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Possible Involvement of Multiple Cytochrome P450S in Fentanyl and Sufentanil Metabolism as Opposed to Alfentanil

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ABSTRACT. Fentanyl, sufentanil, and alfentanil are commonly used as opioid analgesics. Alfentanil clearance has previously been shown to exhibit an important interindividual variability, which was not observed for fentanyl or sufentanil. Differences in pharmacokinetic parameters of alfentanil have previously been associated with the wide distribution of CYP3A4, the only known hepatic cytochrome P450 monooxygenase (CYP) involved in the conversion of alfentanil to noralfentanil. Little is known about the involvement of CYP enzymes in the oxidative metabolism of fentanyl and sufentanil. Microsomes prepared from different human liver samples were compared for their abilities to metabolize fentanyl, sufentanil and alfentanil, and it was found that disappearance of the three substrates was well correlated with immunoreactive CYP3A4 contents but not with other CYPs, including CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2D6 and CYP2E1. Specific known inhibitors of CYP enzymes gave similar results, whereas the use of recombinant human CYP enzymes expressed in yeast provided information about the possible involvement of other CYPs than CYP3A4 in the biotransformation of fentanyl and sufentanil. The possible *in vivo* interaction of fentanyl and sufentanil with other drugs catalyzed by CYP3A4 is also discussed. BIOCHEM PHARMACOL 53;11:1613–1619, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. fentanyl; sufentanil; alfentanil; analgesic drugs; drug metabolism; CYP3A4

FENTANYL, alfentanil, and sufentanil are synthetic opioids widely used in human clinical practice, especially for induction and maintenance of anesthesia, analgesia, or sedation [1]. Fentanyl, which is chemically related to meperidine, was introduced in therapeutics in the early 1960s. Alfentanil and sufentanil are fentanyl derivatives and have some helpful pharmacological particularities. For example, sufentanil is 5 to 10 times more potent than fentanyl and has a shorter duration of action. Alfentanil has a faster onset of action than fentanyl and is approximately one fourth as potent [1, 2]. Alfentanil also has the shortest duration of action of the group [1].

It has previously been shown that there is considerable interindividual variability in the elimination clearance of alfentanil, resulting in a marked difference in the rate of infusion [3]. This intersubject variability in alfentanil metabolism is independent of the well-known polymorphic pharmacogenetic determinant, *i.e.* the genetic polymorphism of CYP2D6 [4, 5]. Because CYP3A4 is the main form involved in the biotransformation of alfentanil to noralfentanil [6, 7], it appears that the wide individual variability in metabolic clearance of alfentanil is associated with the broad unimodal distribution of CYP3A4 [8].

Fentanyl, sufentanil, and alfentanil have a common structure and depend on similar oxidative pathways such as *N*-dealkylation, *O*-dealkylation, and hydroxylation [9, 10]. Like alfentanil, fentanyl and sufentanil need to be metabolized to be eliminated from the body [1, 11]. However, fentanyl and sufentanil present some different features *in vivo* from alfentanil. Unlike alfentanil and sufentanil, the kinetics of fentanyl is not altered in the presence of propofol [12, 13]. Erythromycin, a well-known substrate catalyzed by CYP3A4 [14], has no effect on sufentanil metabolism while metabolism of alfentanil has been found to be prolonged [15]. Sufentanil appears to have pharmacokinetic properties intermediate to those of alfentanil and fentanyl [2]. Thus, it is of particular pharmacological and toxicological importance to identify which CYPs are responsible for the biotransformation of pharmacological

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Abbreviations: CYP, cytochrome P450 monooxygenase; G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; GC-MS, gas chromatographic mass-spectrum; SDS, sodium dodecyl sulfate.

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agents; this information could help predict the influence of medical, genetic, or environmental factors [16].

The purpose of this study was, therefore, to define the human liver CYP enzymes involved in the *in vitro* oxidative metabolism of fentanyl and sufentanil, by using several techniques (chemical inhibitions, immunoblots, yeast expressing specific human CYP enzymes etc.) and to compare these results with available information on alfentanil metabolism.

MATERIALS AND METHODS

Drugs and Chemicals

Pure fentanyl (citrate), sufentanil (citrate), and alfentanil (hydrochloride) were obtained from Janssen Pharmaceutica N.V. (Beerse, Belgium). Internal standard (R 38 527) used to quantify the three substrates was obtained from Janssen Biotech N.V. (Beerse, Belgium). Coumarin, sulfaphenazole, quinidine, diethyldithiocarbamate, troleandomycin, 7-8 benzoflavone, glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6PDH), and nicotinamide adenine dinucleotide phosphate ($NADP^+$) were purchased from Sigma (St. Quentin Fallavier, France). Midazolam was supplied by Roche (Neuilly sur Seine, France). All sodium dodecyl sulfate-polyacrylamide gel electrophoresis reagents were obtained from Bio-Rad (Paris, France). All other chemicals were of the highest purity grade available and were obtained from commercial sources.

