Pharmacogenetic Determinants of Human Liver Microsomal Alfentanil Metabolism and the Role of Cytochrome P450 3A5

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Background: There is considerable unexplained interindividual variability in the clearance of alfentanil. Alfentanil undergoes extensive metabolism by cytochrome P450 3A4 (CYP3A4). CYP3A5 is structurally similar to CYP3A4 and metabolizes most CYP3A4 substrates but is polymorphically expressed. Livers with the CYP3A5*1 allele contain higher amounts of the native CYP3A5 protein than livers homozygous for the mutant CYP3A5*3 allele. This investigation tested the hypothesis that alfentanil is a substrate for CYP3A5 and that CYP3A5 pharmacogenetic variability influences human liver alfentanil metabolism.

Methods: Alfentanil metabolism to noralfentanil and N-phenylpropionamide was determined in microsomes from two groups of human livers, characterized for CYP3A4 and CYP3A5 protein content: low CYP3A5 (2.0–5.2% of total CYP3A, n = 10) and high CYP3A5 (46–76% of total CYP3A, n = 10). Mean CYP3A4 content was the same in both groups. The effects of the CYP3A inhibitors troleandomycin and ketoconazole, the latter being more potent toward CYP3A4, on alfentanil metabolism were also determined.

Results: In the low versus high CYP3A5 livers, respectively, noralfentanil formation was 77 ± 31 versus 255 ± 170 pmol·min⁻¹·mg⁻¹, N-phenylpropionamide formation was 8.0 ± 3.1 versus 20.5 ± 14.0 pmol·min⁻¹·mg⁻¹, and the metabolite ratio was 9.5 ± 0.4 versus 12.7 ± 1.4 (P < 0.05 for all). There was a poor correlation between alfentanil metabolism and CYP3A4 content but an excellent correlation when CYP3A5 (i.e., total CYP3A content) was considered (r² = 0.81, P < 0.0001). Troleandomycin inhibited alfentanil metabolism similarly in the low and high CYP3A5 livers; ketoconazole inhibition was less in the high CYP3A5 livers.

Conclusion: In microsomes from human livers expressing the CYP3A5*1 allele and containing higher amounts of CYP3A5 protein, compared with those with the CYP3A5*3 allele and low CYP3A5, there was greater alfentanil metabolism, metabolite ratios more closely resembled those for expressed CYP3A5, and inhibitors with differing CYP3A4 and CYP3A5 selectivities had effects resembling those for expressed CYP3A5. Therefore, alfentanil is metabolized by human liver microsomal CYP3A in addition to CYP3A4, and pharmacogenetic variability in CYP3A5 expression significantly influences human liver alfentanil metabolism in vivo. Further investigation is warranted to assess whether the CYP3A5 polymorphism is a factor in the interindividual variability of alfentanil metabolism and clearance in vivo.

There is considerable interindividual variability in the disposition of alfentanil, which still remains incompletely explained.¹ For example, one investigation reported a 48% coefficient of variation in alfentanil clearance, even after correcting for age, body weight, and sex.² Alfentanil is a low- to moderate-extraction drug cleared exclusively by hepatic metabolism, with less than 1% excreted unchanged.³ Alfentanil systemic clearance is therefore proportional to alfentanil hepatic metabolism, and interindividual variability in clearance therefore attributed to variability in alfentanil metabolism.⁴–⁶ Nonetheless, the mechanism of variability remains incompletely elucidated.

Alfentanil is metabolized in vivo via N-dealkylation to two major metabolites; noralfentanil and N-phenylpropionamide (fig. 1).⁷–¹⁰ N-phenylpropionamide is formed primarily from alfentanil, not by further metabolism of noralfentanil.¹⁰ Both major pathways of human liver microsomal alfentanil metabolism, noralfentanil and N-phenylpropionamide formation, were catalyzed predominantly by cytochrome P450 3A4 (CYP3A4).¹⁰ Subsequent clinical investigations confirmed that human alfentanil metabolism and clearance in vivo are also determined predominantly by CYP3A activity.⁴–⁶ Indeed, because of the considerable dependence of alfentanil clearance on CYP3A, alfentanil has been used as an in vivo probe for CYP3A activity and drug interactions.⁵–⁶,¹¹–¹⁵

