with commensurate changes in elimination half-lives. There was no significant difference in $V_{dss}$ among the three phases of the investigation.

Anesthesiology, V 87, No 1. Jul 1997
Table 1. Midazolam Noncompartmental Pharmacokinetic Parameters

<table>
<thead>
<tr>
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<th>Control</th>
<th>Rifampin-treated</th>
<th>Troleandomycin-treated</th>
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<tbody>
<tr>
<td>CI (ml·kg⁻¹·min⁻¹)</td>
<td>3.3 ± 1.0</td>
<td>8.7 ± 1.4*</td>
<td>0.80 ± 0.28*</td>
</tr>
<tr>
<td>Vd∞ (L/kg)</td>
<td>0.97 ± 0.38</td>
<td>0.88 ± 0.24</td>
<td>0.84 ± 0.20</td>
</tr>
<tr>
<td>Elimination t₁/₂ (min)</td>
<td>352 ± 160</td>
<td>95 ± 42</td>
<td>392 ± 376*</td>
</tr>
<tr>
<td>Unconjugated 1'-OH/midazolam</td>
<td>0.128 ± 0.029</td>
<td>0.052 ± 0.028*</td>
<td>0.011 ± 0.003*</td>
</tr>
<tr>
<td>Total 1'-OH/midazolam</td>
<td>1.73 ± 0.42</td>
<td>3.36 ± 1.42*</td>
<td>0.122 ± 0.042*</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.
Concentration ratios of unconjugated 1'-OH midazolam/midazolam and total 1'-OH midazolam/midazolam were determined in samples obtained approximately 150 min after midazolam injection.

* Significantly different from control (P < 0.05).

Alfentanil plasma data in untreated and rifampin-treated men were well described by a simple three-compartment model. Rapidly diminishing alfentanil concentrations in rifampin-induced participants were also well described by a two-compartment model with minimal compromise in the goodness-of-fit; however, the three-compartment model was chosen to allow comparison of k₁₀ using the same model for all three treatment conditions. Alfentanil concentrations in troleandomycin-treated men could also be described by a simple three-compartment model, but closer inspection revealed a systematic deviation from a simple triexponential elimination curve (fig. 3). After the redistribution phases, alfentanil concentrations remained relatively constant until approximately 2 h after injection (4.5–5 h after troleandomycin ingestion), after which they decreased exponentially. Analysis using a simple three-compartment model resulted in ill-conditioned variance-covariance matrices and systematic bias in the predicted concentrations (fig. 4A).

Therefore, the three-compartment model for alfentanil disposition in troleandomycin-treated men was refined. Troleandomycin is a mechanism-based, noncompetitive inhibitor requiring P450 3A4-catalyzed troleandomycin metabolism and formation of an enzyme-metabolite complex that is catalytically inactive and stable (albeit reversible under specific conditions in vitro). Because troleandomycin is a substrate for P450 3A4, it may also act as a competitive inhibitor of alfentanil metabolism soon after the time of dosing, when troleandomycin concentrations are highest. P450 was therefore assumed to exist as either active or inactive (noncompetitively or competitively inhibited) enzyme. Restoration of enzyme activity occurs after elimination of the inhibitor and resynthesis of active enzyme. Therefore, the apparent k₁₀ was modeled as a time-dependent parameter k₁₀(t). The proportion of inhibited P450 was represented by Φ. The time-dependent change in Φ was described by the rate constant α and the lag time t₀, which can be conceptualized as collectively describing

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Fig. 2. Effect of P450 3A4 activity on alfentanil disposition. (Left) Alfentanil plasma concentrations in a typical participant. Disposition of alfentanil (20 μg/kg given intravenously) is shown for the control (no treatment; triangles), rifampin treatment (squares), and troleandomycin treatment (circles). Symbols depict measured concentrations, and lines represent predicted concentrations using microconstant parameters derived from a three-compartment model. (Right) Average (± SE) alfentanil plasma concentrations for all nine men. Table 2 shows the average pharmacokinetic parameters for all nine men.
the washout of troleandomycin (reducing the competitive component of P450 inhibition) and resynthesis of new enzyme (reducing the noncompetitive component). One attractive feature of this model is that three compartments are retained, and thus the microconstants derived for the troleandomycin-treated participants can be compared with those from control and rifampin-induced participants.

