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Simultaneous Assessment of Drug Interactions with Low- and High-Extraction Opioids

Application to Parecoxib Effects on the Pharmacokinetics and Pharmacodynamics of Fentanyl and Alfentanil

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Background: **Parecoxib is a parenteral cyclooxygenase-2 (COX-2) inhibitor intended for perioperative analgesia. It is an inactive prodrug hydrolyzed *in vivo* to the active inhibitor valdecoxib, a substrate for hepatic cytochrome P450 3A4 (CYP3A4); hence, a potential exists for metabolic interactions with other CYP3A substrates. This study determined the effects of parecoxib on the pharmacokinetics and pharmacodynamics of the CYP3A substrates fentanyl and alfentanil compared with the CYP3A inhibitor troleandomycin. Alfentanil is a low-extraction drug with a clearance that is highly susceptible to drug interactions; fentanyl is a high-extraction drug and, thus, is theoretically less vulnerable. We therefore also tested the hypothesis that the extraction ratio influences the consequence of altered hepatic metabolism of these opioids.**

Methods: **After Institutional Review Board–approved, written, informed consent was obtained, 12 22- to 40-yr-old healthy volunteers were enrolled in the study. The protocol was a randomized, double-blinded, balanced, placebo-controlled, three-session (placebo, parecoxib, or troleandomycin pretreatment) crossover. Subjects received both alfentanil (15 μg/kg) and fentanyl (5 μg/kg; 15-min intravenous infusion) 1 h after placebo, parecoxib (40 mg intravenously every 12 h), or troleandomycin (every 6 h). Study sessions were separated by 7 or more days. Opioid concentrations in venous blood were determined by liquid chromatography–mass spectrometry. Pharmacokinetic parameters were determined by noncompartmental analysis. Opioid effects were determined by pupillometry, respiratory rate, and Visual Analog Scale scores.**

Results: **There were no significant differences between the placebo and parecoxib treatments in alfentanil or fentanyl plasma concentration, maximum observed plasma concentration, area under the plasma time–concentration time curve, clearance, elimination half-life, or volume of distribution. However, disposition of alfentanil, and to a lesser extent fentanyl, was significantly altered by troleandomycin. Clearances were reduced to 12% (0.64 ± 0.25 ml · kg^{-1} · min^{-1}) and 61% (9.35 ± 3.07) of control (5.53 ± 2.16 and 15.3 ± 5.0) for alfentanil and fentanyl ($P < 0.001$). Pupil diameter *versus* time curves were similar between placebo and parecoxib treatments but were significantly different after troleandomycin.**

Conclusions: **Single-dose parecoxib does not alter fentanyl or alfentanil disposition or clinical effects and does not appear to cause significant CYP3A drug interactions. CYP3A inhibition decreases alfentanil clearance more than fentanyl clearance, confirming that the extraction ratio influences the consequence of altered hepatic drug metabolism. Modified cassette, or "cocktail," dosing is useful for assessing drug interactions in humans.**

PARECOXIB is a highly selective cyclooxygenase-2 (COX-2) inhibitor undergoing clinical development, with intended perioperative analgesic and antiinflammatory use.[1,2] Parecoxib is a parenterally administered inactive prodrug, which rapidly hydrolyzes *in vivo* to the pharmacologically active COX-2 inhibitor valdecoxib.[2] Valdecoxib is a substrate for hepatic cytochrome P450 3A4 (CYP3A4). CYP3A4 is the most abundant cytochrome P450 in the human liver and is highly susceptible to drug interactions.[3–5] Thus, a potential exists for parecoxib (valdecoxib) interactions with other CYP3A substrates.

Alfentanil is metabolized by CYP3A enzymes, and alfentanil systemic clearance is markedly affected by alterations in CYP3A activity. Fentanyl is also a CYP3A substrate and is also susceptible (although less so) to CYP3A drug interactions. Furthermore, alfentanil is a sensitive, validated probe for CYP3A activity.[6] Assessment of a potential interaction between parecoxib and alfentanil or fentanyl is important because (1) unexpected interference with opioid clearance might unacceptably prolong clinical effects, (2) fentanyl is the most commonly used perioperative opioid, (3) alfentanil is highly susceptible to pharmacokinetic drug interactions, and (4) alfentanil clearance is an excellent probe for CY3A drug interactions in general. Therefore, the first purpose of this investigation was to examine the effects of parecoxib on the pharmacokinetics (systemic clearance) and pharmacodynamics (clinical effects, recovery profile) of fentanyl and alfentanil.

Traditional pharmacokinetic theory predicts that the clearance of high-extraction drugs will be affected by changes in hepatic blood flow and will be relatively unaffected by changes in intrinsic clearance (metabolism), while low-extraction drugs will be insensitive to hepatic blood flow changes and will be very dependent

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on intrinsic clearance. Synthetic opioids provide a unique paradigm with which to test this assumption. Although both fentanyl and alfentanil are metabolized by CYP3A, alfentanil is a low-extraction drug (extraction ratio $\approx$ 0.14),[7] and fentanyl is a high-extraction drug (extraction ratio, 0.7–1.0 in volunteers).[8–11] Theory predicts that fentanyl clearance will be less affected by CYP3A inhibition. Nevertheless, the influence of extraction ratio on the alterations in opioid disposition caused by altered intrinsic clearance is unknown. Hence, the second purpose of this investigation was to compare the effect of CYP3A inhibition on fentanyl and alfentanil disposition and systemic clearance. The known CYP3A inhibitor troleandomycin was used to test the hypothesis.