Human Liver Microsomes

Fresh human liver samples were obtained from Caucasian patients who had undergone a partial hepatectomy. As excess material was removed during surgery on the liver, morphologically normal fragments were recovered and prepared as reported previously [17]. It took less than 10 min from the removal of the liver until the collection and freezing of a sample in liquid nitrogen. Patients with acute or chronic hepatitis and with cirrhosis were not included in the study. Approval for the study was obtained from the local ethics committee. For the correlation studies, twelve different human liver samples were used.

Microsomes were prepared by differential centrifugation as described elsewhere [18]. Microsomal fractions were aliquoted in small volumes and stored at −80°C in potassium phosphate buffer (200 mM, pH 7.4) containing 1 mM EDTA and glycerol (20%, v/v) until use.

The microsomal protein content was measured by a modified Lowry's method using bovine serum albumin as standard [19]. Total CYP content was assayed by the method of Omura and Sato using a molar absorption coefficient of 91 cm^{-1} mM^{-1} [20].

Determinations of Enzymatic Activities

Incubations were performed at 37°C in a final volume of 1 mL containing 0.1 M potassium phosphate buffer (pH 7.4) with an NADPH-regenerating system (0.5 mM $NADP^+$, 5 mM G6P, and 1 unit of G6PDH) and microsomal fractions (0.7–0.9 mg of protein). After a 3-min preincubation, the reaction was initiated by adding substrate (final concentration 50 μM fentanyl, sufentanil, or alfentanil) and terminated after 10 min by adding 200 μL of sodium hydroxyde 1N. One hundred μL of the internal standard (30 μM) were immediately added to the reaction mixture. The basic mixture was immediately extracted as previously described [11]. Preliminary studies indicated that the reactions were linear under the conditions used.

Because all *N*-dealkylated products were not available as authentic standards, total fentanyl, sufentanil, or alfentanil metabolism was determined from the disappearance of the substrate on a capillary gas chromatography-mass spectrometry (GC-MS; Model GCD, Hewlett Packard, Palo Alto, CA, USA) system. GC-MS analysis was performed on an HP-1 (25m × 0.20 mm × 0.33 μm film thickness) capillary column and MS was conducted in the electron impact mode at an ionizing voltage of 70 eV. Fentanyl, sufentanil, alfentanil, and the internal standard were detected by selected ion monitoring at mass/charge ratios of 289 (alfentanil-sufentanil), 245 (fentanyl) and 282 (R 38 527) according to Kharasch *et al.* [11], and quantified by comparison with authentic standards. The coefficients of variation for the measurement of fentanyl, sufentanil, and alfentanil by this method were <5%. Preliminary studies were also performed to determine the structure of the different metabolites produced under our conditions by comparing the principal fragment ions for the metabolite with published data [10, 21, 22], as described previously.

The effect of substrate concentration on the rate of reaction was evaluated with a concentration of fentanyl, sufentanil, and alfentanil from 3 different microsomal fractions ranging from 5 μM to 100 μM. The Michaelis-Menten parameters were calculated from Lineweaver–Burk plots by linear regression analysis. All assays were performed in duplicate or triplicate.

Coumarine 7-hydroxylase and midazolam hydroxylase activities were assessed in microsomes as described by Maurice *et al.* [23] and Kronbach *et al.* [24], respectively. Midazolam disappearance was followed using the same GC-MS apparatus under previously reported analytical conditions [25].

Fentanyl, sufentanil, and alfentanil were incubated in microsomes from yeast expressing one specific human form of CYP under conditions classically performed in our laboratory, with the same substrate concentrations as those used with microsomal fractions [26]. The yeast strains used in this study were transfected with a human cDNA coding for one of the following forms: CYP1A1, 1A2, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, or 3A5.

Inhibition Studies

CYP-dependent activities of fentanyl, sufentanil, and alfentanil were inhibited by effective chemical inhibitors of

specific CYPs. Chemical inhibitions were performed by simultaneously adding the inhibitor and the substrate, except when an initial bioactivation was required; the inhibitor was preincubated with microsomes and the NADPH-generating system at 37°C for 20 min before addition of the substrate, and the reaction was continued for an additional 10 minutes. Inhibitors were present at concentrations known to suppress more than 80% of specific CYP activities (7–8 benzoflavone (10 μM), coumarin (100 μM), diethyldithiocarbamate (100 μM), quinidine (20 μM), midazolam (100 μM), sulfaphenazole (50 μM) and troleandomycin (100 μM)) [27]. Each inhibited activity was compared to a control activity conducted under similar conditions, with the same amount of methanol needed for solubilization of the inhibitor. Three liver samples were used for these experiments: HL 3, 5 and 7, on the basis of their high fentanyl (sufentanil or alfentanil) metabolizing activity.