The human CYP3A subfamily is comprised of CYP3A4, CYP3A5, the fetal and minor adult form CYP3A7, and CYP3A45 (which contributes negligibly to drug metabolism).¹¹ CYP3A4 is the most quantitatively abundant CYP in human liver, accounting for 50–50% of total CYP, and in human intestine, accounting for more than 70% of total CYP.¹⁵–¹⁷ CYP3A5 shares considerable sequence homology and qualitatively similar substrate selectivity with CYP3A4.¹⁸ The relative quantitative metabolic activities of CYPs 3A4 and 3A5, however, are substrate dependent and regioselective. In general, for most CYP3A substrates, in vitro intrinsic clearance values for CYP3A4 are greater than for CYP3A5.¹⁹–²³ In contrast, midazolam is avidly metabolized by CYP3A5, and midazolam 1'-hydroxylation by CYP3A5 can be twofold to threefold greater than by CYP3A4.¹⁹,²⁴–²⁶ CYP3A5 is polymorphically expressed.¹⁸,²⁷ The wild-type allele, CYP3A5*1, encodes functional CYP3A5 pro-

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protein. The most common and functionally significant variant allele, CYP3A5*3, results in the production of improperly spliced messenger RNA (mRNA) and a small amount of correctly spliced mRNA. The consequence is the formation of a nonfunctional protein that is truncated at amino acid 102, and the generation of a small amount of wild-type functional CYP3A5, so that CYP3A5*3 is not a truly null allele. Individuals with at least one CYP3A5*1 allele (genotypically homozygous CYP3A5*1/*1 or heterozygous CYP3A5*1/*3) express greater amounts of CYP3A5 protein, whereas those with other variants (most commonly homozygous CYP3A5*3) do not, although the absolute magnitude of CYP3A5 content is disputed. Some reports suggest that in CYP3A5*1 carriers, CYP3A5 accounts for more than 50% of total hepatic and intestinal CYP3A protein, and a significant fraction of total intestinal CYP, whereas others suggest it is substantially lower. Although CYP3A5 genetic variability has been well characterized, less information is available about the influence of CYP3A5 expression on human liver microsomal metabolism, and the relative contribution of CYP3A5 to total CYP3A metabolism remains disputed.

Identification of CYP3A4 as the predominant catalyst of human liver microsomal alfentanil metabolism occurred before the role of CYP3A5 in the biotransformation of some CYP3A substrates was recognized. Despite the increasingly recognized catalytic role for CYP3A5, there is little information regarding CYP3A5 and human liver alfentanil metabolism. The growing interest in alfentanil as a CYP3A probe suggests that this ambiguity be clarified. An early investigation with CYP3A5 expressed in yeast suggested that it did not catalyze alfentanil metabolism. In contrast, experiments from our laboratory using complementary DNA (cDNA)–expressed human CYPs showed that CYPs 3A4 and 3A5 were equieffective at alfentanil metabolism. Therefore, the purpose of this investigation was to test the hypothesis that alfentanil is a substrate for human liver microsomal CYP3A5 and that pharmacogenetic variability in hepatic CYP3A5 expression confers differences in alfentanil metabolism.

Materials and Methods

Chemicals
Noralfentanyl was a gift from Janssen Research Foundation (Piscataway, NJ). The alfentanil analog R38527 (internal standard for noralfentanil and alfentanil) was purchased from Research Diagnostics (Flanders, NJ). Alfentanil was prepared at Research Triangle Institute (Research Triangle Park, NC) and provided by the National Institute on Drug Abuse (Rockville, MD). N-phenylpropionamide and ring-labeled pentadeuterated N-phenylpropionamide (d5-N-phenylpropionamide) were synthesized in our laboratory. Ketoconazole was obtained from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA). Acetonitrile and ammonium hydroxide were from JT Baker (Phillipsburg, NJ). All other reagents were from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific, Inc. (Pittsburgh, PA). All buffers and reagents were prepared with high-purity (18.2 MΩ · cm) water (Milli-Q; Millipore, Bedford, MA).

Microsomal Incubations
Human liver microsomes characterized for their respective CYP3A4 and CYP3A5 content were prepared from livers, obtained from human organ donors, in the University of Washington School of Pharmacy Human Tissue Bank. CYP3A4 and CYP3A5 contents were assessed by Western blot analysis, and CYP3A5 genotype was determined as described previously.

