Anesthesiology
77:467–474, 1992

Identification of the Pharmacogenetic Determinants of Alfentanil Metabolism: Cytochrome P-450 3A4

An Explanation of the Variable Elimination Clearance

Chul-Ho Yun, Ph.D., Margaret Wood, M.B.Ch.B., F.F.A.R.C.S.,† Alastair J. J. Wood, M.B.Ch.B., F.R.C.P.(Edin),‡ F. Peter Guengerich, Ph.D.§*

There is considerable variability in the elimination clearance of the opioid analgesic alfentanil. It has been shown previously that alfentanil clearance is independent of the polymorphic debrisoquine hydroxylase (P-450 2D6), and it is therefore of interest to identify the human cytochrome P-450 enzymes involved in noralfentanil formation, the primary reaction involved in the oxidative N-dealkylation at the piperidine nitrogen. Purified human P-450 3A4 showed appreciable catalytic activity, and yeast recombinant P-450 3A4 also showed alfentanil oxidation activity. When microsomes prepared from different human liver samples were compared, noralfentanil formation activity was well correlated ($r = 0.95$, $P < 0.005$) with nifedipine oxidation (a P-450 3A4 marker) but not with markers of other P-450s, including phenacetin O-deethylation (P-450 1A2), chlorzoxazone 6-hydroxylation (P-450 2E1), and (*S*)-mephenytoin 4′-hydroxylation (a P-450 2C enzyme). Using antibodies that recognize specific human P-450 enzymes (immunoinhibition techniques), it was possible to demonstrate that anti–P-450 3A4 nearly completely inhibited alfentanil oxidation activity in the human liver microsomes, but no other antibodies showed a measurable inhibitory effect. Selective chemical inhibitors of P-450 3A4, gestodene and troleandomycin, inhibited as much as 90% of the microsomal noralfentanil formation activity, but other chemical inhibitors did not show a detectable inhibitory effect. 7,8-Benzoflavone inhibited as much as 90% of the alfentanil oxidation activity of the microsomal or reconstituted P-450 3A4 system. This work indicates that P-450 3A4 contributes significantly to human liver microsomal alfentanil oxidation, whereas P-450 2D6 does not contribute. Human P-450 3A4 metabolizes many clinically important substrates, including nifedipine, cyclosporin, midazolam, lidocaine, and quinidine, and is induced by barbiturates and some antibiotics. Therefore, the possibility exists for pharmacokinetic interactions between alfentanil and other concomitantly administered drugs that are also substrates for, or inducers of, P-450 3A4. (Key words: Analgesics: alfentanil. Anesthetics, intravenous: alfentanil. Biotransformation: alfentanil. Liver: microsomes. Metabolism: cytochromes P-450, P-450 3A4, P-450 2D6; genetic factors; immunoinhibition.)

This article is accompanied by an editorial. Please see: Gonzalez FJ: *In vitro* systems for prediction of rates of drug clearance and drug interactions. ANESTHESIOLOGY 77:413–415, 1992.

ALTHOUGH THE USE of the synthetic opioid alfentanil has become well established in anesthetic practice, there is considerable interindividual variability in the plasma concentration time profile among patients, resulting in marked difference among individuals in the rate of alfentanil infusion required.[1] Using alfentanil population pharmacokinetic parameters, Maitre *et al.* determined that after taking into account age, weight, and gender, 48% of the variability in alfentanil clearance remained unexplained.[2] Although intersubject variability in drug metabolism has been known for many years, it is only recently that the pharmacogenetic determinants of drug oxidation have been defined.[3] A defect in the ability to hydroxylate the antihypertensive agent debrisoquine is present in 5–10% of Caucasians,[4] and individuals can be classified as either extensive metabolizers or poor metabolizers because of genetic polymorphism.

The clinical importance of this genetically determined impairment of debrisoquine oxidation stems from the wide spectrum of drugs the metabolism of which is also impaired in poor metabolizers of debrisoquine. However, Henthorn *et al.*[5] demonstrated that alfentanil clearance is not altered in poor metabolizers of debrisoquine and is thus independent of the polymorphic enzyme debri-

* Research Associate, Biochemistry.

† Professor of Anesthesiology; Associate Professor of Pharmacology.

‡ Professor of Medicine; Professor of Pharmacology.

§ Professor of Biochemistry; Director, Center in Molecular Toxicology.

Received from the Departments of Anesthesiology, Pharmacology, and Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee. Accepted for publication April 1, 1992. Supported in part by United States Public Health Service grants CA 44353 and ES 00267 (FPG) and GM 31304 (AJJW, MW).

Address reprint requests to Dr. Wood: Department of Anesthesiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2125.

soquine 4-hydroxylase (cytochrome P-450 2D6).¶ In contrast to the bimodal distribution of enzyme function associated with debrisoquine genetic polymorphism, it appears that the wide interindividual activity of the enzyme cytochrome P-450 3A4, which has been shown to be pivotal for the metabolism of a wide variety of drugs, including nifedipine, midazolam, lidocaine, cyclosporin and erythromycin, exhibits a broad unimodal distribution of enzyme activity without a distinct subgroup.[6]

Because the variability of alfentanil pharmacokinetics demonstrated by Maitre *et al.*[2] also did not appear to be polymorphic, we hypothesized that alfentanil oxidation was more likely to be catalyzed by the enzyme cytochrome P-450 3A4. With the use of antibodies that recognize different human cytochrome P-450 enzymes, it is possible to identify the specific enzymes involved in reactions of alfentanil metabolism. Thus, the goal of the present study was to define the enzymes in human liver that are responsible for alfentanil oxidation by using immunoinhibition and recombinant techniques, including expression of the cytochrome P-450 of interest in yeast.