Drug interactions in humans are conventionally assessed individually (*i.e.*, the effect of one drug on another). A more cost-effective and efficient approach would be a modified cassette dosing strategy, similar to that used in animals, in which the disposition of multiple substrates is evaluated concomitantly, as is also the effects of a candidate drug on their disposition.[12] We therefore utilized simultaneous administration of fentanyl and alfentanil in a single session and assessed the efficacy of this approach. Lastly, pupillometry has recently been used as a noninvasive measure of alfentanil disposition, clearance, and hepatic CYP3A activity.[13] The third purpose of this investigation was to evaluate pupillometry as a noninvasive surrogate for opioid disposition following combination fentanyl and alfentanil administration and also to assess the effects of parecoxib on opioid pharmacodynamics.

Materials and Methods

Protocol

After obtaining Institutional Review Board (University of Washington, Seattle, Washington)–approved, written, informed consent, 13 healthy subjects (six men and seven women aged 30 $\pm$ 5 yr) within 30% of normal body weight (74 $\pm$ 15 kg) were enrolled in the study. Individuals were excluded if they were pregnant or if they were taking benzodiazepines, barbiturates, opioids, nonsteroidal antiinflammatory drugs, or drugs known to cause induction or inhibition of hepatic P450 enzymes. All subjects fasted for a minimum of 6 h prior to opioid administration. Sample sizes were based on alfentanil systemic clearance, which was the primary outcome variable. In order to detect a 30% difference in alfentanil clearance between parecoxib and placebo with 80% power at a significance level of 0.05, a sample size of 12 subjects was needed.[14,15]

The design was a randomized, placebo-controlled, double-blinded, balanced, two-sequence, three-session (control, parecoxib, or troleandomycin pretreatment) crossover. Each subject served as his or her own control and underwent physical and laboratory examination (hematology, biochemistry, urinalysis, hepatitis B surface antigen test, drug toxicology tests) prior to and after the completion of the study. Subjects were randomized to one of two sequences: parecoxib, placebo, and troleandomycin pretreatments, in that order, each separated by 7 days, or placebo, parecoxib, and troleandomycin pretreatments. This sequence was used because a longer washout was needed after troleandomycin than after placebo or parecoxib. Placebo (normal saline) or intravenous parecoxib (40 mg) was administered 1 h before the opioid infusion and again 12 h later. The dose and timing of parecoxib administration was selected to mimic intended clinical use (typically an hour before induction of anesthesia). Oral troleandomycin (500 mg) was given 1.75 h prior to opioid infusion followed by an additional 250-mg dose every 6 h relative to the first dose (four troleandomycin doses total) to ensure maximal CYP3A inhibition. Parecoxib and placebo pretreatments were double blinded. Since troleandomycin was administered orally, it was not blinded.

For each study session, a peripheral intravenous catheter was inserted in each arm for drug administration and blood sampling. Supplemental oxygen and monitoring (electrocardiography, blood pressure measurement, pulse oximetry) were provided for all subjects. A trained independent observer, who was blinded to the purpose of the investigation and the identity of the drug pretreatment, was present throughout the study period to record hemodynamic and other effect data and to administer the psychomotor tests. Subjects received a 15-min opioid infusion (15 μg/kg alfentanil and 5 μg/kg fentanyl) after the pretreatment. Droperidol (0.625-mg intravenous bolus) was administered at the initiation of the opioid infusion. The end of the opioid infusion was designated as time zero. Venous blood samples for opioid measurement were obtained at baseline, 0 (end infusion), 1, 3, 5, 10, 15, 30, 45, 60, 90, 120, 240, 360, 480, 600, 720, 960, 1,200, and 1,440 min after opioid administration. Venous blood samples for parecoxib, valdecoxib (SC-65872), and 1-hydroxyvaldecoxib (SC-66905) concentrations were drawn during the placebo or parecoxib session at predose, 15, 30, 60 (prior to opioid infusion), 240, 480, and 720 (prior to evening parecoxib/placebo dose) min after parecoxib. The samples were centrifuged, and the plasma was removed and stored at -20°C until analysis.

Analytical Methods

Fentanyl and alfentanil assays were performed at Triangle Laboratories (Durham, North Carolina). Alfentanil, fentanyl, and the internal standard (fentanyl-d5) were removed from the plasma by solid phase extraction and were analyzed using high-pressure liquid chromatography–tandem mass spectrometry with multiple reaction monitoring. Quantitation was performed using separate weighted ($1/x^2$) linear least-squares regression lines gen-

erated from plasma calibration samples. The method demonstrated acceptable linearity, precision, and accuracy in the ranges of 0.25–50 ng/ml for alfentanil and 0.05–10 ng/ml for fentanyl.