Figure 3 shows application of the time-dependent inhibition model, which depicts actual and predicted alfentanil concentrations and the time-dependent $k_{10}(t)$ for one representative participant. There was an excellent fit between observed alfentanil concentrations and those predicted using this $k_{10}(t)$ model. Tables 2 and 3 summarize derived kinetic parameters for time-dependent troleandomycin inhibition of P450 3A4 activity and alfentanil elimination. The uninhibited $k_{10}$ for the troleandomycin-inhibited men (0.080 ± 0.053 min⁻¹; lognormal mean, 0.073 min⁻¹) was not significantly different from the $k_{10}$ in untreated men (0.16 ± 0.21 min⁻¹; lognormal mean, 0.096 min⁻¹). This provided additional support for the time-dependent $k_{10}(t)$ model. The initial extent of P450 inhibition ($\phi$) averaged 71%, and the apparent lag time for reappearance of enzyme activity was 220 min after troleandomycin ingestion. Figure 4B shows prediction accuracy of the $k_{10}(t)$ model in troleandomycin-treated men. Data from 0–600 min after alfentanil injection were well fit by the time-dependent inhibition model, and thus parameters summarized in tables 2 and 3 were derived using only these data. In contrast, data from 600–1,440 min showed substantial deviations from the model. In fact, these deviations were not unexpected and may be explained: Participants received a second troleandomycin dose approximately 12 h after the initial dose (about 600 min after alfentanil injection), which would have been followed by renewed P450 inhibition and alfentanil concentrations expected higher than those predicted after recovery from a single dose of troleandomycin. Indeed, the positive deviation between measured and predicted concentrations after 600 min (fig. 4B) supports re-inhibition of P450 3A4 after the second troleandomycin dose. Insufficient sampling between 600–1,440 min, however, precluded model incorporation of a second time-dependent inhibition of $k_{10}(t)$.

Multicompartmental modeling also provided evidence for significant alterations in alfentanil elimination by manipulating P450 3A4 activity (table 2). For trolean-
ALFENTANIL DISPOSITION DEPENDS ON P450 3A4

Fig. 3. Time-dependent inhibition of alfentanil elimination by troleandomycin. The main figure shows alfentanil (20 μg/kg) disposition in a man treated with troleandomycin. Symbols depict measured concentrations, and lines represent predicted concentrations using parameters derived from a three-compartment model incorporating the time-dependent parameter k_{10}(t). The inset shows the time-dependent value of k_{10}(t).

Fig. 4. Prediction accuracy of three-compartment models for alfentanil disposition in men treated with troleandomycin. Data were fit to (A) conventional [k_{10}] and (B) time-dependent inhibition [k_{10}(t)] models. Each line represents data from a single participant. A perfect model fit would result in a straight line at 1. There was systematic bias and inaccuracy using the conventional model. Data fitting was improved by the k_{10}(t) model, but did deviate at times after the second dose of troleandomycin (arrow).

domycin-treated men, alfentanil clearance was calculated from the average (over time) k_{10}. Minimal and maximal clearances in these participants was also calculated from the maximally inhibited and uninhibited k_{10}(t). Alfentanil clearance was increased threefold in rifampin-induced men and decreased threefold in troleandomycin-treated men. Log-normal averages also showed a ninefold range in alfentanil clearances between rifampin- and troleandomycin-treated men. The apparent central compartment volume V_{1} was somewhat diminished after troleandomycin, as expected, because the lower clearance resulted in higher initial drug concentrations and the inverse trend was observed in men treated with rifampin. There were no differences in V_{1}. There was excellent agreement between noncompartmental and multicompartmental (with time-dependent k_{10} for troleandomycin-treated men) clearances.

Significant effects of alterations in P450 3A4 activity were also apparent from direct measures of alfentanil metabolism. Noralfentanil formation clearance, calculated from urine noralfentanil recovery and noncompartmental alfentanil clearances, was reduced from 0.65 ml·kg⁻¹·min⁻¹ in controls to 0.21 ml·kg⁻¹·min⁻¹ in men treated with troleandomycin (P < 0.05; table 4). Apparent noralfentanil formation clearance in rifampin-induced participants was not statistically different from controls.