Materials and Methods

Chemicals

[^{3}H]-Alfentanil (12.3 Ci/mmol), labeled with ^{3}H at the 3-position of the phenyl ring, and its unlabeled metabolites were obtained from Janssen Research Foundation (Beerse, Belgium); [^{3}H]-alfentanil was > 99.9% radiochemically pure as judged by reverse-phase high performance liquid chromatography (HPLC) and was used without further purification. 7,8-Benzoflavone and quinidine were purchased from the Aldrich Chemical Co. (Milwaukee, WI). Gestodene was provided by Professor H. Kuhl (University of Frankfurt). Troleandomycin was a gift of Professor P. H. Beaune (Necker Institut National de la Santé et de la Recheruhe Médicale, Paris). Sulfaphenazole was obtained as a gift from the Meiji Yakuhin Co. Ltd. (Tokyo). Other chemicals were of the highest grade commercially available.

Enzyme Preparations and Antibodies

Human liver samples were obtained from organ donors through Tennessee Donor Services (Nashville, TN; denoted "HL" for human liver and including a code number). Microsomes were prepared as described elsewhere.[7]

Human P-450 3A4,[8] P-450 2C8,[9] P-450 $2C_{MP}$ (a P-450 2C enzyme with (*S*)-mephenytoin 4′-hydroxylase),[10] P-450 2E1,[11] P-450 1A2,[12] and P-450 2D6,[12] were prepared as described elsewhere. Rabbit liver NADPH–P-450 reductase was purified essentially as described elsewhere.[13]

Rabbit anti–P-450 3A4,[8] anti–P-450 2C,[14] anti–P-450 2E1,[15] anti–P-450 1A2,[12] and anti–P-$450_{UT\text{-}H}$ (2D1)[16] preparations have been characterized previously.

Yeast (*Saccharomyces cerevisiae*) expressing P-450 3A4 came from previous work,[17] and the protein was partially purified using slight modifications of a method described elsewhere.[8,17]

Assays

Protein was assayed using a bicinchoninic acid procedure according to the manufacturer's directions (Pierce Chemical Co., Rockford, IL). P-450 concentrations were determined by Fe^{2+}-CO *versus* Fe^{2+} difference spectroscopy.[18]

Noralfentanil formation was analyzed with the procedure of Lavrijsen *et al.*[19] with slight modifications. Each of the purified P-450s (10–50 pmol) was reconstituted immediately before use in the enzyme assays in 0.5 ml of 50 mM potassium phosphate buffer (*p*H 7.4) containing 30 μM L-α1,2-dilauroyl-*sn*-glycerol-3-phosphocholine and a 2-fold molar excess of rabbit liver NADPH–P-450 reductase. In the case of microsomal assays, the microsomal protein (100 μg) was incubated in 0.5 ml 50 mM potassium phosphate buffer (*p*H 7.4). After a 3-min preincubation in the presence of alfentanil (10 μM), the reactions were initiated by the addition of an NADPH-generating system using open glass vials in a shaking water bath at 37° C. The reactions were stopped (usually after 5–10 min) by the addition of 1.0 ml CH_2Cl_2 with mixing, using a vortex device.

After separation and concentration of the organic phase to dryness under N_2 gas, the residue was dissolved in 50 μl CH_3OH and aliquots (20 μl) were analyzed immediately by HPLC. Metabolite analysis was performed on a Zorbax octadecylsilane (C_{18}) HPLC column (4.6 × 150 mm; MacModd, Chadds Ford, PA). The solvents used were 0.1 M ammonium acetate (*p*H 6.2) (solvent A) and a mixture of 1.0 M ammonium acetate (*p*H 6.2)/acetonitrile/methanol/tetrahydrofuran (10:30:20:40, v/v/v/v) (sol-

¶ The nomenclature used here for the P-450 enzymes is that described by Nebert *et al.*[34] For reviews of human P-450 enzymes see references 51–54. Four different human P-450 enzymes in the P-450 3A family have been described,[34] and all are probably recognized by the antibody used in the current study. There is evidence that P-450 3A4 is the major one of these enzymes expressed in adult human liver: P-450 3A7 appears to be restricted primarily to fetal liver,[55] P-450 3A5 is expressed in only about 25% of the population and usually at levels approximately one-third that of P-450 3A4[56,57] and P-450 3A3 was shown to be expressed at a level <5% of that of P-450 3A4 in 12 different liver samples.[42,58] The number of genes in the human P-450 3A family is probably not greater, since only three genomic fragments were recognized in common by long, nonoverlapping complementary DNA probes.[59] The total size of the hybridizing gene fragments is in the range of 50–60 kb,[59] and Kamataki and associates recently have characterized the P-450 3A4 and 3A7 genes and found both to be 27 kb in length (personal communication).

vent B) with a linear gradient running from 75% solvent A and 25% solvent B to 50% solvent A and 50% solvent B (v/v) over 20 min. The flow rate was 1 ml/min. The eluent was monitored at 230 nm. Quantitation of products was done using a Radiometric Flo-one counter (Radimatic, Tampa, FL) after the effluent was mixed with liquid scintillation cocktail (Flo-scint II, Radiomatic).

Inhibition Studies

Immunoinhibition studies were done by incubating human liver microsomes with varying amounts of antibodies (immunoglobulin G fraction) for 20 min at 23° C before addition of other components required for catalytic activity. In control experiments, varying levels of the preimmune immunoglobulin G were used.