Incubations (final volume 1.0 ml) contained 50 μg/ml microsomal protein and 1 μM alfentanil in 100 mM potassium phosphate buffer (pH 7.4). After preincubation (37°C for 3 min), the reaction was initiated by adding an NADPH regenerating system (final concentrations in incubation: 10 mM glucose-6-phosphate, 1 mM NADPH, 1 U/ml glucose-6-phosphate dehydrogenase, and 5 mM magnesium chloride) and terminated after 10 min by adding 20 μl phosphoric acid and placing on ice. Four of the 20 human liver microsomal incubations had more than 10% substrate depletion and were consequently repeated with a 4-min incubation time. Preliminary experiments established linearity of product formation with time and protein content.

Microsomes from six human livers (three each with low [2–3%] and high [54–60% of total] CYP3A content) were used to evaluate the effect of CYP3A inhibition by ketoconazole (0.05 μM) or troleandomycin (0.5 μM). Ketoconazole exhibits significantly greater potency and
which were then applied to Oasis MCX (1 ml, 30 mg, (5.6 ng R38527 and 2.8 ng d5-N-phenylpropionamide), reaction mixtures were added the internal standards to quenched were quantified by liquid chromatography–mass spectrometry after solid phase extraction. To added alfentanil (1 μM) microsomes were preincubated with inhibitor and the NADPH regenerating system for 15 min and then initiated by adding alfentanil (1 μM).

**Analytical Methods**

Alfentanil, noralfentanil, and N-phenylpropionamide were quantified by liquid chromatography–mass spectrometry after solid phase extraction. To quenched reaction mixtures were added the internal standards (5.6 ng R38527 and 2.8 ng d5-N-phenylpropionamide), which were then applied to Oasis MCX (1 ml, 30 mg, 30 μm) solid-phase extraction cartridges (Waters Corp., Milford, MA) previously conditioned with 1 ml methanol and 1 ml deionized water. Cartridges were washed with 1 ml hydrochloric acid, 0.1 N, eluted into polypropylene tubes (used because N-phenylpropionamide recovery was better than with glass) with 1 ml 5% ammonium hydroxide in methanol, and the eluent evaporated to dryness under nitrogen at 65°C. Samples were reconstituted with 50 μl acetone/ethanol, 18%, in 20 μl formic acid.

Noralfentanil, N-phenylpropionamide, and alfentanil concentrations were measured by liquid chromatography–mass spectrometry using selected ion monitoring. The instrument was an Agilent 1100 liquid chromatograph–mass spectrometer using a Zorbax Eclipse XBD C18 column (2.1 × 50 mm, 5 μm) with a Zorbax Eclipse C8 guard column (2.1 × 12.5 mm, 5 μm) (Agilent, Palo Alto, CA). The mobile phase (0.25 ml/min) gradient started at 18% acetonitrile in 20 μl formic acid for 30 s, increased to 24% (4 min) and 75% (7 min) acetonitrile, and held at 75% for 1 min before reequilibrating at 18% acetonitrile. Injections were 12 μl. Under these conditions, N-phenylpropionamide, d5-N-phenylpropionamide, noralfentanil, alfentanil, and R28527 eluted at 4.3, 4.2, 3.7, 6.2, and 6.5 min and were monitored at mass:charge ratios (m/z) of 150, 155, 277, 417, and 431, respectively. The nitrogen drying gas was at 325°C and 6 l/min, fragmentor at 70 V, nebulizer pressure 25 psi, and the capillary at 2,500 V. Analytes were quantified using standard curves of peak area ratios. Standard curves were linear (average \( r^2 > 0.99 \)) over the ranges of 0.3–500 ng/ml noralfentanil, 0.3–100 ng/ml N-phenylpropionamide, and 0.2–1 μM alfentanil.

**Statistical Analysis**

Results are expressed as mean ± SD. Differences between groups were compared using the Student t test.

**Results**

Microsomes were prepared from 10 human livers with low CYP3A5 content and 10 with high CYP3A5 content (2.0–5.2 and 46–76% of total CYP3A, respectively; table 1). All of the low CYP3A5 livers had the CYP3A5*3/*3 genotype. Nine of the high CYP3A5 livers had the CYP3A5*1/*3 genotype, and one was CYP3A5*1/*1.