To establish further the role of P450 3A4 in alfentanil disposition, alfentanil systemic clearance was compared

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Log-normal Average</th>
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<tbody>
<tr>
<td>k_{10}</td>
<td>0.080 ± 0.033</td>
<td>0.047–0.118</td>
<td>0.073</td>
</tr>
<tr>
<td>φ</td>
<td>0.71 ± 0.16</td>
<td>0.55–1.0</td>
<td>0.69</td>
</tr>
<tr>
<td>t_{0} (min)</td>
<td>223 ± 83</td>
<td>89–359</td>
<td>207</td>
</tr>
<tr>
<td>α (min⁻¹)</td>
<td>0.036 ± 0.012</td>
<td>0.0006–0.1</td>
<td>0.012</td>
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Anesthesiology, V 87, No 1, Jul 1997
Table 4. Noralfentanil Metabolite Kinetics

<table>
<thead>
<tr>
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<th>Control</th>
<th>Rifampin-treated</th>
<th>Troleandomycin-treated</th>
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<tr>
<td>CL (ml·kg⁻¹·min⁻¹)</td>
<td>0.63 ± 0.36</td>
<td>0.47 ± 0.25</td>
<td>0.21 ± 0.23†</td>
</tr>
<tr>
<td>Metabolite ratio*</td>
<td>0.049 ± 0.014</td>
<td>0.047 ± 0.018</td>
<td>0.003 ± 0.001†</td>
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* The plasma noralfentanil/alfentanil concentration ratio was determined 30 min after alfentanil injection. Noralfentanil assay insensitivity precluded ratio assessment at later time points.
† Significantly different from control (P < 0.05).

with P450 3A4 activity, assessed as the clearance of midazolam, in control, rifampin-treated, and troleandomycin-treated participants. There was an excellent correlation (r = 0.97, P < 0.001) between the noncompartmental systemic clearances of alfentanil and midazolam (fig. 5). There was also an excellent correlation (r = 0.96, P < 0.001) between the systemic clearances of alfentanil and midazolam determined by multicompartmental modeling (data not shown).

The ratio of plasma metabolite to parent drug concentration was assessed as a potential simple measure of alfentanil or midazolam clearance and P450 3A4 activity. Plasma noralfentanil/alfentanil concentration ratios were determined during the postdistribution phase (30 min after alfentanil injection). The ratio was decreased significantly in troleandomycin-treated men (table 4) and was < 0.005 in all of them after P450 3A4 inhibition, but the ratio was unchanged in rifampin-induced participants. There was a significant linear correlation between the noralfentanil/alfentanil ratio and systemic alfentanil clearance (r = 0.70, P < 0.001), although there was substantial overlap in the ratio between controls and rifampin-induced participants (fig. 6). Plasma 1'-hydroxymidazolam/midazolam ratios were measured in samples obtained approximately 150 min after injection. The metabolite ratio for unconjugated 1'-hydroxymidazolam was decreased in troleandomycin-treated participants but also diminished in men treated with rifampin (table 1). It is known that 1'-hydroxymidazolam circulates primarily as the glucuronide conjugate. Rifampin induction of metabolite glucuronidation or systemic elimination might confound the ratio, and thus unconjugated 1'-hydroxymidazolam and 1'-hydroxymidazolam-glucuronide concentrations were remeasured. Indeed, rifampin increased the percentage of 1'-hydroxymidazolam glucuronide and accelerated the disappearance of unconjugated 1'-hydroxymidazolam (fig. 1). The total (unconjugated and glucuronidated) 1'-hydroxymidazolam/midazolam ratio was decreased after troleandomycin and increased after rifampin treatment (table 1). There was a significant linear correlation between the ratio and systemic midazolam clearance (r = 0.84, P < 0.001; fig. 6), and the ratio was less than 0.02 in all participants after P450 3A4 inhibition. Nevertheless, there was overlap in the ratio between untreated and P450 3A4-induced participants, and only 70% of the variance in the ratio was due to systemic clearance.

Discussion

This investigation tested the hypothesis that cytochrome P450 3A4 is the predominant enzyme responsible for human alfentanil metabolism and clearance in vivo. P450 3A4 activity was induced and inhibited by
Fig. 6. Relation between plasma metabolite ratios and systemic clearance for alfentanil and midazolam. The noralfentanil:alfentanil ratio was determined 30 min after alfentanil injection. The ratio of total 1'-hydroxymidazolam to midazolam concentrations was determined in samples obtained approximately 150 min after midazolam injection. Clearances were determined by noncompartmental methods. Symbols denote control (triangles), rifampin-treated (squares), and troleandomycin-treated (circles) participants. The correlation was significant (P < 0.001) for both alfentanil and midazolam.