Immunoquantitation of CYP enzyme Levels in Human Hepatic Microsomes

Hepatic microsomes (2–100 μg of protein) were submitted to electrophoresis on 12% polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS) and electrophoretically transferred to nitrocellulose sheets as described previously [28]. The nitrocellulose sheets were incubated with appropriately diluted polyclonal antibodies. All polyclonal antibodies were produced in female New Zealand rabbits after immunization by pure human CYP1A2, 2C8, 2C9, 2D6, 2E1, or 3A4, which were obtained in our laboratory using heterologous expression of the corresponding cDNA in bacteria [29]. The specificity and sensitivity of these antibodies have already been reported [28, 29]. Specific binding was visualized with a peroxydase-labeled secondary antibody with 4-chloro-1-naphtol for color development. The optical density of each stained band was determined by scanning with a densitometer (Scan Jet 2C, Hewlett Packard) linked to a microcomputer running (Scan Analysis®) software. Various concentrations of liver microsomes were loaded on each gel to establish a standard curve. The amount of each CYP enzyme was expressed in arbitrary units by comparison with the specific content of the CYP enzyme of the HL 5 sample, which was considered to 100%.

Statistical Analyses

Regression analyses were performed using Pearson's coefficient of correlation accepting $P < 0.05$ as significant. Statistical significance of inhibition studies was assessed by Student's t test with $P < 0.05$. Kinetic parameters were obtained by linear relationships of the double reciprocal plots calculated by linear regression.

RESULTS AND DISCUSSION

Hepatic Microsomal Activities

Fentanyl, sufentanil, and alfentanil were rapidly metabolized in human liver microsomes yielding different metabolites (data not shown). The main metabolite obtained after incubation of these synthetic opioids in microsomal fractions was always the corresponding *N*-dealkylated metabolite, at the piperidine nitrogen, produced from the parental molecule, leading to norfentanyl and noralfentanil for the oxidative metabolism of fentanyl and sufentanil-alfentanil, respectively. This metabolite represents more than 70% of total *in vitro* metabolism and has been previously observed for all 3 compounds [4, 6, 9, 10, 30]. Because of the existence of a predominant metabolite, a GC-MS analysis based on the disappearance of the substrate is sufficient to access to the specific activity enzyme [11]. No metabolite was obtained if incubations were performed without the NADPH-generating system. The rate of the substrate disappearance was 2.38 ± 0.37, 3.72 ± 0.71, and 2.98 ± 1.24 nmol min^{-1} mg^{-1} for fentanyl, sufentanil, and alfentanil respectively. There was a 1.5-, 1.7-, and 7.5-fold variation in the reaction rate of the metabolism of sufentanil (2.9–5.0 nmol min^{-1} mg^{-1}), fentanyl (1.7–3.2 nmol min^{-1} mg^{-1}), and alfentanil (0.7–5.2 nmol min^{-1} mg^{-1}), respectively. The wide distribution observed in alfentanil metabolism can be compared with previous reports [6, 7]. Early studies have established that interindividual variability in the plasma concentration time profile of alfentanil [3] is independent of debrisoquine genetic polymorphism (CYP2D6) [4, 5] but can be attributed to the wide interindividual expression of CYP3A4 [6, 7]. The apparent kinetic constants were Km = 35.5 ± 3.6 μM and Vm = 3.6 ± 0.01 nmol min^{-1} mg^{-1}, Km = 33.6 ± 3.9 μM and Vm = 9.6 ± 0.9 nmol min^{-1} mg^{-1}, and Km = 31 ± 3.5 μM and Vm = 7.6 ± 1.7 nmol min^{-1} mg^{-1}, for fentanyl, sufentanil, and alfentanil, respectively. The kinetic parameters are comparable to published data for alfentanil and sufentanil [10].