Inhibition of noralfentanil formation by gestodene, troleandomycin, sulfaphenazole, diethyldithiocarbamate, and quinidine were determined using human liver microsomes; the general methods have been presented elsewhere.[20] Briefly, microsomes were incubated with the compound under consideration and an NADPH-generating system in a small volume (50 μl) for 10 min and then diluted 10-fold for assay of catalytic activity. The same approach was used with 7,8-benzoflavone, which can either inhibit or stimulate different P-450 enzymes.[15,17]

Results

Alfentanil Oxidation Activities of Purified Human Liver P-450s and Comparison to Human Liver Microsomes and Recombinant P-450 3A4

Alfentanil was rapidly metabolized in human liver microsomes, yielding noralfentanil as the main metabolite, as reported previously.[19] The human liver microsomes prepared from sample HL 110, known to be high in P-450 3A4, had the highest alfentanil oxidation activity (table 1). The catalytic activities of several purified human liver P-450s were measured and compared to the most active microsomal preparation (from HL 110) (table 1). Only the P-450 3A4 preparation had alfentanil oxidation activity. Also, yeast microsomes expressing P-450 3A4 showed catalytic activity. Purified P-450 3A4 from these yeast also showed activity. Neither purified P-450 1A2, P-450 2C8, P-450 2D6, P-450 2E1, nor P-450 $2C_{MP}$ catalyzed alfentanil oxidation (data not shown).

Correlation of Rates of Noralfentanil Formation with Other Enzymatic Activities in Human Liver Microsomes

Noralfentanil formation rates ranged from 0.14 to 4.38 (nmol product formed) · min^{-1} · (mg microsomal protein)$^{-1}$ in microsomes prepared from 15 different human liver samples, a difference of 31-fold (fig. 1). Importantly, good correlation was observed between rates of noralfentanil formation and nifedipine oxidation, a marker of P-450 3A4 in different human liver microsomes ($r = 0.95$, $P < 0.0005$; fig. 1A).

No significant correlation ($P > 0.10$) of rates of noralfentanil formation was seen with rates of phenacetin O-deethylation (P-450 1A2) (fig. 1B), chlorzoxazone 6-hydroxylation (P-450 2E1) (fig. 1C), or (*S*)-mephenytoin 4′-hydroxylation (P-450 $2C_{MP}$) (fig. 1D). Previous studies had shown no correlation with P-450 2D6.[19,21]

TABLE 1. Noralfentanil Formation by Human Liver Microsomes and Purified P-450s

Sample	Noralfentanil Formation (nmol product formed · min^{-1} · nmol P-450^{-1})
Human liver microsomes	
HL 110	5.15
Purified human liver P-450s	
1A2	<0.01
2C8	<0.01
$2C_{MP}$	<0.01
2D6	<0.01
2E1	<0.01
3A4	0.86
Yeast recombinant P-450s	
3A4 (yeast microsomes)	0.31
3A4 (partially purified from yeast)	0.58

Immunoinhibition of Alfentanil Oxidation Activity by Antibodies in Human Liver Microsomes

To determine which P-450 is primarily responsible for alfentanil oxidation, immunoinhibition studies with anti–P-450 3A4, anti–P-450 2C, anti–P-450 2D1, anti–P-450 1A2, and anti–P-450 2E1 were carried out. Only anti–P-450 3A4 inhibited the alfentanil oxidation activity in human liver sample HL 110 microsomes (fig. 2). None of the other antibodies inhibited the alfentanil oxidation activity. These results strongly suggest that P-450 3A4 is responsible for almost all of the alfentanil oxidation activity in human liver.

Inhibition of Alfentanil Oxidation Activity by Chemicals in Human Liver Microsomes

Gestodene[22] and troleandomycin[23,24] are effective inhibitors of P-450 3A enzymes, whereas sulfaphenazole inhibits tolbutamide hydroxylation (P-450 2C9 and 2C10) in human liver microsomes and *in vivo*.[25,26] Diethyldithiocarbamate[15] and quinidine[27] are selective inhibitors of P-450 2E1 and P-450 2D6, respectively. Neither sulfaphenazole, diethyldithiocarbamate, nor quinidine had