Plasma concentrations of parecoxib, valdexocib, and 1-hydroxyvaldecoxib were determined by liquid chromatography–tandem mass spectrometry using a validated assay. After adding the $^{13}C_6$ respective internal standards, analytes were removed using a C8 solid phase extraction column. Analytes (> 98% recovery) were separated by reversed-phase high-pressure liquid chromatography on a C18 column, detected by multiple reaction monitoring, and quantified using standard curves of peak area ratios (*vs.* respective internal standards). Assay ranges were 0.5–200 ng/ml for valdecoxib and 1-hydroxyvaldecoxib and 5–2,000 ng/ml for parecoxib. Coefficients of variation for valdecoxib, 1-hydroxyvaldecoxib, and parecoxib were 10, 9, and 3% (interday) and 14, 9, and 12% (intraday), respectively, at the limit of quantitation.

Clinical Effects

Subjective self-assessment of feelings or mood states and quality of recovery was quantified by Visual Analog Scale (VAS). Attributes assessed (and scored from 0 to 100) included level of alertness or sedation (almost asleep to wide awake), energy level (no energy to full of energy), confusion (confused to clear headed), clumsiness (extremely clumsy to well coordinated), anxiety (calm and relaxed to extremely nervous), and nausea (no nausea to worst nausea). This test was given at baseline (prior to parecoxib–placebo), prior to alfentanil–fentanyl infusion, and at 0 (end of infusions), 15, 30, 60, 120, 240, and 360 min after alfentanil–fentanyl infusion.

Pupil size permits easy, reproducible, and noninvasive measurement of opioid pharmacodynamic effect.[13] Dark-adapted pupil diameters were measured using infrared pupillometry (PupilScan, model 6; Fairville Medical Optics, Inc., Newark, NJ) prior to the parecoxib or placebo dose, prior to opioid infusion, and at 0 (end of infusions), 5, 15, 30, 45, 60, 90, 120, 180, 240, and 360 min after opioid infusion. The reported measurements were the average of three pupil diameter readings. The pupil diameter measurement obtained prior to the opioid infusion was used as the baseline value.

Vital signs (blood pressure, respiration rate, heart rate) were measured prior to the fentanyl and alfentanil infusions and at 0 (end of infusion), 15, 30, 60, 120, and 240 min after the infusion.

Data Analysis

Fentanyl and alfentanil pharmacokinetic parameters for each subject were determined by noncompartmental analysis with an intravenous infusion model using WinNonlin (Pharsight, Palo Alto, CA). Area under the plasma time–concentration time curve extrapolated to infinity ($AUC_{0\text{-(infinity)}}$), area under the plasma time–concentration time curve to the last quantifiable concentration ($AUC_{0\text{-loq}}$), maximum observed plasma concentration (C_{MAX}), time to maximum plasma concentration (T_{MAX}), terminal elimination half-life (T1/2), terminal elimination rate constant (Kel), plasma clearance (CL), distribution volume (VDss), and area under the effect curve (AUEC; determined as the area under percent decrement in pupil diameter from baseline to the time of the last measurement, using the trapezoidal rule) were compared by parametric or nonparametric (for nonnormal variance) repeated measures analysis of variance (RMANOVA) using SigmaStat (SPSS Inc., Chicago, IL) or SAS 6.12 (SAS Institute Inc., Cary, NC). In addition, in the ANOVA model, the sources of variation included were sequence (one or two), subjects nested within sequence, period (one or two), and treatment (placebo *vs.* parecoxib). Effects due to subject were random, while all other effects were fixed. Sequence effect was tested by subject nested within sequence as the between-subject error term in the denominator of the F statistic. All other effects were tested by the within-subject mean square error from the ANOVA model. Within the ANOVA, pairwise comparison was performed to assess the effects of parecoxib on the pharmacokinetics of fentanyl or alfentanil. A point estimate and 90% confidence intervals (CIs) were obtained for the difference in treatment natural logarithmic means. This point estimate and the lower and upper limits of the CIs were exponentiated to obtain an estimate of the relative ratios. Repeated measures ANOVA was performed on respiratory rate and pupil diameter using treatment groups and measurement times as factors and their interactions. Student–Newman–Keuls method for multiple comparisons was used. One-way ANOVA or Kruskal–Wallis one-way ANOVA on ranks was performed on VAS measurements, and Bonferroni correction was applied. All results are reported as mean ± SD (SD). All statistical tests were performed at $\alpha = 0.05$.

Pharmacokinetic Theory

The theoretical effect of altered hepatic intrinsic clearance (CL_{int}) on drug disposition, expressed as the plasma AUC, was calculated as described by Lin and Lu.[16] To calculate CL_{int} for any drug, based on its extraction ratio (ER, or E_H):

$$E_H = \frac{CL_{int}}{Q_H + CL_{int}}$$

hence

$$CL_{int} = \frac{E_H \cdot Q_H}{1 - E_H}$$

Thus, for example, assuming hepatic blood flow (Q_H) is 1,500 ml/min, CL_{int} for a drug with $E_H = 0.1$ is 165 ml/min, and CL_{int} for $E_H = 0.85$ is 8,500 ml/min.