Immunoblots

Rates of fentanyl, sufentanil, and alfentanil oxidation were compared with microsomal antigenic CYP enzyme contents of the human liver samples. There was a highly significant correlation between the disappearance of these anesthetic drugs and the relative amounts of CYP3A4 which exhibited a 4-fold variation between the individual livers (Fig. 1). A highly significant correlation was also observed between midazolam metabolism, a probe of CYP3A4 activity [31], and the disappearance of fentanyl ($r = 0.85$), sufentanil ($r = 0.90$), and alfentanil ($r = 0.93$). There was no significant correlation between the immunoquantified content of CYP1A2, 2C8, 2C9 2D6, and 2E1, between the coumarin 7-hydroxylase activity, a marker of CYP2A6 activity [32], or between the total CYP content and the rate of fentanyl, sufentanil, and alfentanil (Table 1). The mean

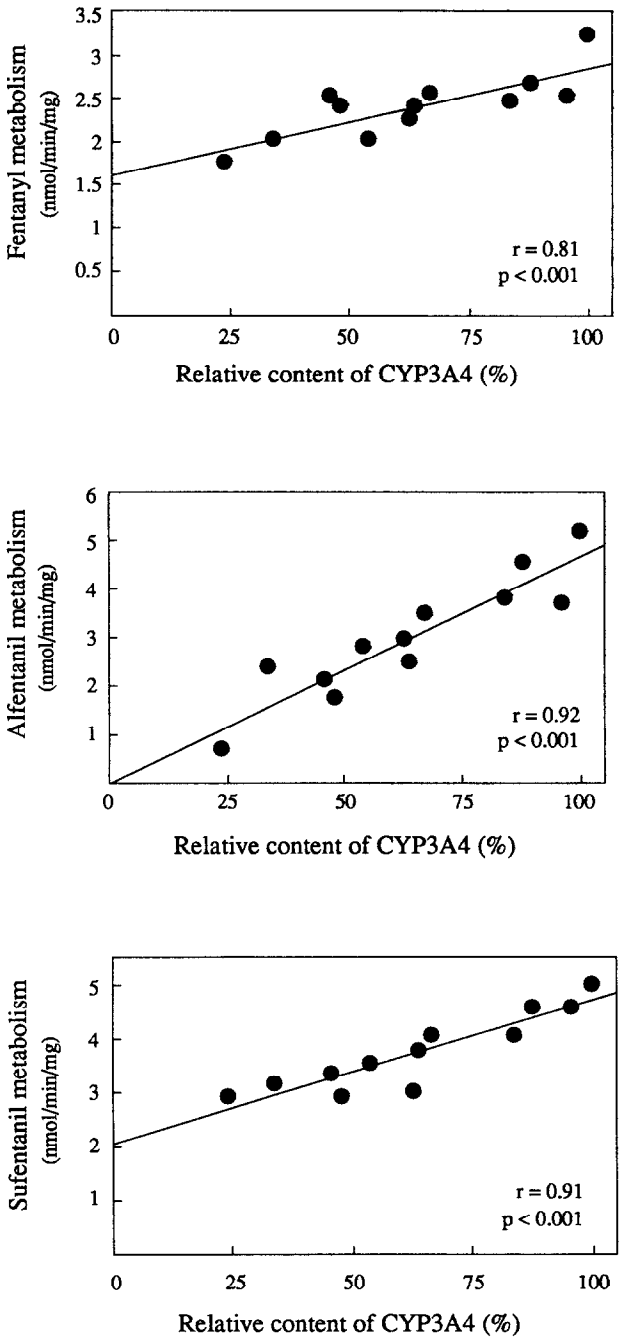

FIG. 1. Relationships between microsomal antigenic CYP3A4 content and fentanyl (top), alfentanil (medium), and sufentanil (bottom) oxidation. Each data point represents the mean of duplicate or triplicate determinations from each human liver.

value for CYP content was found to be 0.36 ± 0.09 nmol mg^{-1} of microsomal protein with a 3-fold variation in the total CYP content. These results, consistent with earlier reported values from human liver microsomal samples [33], strongly suggest that the CYP3A4 enzyme is responsible for the majority of the fentanyl, sufentanil, and alfentanil oxidation in human liver.

Chemical Inhibition

To assess the involvement of the major constitutive CYP enzymes in the metabolism of these opioid drugs, 7–8 benzoflavone, coumarin, diethyldithiocarbamate, quinidine, sulfaphenazole and troleandomycin were used as selective inhibitors for CYP1A2, 2A6, 2E1, 2D6, 2C8/9, and 3A4-dependent activities [27], respectively (Table 2). Metabolism of the three anesthetic drugs was highly diminished after microsomal incubation with troleandomycin for fentanyl and alfentanil, and somewhat diminished for sufentanil. These results were similar to those obtained with midazolam as a competitive inhibitor. This suggests that among the explored CYPs, only CYP3A4 significantly contributes to the metabolism of these anesthetic drugs.