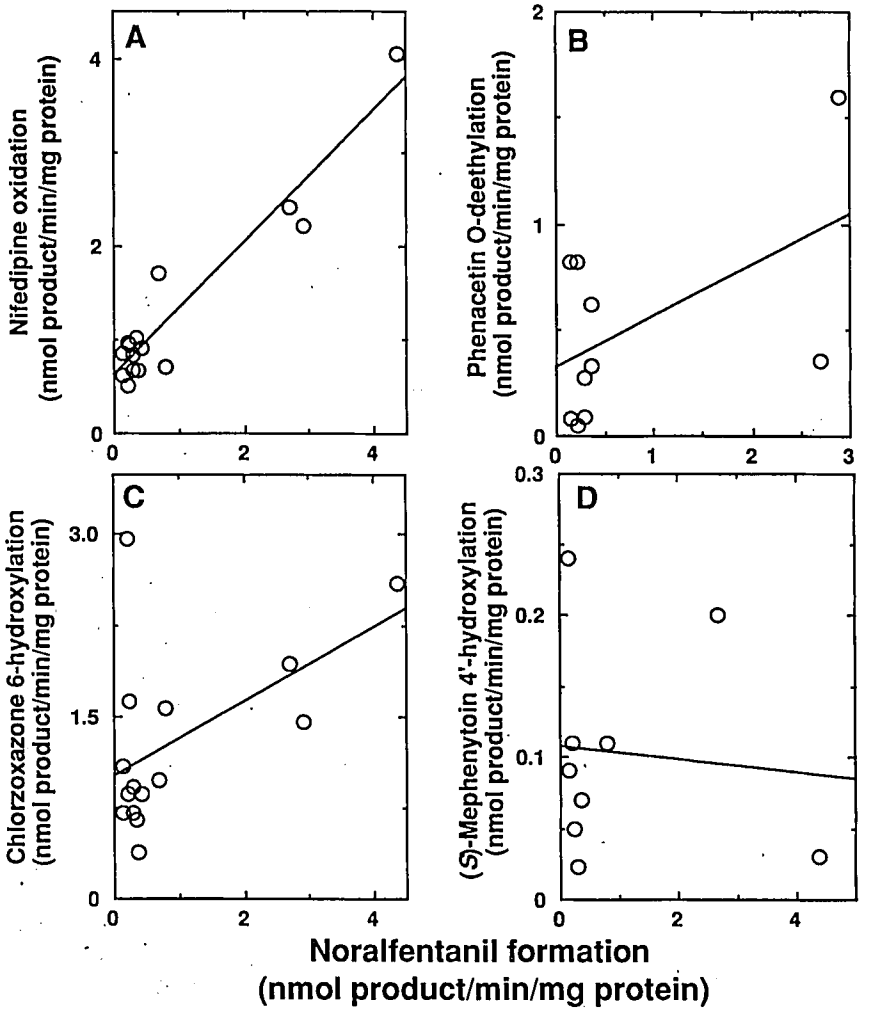

FIG. 1. Relationships between nifedipine oxidation (*A*), phenacetin O-deethylation (*B*), chlorzoxazone 6-hydroxylation (*C*), and (*S*)-mephenytoin 4'-hydroxylation (*D*) and noralfentanil formation in different human liver microsomes. The respective correlation coefficients (r^2) obtained in these relations were as follows: *A*, 0.90; *B*, 0.29; *C*, 0.29; *D*, 0.01 (rates of phenacetin O-deethylation and (*S*)-mephenytoin 4'-hydroxylation were not available for all 15 of the samples).

any measurable effect. In contrast, gestodene and troleandomycin (50 μM), inhibitors of P-450 3A enzymes, inhibited 69–88% of the alfentanil oxidation activity in the most active human liver microsomal sample (HL 110) among 15 microsomal preparations (fig. 3). This finding is consistent with the results of the antibody work described in the previous section, which suggest that the major P-450 enzymes catalyzing alfentanil oxidation are P-450 3A4 or other enzymes in the P-450 3A subfamily.

Effect of 7,8-Benzoflavone on Oxidation Activity Catalyzed by Human Liver Microsomes of Purified P-450 3A4

7,8-Benzoflavone was added to the human liver microsomal samples from HL 110 to examine its effect on the alfentanil oxidation activity of P-450 3A4, since some activities of this enzyme are stimulated by this compound.[28,29] At a concentration of 50 μM 7,8-benzoflavone, the alfentanil oxidation activity in the microsomal system from HL 110 was inhibited by 89% (fig. 4). In the case of reconstituted purified P-450 3A4, 7,8-benzoflavone (50 μM) inhibited 78% of the alfentanil oxidation activity.

Discussion

This study has clearly demonstrated that the liver enzyme principally responsible for the oxidation of alfentanil is P-450 3A4. The main metabolic pathway for alfentanil is oxidation (N-dealkylation) with the formation of the metabolite noralfentanil.[19,21] We determined the extent of oxidation of alfentanil to its major metabolite noralfentanil by an HPLC assay in human liver microsomes. Antibody specific to P-450 3A enzymes completely inhibited alfentanil oxidation to noralfentanil, whereas the antibodies inhibitory to other P-450 enzymes (including P-450 2D6, the debrisoquine 4-hydroxylase) exhibited no measurable effect. In addition, the selective inhibitors of P-450 3A4 gestodene and troleandomycin inhibited as

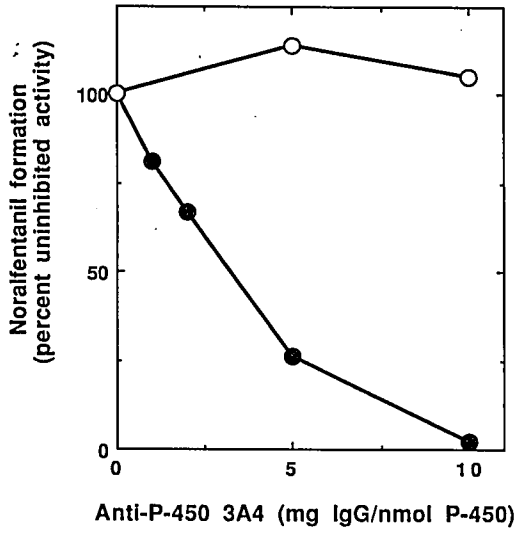

FIG. 2. Effect of anti-P-450 3A4 on noralfentanil formation activity. Microsomes from sample HL 110 were preincubated with preimmune IgG (open circles) or anti-P-450 3A4 IgG (filled circles).

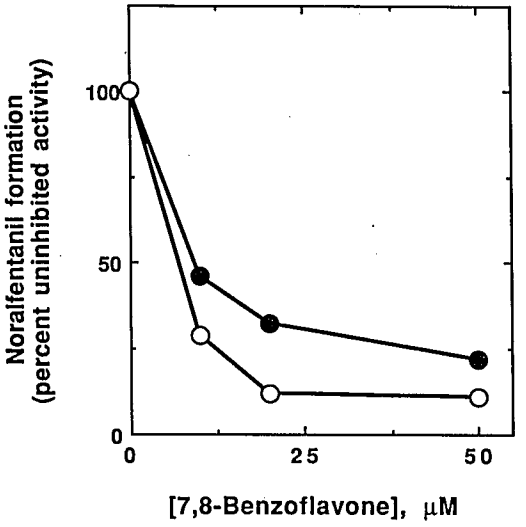

FIG. 4. Effect of 7,8-benzoflavone on noralfentanil formation activities catalyzed by human liver microsomal sample HL 110 (open circles) and purified P-450 3A4 (filled circles).

much as 90% of microsomal noralfentanil formation. Thus, our data indicate that P-450 3A4 catalyzes the metabolism of alfentanil to noralfentanil.

