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# In Vitro *Inhibition of a Polymorphic Human Liver P-450 Isozyme by Narcotic Analgesics*

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**Genetically determined differences in the ability of individuals to oxidize certain drugs by hepatic P-450 processes has gained increasing attention. The so-called debrisoquin hydroxylase, which is defective in approximately 5–10% of whites, is known to be of major importance for the metabolism of several drugs. The possible relation of this isozyme to the metabolism of narcotics eliminated by oxidative biotransformation has not been studied. Several narcotics were screened for *in vitro* interaction with this P-450 isozyme. This was accomplished by testing for competitive inhibition by narcotics of the 2-hydroxylation of desmethylimipramine in a human liver microsomal preparation. Alfentanil, fentanyl, and dextropropoxyphene were found to competitively inhibit this pathway demonstrating an interaction with this polymorphic isozyme. No interaction was found for codeine, meperidine, methadone, morphine, or nalbuphine. These results suggest that a genetic defect may be important for elimination clearance by metabolism for dextropropoxyphene, alfentanil, and fentanyl and that *in vivo* investigation is warranted. (Key words: Anesthetics, intravenous: alfentanil; fentanyl; meperidine; methadone; morphine; nalbuphine. Metabolism, genetic factors. Metabolism: alfentanil; codeine; dextropropoxyphene; fentanyl; meperidine; methadone; morphine; nalbuphine.)**

WIDE INTERSUBJECT variability in oxidative drug metabolism and its capacity to affect response to drug therapy is now well recognized.[1] A defect in the hydroxylation of debrisoquin is present in approximately 5–10% of whites and is inherited as an autosomal recessive trait with a mutant allele frequency of 35–43%.[2,3] Individuals who are poor metabolizers of debrisoquin are poor oxidizers of other drugs as well. Such patients taking these drugs may suffer therapeutic consequences resulting from attaining higher than expected plasma concentrations, such as orthostatic hypotension with debrisoquin, excessive β-blockade with metoprolol, propranolol, and bufuralol, and CNS toxic side effects with nortriptyline or, because of failure to produce the active metabolite, there may be a lack of, or reduction in, the therapeutic effect, as with the antiarrhythmic encainide.[4,5] Phenotypic consistency with defective debrisoquin hydroxylation, both *in vivo* and *in vitro* using human liver microsomes, has been demonstrated for the P-450 oxidation of sparteine and desmethylimipramine.[6–8]

With the advent of the human liver bank it has become easier to study human microsomal drug metabolic processes *in vitro*.[9] *In vitro* demonstration of competitive inhibition of the formation of 2-hydroxydesmethylimipramine (2-OH-DMI) from desmethylimipramine (DMI) by a drug of interest indicates that the genetically variable debrisoquin hydroxylase isozyme may be important in the metabolism of the drug being tested.[10,11] With such *in vitro* techniques, a relatively large number of drugs can be studied inexpensively to identify those drugs likely to have polymorphic drug metabolism.

Large interindividual differences in elimination clearance for some narcotics that undergo oxidative metabolism in the liver have been reported.[12–14] The possible contribution of a clearly defined monogenetic factor accounting for differences in the oxidative metabolism of narcotic drugs has not been established. We, therefore, sought to demonstrate *in vitro* competitive inhibition of DMI 2-hydroxylation by several narcotics.

## Methods

### CHEMICALS AND REAGENTS

DMI hydrochloride and N-desmethylclomipramine (internal standard) were obtained from Ciba-Geigy (Basel, Switzerland), 2-OH-DMI from Regis Chemical Company (Chicago, Illinois), pig heart isocitric dehydrogenase from Boehriger and Soehne (Mannheim, West Germany) and

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TABLE 1. Effect of Various Narcotics on 2-Hydroxylation of Desmethylimipramine by Human Liver In Vitro

| Test Drug | Highest Test Drug Concentration (μM) | % of Control 2-OH-DMI Formation Rate | $K_i$* (μM) |
|---|---|---|---|
| Control | 0 | 100 | — |
| Alfentanil | 100 | 51 | 176 |
| Codeine | 500 | 96 | — |
| Dextropropoxyphene | 50 | 28 | 2.5 |
| Fentanyl | 100 | 37 | 21 |
| Meperidine | 500 | 94 | — |
| Methadone | 500 | 95 | — |
| Morphine | 500 | 100 | — |
| Nalbuphine | 500 | 100 | — |

* Inhibition rate constant.

NADPH and d,l-isocitrate from Sigma Chemical Company (St. Louis, Missouri). The drugs came from their respective manufacturers. Analytic grade chemicals and solvents were from Merck (Darmstadt, West Germany).

### HUMAN LIVER MICROSOMES

Adult human liver specimens came from the liver bank at the Karolinska Institute.[9] These liver specimens were obtained from a single kidney transplant donor with total cerebral infarction shortly after circulatory arrest, and the samples were frozen as small cubes in liquid nitrogen within half an hour after the patient's death. This procedure was approved by the Swedish Board of Health and Welfare. Microsomes were prepared as previously described[9] and assayed for protein content.[15] The microsomes were stored at −80° C at a protein concentration of 10 mg/ml.