Mean CYP3A4 content in the two groups was identical. For livers with low CYP3A5 content, noralfentanil and N-phenylpropionamide formation ranged from 35 to 120 pmol · min\(^{-1} \) · mg\(^{-1} \) and 3.9 to 12.0 pmol · min\(^{-1} \) · mg\(^{-1} \), respectively. For livers with high CYP3A5 content, noralfentanil and N-phenylpropionamide formation ranged from 78 to 557 pmol · min\(^{-1} \) · mg\(^{-1} \) and from 6.2 to 50.5 pmol · min\(^{-1} \) · mg\(^{-1} \), respectively. The noralfentanil:N-phenylpropionamide metabolite formation ratios for the low and high CYP3A5 livers were significantly different (9.5 ± 0.4 vs. 12.7 ± 1.4, respectively; \( P < 0.05 \)). Although there was within-group variability in absolute rates of metabolite formation, the metabolite formation ratio was relatively consistent.

The relation between alfentanil metabolism and CYP3A isoform content was determined in the 20 liver microsomes. There was a relatively poor correlation between noralfentanil formation and CYP3A4 content (\( r^2 = 0.31 \)) and a somewhat better correlation between N-phenylpropionamide formation and CYP3A4 content (\( r^2 = 0.38 \)) (fig. 2). When CYP3A5 content was added to both analyses, the correlation between total 3A content (CYP3A4 and CYP3A5) and noralfentanil and N-phenylpropionamide formation improved significantly (\( r^2 = 0.81 \) and \( r^2 = 0.72 \), respectively; fig. 3). There was a significant correlation between the noralfentanil:N-phenylpropionamide metabolite formation ratio and liver microsomal CYP3A5 content (fig. 4).

Six of the human liver microsomes, three each with low and high CYP3A5 content, were used to study the effect of troleandomycin and ketoconazole inhibition of CYP3A on alfentanil metabolism (table 2). In both the

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**Table 1. Genetic Determinants of Human Liver Microsomal Alfentanil Metabolism**

<table>
<thead>
<tr>
<th>CYP Content, pmol/mg</th>
<th>Metabolite Formation, pmol · min(^{-1} ) · mg(^{-1} )</th>
<th>Metabolite Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A5</td>
<td>CYP3A4</td>
<td>Total CYP3A</td>
</tr>
<tr>
<td>Low-CYP3A5</td>
<td>3.6 ± 1.0</td>
<td>93 ± 36</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(2.1–5.0)</td>
<td>(52–171)</td>
</tr>
<tr>
<td>High-CYP3A5</td>
<td>117 ± 68</td>
<td>93 ± 39</td>
</tr>
</tbody>
</table>

Alfentanil concentration was 1 μM. Results are presented as mean ± SD (range).

Selectivity for CYP3A4 than CYP3A5, whereas troleandomycin shows much less selectivity. Microsomes were preincubated with inhibitor and the NADPH regeneration system for 15 min and then initiated by adding alfentanil (1 μM).
low- and high-CYP3A5 microsomes, 0.5 μM troleandomycin decreased both noralfentanil and N-phenylpropionamide formation to 58–64% of control. In contrast, ketoconazole inhibition was influenced by CYP3A5 content. Noralfentanil and N-phenylpropionamide formation was reduced to 20–24% of control in low-CYP3A5 liver microsomes but only to 32–33% of control in high-CYP3A5 liver microsomes (\( P < 0.05 \) for both metabolites).

Discussion

The results of this investigation show that alfentanil is a substrate for human liver microsomal cytochrome P4503A content. Each data point represents an individual liver and the mean of duplicates. Shown are correlations of CYP3A4 content with noralfentanil formation and N-phenylpropionamide (AMX) formation.

Livers were matched for CYP3A4 based on protein content. Metabolism of itraconazole is catalyzed exclusively by CYP3A4 and not at all by CYP3A5; thus, characterization of itraconazole metabolism by liver microsomes permits matching by CYP3A4 catalytic activity in addition to content.\(^ {34} \) When liver microsomal alfentanil metabolism was normalized for CYP3A4 activity, the adjusted rates of noralfentanil and N-phenylpropionamide formation were 58–64% of controls. In contrast, ketoconazole inhibition was influenced by CYP3A5 content. Noralfentanil and N-phenylpropionamide formation was reduced to 20–24% of control in low-CYP3A5 liver microsomes but only to 32–33% of control in high-CYP3A5 liver microsomes (\( P < 0.05 \) for both metabolites).