We suspected that 7,8-benzoflavone might enhance the formation of noralfentanil, because some P-450 3A4 reactions are stimulated by this compound.[17,25,28,29] However, inhibition was observed (fig. 4). This observation is not inconsistent with a role for P-450 3A4, because we recently have demonstrated that the enzyme can oxidize aflatoxin B_1 to both the 8,9-expoxide and the 3α-alcohol: the former reaction is stimulated by 7,8-benzoflavone, whereas the latter reaction is inhibited.[30] These observations were made with the yeast recombinant enzyme as well as in human liver microsomes and have been interpreted in terms of an allosteric mechanism.[30] Thus, caution is needed in the interpretation of effects of selective modifiers of P-450–catalyzed reactions.

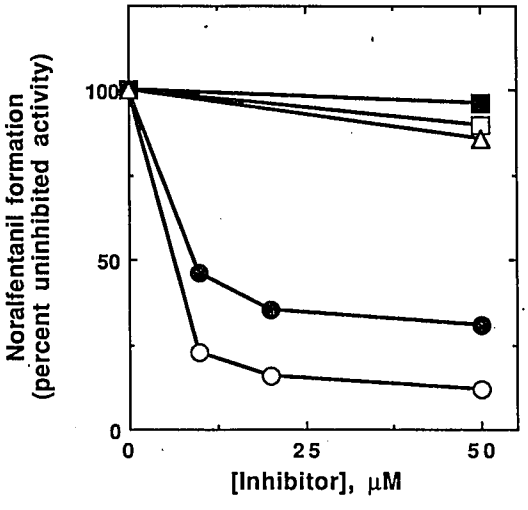

FIG. 3. Effects of gestodene (open circles), troleandomycin (filled circles) (inhibitor of P-450 3A enzymes), sulfaphenazole (open squares; inhibitor of P-450 2C8, 2C9, and 2C10), quinidine (filled squares; inhibitor of P-450 2D6), and diethyldithiocarbamate (triangles; inhibitor of P-450 2E1) on rates of noralfentanil formation by human liver microsomal sample HL 110.

Genetic polymorphism for the pathways of acetylation and hydrolysis (*e.g.*, succinylcholine) have been recognized for many years; however, only a small number of drugs are metabolized by either of these pathways. Most drugs are metabolized by oxidative pathways, and increased knowledge of genetic polymorphism for the oxidative pathways has been one of the most important recent advances in understanding interindividual variability in drug metabolism and pharmacokinetics. Genetic defects in the metabolism of sparteine and debrisoquine were the first oxidation defects to be identified.[4,31] Subsequently, these were shown to reflect alterations in the activity of the same P-450 enzyme.[32,33] Multiple genetically determined P-450 cytochrome enzymes exist, and each enzyme is assigned to families and subfamilies according to the degree of similarity of the amino acid sequence of the P-450 isozyme.[34] P-450 2D6, the locus of the debrisoquine drug oxidation polymorphism, is perhaps the most well known subfamily with regard to genetic polymorphism, and the enzyme has been shown to exhibit marked stereoselective metabolism and a bimodal distribution.[35] However, many commonly administered drugs have now been identified as substrates for another important enzyme, P-450 3A4, namely nifedipine,[8] cyclosporin,[36,37] erythromycin,[38,17] midazolam,[39] quinidine,[40] and lidocaine.[41]

The current study has now shown that P-450 3A is also responsible for the oxidation of the intravenous opioid alfentanil. There appears to be a broad unimodal distribution of enzyme activity for P-450 3A with a wide interindividual variability (more than one order of magnitude), and a distinct subgroup has not been identified.[6] This results in marked interindividual variability in the pharmacokinetics of drugs metabolized by this enzyme, as indeed has been shown to be the case for alfentanil. P-450 3A4 is the enzyme principally responsible for the metabolism of the immunosuppressive drug cyclosporin,[36,37] and the renal toxicity of cyclosporin is well recognized. To minimize toxicity by better predicting cyclosporin pharmacokinetics, the ^{14}C-erythromycin breath test has been used as a measure of P-450 3A activity *in vivo* because P-450 3A enzymes also catalyze the N-demethylation of erythromycin.[42–44] Thus, the production of $^{14}CO_2$ in the expired breath has been shown to predict cyclosporin blood levels.[44,45]

Because so many drugs have now been shown to be substrates for P-450 3A, the possibility of important clinical pharmacokinetic drug interactions exist. It is interesting that erythromycin, an antibiotic frequently administered preoperatively, has been shown to inhibit the metabolism of alfentanil.[46] Erythromycin administration for 7 days significantly increased alfentanil elimination half-life and decreased clearance,[46] indicating that erythromycin inhibits the metabolism of alfentanil. Subjects differed in their sensitivity to erythromycin (in keeping with the demonstrated wide interindividual variation in activity of cytochrome P-450 3A4), so that alfentanil elimination is not predictable. A case report has described unconsciousness associated with midazolam and erythromycin in an 8-yr-old boy with a ventricular-septal defect given erythromycin for antibiotic prophylaxis before adenoidectomy.[47] The plasma concentration of midazolam was greater in this patient than in six other children who did not receive erythromycin.[47] Such interactions might also be predicted to occur for alfentanil.