pretreatment of participants with rifampin and troleandomycin, respectively. Midazolam was used as a validated noninvasive probe to confirm P450 3A4 activity.21 Rifampin induction of P450 3A4 activity resulted in a threefold increase in alfentanil systemic clearance, and troleandomycin inhibition of P450 3A4 activity resulted in a four- to fivefold decrease in alfentanil clearance. Troleandomycin also decreased noralfentanil formation clearance to one third of control and decreased the plasma noralfentanil:alfentanil concentration ratio by 95%. There was a highly significant linear correlation between alfentanil systemic clearance and P450 3A4 activity, measured as midazolam systemic clearance, over a 25-fold range in P450 3A4 activity. These results show unambiguously that P450 3A4 is the predominant P450 isoform responsible for alfentanil systemic clearance and alfentanil metabolism in humans in vivo. Alfentanil systemic clearance is established as a noninvasive metabolic probe for P450 3A4 activity in vivo.

Cytochrome P450 3A4 Activity

Troleandomycin is used extensively as an isoform-selective inhibitor of human P450 3A4 in vitro (for review, see Newton et al.25). It is metabolized to an intermediate that binds to P450 3A4, forming an inactive enzyme-metabolite complex.26 Microsomes prepared from livers of patients treated with troleandomycin for 7 days showed in vitro evidence of substantial P450 3A4 inactivation.26 The present investigation clearly establishes troleandomycin as a highly effective inhibitor of human P450 3A4 in vivo. Systemic clearance of the P450 3A4 probe midazolam was diminished by 76% after troleandomycin, and clearance of alfentanil was diminished by 79%. Further, only a single dose of troleandomycin, administered 2 h before the test probe, was required to inhibit P450 3A4 activity. The duration of P450 3A4 inactivation after a single troleandomycin dose is unknown, and thus we administered additional doses at 12 h intervals. Evidence was obtained, however, that complete inhibition of a single troleandomycin dose may last less than 12 h, because a second troleandomycin dose appeared to increase the degree of P450 3A4 inhibition (fig. 4). In a previous human investigation, 7 days of troleandomycin treatment somewhat diminished the clearance of antipyrine, but a single dose of troleandomycin had no effect on antipyrine clearance.28 The single troleandomycin dose may have failed to diminish antipyrine clearance because several P450 isoforms in addition to P450 3A4 metabolize this drug.29 Troleandomycin inhibition of human P450 3A4 activity in vitro explains the pharmacokinetic interactions observed between this antibiotic and several P450 3A4 substrates (steroids, theophylline, carbamazepine, triazolam, cyclosporine).31,32 Troleandomycin is an effective in vivo probe to assess the role of P450 3A4 in human drug disposition.

There was excellent agreement between troleandomycin inhibition of P450 3A4 activity observed in vivo and effects on human liver microsomal metabolism in vitro reported previously. Troleandomycin inhibited 60-90% of P450 3A4 activity29 and alfentanil metabolism in human liver microsomes in vitro.17,18 Similarly, we calculated an average 71% (range, 55-100%) inhibition of hepatic P450 3A4 activity in vivo by a single dose of troleandomycin, using the time-dependent model of P450 3A4 inhibition.

Anesthesiology. V 87, No 1, Jul 1997
Rifampin is a known inducer of P450 3A4 in humans. P450 3A4 protein content and catalytic activity were increased two and five times, respectively, in microsomes prepared from livers of patients receiving rifampin for 7 days. Rifampin enhances the elimination of many P450 3A4 substrates, but most studies have examined rifampin effects on the clearance of oral drugs, and induction of intestinal P450 3A4 may account for a significant fraction of total gastrointestinal first-pass metabolism and the apparent induction of hepatic P450 3A4. Information regarding rifampin effects specific to human hepatic P450 3A4 activity, using intravenously administered P450 3A4 substrates, is surprisingly minimal and limited primarily to steroids. The present results clearly show that rifampin increased hepatic P450 3A4 activity, midazolam systemic clearance, and alfentanil systemic clearance 2.5–3 times. Thus rifampin induces both intestinal and hepatic P450 3A4 and increases clearance of oral and intravenous midazolam. Rifampin induction is not, however, limited exclusively to the 3A4 isoform of P450, so rifampin induction of metabolism alone is insufficient to implicate P450 3A4 in the biotransformation of a drug.