Activities in Microsomes from Transfected Yeast

Metabolism of fentanyl, sufentanil and alfentanil was also performed in microsomes from transfected yeast, and activities in these microsomes are reported in Table 3. Yeast-recombinant CYP3A4 exhibited a high activity of fentanyl, sufentanil, and alfentanil biotransformation. However, other yeast strains also accounted for significant activity. This was mainly the case for CYP1A1 in all three substrates, CYP2C9, 2D6 and 3A5 in fentanyl metabolism, and CYP 2D6 in sufentanil metabolism.

General Discussion

This study clearly demonstrates that CYP3A4 is primarily responsible for the oxidative *N*-dealkylation of fentanyl, sufentanil, and alfentanil, which is considered to be the major metabolic pathway [9, 10]. These results are also in a good agreement with those previously published showing that alfentanil was predominantly, if not exclusively, metabolized by CYP3A4 [6, 21]. A recent similar study also

TABLE 1. Correlation between fentanyl, sufentanil, and alfentanil disappearance and relative CYP enzyme contents in microsomes from human livers

	CYPs							
Substrate	CYP	CYP1A2	CYP2A6	CYP2C8	CYP2C9	CYP2D6	CYP2E1	CYP3A4
Fentanyl	0.19	0.14	0.52	0.23	0.51	0.21	0.41	0.81*
Sufentanil	0.12	0.08	0.35	0.45	0.13	0.55	0.12	0.91*
Alfentanil	0.00	0.15	0.36	0.35	0.28	0.43	0.11	0.92*

Values are expressed as Pearson's correlation coefficients. *$P < 0.001$.

TABLE 2. Inhibition percentage of fentanyl, sufentanil, and alfentanil metabolism after microsomal incubation in the presence of specific CYP enzyme inhibitors

	Inhibited CYP enzymes					
Substrate	CYP 1A2	CYP 2A6	CYP 2C8/9	CYP 2D6	CYP 2E1	CYP 3A4
Fentanyl	12 ± 3	14 ± 8	3 ± 2	2 ± 2	0	82 ± 7
Sufentanil	10 ± 2	10 ± 5	3 ± 2	9 ± 3	18 ± 3	58 ± 5
Alfentanil	17 ± 4	12 ± 3	5 ± 2	0	0	85 ± 4

Inhibitor concentrations and incubation conditions are described in Materials and Methods. Experiments were realized from three human livers. Results represent the mean of duplicate determinatsion.

identified the participation of CYP3A4 in the metabolism of fentanyl and sufentanil [34]. However, this study provided some evidence as to the participation of other forms of CYP than CYP3A4 in the metabolism of fentanyl and sufentanil. Indeed, the low *in vitro* variability in the metabolism of fentanyl (1.5-) and sufentanil (1.7-) compared to alfentanil (7.5-fold), the moderate inhibition of sufentanil oxidase activity by troleandomycin (Table 2), and the marked displacement from the x-intercept along the y-axis of the linear regression between fentanyl and sufentanil *N*-dealkylase activities and immunoreactive CYP3A4 content (Fig. 1) suggest the intervention of one or more CYPs in this oxidative metabolism associated with CYP3A4. Moreover, yeast expressing CYP1A1, 2C8, 2C9, 2D6, and 3A5 exhibited significant fentanyl and sufentanil (except CYP3A5) *N*-dealkylase activities. Unfortunately, these results were not supported by studies with human liver microsomes (Table 1 and 2), probably as a consequence of the high CYP3A4-dependent activity of these microsomes. Thus, the characterization of other CYPs than CYP3A4 in the oxidative metabolism of fentanyl and sufentanil does not appear clearly. However, the use of a single CYP expressed in heterologous systems yields evidence as to the intrinsic capacity of this form to catalyze the reaction [35]. Therefore, the *in vivo* participation of these forms in the metabolic pathway depends on the importance of these forms in liver microsomes and the hepatic level of the drug observed after the administration of a therapeutic dose [36]. Among these forms, only CYP2C8, 2C9 and 2D6

TABLE 3. Metabolism of fentanyl, sufentanil, and alfentanil after incubations in yeast expressing human CYP enzymes

Yeast expressing human CYP enzyme	Fentanyl	Sufentanil	Alfentanil
CYP 1A1	1.90	5.13	1.23
CYP 1A2	<0.01	<0.01	<0.01
CYP 2C8	0.43	0.30	<0.01
CYP 2C9	1.03	0.37	<0.01
CYP 2C18	<0.01	<0.01	0.30
CYP 2C19	<0.01	<0.01	0.17
CYP 2D6	0.77	0.63	<0.01
CYP 2E1	<0.01	0.23	0.23
CYP 3A4	1.03	1.00	0.93
CYP 3A5	1.00	0.27	0.17

