

## Alfentanil Clearance is Independent of the Polymorphic Debrisoquin Hydroxylase

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Because alfentanil has been shown to inhibit debrisoquin hydroxylase *in vitro*, and there is considerable variability in the reported elimination clearance of alfentanil, the possible influence of the debrisoquin metabolic phenotype on the elimination clearance of alfentanil was studied. The disposition of alfentanil was determined after rapid intravenous administration to four extensive debrisoquin metabolizers and three poor debrisoquin metabolizers. Debrisoquin hydroxylation phenotype was determined using the urinary dextromethorphan/dextrorphan metabolic ratio test. The disposition of alfentanil was characterized by a three-compartment open mammillary model. There was no relationship between the dextromethorphan/dextrorphan metabolic ratio and the elimination clearance of alfentanil despite a nearly seven hundred-fold range of the metabolic ratio in the seven volunteers. This indicates that the variability in the elimination clearance of alfentanil is not due to the polymorphism of debrisoquin hydroxylase. Nor is this variability due to variable hepatic blood flow because in this study alfentanil clearance was not related to indocyanine green clearance. (Key words: Analgesics: opioid, alfentanil. Metabolism, genetic factors: debrisoquin, dextromethorphan. Pharmacokinetics: alfentanil.)

WIDE INTERSUBJECT VARIABILITY in the ability to oxidatively metabolize drugs and the capacity of this variability to affect response to drug therapy are now well recognized. A defect in the hydroxylation of debrisoquin is present in 5-10% of Caucasians and is thought to be inherited as an autosomal recessive trait.<sup>1</sup> Poor metabolizers of debrisoquin have been shown to be poor oxidizers of other drugs as well, often with important therapeutic consequences.<sup>1</sup> The fact that the P-450 enzyme referred to as debrisoquin hydroxylase is also responsible for the oxidative metabolism of other drugs has provided the basis for using sparteine,<sup>2</sup> desmethylimipramine,<sup>3</sup> and dextromethorphan<sup>4,5</sup> as probes to classify the debrisoquin hydroxylator phenotypes of individuals as either extensive metabolizers (EM) or poor metabolizers (PM).

Debrisoquin hydroxylase catalyzes oxidative reactions such as N-dealkylation and O-demethylation.<sup>1</sup> Alfentanil is metabolized by oxidative reactions that include N-dealkylation and O-demethylation.<sup>6</sup> Henthorn *et al.*<sup>7</sup> reported that alfentanil competitively inhibits debrisoquin hydroxylase *in vitro*. The polymorphic debrisoquin hydroxylase has been invoked as a possible explanation for observations of prolonged respiratory depression following infusions of alfentanil,<sup>8</sup> abnormal alfentanil kinetics,<sup>9</sup> and even reduced clearance of sufentanil in patients.<sup>10</sup>

The purpose of this study was to determine the disposition of alfentanil in healthy volunteers of known debrisoquin oxidation phenotype in order to evaluate the effect of this genetic polymorphism on the pharmacokinetics of alfentanil.

### Methods

From a larger population, seven healthy volunteers (four males and three females) were chosen to include individuals of both extensive and poor debrisoquin hydroxylator phenotypes (table 1) and gave institutionally approved written informed consent. No subject smoked cigarettes or was currently taking medication. Each volunteer was phenotyped with a single 30-mg oral dose of dextromethorphan hydrobromide (Robitussin DM®, A. H. Robins Co., Richmond, VA) taken just before bedtime. The ratio between the parent drug and the O-demethylated metabolite, dextrorphan, was determined from an aliquot of an 8-h urine collection following the dose. Dextromethorphan and dextrorphan were deter-

TABLE 1. Subject Characteristics

Subject	Age	Weight (kg)	Sex	Dextromethorphan Metabolic Ratio*	ICG Cl <sub>2</sub> (ml/min)
1	43	85	M	0.025	1158
2	35	78	M	0.023	1328
3	26	55	F	0.008	982
4	36	58	M	0.120	828
5	34	61	F	3.966†	1165
6	24	51	F	5.605†	969
7	32	85	M	2.980†	1042

\* Dextromethorphan/dextrorphan in an 8-h urine collection following a 30-mg dose of dextromethorphan.