Previous *in vitro* work, using the dehydroxylation of desmethylimipramine as a probe for debrisoquine hydroxylase activity, had suggested that alfentanil competitively inhibits the enzyme P-450 2D6 (debrisoquine hydroxylase).[48] However, other *in vitro* studies have shown that debrisoquine is only a weak noncompetitive inhibitor of alfentanil metabolism and of the formation of its major metabolites.[9] Although drugs that are metabolized by the same enzyme both bind to that enzyme, drugs may also bind to the enzyme but not themselves be metabolized by the enzyme. Thus, for example, quinidine binds tightly to P-450 2D6 and inhibits the clearance of drugs that are also metabolized by P-450 2D6, such as debrisoquine and propranolol.[49,50] However, quinidine itself is not metabolized by this enzyme, so that *in vivo*, the metabolism of quinidine does not cosegregate with that of debrisoquine because it is metabolized not by P-450 2D6 but by P-450 3A4.[40] There is, therefore, no difference in quinidine metabolism in poor and extensive metabolizers of debrisoquine.

Similarly, for alfentanil, alfentanil clearance *in vivo* is not altered in poor metabolizers of debrisoquine.[5] We interpret these previous studies to imply that alfentanil binds to P-450 2D6 and hence inhibits the metabolism *in vitro* of debrisoquine. Because alfentanil is not itself metabolized by P-450 2D6 but rather, as we have shown, by P-450 3A4, debrisoquine will not competitively inhibit alfentanil clearance *in vitro*. Our demonstration that P-450 3A4 makes a major contribution to human liver microsomal alfentanil oxidation and that P-450 2D6 does not, therefore resolves the previous apparent conflict between the *in vitro* and *in vivo* data for alfentanil metabolism.

In summary, we have shown that P-450 2D6 (the debrisoquine 4-hydroxylase) is not responsible for the oxidation of alfentanil to noralfentanil, but that another enzyme, P-450 3A4, has the major role in the oxidation of alfentanil. Human cytochrome P-450 3A enzymes are known to metabolize many clinically important substrates, namely nifedipine, cyclosporin, midazolam, lidocaine, quinidine, and now alfentanil. Thus, the possibility exists for pharmacokinetic interactions between alfentanil and other concomitantly administered drugs that are also substrates for P-450 3A4. Alfentanil oxidation by P-450 3A4 *in vivo* would in large part explain the wide interindividual variation in alfentanil kinetics demonstrated perioperatively. Further research is required to demonstrate that alfentanil metabolism *in vivo* correlates with P-450 3A4 functional activity.

The authors are grateful to J. Heykants, Ph.D. and Janssen Research Foundation for the gift of [^{3}H]-alfentanil.

References

1. Maitre PO, Ausems ME, Vozeh S, Stanski DR: Evaluating the accuracy of using population pharmacokinetic data to predict plasma concentrations of alfentanil. ANESTHESIOLOGY 68:59–67, 1988
2. Maitre PO, Vozeh S, Heykants J, Thomson DA, Stanski DR: Population pharmacokinetics of alfentanil: The average dose–plasma concentration relationship and interindividual variability in patients. ANESTHESIOLOGY 66:3–12, 1987
3. Brosen K: Recent developments in hepatic drug oxidation: Implications for clinical pharmacokinetics. Clin Pharmacokinet 18: 220–239, 1990
4. Mahgoub A, Idle JR, Dring LA, Lancaster R, Smith RL: Polymorphic hydroxylation of debrisoquine in man. Lancet 2:584–586, 1977
5. Henthorn TK, Avarm MJ, Krejcie TC: Alfentanil clearance is independent of the polymorphic debrisoquin hydroxylase. ANESTHESIOLOGY 71:635–639, 1989