P450 3A4 is the most abundantly expressed P450 isoform, comprising 20–60% of total hepatic P450, and has the broadest substrate profile of any known P450 isoform, and is considered responsible for metabolizing more than one half of all currently used therapeutic drugs. There is considerable interest in identifying a noninvasive pharmacologic probe for human P450 3A4 activity. Human liver microsomal P450 3A4 metabolizes cortisol to 6β-hydroxycortisol, and the urine 6β-hydroxycortisol/cortisol concentration ratio has been suggested as a noninvasive marker of P450 3A4 activity. Therefore, effects of P450 3A4 induction and inhibition on the urine 6β-hydroxycortisol/cortisol ratio were also examined in participants for whom urine was available. The ratio was measured in the baseline urine sample in controls and rifampin-treated men (after 5 days of rifampin), and after 48 h of troleandomycin treatment (fig. 7). There was no significant or consistent change in the ratio in pretreated participants compared with controls, and there was no relation between this ratio and P450 3A4 activity. In contrast, Ged et al. and Horsmans et al. observed a fivefold increase in the ratio in rifampin-induced participants and a significant correlation between the ratio and P450 3A4 protein content in microsomes from liver biopsy samples. Reasons for the difference in results are not apparent, but previous investigators have questioned the utility of the urinary 6β-hydroxycortisol ratio as a noninvasive probe of hepatic P450 3A4 activity. In contrast, the present results showed excellent agreement between the systemic clearances of midazolam and alfentanil. Both drug clearances appear to be superior probes of P450 3A4 activity compared with the urine metabolite ratio of the endogenous substrate cortisol.

Determination of systemic and metabolite formation clearances requires repetitive plasma sampling, quantitative urine collection, and considerable sample analysis. There is considerable interest in developing single-sample plasma or urine measures of drug metabolism, clearance, and P450 activity. The plasma metabolite:parent drug ratio was thus examined as a surrogate marker for clearance and P450 3A4 activity. Although the metabolite ratio and systemic clearance were significantly correlated for both alfentanil and midazolam, there was considerable scatter. For midazolam, the correlation applied to total 1-hydroxymidazolam, but not to unconjugated 1′-hydroxymidazolam, due to both increased metabolite clearance and glucuronidation. For alfentanil, both the noralfentanil ratio and noralfentanil formation clearance were diminished by troleandomycin, however rifampin induction of P450 3A4 also decreased both measures. This unexpected finding may be attributable to enhanced parent drug elimination by pathways other than those reflected by noralfentanil.
or more likely to increased clearance of noralfentanil, analogous to midazolam. There was no evidence of noralfentanil glucuronidation. Although the metabolite ratio for both midazolam and alfentanil accurately reflected systemic clearance and P450 3A4 activity after troleandomycin inhibition, and may serve as a probe for P450 3A4 inhibition, the ratio was not accurate with normal or increased P450 3A4 activity (alfentanil) and predicted only 50-70% of clearance variability (midazolam). Thus the plasma metabolite:parent drug ratio for these P450 3A4 substrates is not a robust surrogate marker for P450 3A4 induction and inhibition status.

Alfentanil Disposition and P450 3A4

Demonstration of P450 3A4 as the predominant P450 isoform responsible for alfentanil metabolism in vivo confirms the findings of previous investigations that identified P450 3A4 as the predominant P450 isoform catalyzing human liver microsomal alfentanil metabolism in vitro. In a bank of microsomes from 20 human livers, there was a highly significant correlation between P450 3A4 activity and alfentanil metabolism in vitro to noralfentanil (r = 0.85) and to N-phenylproponamide (r = 0.84), the predominant routes of biotransformation. In the present investigation, there was a highly significant correlation between P450 3A4 activity and alfentanil systemic clearance (r = 0.97) in vivo. This investigation clearly establishes human liver microsomal alfentanil metabolism in vitro as an excellent model for human alfentanil metabolism and disposition in vivo.

Although the present results show the influence of P450 3A4 activity on disposition of an alfentanil bolus, they also apply to an alfentanil infusion. Computers have simulated opioid plasma concentrations during and after an infusion and predicted the recovery times after terminating infusions of varying duration. The time required for a 50% decrease in drug concentration after termination of an infusion designed to maintain a constant drug concentration has been called the “context-sensitive half-time.” Alfentanil kinetics and the context-sensitive half-time have been predicted by several investigators and used to simulate clinical responses and the effects of drug interactions. The accuracy of predicted alfentanil context-sensitive half-times was recently confirmed by comparing predicted with measured context-sensitive half-times after a 3-h alfentanil infusion. Therefore we predicted alfentanil context-sensitive half-times under conditions of normal, low, and high P450 3A4 activity (fig. 8). The half-time for normal (control) patients of 38 min corresponded closely with the 41 min determined using the kinetic parameters for venous plasma concentrations as adapted to patients younger than 40 yr.