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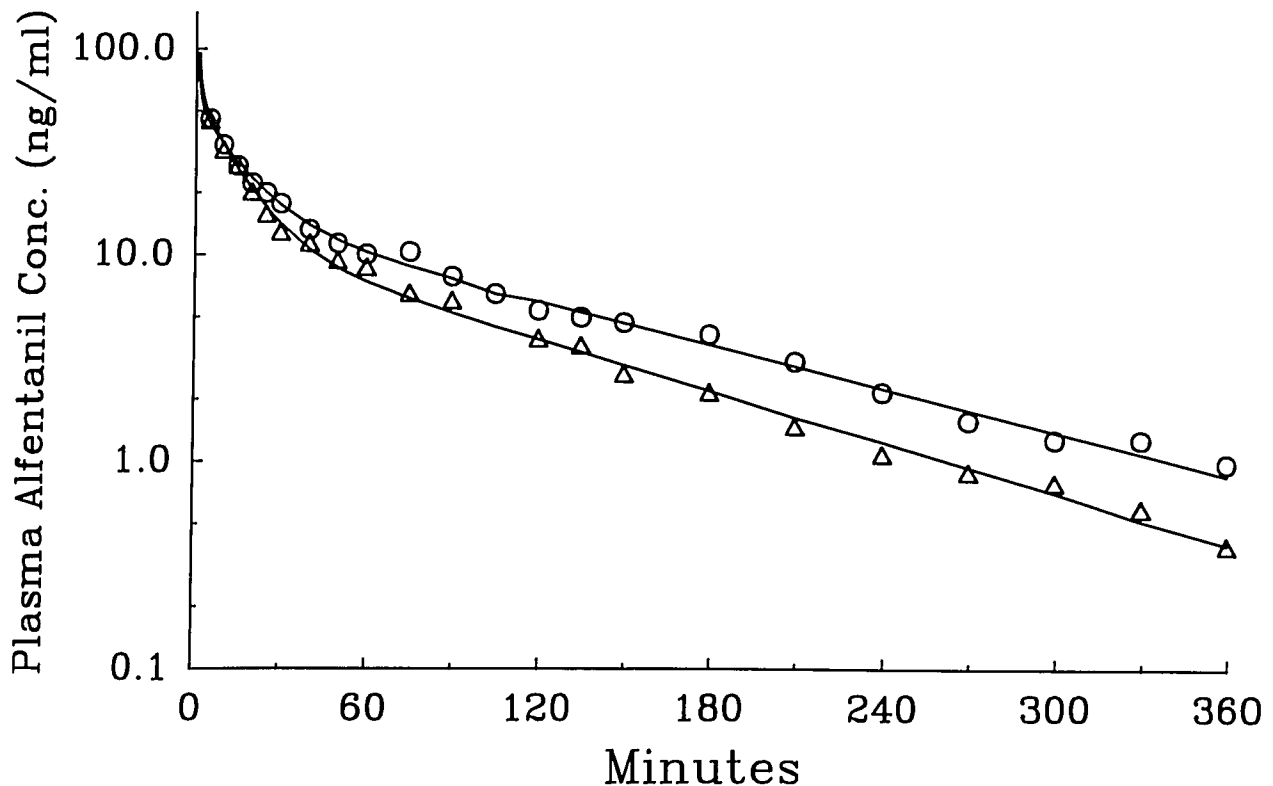


FIG. 1. Alfentanil arterial plasma concentration *versus* time relationship in a volunteer with an extensive metabolizer debrisoquin phenotype (subject 3, circles) and a volunteer with a poor metabolizer debrisoquin phenotype (subject 6, triangles). The lines are computer derived nonlinear least squares regressions for the three-compartment alfentanil model.

mined by gas chromatography on a DB-17 megabore column (J & W Scientific, Folsom, CA) with nitrogen detection, a method modified from that of Schmid *et al.*,<sup>5</sup> after solid phase extraction (CN Bond Elut®, Analytichem International, Harbor City, CA) of the hydrolysate. The assays for dextromethorphan and dextrorphan were linear over the range of 0.10–10.0  $\mu\text{g}/\text{ml}$  with coefficients of variation of less than 10%. Blood samples were obtained for CBC and routine clinical chemistries and subjects were instructed to report to the Clinical Research Center of Northwestern University Medical School following an overnight fast.

A radial artery was cannulated with a 20-G catheter-over-needle in each subject for blood sampling. Volunteers were supine for at least 1 h before and 1.5 h after drug administration. Subjects were then fed a standardized breakfast (coffee, orange juice, and an Egg McMuffin®, McDonalds Corp., Oakbrook, IL) and allowed to assume a sitting position.

Alfentanil HCl (Alfenta®, Janssen Pharmaceutica, Piscataway, NJ), 10  $\mu\text{g}/\text{kg}$ , was injected over 15 s into the stream of a rapidly running intravenous infusion. Arterial blood sampling began after complete intravascular mixing.<sup>11</sup> Thirty-one blood samples were obtained over the subsequent 6 h.

Because of the low plasma alfentanil concentrations following the administration of this small alfentanil dose to volunteers, the drug concentrations were measured by a modification of the direct specific radioimmunoassay method of Michiels *et al.*<sup>12</sup> The sample was added as 200  $\mu\text{l}$  of plasma in the manner of the fentanyl RIA<sup>13</sup> rather than as a 1:10 or 1:100 plasma dilution<sup>12</sup> because it was not necessary to dilute the samples to get them in the linear range of the RIA.