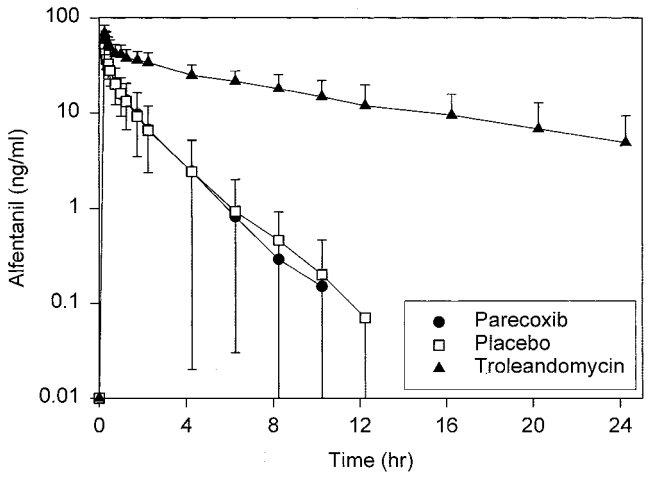

Fig. 1. Alfentanil plasma concentrations after placebo (□), parecoxib (●), and troleandomycin (▲) pretreaments. Time zero designates the beginning of the opioid infusion.

For an intravenously administered drug, using the well-stirred model, and ignoring the effects of protein binding (assuming fu = 1),

$$\text{AUCiv} = \frac{\text{dose}}{\text{CL}_\text{H}} = \frac{\text{dose}}{\frac{[\text{Q}_\text{H} \cdot \text{CL}_\text{int}]}{[\text{Q}_\text{H} + \text{CL}_\text{int}]}}$$

where CL_H and CL_{int} are hepatic clearance and intrinsic clearance, respectively. To compare the AUCs after induction or inhibition (AUCx) to that of control (AUCc):

$$\frac{\text{AUCx}}{\text{AUCc}} = \frac{\text{dose}/\text{CL}_\text{Hx}}{\text{dose}/\text{CL}_\text{Hc}} = \frac{\text{CL}_\text{Hc}}{\text{CL}_\text{Hx}} = \frac{\frac{\text{Q}_\text{H} \cdot \text{CL}_\text{intc}}{\text{Q}_\text{H} + \text{CL}_\text{intc}}}{\frac{\text{Q}_\text{H} \cdot \text{CL}_\text{intx}}{\text{Q}_\text{H} + \text{CL}_\text{intx}}}$$

For any drug, one can calculate a family of AUCx/AUCc values based on varying degrees of inhibition (or induction) of CL_{int}, expressed as CL_{intx}/CL_{intc}.

Results

Thirteen subjects were enrolled in the study. One woman voluntarily withdrew after one (parecoxib) session because of opioid-induced side effects. Twelve subjects (six men and six women) completed the study. Data from subjects who completed all treatment periods were included in the statistical analyses (N = 12).

Alfentanil plasma concentrations in the control and parecoxib-treated subjects were superimposable (fig. 1). There was no significant difference between parecoxib and control in any alfentanil pharmacokinetic parameter (table 1). The ratios of the alfentanil geometric least-squares means (parecoxib–placebo) for plasma CMAX, $AUC_{0\text{-(infinity)}}$, and CL ranged from 1.00 to 1.04, and the 90% CIs contained the equality point 1.0. The *P* values comparing the geometric least-squares means were ≥ 0.65, indicating that the two treatments were not statistically significantly different.

Fentanyl plasma concentrations in the control and parecoxib-treated subjects were also indistinguishable (fig. 2). There was no significant difference between parecoxib and control in any fentanyl pharmacokinetic parameter (table 1). The ratios of fentanyl geometric least-squares means (parecoxib–placebo) for plasma CMAX, $AUC_{0\text{-(infinity)}}$, $AUC_{0\text{-loq}}$, and CL were 0.94, 1.02, 1.05, and 0.99, respectively, and the 90% CIs contained the equality point 1.0. The *P* values comparing the geometric least-squares means were ≥ 0.27, indicating that

Table 1. Alfentanil and Fentanyl Pharmacokinetic Parameters

	Placebo (n = 12)	Parecoxib (n = 12)	Troleandomycin (n = 12)
Alfentanil			
$AUC_{0\text{-}\infty}$, $h \cdot ng^{-1} \cdot m^{-1}$	54.4 ± 29.2	55.5 ± 32.6	469 ± 244*
$AUC_{0\text{-loq}}$, $h \cdot ng^{-1} \cdot ml^{-1}$	53.4 ± 28.8	54.4 ± 32.1	393 ± 149*
Cmax, ng/ml	54.5 ± 13.7	55.4 ± 14.3	71.7 ± 14.3*
Tmax, h	0.26 ± 0.02	0.27 ± 0.01	0.26 ± 0.02
Kel, l/h	0.65 ± 0.20	0.66 ± 0.20	0.10 ± 0.04*
T1/2, h	1.19 ± 0.49	1.15 ± 0.39	7.98 ± 3.38*
CL, $ml \cdot kg^{-1} \cdot min^{-1}$	5.53 ± 2.16	5.76 ± 2.73	0.64 ± 0.25*
VDss, ml/kg	401 ± 76	408 ± 121	368 ± 76
Fentanyl			
$AUC_{0\text{-}\infty}$, $h \cdot ng^{-1} \cdot m^{-1}$	6.04 ± 2.19	6.08 ± 2.17	9.94 ± 3.77*
$AUC_{0\text{-loq}}$, $h \cdot ng^{-1} \cdot ml^{-1}$	5.08 ± 1.51	5.29 ± 1.83	7.59 ± 2.19*
Cmax, ng/ml	3.6 ± 1.1	3.2 ± 1.2	3.1 ± 1.0
Tmax, h	0.26 ± 0.02	0.26 ± 0.03	0.26 ± 0.02
Kel, l/h	0.117 ± 0.073	0.125 ± 0.077	0.061 ± 0.015*
T1/2, h	8.14 ± 4.1	7.44 ± 3.6	12.1 ± 3.3*
CL, $ml \cdot kg^{-1} \cdot min^{-1}$	15.3 ± 5.0	14.9 ± 4.2	9.35 ± 3.07*
VDss, ml/kg	7,200 ± 2,400	7,120 ± 2,470	7,670 ± 2,400