6. Schellens JHM, Soons PA, Briemer DD: Lack of bimodality in nifedipine plasma kinetics in a large population of healthy subjects. Biochem Pharmacol 37:2507–2510, 1988
7. Wang PP, Beaune P, Kaminsky LS, Dannan GA, Kadlubar FF, Larrey D, Guengerich FP: Purification and characterization of six cytochrome P-450 isozymes from human liver microsomes. Biochemistry 22:5375–5383, 1983
8. Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T, Waxman DJ: Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. J Biol Chem 261:5051–5060, 1986
9. Ged C, Umbenhauer DR, Bellew TM, Bork RW, Srivastava PK, Shinriki N, Lloyd RS, Guengerich FP: Characterization of cDNAs, mRNAs, and proteins related to human liver microsomal cytochrome P-450 (*S*)-mephenytoin 4′-hydroxylase. Biochemistry 27:6929–6940, 1988
10. Srivastava PK, Yun C-H, Beaune PH, Ged C, Guengerich FP: Separation of human liver tolbutamide hydroxylase and (*S*)-mephenytoin 4′-hydroxylase cytochrome P-450 enzymes. Mol Pharmacol 40:69–79, 1991
11. Wrighton SA, Thomas PE, Ryan DE, Levin W: Purification and characterization of ethanol-inducible human hepatic cytochrome P-450HLj. Arch Biochem Biophys 258:292–297, 1987
12. Distlerath LM, Reilly PEB, Martin MV, Davis GG, Wilkinson GR, Guengerich FP: Purification and characterization of the human liver cytochromes P-450 involved in debrisoquine 4-hydroxylation and phenacetin O-deethylation, two prototypes for genetic polymorphism in oxidative drug metabolism. J Biol Chem 260: 9057–9067, 1985
13. Yasukochi Y, Masters BSS: Some properties of a detergent-solubilized NADPH-cytochrome c (cytochrome P-450) reductase purified by biospecific affinity chromatography. J Biol Chem 215:5337–5344, 1976
14. Shimada T, Misono KS, Guengerich FP: Human liver microsomal cytochrome P-450 mephenytoin 4-hydroxylase, a prototype of genetic polymorphism in oxidative drug metabolism: Purification and characterization of two similar forms involved in the reaction. J Biol Chem 261:909–921, 1986
15. Guengerich FP, Kim D-H, Iwasaki M: Role of human cytochrome P-450 IIE1 in the oxidation of several low molecular weight cancer suspects. Chem Res Toxicol 4:168–179, 1991
16. Larrey D, Distlerath LM, Dannan GA, Wilkinson GR, Guengerich FP: Purification and characterization of the rat liver microsomal cytochrome P-450 involved in the 4-hydroxylation of debrisoquine, a prototype for genetic variation in oxidative drug metabolism. Biochemistry 23:2787–2795, 1984
17. Brian WR, Sari M-A, Iwasaki M, Shimada T, Kaminsky LS, Guengerich FP: Catalytic activities of human liver cytochrome P-450 IIIA4 expressed in *Saccharomyces cerevisiae*. Biochemistry 29: 11280–11292, 1990
18. Omura T, Sato R: The carbon monoxide-binding pigment of liver microsomes: I. Evidence for its hemoprotein nature. J Biol Chem 239:2370–2378, 1964
19. Lavrijsen KLM, Van Houdt JMG, Van Dyck DMJ, Hendrickx JJJM, Woestenborghs RJH, Lauwers W, Meuldermans WEG, Heykants JJP: Is the metabolism of alfentanil subject to debrisoquine polymorphism? A study using human liver microsomes. ANESTHESIOLOGY 69:535–540, 1988
20. Guengerich FP: Oxidation of 17α-ethynylestradiol by human liver cytochrome P-450. Mol Pharmacol 33:500–508, 1988
21. Meuldermans W, Van Peer A, Hendrickx, Woestenborghs R, Lauwers W, Heykants J, Bussche GV, Van Craeyvelt H, Van Der Aa P: Alfentanil pharmacokinetics and metabolism in humans. ANESTHESIOLOGY 69:527–534, 1988
22. Guengerich FP: Mechanism-based inactivation of human liver cytochrome P-450 IIIA4 by gestodene. Chem Res Toxicol 3:363–371, 1990
23. Pessayre D, Tinel M, Larrey D, Cobert B, Funck-Brentao C, Babany G: Inactivation of cytochrome P-450 by a troleandomycin metabolite. Protective role of glutathione. J Pharmacol Exp Ther 224:685–691, 1983
24. Guengerich FP, Shimada T: Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chem Res Toxicol 4:391–407, 1991
25. Brian WR, Srivastava PK, Umbenhauer DR, Lloyd RS, Guengerich FP: Expression of a human liver cytochrome P-450 protein with tolbutamide hydroxylase activity in *Saccharomyces cerevisiae*. Biochemistry 28:4993–4999, 1989
26. Miners JO, Smith KJ, Robson RA, McManus ME, Veronese ME, Birkett DJ: Tolbutamide hydroxylation by human liver microsomes: kinetic characterization and relationship to other cytochrome P-450 dependent xenobiotic oxidations. Biochem Pharmacol 37:1137–1144, 1988
27. Inaba T, Jurima M, Mahon WA, Kalow W: In vitro inhibition studies of two isozymes of hyman liver cytochrome P-450: Mephenytoin p-hydroxylase and sparteine monooxygenase. Drug Metab Dispos 13:443–448, 1985
28. Shimada T, Iwasaki M, Martin MV, Guengerich FP: Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by *umu* gene response in *Salmonella typhimurium* TA1535/pKS1002. Cancer Res 49: 3218–3228, 1989
29. Shimada T, Martin MV, Pruess-Schwartz D, Marnett LJ, Guengerich FP: Roles of individual human cytochrome P-450 enzymes in the bioactivation of benzo(*a*)pyrene, 7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene, and other dihydrodiol derivatives of polycyclic aromatic hydrocarbons. Cancer Res 49:6304–6312, 1989
30. Raney KD, Shimada T, Kim D-H, Groopman JD, Harris TM, Guengerich FP: Oxidation of aflatoxin B_1 and related dihydrofurans by human liver microsomes: Significance of aflatoxin Q_1 as a detoxication product. Chem Res Toxicol 5:202–210, 1992
31. Eichelbaum M, Bertilsson L, Sawe J, Zekorn C: Polymorphic oxidation of sparteine and debrisoquine: Related pharmacogenetic entities. Clin Pharmacol Ther 31:184–186, 1982
32. Gonzalez FJ, Skoda RC, Kimura S, Umeno M, Zanger UM, Nebert DW, Gelboin HV, Hardwick JP, Meyer UA: Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. Nature 331:442–446, 1988
33. Eichelbaum M, Baur MP, Dengler HJ, Osikowska-Evers B, Tieves G, Zekorn C, Rittner C: Chromosomal assignment of human cytochrome P-450 (debrisoquine/sparteine type) to chromosome 22. Br J Clin Pharmacol 23:455–458, 1987
34. Nebert DW, Nelson DR, Coon MJ, Estabrook RW, Feyereisen R, Fujii-Kuriyama Y, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Loper JC, Sato R, Waterman MR, Waxman DJ: The P450 superfamily: Update on new sequences, gene mapping and recommended nomenclature. DNA Cell Biol 10:1–14, 1991
35. Eichelbaum M: Pharmacokinetic and pharmacodynamic consequences of stereoselective drug metabolism in man. Biochem Pharmacol 37:93–96, 1988
36. Kronbach T, Fischer V, Meyer UA: Cyclosporine metabolism in human liver: Identification of a cytochrome P-450III gene family as the major cyclosporine-metabolizing enzyme explains interactions of cyclosporine with other drugs. Clin Pharmacol Ther 43:630–635, 1988
37. Combalbert J, Fabre I, Fabre G, Dalet I, Derancourt J, Cano JP, Maurel P: Metabolism of cyclosporin A. IV. Purification and identification of the rifampicin-inducible human liver cytochrome P-450 (cyclosporin A oxidase) as a product of P450 IIIA gene subfamily. Drug Metab Dispos 17:197–207, 1989