Results are expressed in nmol · min^{-1} · $nmol^{-1}$ of specific CYP content and represent the mean of 2 separate assays.

are well-expressed in human liver [37, 38], whereas CYP1A1 is not constitutively expressed in human liver [39] and CYP3A5 is expressed in *ca.* 20% of adult livers and does not usually exhibit a marked specificity for substrates compared to CYP3A4 [40]. Similarly, fentanyl has been reported to competitively inhibit desmethylimipramine oxydation with a 21 μM Ki value [5]. This suggests that fentanyl may be mediated by CYP2D6 even if quinidine failed to demonstrate this in our results. Therefore, fentanyl and sufentanil do not seem to exhibit interindividual variability in their pharmacokinetic parameters [41] compared to alfentanil [3, 6, 7] while they are both widely metabolized, since less than 6% of the body clearance of fentanyl, and everless for sufentanil, is exclusively due to renal mechanisms [1, 2]. The low variability of the *in vitro* metabolism of sufentanil can probably be related to the homogeneity of sufentanil pharmacokinetics between different subjects [41]. Further, a prior administration of erythromycin was not associated with a modification of the pharmacokinetic parameters of sufentanil even in subjects who were sensitive to the interaction between this macrolide and alfentanil [41, 42]. Since many drugs have been shown to be substrates for CYP3A4, the potential for important clinical drug interactions exists. This kind of interaction has been identified for alfentanil [43], midazolam [44], or cyclosporin [45] but not for sufentanil [41] or fentanyl [46], whereas the last two are both metabolized by CYP3A4. Some investigators explain the lesser variability in sufentanil pharmacokinetics and the relative resistance of sufentanil to drug interaction compared to alfentanil by the difference in their hepatic extraction ratio [1, 6, 34]. With a low-to-intermediate extraction ratio [47–48], alfentanyl appears to be largely dependent on intrinsic clearance, whereas fentanyl and sufentanil seem to be only related to the hepatic blood flow [2, 49]. However, participation of other forms of CYP in the oxidative metabolism of fentanyl and sufentanil as opposed to alfentanil, which could supply the lack of CYP3A4 activity in some subjects, could also contribute to the low variability observed *in vivo*. In conclusion, this study brings some evidence as to the participation of other CYPs than CYP3A4 in fentanyl and sufentanil *N*-dealkylase activities as compared to the oxidative metabolism of alfentanil, which relies solely on CYP3A4. The probable involvement of the minor forms, represented by CYP2C8, 2C9 and/or 2D6, may contribute

to the low interindividual variability observed with these two drugs, although the efficiency of these enzymatic pathways was not evaluated.