* $P < 0.05$ *vs.* placebo.

$AUC_{0\text{-}\infty}$ = area under the plasma time–concentration time curve extrapolated to infinity; $AUC_{0\text{-loq}}$ = area under the plasma time–concentration time curve to the last quantifiable concentration; CL = plasma clearance; Cmax = time to maximum plasma concentration; Kel = terminal elimination rate constant; T1/2 = terminal elimination half-life; Tmax = time to maximum plasma concentration; VDss = distribution volume.

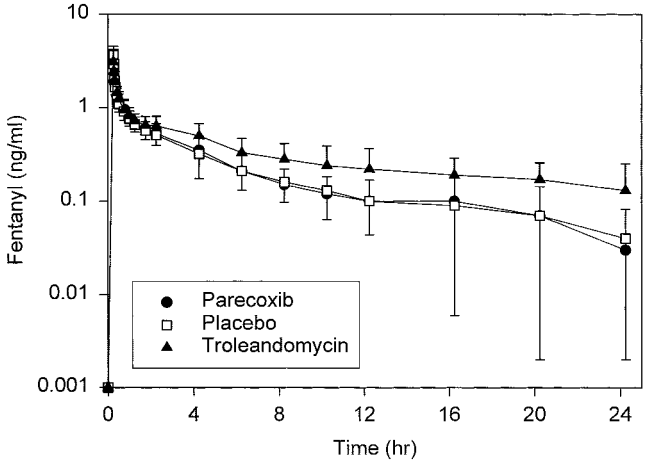

Fig. 2. Fentanyl plasma concentrations after placebo (□), parecoxib (●), and troleandomycin (▲). Time zero designates the beginning of the opioid infusion.

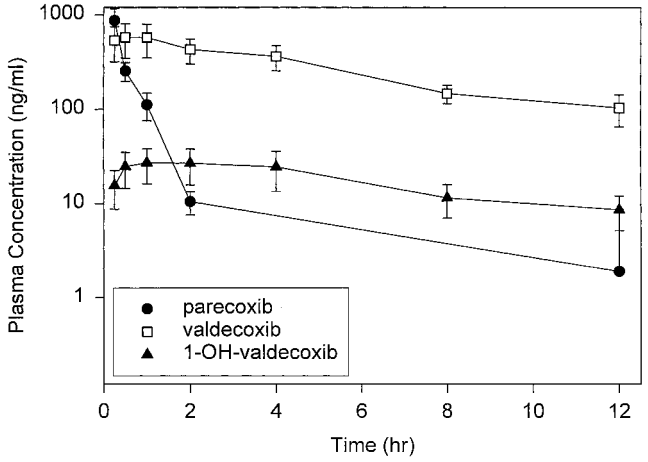

Fig. 3. Plasma concentrations of parecoxib and metabolites. Time zero denotes parecoxib injection.

the two treatments were not statistically significantly different.

In the troleandomycin-treated subjects, mean alfentanil concentrations were substantially higher (fig. 1), and alfentanil CL was significantly lower compared with placebo (table 1). The ratios of alfentanil geometric least-squares means (troleandomycin–placebo) for plasma C_{MAX}, $AUC_{0\text{-(infinity)}}$, and CL were 1.31, 8.83, and 0.12, respectively. None of the 90% CIs contained the equality point 1.0, and the *P* values comparing the geometric least-squares means were all < 0.001, indicating that the two treatments were significantly different. Fentanyl plasma concentrations, $AUC_{0\text{-(infinity)}}$, and $AUC_{0\text{-loq}}$ were significantly higher (fig. 2), and CL was significantly diminished (table 1) by troleandomycin inhibition of hepatic CYP3A. The ratios of fentanyl geometric least-squares means (troleandomycin–placebo) for plasma $AUC_{0\text{-(infinity)}}$, $AUC_{0\text{-loq}}$, and CL were 1.66, 1.51, and 0.62, respectively. The 90% CI for these parameters did not contain the equality point, and the *P* value comparing the geometric least-squares means was < 0.001, indicating that they were significantly different between groups.

Parecoxib and metabolite concentrations are shown in figure 3. Opioid effects on pupil diameters are shown in figure 4. Compared with placebo, parecoxib had no effect on the time course of miosis after opioid infusion, and summary statistics for pupil diameters were not different between parecoxib and placebo for any parameter (table 2). In contrast, the time course of miosis in troleandomycin-treated subjects was significantly different from placebo, and the AUEC was significantly greater, without a change in maximal effect.