38. Watkins PB, Wrighton SA, Maurel P, Schuetz EG, Mendez-Picon G, Parker GA, Guzelian PS: Identification of an inducible form of cytochrome P-450 in human liver. Proc Natl Acad Sci USA 82:6310–6314, 1985
39. Kronbach T, Mathys D, Umeno M, Gonzalez FJ, Meyer UA: Oxidation of midazolam and triazolam by human liver cytochrome P450 IIIA4. Mol Pharmacol 36:89–96, 1989
40. Guengerich FP, Müller-Enoch D, Blair IA: Oxidation of quinidine by human liver cytochrome P-450. Mol Pharmacol 30:287–295, 1986
41. Bargetzi MJ, Aoyama T, Gonzalez FJ, Meyer UA: Lidocaine metabolism in human liver microsomes by cytochrome P450 IIIA4. Clin Pharmacol Ther 46:521–527, 1989
42. Kolars JC, Murray SA, Peters KM, Watkins PB: Differential regulation of liver P-450III cytochromes in choline-deficient rats: Implications for the erythromycin breath test as a parameter of liver function. Hepatology 12:1371–1378, 1990
43. Watkins PB, Murray SA, Winkelman LG, Heuman DM, Wrighton SA, Guzelian PS: Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochromes P-450: Studies in rats and patients. J Clin Invest 83:688–697, 1989
44. Watkins PB, Hamilton TA, Annesley TM, Ellis CN, Kolars JC, Voorhees JJ: The erythromycin breath test as a predictor of cyclosporine blood levels. Clin Pharmacol Ther 48:120–129, 1990
45. Lucey MR, Kolars JC, Merion RM, Campbell DA, Aldrich M, Watkins PB: Cyclosporin toxicity at therapeutic blood levels and cytochrome P-450 IIIA. Lancet 335:11–15, 1990
46. Bartowski RR, Goldberg ME, Larijani GE, Boerner T: Inhibition of alfentanil metabolism by erythromycin. Clin Pharmacol Ther 46:99–102, 1989
47. Hiller A, Olkkola KT, Isohanni P, Saarnivaara L: Unconsciousness associated with midazolam and erythromycin. Br J Anaesth 65: 826–828, 1990
48. Henthorn TK, Spina E, Dumont E, von Bahr C: In vitro inhibition of a polymorphic human liver P-450 isozyme by narcotic analgésics. ANESTHESIOLOGY 70:339–342, 1989
49. Inaba T, Tyndale RE, Mahon WA: Quinidine: Potent inhibition of sparteine and debrisoquine oxidation in vivo (letter to the editor). Br J Clin Pharmacol 22:199–200, 1986
50. Hong-Hao Z, Anthony LB, Roden DM, Wood AJJ: Quinidine reduces clearance of (+)-propranolol more than (−)-propranolol through marked reduction in 4-hydroxylation. Clin Pharmacol Ther 47:686–693, 1990
51. Guengerich FP: Characterization of human microsomal cytochrome P-450 enzymes. Ann Rev Pharmacol Toxicol 29:241–264, 1989
52. Distlerath LM, Guengerich FP: Enzymology of human liver cytochromes P-450, Mammalian Cytochromes P-450. Volume 1. Edited by Guengerich FP. Boca Raton, CRC Press, 1987, pp 133–198
53. Guengerich FP: Characterization of human cytochrome P-450 enzymes. FASEB J 6:745–748, 1992
54. Gonzalez FJ: The molecular biology of cytochrome P-450s. Pharmacol Rev 40:243–288, 1989
55. Komori M, Nishio K, Kitada M, Shiramatsu K, Muroya K, Soma M, Nagashima K, Kamataki T: Fetus-specific expression of a form of cytochrome P-450 in human livers. Biochemistry 29: 4430–4433, 1990
56. Wrighton SA, Ring BJ, Watkins PB, Vandenbranden M: Identification of a polymorphically expressed member of the human cytochrome P-450III family. Mol Pharmacol 86:97–105, 1989
57. Wrighton SA, Brian WR, Sari MA, Iwasaki M, Guengerich FP, Raucy JL, Molowa DT, Vandenbranden M: Studies on the expression and metabolic capabilities of human liver cytochrome P450IIIA5 (HLp3). Mol Pharmacol 38:207–213, 1990
58. Bork RW, Muto T, Beaune PH, Srivastava PK, Lloyd RS, Guengerich FP: Characterization of mRNA species related to human liver cytochrome P-450 nifedipine oxidase and the regulation of catalytic activity. J Biol Chem 264:910–919, 1989
59. Beaune PH, Umbenhauer DR, Bork RW, Lloyd RS, Guengerich FP: Isolation and sequence determination of a cDNA clone related to human cytochrome P-450 nifedipine oxidase. Proc Natl Acad Sci USA 83:8064–8068, 1986