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References

1. Clotz MA and Nahata MC, Clinical uses of fentanyl, sufentanil, and alfentanil. *Clin Pharmacy* **10:** 581–593, 1991.
2. Mather LE, Clinical pharmacokinetics of fentanyl and its newer derivatives. *Clin Pharmacokinet* **8:** 422–446, 1983.
3. Maitre PO, Vozeh S, Heykants J, Thomson DA and Stanski DR, Population pharmacokinetics of alfentanil: The average dose-plasma concentration relationship and interindividual variability in patients. *Anesthesiology* **66:** 3–12, 1987.
4. Lavrijsen KM, Van Houdt JMG, Van Dyck MJ, Hendrickx JJJM, Woestenborghs RJH, Lauwers W, Meuldermans WEG and Heykants JJP, Is the metabolism of alfentanil subject to debrisoquine polymorphism? *Anesthesiology* **69:** 535–540, 1988.
5. Henthorn TK, Spina E, Dumont E and Von Bahr C, *In vitro* inhibition of a polymorphic human liver P-450 isozyme by narcotic analgesics. *Anesthesiology* **70:** 339–342, 1989.
6. Kharasch ED and Thummel KE, Human alfentanil metabolism by cytochrome P450 3A3/4. An explanation for the interindividual variability in alfentanil clearance? *Anesth Analg* **76:** 1033–1039, 1993.
7. Yun CH, Wood M, Wood AJ-J and Guengerich FP, Identification of the pharmacogenetic determinants of alfentanil metabolism: Cytochrome P-450 3A4. *Anesthesiology* **77:** 467–474, 1992.
8. Schellens JHM, Soons PA and Briemer DD, Lack of bimodality in nifedipine plasma kinetics in a large population of healthy subjects. *Biochem Pharmacol* **37:** 2507–2510, 1988.
9. Meuldermans W, Hendrickx J, Lauwers W, Hurkmans R, Swysen E, Thijssen J, Timmerman P, Woestenborghs R and Heykants J, Excretion and biotransformation of alfentanil and sufentanil in rats and dogs. *Drug Metab Dispos* **15:** 905–913, 1987.
10. Lavrijsen K, Van Houdt J, Van Dick D, Hendrickx J, Lauwers W, Hurkmans R, Bockx M, Janssen C, Meuldermans W and Heykants J, Biotransformation of sufentanil in liver microsomes of rats, dogs, and humans. *Drug Metab Dispos* **18:** 704–710, 1990.
11. Kharasch ED, Hill HF and Eddy AC, Influence of dexmedetomidine and clonidine on human liver microsomal alfentanil metabolism. *Anesthesiology* **75:** 520–524, 1991.
12. Janicki PK, James MFM and Erskine WAR, Propofol inhibits enzymatic degradation of alfentanil and sufentanil by isolated liver microsomes *in vitro*. *Br J Anaesth* **68:** 311–312, 1992.
13. Gill SS, Wright EM and Reilly CS, Pharmacokinetic interaction of propofol and fentanyl: Single bolus injection study. *Br J Anaesth* **65:** 760–765, 1990.
14. Watkins PB, Turgeon DK, Saenger P, Lown KS, Kolars JC, Hamilton T and Guzelian PS, Comparaison of urinary 6β-cortisol and the erythromycin breath test as measures of hepatic P450IIIA activity. *Clin Pharmacol Ther* **52:** 265–273, 1992.
15. Bartkowski RR, Goldberg ME, Huffnagle S and Epstein RH, Effect of erythromycin on sufentanil metabolism: Differences from alfentanil. *Anesth Analg* **70:** S 16, 1990.
16. Gonzalez FJ, *In vitro* systems for prediction of rates of drugs clearance and drug interactions. *Anesthesiology* **77:** 413–415, 1992.
17. Kobayashi K, Chiba K, Tani M, Kuroiwa Y and Ishizaki T, Development and preliminary application of a high-performance liquid chromatographic assay for omeprazole metabolism in human liver microsomes. *J Pharmaceutic Biomed Analysis* **12:** 839–844, 1994.
18. Kremers P, Beaune P, Cresteil T, De Graeve J, Columelli S, Leroux JP and Gielen JE, Cytochrome P450 monooxygenase activities in human and rat liver microsomes. *Eur J Biochem* **118:** 599–606, 1981.
19. Hartree EF, Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal Biochem* **48:** 422–427, 1972.
20. Omura T and Sato R, The carbon monoxide binding pigment of liver microsomes. Evidences for its hematoprotein nature. *J Biol Chem* **239:** 2370–2378, 1964.
21. Labroo RB, Thummel KE, Kunze KL, Podoll T, Trager WF and Kharasch ED, Catalytic role of cytochrome P450 3A4 in multiple pathways of alfentanil metabolism. *Drug Metab Dispos* **23:** 490–496, 1995.
22. Goromaru T, Katashima M, Matsuura H and Yoshimura N, Metabolism of fentanyl in isolated hepatocytes from rat and guinea pig. *Chem Pharmaceutic Bull* **33:** 3922–3928, 1985.
23. Maurice M, Emiliani S, Dalet-Beluche I, Delancourt J and Lange R, Isolation and characterization of a cytochrome P450 of the IIA subfamily from human liver microsomes. *Eur J Biochem* **200:** 508–517, 1991.
24. Kronbach T, Mathys D, Umeno M, Gonzalez FJ and Meyer UA, Oxidation of midazolam and triazolam by human liver cytochrome P450IIIA4. *Mol Pharmacol* **36:** 89–96, 1989.
25. Drouet-Coassolo C, Aubert C, Coassolo P and Cano JP, Capillary gas chromatographic-mass spectrometric method for the identification and quantification of some benzodiazepines and their unconjugated metabolites in plasma. *J Chromatogr Biomed Appl* **487:** 295–311, 1989.
26. Lopez-Garcia MP, Dansette PM, Valadon P, Amar C, Beaune PH, Guengerich FP, Mansuy D, Human-liver cytochromes P-450 expressed in yeast as tools for reactive-metabolite formation studies: Oxidative activation of tienilic acid by cytochromes P-450 2C9 and 2C10. *Eur J Biochem* **213:** 223–232, 1993.
27. Newton DJ, Wang RW, Lu AYH, Cytochrome P450 inhibitors. Evaluation of specificities in the *in vitro* metabolism of therapeutic agents by human liver microsomes. *Drug Metab Dispos* **23:** 154–158, 1995.
28. De Waziers P, Cugnenc PH, Yang CS, Leroux JP, Beaune PH, Cytochrome P450 isozymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. *J Pharmacol Exp Therap* **253:** 387–394, 1990.
29. Belloc C, Baird S, Cosme J, Lecoeur S, Gautier J-C, Challine D, De Waziers I, Flinois J-P, Beaune P, Human cytochromes P450 expressed in *Escherichia coli*: Production of specific antibodies. *Toxicology* **106:** 207–219, 1996.
30. Goromaru T, Matsuura H, Yoshimura N, Miyawaki T, Sameshima T, Miyao J, Furuta T, Baba S, Identification and quantitative determination of fentanyl metabolites in patients by gas chromatography-mass spectrometry. *Anesthesiology* **61:** 73–77, 1984.
31. Lown KS, Thummel KE, Benedict PE, Shen DD, Turgeon DK, The erythromycin breath test predicts the clearance of midazolam. *Clin Pharmacol Therap* **57:** 16–24, 1995.
32. Pearce R, Greenway D, Parkinson A, Species differences and interindividual variation in liver microsomal cytochrome