Parecoxib had no influence on opioid effects. No significant differences in VAS scores were observed for any of the clinical effect parameters (anxiety, clumsiness, confusion, energy level, or sedation) at any time point (data not shown). Similarly, VAS scores after troleandomycin were not different from those after placebo. The respiratory rate decreased from baseline means of 12–14 breaths/min to 8 breaths/min at the end of the opioid infusion but did not differ among parecoxib-treated, placebo-treated, and troleandomycin-treated subjects. Respiratory rates of less than 5 breaths/min were common in all groups at the end of the opioid infusion but no longer occurred at the 15-min postdose time point. Occasional apnea was observed at the end of the opioid infusion; however, all subjects breathed in response to verbal reminders. Nausea was common (one third of placebo-treated and parecoxib-treated subjects and twofold greater in troleandomycin-treated subjects); however, emesis only occurred in one (troleandomycin-treated) subject.

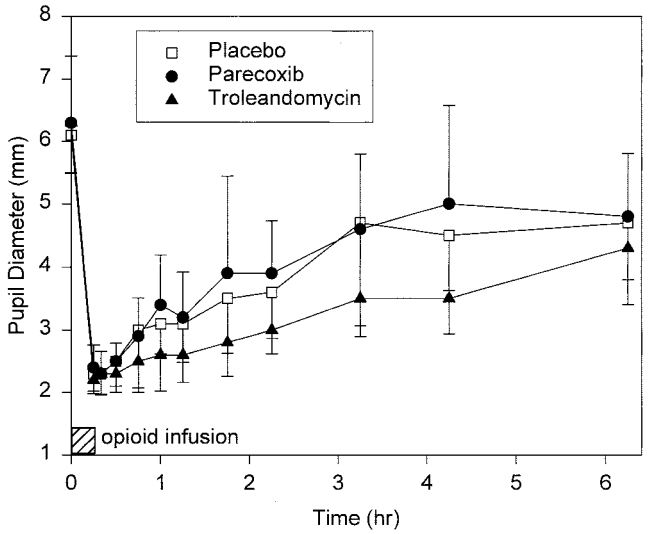

Fig. 4. Time course of opioid effects on pupil diameter after placebo (□), parecoxib (●), and troleandomycin (▲) pretreatments. Time zero designates the beginning of the opioid infusion. Pretreatment with troleandomycin was significantly different from placebo or parecoxib ($P < 0.05$).

Table 2. Summary Statistics of Pupil Diameter Measurements

Parameter	Placebo	Parecoxib	Troleandomycin
AUEC, % decrement/h	206 ± 67	202 ± 102	298 ± 53*
EMAX, % decrement	64 ± 3	65 ± 6	66 ± 5
Time to EMAX, h	0.36 ± 0.11	0.37 ± 0.15	0.47 ± 0.35
Time to return-to-baseline, h	4.8 ± 2.2	4.0 ± 1.9	6.2†

* $P = 0.005$. † Only one subject returned to baseline.

AUEC = area under the effect curve; EMAX = maximum observed percent decrement.

Discussion

Drug interactions are traditionally investigated by comparing plasma concentrations and pharmacokinetic parameters (and sometimes drug effects) with and without a potentially interacting compound. In investigating opioid interactions, for example, the effects of troleandomycin on alfentanil disposition[6] and of oral ritonavir on fentanyl disposition[11] were examined in this manner. In contrast, the present investigation utilized simultaneous administration of multiple substrates to a single individual (cassette dosing, "N-in-one" dosing, or a "cocktail" approach) to concomitantly evaluate multiple potential drug interactions.[12,17] Advantages of this approach include the ability to evaluate the pharmacokinetics of several drugs under identical conditions, the elimination of interday variability, a reduction of the risk to subjects by decreasing the number of study sessions, and a reduction of time and expense by minimizing study sessions, blood collection, and sample analysis. This is the first known use of cassette dosing to evaluate drug interactions with multiple opioids in humans.

The cassette dosing strategy was used to assess opioid drug interactions with parecoxib, a COX-2 inhibitor metabolized by CYP3A4. Alfentanil and fentanyl were studied because (1) fentanyl is the most commonly used perioperative opioid, and drug development guidelines require assessment of potential drug interactions; (2) alfentanil is very susceptible (perhaps the most susceptible opioid) to drug interactions, which carry significant clinical consequence; (3) alfentanil is an excellent *in vivo* probe in general for CYP3A drug interactions; and (4) alfentanil is a low-extraction opioid, while fentanyl is a high-extraction opioid, thereby permitting study of the full extremes of opioid disposition. Single-dose parecoxib given 1 h prior to opioid administration (to simulate the approximate timing of preoperative dosing) had no effect on the plasma disposition, pharmacokinetic parameters, time course of effect, or pharmacodynamics (concentration–effect relationship) of alfentanil or fentanyl. These results suggest that parecoxib will not have significant perioperative drug interactions with these opioids, and, in general, parecoxib and its active metabolite, valdecoxib, are not CYP3A4 inhibitors. This finding is consistent with the absence of parecoxib effects on the clearance of midazolam, another widely used CYP3A probe.[18]