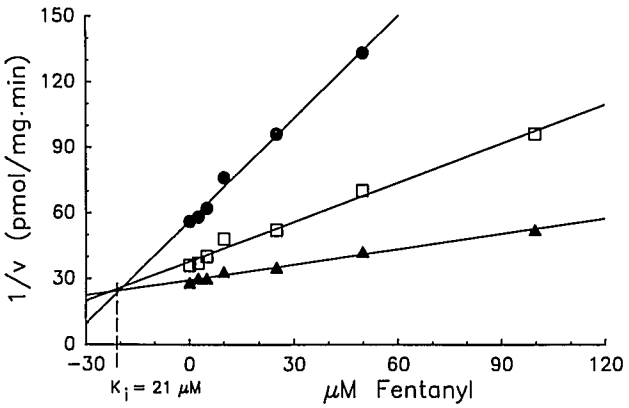


FIG. 1. Dixon plots showing the effects of different concentrations of fentanyl (abscissa) on the formation rate (v = pmol/mg × min; ordinate) of 2-hydroxydesmethylimipramine from different concentrations of desmethylimipramine (10 μM ● —— ●, 25 μM □ —— □, and 50 μM ▲ —— ▲). Solid lines represent best fit least squares linear regression lines. $K_i$ was taken as the geometric midpoint of the triangle formed by the intersection of the three regression lines.

### EXPERIMENTAL PROTOCOL

The rate of hydroxylation of DMI in the livers was determined in a system containing microsomes (0.5 mg protein/ml), 50 mM Tris-HCl (*p*H 7.5), 5 mM $MgCl_2$, 1 mM $NADP^+$, 5 mM d,l-isoctrate, and 0.5 mg pig heart isocitric dehydrogenase per ml incubation mixture. Incubations were performed at 37° C in air for 20 min with different inhibitor drug concentrations (alfentanil, codeine, dextropropoxyphene, fentanyl, meperidine, methadone, morphine, and nalbuphine; one incubation at each concentration) at three fixed concentrations of DMI (10, 25, and 50 μM). Prior to this step the narcotic compounds were screened by testing concentrations up to 1.0 mM for the presence of an ability to inhibit the hydroxylation of DMI (25 μM). The reaction was started by the addition of microsomes and stopped with 300 μl carbonate buffer, *p*H 10.9. The concentration of 2-OH-DMI was measured in duplicate using a modification[8] of the high performance liquid chromatographic method of Sutfin and Jusko[16] using electrochemical detection at 0.45 V. This method is able to separate and measure DMI and 2-OH-DMI concentrations in plasma, urine, and microsomal preparations.[8] Standard curves were prepared by adding 2-OH-DMI to microsomes and co-factors. Carbonate buffer was added before the microsomes to ensure that no metabolism occurred. None of the drugs tested interferes with the this assay technique. The coefficient of variation was 1.3% for DMI to 800 nM and 3.5% for 2-OH-DMI to 600 nM. The method of Dixon[17] was used to compare the formation rates of 2-OH-DMI at various inhibitor concentrations to detect competitive inhibition and estimate the inhibitory rate constant $K_i$.

## Results

The rate of 2-hydroxylation of DMI was linear for at least 20 min and to 1 mg/ml of microsomal protein. The apparent $K_M$ was approximately 20 μM. Table 1 shows the effect of the narcotics tested on the 2-hydroxylation of DMI in human liver microsomes *in vitro*. Dextropropoxyphene, fentanyl, and alfentanil competitively inhibited the hydroxylation while nalbuphine, meperidine, morphine, codeine, and methadone did not. Figure 1 is a Dixon plot with representative competitive inhibition and demonstrates that fentanyl is a potent inhibitor (apparent inhibition constant $K_i$ = 21 μM). Dextropropoxyphene was an even more potent inhibitor ($K_i$ = 2.5 μM), whereas the inhibition seen with alfentanil was less ($K_i$ = 176 μM).

## Discussion

Previous studies of the 4-hydroxylation of debrisoquin and 2-hydroxylation of DMI *in vivo* and in human liver

microsomes have demonstrated that the two drugs are hydroxylated by the same cytochrome P-450 isozyme, the so-called debrisoquin hydroxylase.[8] Further studies have shown that drugs that depend on the debrisoquin phenotype for their metabolism inhibit the 2-hydroxylation of DMI *in vitro*.[11] Others have shown that similar inhibition tests with debrisoquin,[18] sparteine,[10] and bufuralol[19] could also be used as screening tests to identify drugs that interact with the debrisoquin hydroxylase. We studied representative narcotic agents for their ability to inhibit DMI 2-hydroxylation *in vitro* to either confirm or deny the possibility that certain narcotics depend on this genetically variable P-450 isozyme for their elimination clearance.