Plasma alfentanil concentration *versus* time data were fit to a three-compartment open mammillary model (*i.e.*, one in which there is a central plasma equivalent volume,  $V_C$ , from which drug is eliminated and is distributed to both a rapidly equilibrating plasma equivalent volume,  $V_F$ , and a slowly equilibrating plasma equivalent volume,  $V_S$ ) using the CONSAM/SAAM29§ program.<sup>14</sup> The data were weighted in proportion to the reciprocal of the estimated standard deviation for each datum; it was assumed that all data had estimated fractional standard deviations of 0.1. CONSAM/SAAM29 provided estimates of the  $V_C$  and the five adjustable rate constants of the model

§ Berman M, Weiss M. SAAM users manual. Bethesda, Maryland: DHEW Publication (NIH), 1978:79–180.

TABLE 2. Alfentanil Kinetics

Subject	Compartmental Volumes (l)				Clearances (l/min)†			t1/2β (min)
	V <sub>C</sub>	V <sub>F</sub>	V <sub>S</sub>	V <sub>SS</sub> *	CL <sub>F</sub>	CL <sub>S</sub>	CL <sub>E</sub>	
1	2.50	5.72	12.67	20.87	3.51	0.52	0.20	84.5
2	3.66	4.85	8.49	17.01	3.24	0.26	0.12	110.8
3	4.20	3.96	10.67	18.83	1.78	0.26	0.19	88.5
4	3.51	4.63	11.25	19.39	2.46	0.23	0.19	94.3
5	4.61	8.53	10.61	23.75	3.40	0.30	0.28	71.8
6	2.96	3.51	10.43	16.91	1.61	0.23	0.23	73.8
7	3.65	7.20	9.80	20.65	4.77	0.36	0.21	76.8
Mean (SD)	3.58 (0.71)	4.85 (1.79)	10.56 (1.28)	19.63 (2.40)	2.97 (1.10)	0.31 (0.10)	0.20 (0.05)	85.8 (13.7)

\* V<sub>SS</sub> is the sum of V<sub>C</sub>, V<sub>F</sub>, and V<sub>S</sub>.

† Clearances are calculated as the product of a volume and the exiting rate constant. See text for details.

V<sub>C</sub> = Central plasma equivalent volume.

V<sub>F</sub> = Rapidly equilibrating plasma equivalent volume.

V<sub>S</sub> = Slowly equilibrating plasma equivalent volume.

CL<sub>F</sub> = Clearance to (from) V<sub>F</sub>.

CL<sub>S</sub> = Clearance to (from) V<sub>S</sub>.

CL<sub>E</sub> = Elimination clearance.

t1/2β = Elimination half life.

that were used to calculate the intercompartmental clearances for the two peripheral compartments (e.g., CL<sub>F</sub> = k<sub>CF</sub>V<sub>C</sub> = k<sub>FC</sub>V<sub>F</sub>). These clearances therefore provided volume independent estimates of drug distribution.<sup>15</sup>

In order to estimate hepatic blood flow, indocyanine green (ICG) (Cardio-Green®, Hyson, Westcott, and Dunning, Baltimore, MD), 0.5 mg/kg, was administered concomitantly with the alfentanil. Sample collection, spectrophotometric blood concentration determination, and data analysis for ICG were performed as previously described.<sup>11</sup>

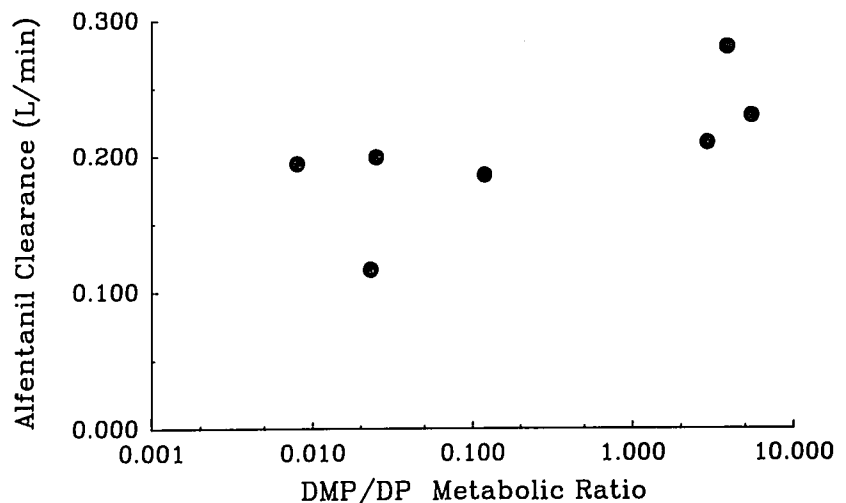
The relationship between alfentanil elimination clearance and the dextromethorphan metabolic ratio was sought using standard least squares linear regression and Spearman rank correlation techniques. The criterion for rejection of the null hypothesis was  $P < 0.05$ .