P450 2A enzymes: effects on coumarin, dicumarol and testosterone oxidation. *Arch Biochem Biophys* **298:** 211–225, 1992.
33. Guengerich FP, Turvey CG, Comparaison of levels of several human microsomal cytochrome P-450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical liver samples. *J Pharmacol Exp Therap* **256:** 1189–1194, 1991.
34. Tateishi T, Krivoruk Y, Ueng Y-F, Wood AJJ, Guengerich FP, Wood M, Identification of human liver cytochrome P-450 3A4 as the enzyme responsible for fentanyl and sufentanil *N*-dealkylation. *Anesth Analg* **82:** 167–172, 1996.
35. Gonzalez FJ, Crespi CL, Gelboin HV, cDNA-expressed human cytochrome P450s: A new age of molecular toxicology and human risk assessment. *Mutat Res* **247:** 113–127, 1991.
36. Kato R, Yamazoe Y, The importance of substrate concentration in determining cytochromes P450 therapeutically relevant *in vivo*. *Pharmacogenetics* **4:** 359–362, 1994.
37. Goldstein JA, De Morais SMF, Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics* **4:** 285–299, 1994.
38. Heim MH and Meyer, UA, Evolution of a highly polymorphic human cytochrome P450 genes cluster: CYP2D6. *Genomics* **14:** 49–58, 1992.
39. Tassaneeyakul W, Birkett DJ, Veronese ME, McManus ME, Tukey RH, Quattrochi LC, Gelboin HV and Miners JO, Specificity od substrate and inhibitor probes for human cytochromes P450 1A1 and 1A2. *J Pharmacol Exp Therap* **265:** 401–407, 1993.
40. Wrighton SA, Brian WR, Sari M-A, Iwasaki M, Guengerich FP, Raucy JL, Molowa DT and Van den Branden M, Studies on the expression and metabolic capabilities of human liver cytochrome P450IIIA5 (HLp3). *Mol Pharmacol* **38:** 207–213, 1990.
41. Bartkowski RR, Goldberg M and Huffnagle S, Sufentanil disposition. Is it affected by erythromycin administration? *Anesthesiology* **78:** 260–265, 1993.
42. Lehmann KA, Sipakis K, Gasparini R and Van Peer A, Pharmacokinetics of sufentanil in general surgical patients under different conditions of anesthesia. *Acta Anesth Scand* **37:** 176–180, 1993.
43. Bartowski RR and McDonnell TE. Prolonged alfentanil effect following erythromycin administration. *Anesthesiology* **73:** 566–568, 1990.
44. Hiller A, Olkkola KT, Isohanni P and Saarnivaara L, Unconsciousness associated with midazolam and erythromycin. *Br J Anaesth* **65:** 826–828, 1990.
45. Lucey MR, Kolars JC, Merion RM, Campbell DA, Aldrich M and Watkins PB, Cyclosporin toxicity at therapeutic blood levels and cytochrome P-450 IIIA. *Lancet* **335:** 11–15, 1990.
46. Mather LE, Clinical pharmacokinetics of fentanyl and its newer derivatives. *Clin Pharmacokinet* **8:** 422–446, 1983.
47. Bower S and Hull CJ, Comparative pharmacokinetics of fentanyl and alfentanil. *Br J Anaesth* **54:** 871–877, 1982.
48. Ferrier C, Marty J, Bouffard Y, Haberer JP, Levron JC and Duvaldestin P, Alfentanil pharmacokinetics in patients with cirrhosis. *Anesthesiology* **62:** 480–484, 1985.
49. Gepts E, Shafer SL, Camu F, Stanski DR, Woestenborghs R, VanPeer A and Heykants JJP, Linearity of pharmacokinetics and model estimation of sufentanil. *Anesthesiology* **83:** 1194–1204, 1995.