Two caveats regarding the cassette dosing strategy merit mention. First, cassette dosing assumes (requires) the absence of interactions between the drugs in the cassette, consequently manifested by concordance between the pharmacokinetic parameters obtained from cassette dosing and from conventional single-drug dosing.[12] In the present investigation, since both alfentanil and fentanyl are metabolized by CYP3A, they may theoretically compete for metabolism and elimination. Nonetheless, since the concentrations of both opioids are at least an order of magnitude lower than their respective Km values for hepatic metabolism, no metabolic pharmacokinetic interaction would be expected.[19] Indeed, the alfentanil and fentanyl clearances observed herein were commensurate with those obtained with individual dosing; hence, the cassette strategy with these opioids is valid. Second, cassette dosing must be safe. Simultaneous fentanyl and alfentanil administration, in doses sufficient to permit analytical quantification and administered as a 15-min infusion, was safe and devoid of significant adverse effects. Clinical effects were mainly sedation, respiratory depression, and nausea. Sedation was limited; some subjects briefly hypoventilated, but this did not require intervention other than a verbal reminder; and nausea was not uncommon. Ondansetron prophylaxis, rather than droperidol, might decrease the incidence of nausea.

Another aspect of this investigation was the assessment of opioid effects and pharmacokinetics. Conventional pharmacokinetic interaction studies, whether single or cassette dosing, require invasive arterial or venous access, frequent blood draws, and expensive analytical techniques (particularly for cassette dosing). The time and expense associated with drug concentration measurements has prompted the search for an accurate, sensitive, noninvasive surrogate for plasma drug concentrations. For example, other pharmacokinetic–pharmacodynamic studies have used electroencephalogram slowing[20] or respiratory depression[21] as a measure of opioid effect. However, high opioid plasma concentrations are required for electroencephalogram effects, with potential for serious side effects, such as respiratory depression and skeletal muscle rigidity. Opioid-induced miosis has been investigated as a surrogate for plasma concentration. Miosis is sensitive to low opioid concentrations; pupil diameter can be measured accurately,

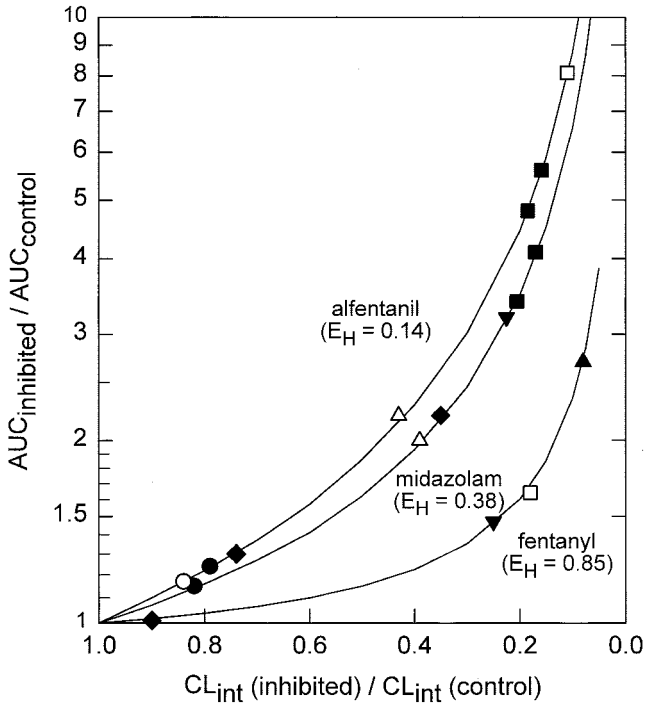

Fig. 5. Role of extraction ratio in the influence of altered intrinsic clearance on drug disposition. Solid lines show the theoretical relationship between the area under the plasma time–concentration time curve (AUC) and the inhibition of intrinsic clearance (expressed as ratios relative to the uninhibited state) for low-extraction drugs (alfentanil; hepatic extraction ratio [E_H] = 0.14), intermediate-extraction drugs (midazolam; E_H = 0.38), and high-extraction drugs (fentanyl; E_H = 0.85, based on values from studies in volunteers[9–11]), calculated as described in Materials and Methods. The clinical consequences (change in AUC) of inhibiting hepatic intrinsic clearance depend on the extraction ratio, with the disposition of high-extraction drugs comparatively less affected by changes in intrinsic clearance. Ratios of plasma AUC (or observed clearance ratios, if AUC data were not available) were determined from published data for erythromycin (◇; the erythromycin–fentanyl interaction has not been studied, so data for sufentanil, also a high-extraction drug, are shown instead),[27,40,41] propofol (○),[42] diltiazem (●),[43] fluconazole (△),[31,44] itraconazole (▼),[10,44] ritonavir (▲),[11] troleandomycin,[6,13] and multidose troleandomycin (this investigation) and from supplemental data for itraconazole provided by Klaus Olkkola, M.D. (Associate Professor, Department of Anaesthesia and Intensive Care Medicine, Helsinki University Central Hospital, Helsinki, Finland; written communication, December 2001). Measured AUC ratios (ordinate) are plotted where they intersect the theoretical lines, which then provides predicted ratios for intrinsic clearance (abscissa). Although the effect of a given inhibitor on AUC will vary with the substrate, the inhibitor should cause similar reductions in intrinsic clearance. This is seen with diltiazem, fluconazole, itraconazole, and troleandomycin.