### Results

Volunteer characteristics are listed in table 1. The three poor debrisoquin hydroxylators were selected because their dextromethorphan metabolic ratios were greater than the mean for poor metabolizers, making their phenotypic status unequivocal. Three of the extensive metabolizers were chosen because their dextromethorphan metabolic ratios were within one standard deviation of the mean for extensive metabolizers. The other extensive metabolizer had a dextromethorphan metabolic ratio near the cut-point separating the two phenotypes.<sup>4</sup>

Representative plasma concentration *versus* time relationships for an extensive and a poor metabolizer of dextromethorphan are illustrated in figure 1. The kinetic variables describing the disposition of alfentanil in the volunteers are presented in table 2.

FIG. 2. Alfentanil elimination clearance plotted as a function of the metabolic ratio of the dextromethorphan phenotyping test. There is clearly no relationship between these variables (Pearson's  $r = 0.6967$ , Spearman's  $\rho = 0.7857$ ).



As illustrated in figure 2, there was no relationship between the elimination clearances of alfentanil in these volunteers and their dextromethorphan metabolic ratios. Nor was there a relationship between the elimination clearance of alfentanil (table 2) and the elimination clearance of ICG (table 1) ( $r = -0.2751$ ).

### Discussion

It was the purpose of the present study to identify a potential source of variability in the elimination clearance of alfentanil: that is, polymorphic metabolism by the P-450 isozyme, debrisoquin hydroxylase. Despite *in vitro* evidence that debrisoquin hydroxylase may metabolize alfentanil<sup>7</sup> and *in vivo* evidence of considerable variability in alfentanil elimination clearance,<sup>8,9</sup> the present findings unequivocally demonstrate that the polymorphic debrisoquin hydroxylase does not influence the elimination clearance of alfentanil. These results are consistent with those of Meuldermans *et al.*<sup>6</sup> who studied the disposition of alfentanil in a single poor metabolizer of debrisoquin. These *in vivo* findings are not inconsistent with *in vitro* evidence suggesting that alfentanil is metabolized by the polymorphic debrisoquin hydroxylase<sup>7</sup> since this *in vitro* method is unable to determine the importance of this enzyme to the *in vivo* metabolism of alfentanil. The *in vitro* results of Lavrijsen *et al.*,<sup>16</sup> indicating that debrisoquin hydroxylase is not an important isozyme for the *in vivo* metabolism of alfentanil are consistent with the present results and those of Meuldermans *et al.*<sup>6</sup>

Only the results of McDonnell *et al.*\*\* conflict with the present *in vivo* findings and those of Meuldermans *et al.*<sup>6</sup> In their study, alfentanil clearance was related to the formation rate of acetaminophen from phenacetin. Although the formation rate of acetaminophen from phenacetin is affected by the polymorphic debrisoquin hydroxylase,<sup>17</sup> it is not possible with current information to distinguish between extensive and poor debrisoquin oxidation phenotypes on the basis of the acetaminophen formation rate as suggested by McDonnell *et al.* Therefore, one cannot conclude from these results that the reduced elimination clearance of alfentanil (and phenacetin) in their patient has a genetic basis.

It is possible that other factors may account for the variability in alfentanil clearance. One or both of the two important alfentanil metabolic pathways (*i.e.*, N-dealkylation and O-demethylation)<sup>6</sup> may be mediated by another polymorphic P-450 isozyme such as mephenytoin hydroxylase,<sup>18</sup> which has recently been shown to affect the

elimination clearance of diazepam,<sup>19</sup> thus explaining the observed variability in alfentanil elimination clearance.<sup>8,9</sup> Bartkowski *et al.*<sup>20</sup> recently reported that some individuals undergo a marked reduction in alfentanil clearance after treatment with erythromycin, presumably *via* inhibition of P-450 by the antibiotic. Although the correlation between the clearances of ICG and alfentanil demonstrated by Chauvin *et al.*<sup>21</sup> suggests the variability in hepatic blood flow may contribute to the wide range of elimination clearances of alfentanil, one would not expect this to be important for a low to intermediate extraction ratio drug like alfentanil.<sup>22</sup> Indeed, the lack of relationship between ICG elimination clearance and that of alfentanil in the present study is consistent with alfentanil being a low extraction ratio drug despite being inconsistent with the results of Chauvin *et al.*<sup>21</sup>

In conclusion, the variability in the elimination clearance of alfentanil is not due to the polymorphism of debrisoquin hydroxylase. In addition, our results indicate that this variability is also not due to variable hepatic blood flow because alfentanil clearance was not related to indocyanine green clearance.

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