The major metabolic pathway for dextropropoxyphene is *via* an N-dealkylation,[20] a reaction that may be mediated by debrisoquin hydroxylase.[4] Dextropropoxyphene was a particularly potent competitive inhibitor of DMI-hydroxylation ($K_i$ = 2.5 $\mu$M), indicating interactions with the same enzymatic site as DMI and, most likely, debrisoquin. This level of *in vitro* inhibition is consistent with *in vivo* observations. Oates *et al.*[21] have reported that treatment with dextropropoxyphene increased the debrisoquin metabolic ratios in eight of nine subjects but not sufficiently to alter phenotypic classification. In addition, Robson *et al.*[22] have demonstrated in volunteers that dextropropoxyphene inhibits distinct P-450 metabolic pathways of certain other drugs (*i.e.*, 8-hydroxylation of theophylline but not its 1-demethylation), indicating that there may be some isozyme specificity. Such correlation between potent *in vitro* inhibition and *in vivo* findings is not unexpected. Quinidine, which we reported to have an even greater competitive inhibitory effect on DMI-hydroxylation ($K_i$ = 0.27)[11] than dextropropoxyphene, has been demonstrated to inhibit debrisoquin hydroxylase *in vivo* enough to alter debrisoquin phenotypic status.[23]

Alfentanil and fentanyl also competitively inhibited DMI-hydroxylation. Although neither bimodal distributions nor metabolic polymorphisms have been demonstrated for either drug, the possibility of polymorphic alfentanil metabolism has been offered to explain the observation that occasionally patients have a reduced clearance for this narcotic.[14,24] Both fentanyl and alfentanil depend on similar oxidative pathways for elimination clearance (fig. 2).[25,26] All of these pathways (N-dealkylation, O-dealkylation, and hydroxylation) may be mediated by debrisoquin hydroxylase.[4] Sufentanil was not studied. However, because of structural similarities with its congeners (fentanyl and alfentanil), the importance of comparable oxidative biotransformations is suggested.

The competitive inhibition of DMI hydroxylation by fentanyl, alfentanil, and dextropropoxyphene demonstrates that these drugs interact with the catalytic enzyme site hydroxylating DMI and, by inference, probably debrisoquin. The degree of inhibition is not an indicator of the importance of the isozyme for the metabolism of the test drug. However, competitive inhibition does not prove that the drug is metabolized by this site, nor does it exclude the possibility that other P-450 isozymes may biotransform these drugs and that these alternate pathways may be important or even dominant. Recent *in vitro* work by Lavrijsen *et al.*[27] found that debrisoquin does not competitively inhibit the formation of the major metabolites of alfentanil, noralfentanil, and N-phenylpropanamide.

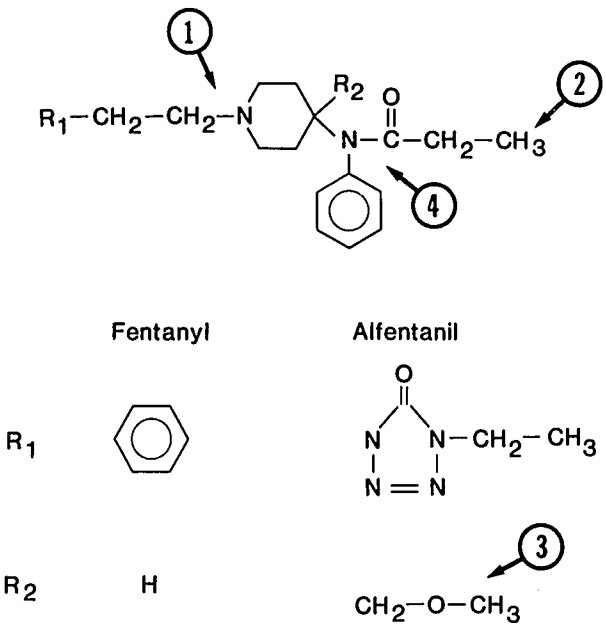


FIG. 2. Molecular structure of fentanyl and alfentanil showing important sites of oxidative metabolism in humans. The major route of biotransformation for both drugs is an N-dealkylation at site 1. Alfentanil undergoes an O-dealkylation at site 3. Fentanyl, or its N-dealkylated metabolite, undergoes hydroxylation at site 2. N-dealkylation at site 4 has also been demonstrated as a minor pathway for fentanyl. Although yet undemonstrated because of shared structure, these latter pathways (2 and 4) may apply to alfentanil as well.

Our results indicate that alfentanil, fentanyl, and dextropropoxyphene interact at a P-450 isozyme with genetic polymorphism. The importance of this genetic factor to the elimination clearance of alfentanil has been reported by Meuldersmans *et al.*[26] in a study of three subjects (one poor metabolizer of debrisoquin) and in a preliminary report of a study of six subjects (two poor metabolizers) by Henthorn *et al.*[28] Both groups found alfentanil clearance to be unaffected by the debrisoquin hydroxylase polymorphism. The importance of this polymorphism to the metabolism of fentanyl and dextropropoxyphene deserves to be investigated *in vivo*. These results also show that it is unlikely that the so-called debrisoquin hydroxylase is responsible for the metabolism of any of the other narcotics tested.

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