frequently, and noninvasively with a commercial infrared pupillometer more easily than analgesia or respiratory depression; and results are obtained in real time. Miosis has been used as a surrogate for plasma alfentanil concentration.[13] The clinical effect and time course of miosis closely approximated plasma alfentanil concentrations, and there was a significant correlation between alfentanil effect AUC and plasma AUC. This suggested that alfentanil effect kinetics might be used as a noninvasive surrogate for conventional plasma pharmacokinetics, and alfentanil effect clearance might be a noninvasive *in vivo* probe for hepatic CYP3A activity.[13] Others have investigated the relationship between miosis and other opioid plasma concentrations. Pupil diameter was significantly correlated with opioid-induced respiratory depression.[22] Decreased plasma morphine concentrations were associated with significantly reduced pupillary effects.[23] An inverse relationship was found between methadone plasma concentrations and pupil diameter.[24] Morphine, morphine-6-glucuronide, and morphine-3-glucuronide were compared using pupil diameter as a measure of central opioid effect.[25,26] The present investigation supports miosis as a reflection of opioid concentrations and pharmacokinetic data and as a noninvasive measure of opioid drug interactions. In concordance with the pharmacokinetic data, parecoxib had no effect on fentanyl–alfentanil miosis, whereas troleandomycin altered the time course of miosis. Since the concentration–response (pharmacodynamic) relationship was unaffected, altered pharmacokinetics explains the troleandomycin effects on miosis. Thus, the present findings suggest that analytical opioid assays may be foregone or at least more selectively targeted if miosis is unchanged. However, if an interaction does alter miosis, then one would proceed with analytical assays to identify and quantify the interaction. Further investigations are required to identify the sensitivity and specificity of miosis as a preanalytic screening method for opioid cassette dosing studies. Nevertheless, such noninvasive prescreening represents an additional potential gain over those achieved by cassette dosing alone for evaluating opioid and CYP3A drug interactions.

The piperidine synthetic opioids all undergo extensive CYP3A-catalyzed metabolism. Whereas alfentanil is very sensitive to drug interactions, fentanyl (and sufentanil) are relatively insensitive, a property classically attributed to differences in extraction ratio.[10,27] Alfentanil disposition is dependent on intrinsic clearance,[6] elimination is independent of hepatic blood flow,[28] alfentanil metabolism *in vitro*[29,30] and *in vivo*[6] is catalyzed predominantly by CYP3A, and alfentanil clearance is affected by the CYP3A inhibitors fluconazole[31] and troleandomycin.[6] Fentanyl is also cleared predominantly by hepatic metabolism.[32,33] CYP3A is the predominant P450 isoform[34,35]; however, fentanyl is a high-extraction drug. Conventional pharmacokinetic theory predicts that fentanyl clearance will be unaffected by changes in intrinsic clearance (metabolism). Consistent with this hypothesis, the strong CYP3A inhibitor itraconazole minimally affected fentanyl pharmacokinetics.[10] In contrast, however, other evidence suggests that alterations in intrinsic hepatic metabolism may have an effect on fentanyl systemic clearance. For example, the potent CYP3A inhibitor ritonavir did reduce fentanyl clearance by 67%.[11] Furthermore, patients receiving anticonvulsant therapy had increased fentanyl requirements and fentanyl sus-

ceptibility to drug interaction.[36] Moreover, the pharmacokinetics of other high-extraction drugs can be affected by changes in hepatic metabolism. For example, the clearance of tirilazad (extraction ratio > 0.9[37]), which is extensively metabolized by CYP3A,[38] is markedly affected by changes in CYP3A activity.[39] Despite years of conjecture, the hypothesis that extraction ratio determines the extent to which altered metabolism affects opioid clearance remains untested. Simultaneous determination of altered CYP3A effects on alfentanil and fentanyl disposition in the present investigation permitted evaluation of the hypothesis. CYP3A inhibition did decrease fentanyl clearance (to 61% of control); however, the extent was much less than the reduction in alfentanil clearance (to 12% of control). Thus, extraction ratio does determine the pharmacokinetic consequence of altered intrinsic clearance.

Theoretical and actual relationships between intrinsic clearance and disposition are shown in figure 5 for fentanyl, alfentanil, and midazolam (for comparison). Based on the well-stirred model, and ignoring the effects of protein binding, clinically significant reductions in systemic clearance (25% increase in AUC) would occur with a 20% decrease in alfentanil intrinsic clearance but not until a 65% decrease with fentanyl. Reduction of intrinsic clearance, such as by diltiazem, fluconazole, itraconazole, and troleandomycin, has markedly less effect on the clearance of fentanyl compared with alfentanil.

In summary, these results show that bolus parecoxib has no effect on the disposition or clinical effects of alfentanil or fentanyl, which represent the spectrum from low- to high-extraction opioids. Parecoxib does not appear to cause significant CYP3A drug interactions. CYP3A inhibition causes a greater decrease in alfentanil clearance compared with fentanyl clearance. Cassette dosing represents a novel and useful approach to assessing clinical drug interactions. Pupillometry may play a role as a surrogate or screening device for plasma concentrations in cassette dosing, as well as in conventional single-drug dosing.

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