The context-sensitive half-time for alfentanil was markedly influenced by P450 3A4 activity. For example, the time required for a 50% decline in concentration after a 3-h infusion was decreased to 14 min by high P450 3A4 activity and markedly increased to 216 min by low P450 3A4 activity. We also compared these P450 3A4-dependent alfentanil half-times with those of fentanyl and remifentanil, determined using reported pharmacokinetic parameters. Alfentanil half-times with high P450 3A4 activity were closer to those for remifentanil than for alfentanil with normal P450 3A4 activity. In contrast, alfentanil half-times with low P450 3A4 activity matched or exceeded those for fentanyl. For example, the normal fentanyl half-time for a 3-h infusion was 140 min, whereas that for alfentanil with low P450

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Fig. 8. Effect of P450 3A4 activity on the time required for a 50% decrease in alfentanil venous plasma concentration after targeted infusions of variable durations. These context-sensitive half-times were determined according to the method of Hughes et al. using the pharmacokinetic parameters in table 2. For low P450 3A4 activity, the average k_e(t) was used. Use of the fully (71%) inhibited k_e(t) would result in even longer half-times. Half-times for fentanyl and remifentanil in normal participants were calculated using reported kinetic parameters.

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55 Treatment of plasma with β-glucuronidase did not increase noralfentanil concentrations.

Anesthesiology, V 87, No 1, Jul 1997
3A4 activity was 216 min. The effect of diminished P450 3A4 activity on alfentanil is even greater after shorter infusions: The half-times for fentanyl and alfentanil are 28 and 199 min, and 14 and 158 min, respectively, after 60- and 30-min infusions. Although these represent relative extremes of P450 3A4 activity, the clinical implications of these differences are obvious and most important for low P450 3A4 activity. A clinician using an alfentanil infusion with a pump programmed for "typical" alfentanil kinetic parameters for a patient with unknown low P450 3A4 activity would, in essence, be using the equivalent of fentanyl (or worse). The consequence would be substantially delayed emergence, prolonged respiratory depression, or both.

Population variability in P450 3A4 activity may be attributed to individual differences in P450 3A4 expression, drug interactions, or both. The clinical effect of alfentanil kinetic dependence on P450 3A4 activity, however, is insensitive to the cause of variable 3A4 activity. Specifically, there is considerable individual genetic heterogeneity in hepatic P450 3A4 protein content and activity. Many surveys have shown differences in P450 3A4 that are routinely 10- to 20-fold and as high as 40-fold. This degree of P450 3A4 variability is sufficient to explain the 10-fold range in alfentanil clearance observed clinically. In addition, P450 3A4 is the P450 isofrom with the broadest substrate profile, responsible for the metabolism of at least 50 therapeutic and investigational drugs. Given the exquisite sensitivity of alfentanil to changes in P450 3A4 activity, alfentanil disposition appears particularly susceptible to drug interactions. The present and previous investigations demonstrate this susceptibility to drug interactions. Further, other known inhibitors of P450 3A4 activity, such as imidazole antifungals (miconazole, ketoconazole, itraconazole, fluconazole), may markedly impair alfentanil clearance. Alfentanil should be used with caution in these patients. This suggests that a significant portion of alfentanil kinetic variability may actually result from perioperative drug interactions. Large population studies are needed to assess the relation between P450 3A4 activity and alfentanil disposition.

The age-related decline in alfentanil clearance, particularly in women, is presently unexplained. This might be explained by an age-related decline in hepatic P450 3A4 activity. Nevertheless, several investigations have shown that hepatic P450 3A4 protein content and activity, and P450 3A4 activity in vivo in adult humans, do not vary age. This presents the intriguing possibility that age- and sex-related changes in alfentanil clearance may actually represent P450 3A4-related drug interactions because the number of such drugs ingested increases with age.

We have shown that cytochrome P450 3A4 is the predominant enzyme responsible for alfentanil metabolism and systemic clearance in vivo. Interindividual variability in alfentanil clearance likely results from differences in hepatic P450 3A4 expression and to P450 3A4-related drug interactions.

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Anesthesiology, V 87, No 1, Jul 1997