Discussion

The results of this investigation show that alfentanil is a substrate for human liver microsomal cytochrome P4503A, that genetic polymorphism in CYP3A5 expression influences hepatic alfentanil metabolism independently of CYP3A4 content, that hepatic microsomal alfentanil metabolism reflects total CYP3A content rather than exclusively CYP3A4 content, and that alfentanil metabolism in vitro to either noralfentanil or N-phenylpropionamide should be considered a probe for total hepatic CYP3A. These conclusions are all based on experiments conducted at clinically relevant alfentanil concentrations and refute the contention that CYP3A5 does not metabolize alfentanil.\(^ {34} \)

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noralfentanil formation in high-CYP3A5 livers were still higher than in the low-CYP3A5 livers (not shown). In addition, there was no significant difference between the high-CYP3A5 and low-CYP3A5 liver microsomes in the mean contents of NADPH cytochrome P450 reductase or cytochrome P₄₅ (not shown). Hence, confounding factors seem not to account for the observed results. Rather, differences in alfentanil metabolism between high- and low-CYP3A5 liver microsomes seem attributable to CYP3A5 expression.

Alfentanil metabolism by livers with low and high CYP3A5 content is consistent with alfentanil metabolism by CYP3A4 and CYP3A5 proteins expressed from human cDNA using a baculovirus expression system, as described previously. The threefold-greater rates of noralfentanil and N-phenylpropionamide formation in CYP3A5*1 livers expressing higher amounts of CYP3A5 protein, compared with low CYP3A5 livers, is consistent with alfentanil turnover by cDNA-expressed CYP3A5 that is approximately equivalent to that by expressed CYP3A4. Specifically, in vitro clearance estimates (Vmax/Km) for expressed CYP3A4 and CYP3A5-catalyzed noralfentanil formation were similar (0.43 and 0.48 ml · nmol⁻¹ · min⁻¹, respectively), as were those for N-phenylpropionamide formation (0.05 and 0.02 ml · nmol⁻¹ · min⁻¹). In addition, the alfentanil metabolite formation ratio (noralfentanil:N-phenylpropionamide) in low-CYP3A5 livers (10 ± 1) was close to that for expressed CYP3A4 (9 ± 1), whereas that in high-CYP3A5 livers (13 ± 1) was intermediate to that for expressed CYP3A4 (9 ± 1) and CYP3A5 (21 ± 1). Equivalent troleandomycin inhibition of alfentanil metabolism by high and low CYP3A5-containing liver microsomes was similar to expressed CYPs, in which troleandomycin IC₅₀ concentrations for inhibition of alfentanil metabolism were relatively comparable for both CYP3A4 and CYP3A5. Lesser troleandomycin inhibition of alfentanil metabolism by high CYP3A5 compared with low CYP3A4 liver microsomes is similar to expressed CYPs, in which the ketoconazole IC₅₀ was an order of magnitude lower for CYP3A4 than for CYP3A5. Therefore, there is excellent concordance between expressed and microsomal CYP3A5-catalyzed alfentanil metabolism.

A major role for CYP3A5 in microsomal metabolism of alfentanil is similar to that for midazolam, which is avidly metabolized by CYP3A5, and different from that for many other CYP3A substrates. Midazolam 1'-hydroxylation was greater for purified CYP3A5 than for CYP3A424,25 and approximately equivalent for the two expressed enzymes.9 Midazolam metabolism was greater in human liver microsomes containing both CYPs 3A5 and 3A4, compared with those expressing only CYP3A4.25,31 The correlation between midazolam 1'-hydroxylation and liver microsomal CYP3A4 content (r = 0.75) was substantially improved when replaced with the summed contents of CYP 3A4 plus 3A5 (r = 0.93).27,51 Midazolam metabolite ratios (1'-hydroxymidazolam:4'-hydroxymidazolam) were higher in livers and intestines containing at least one CYP3A5*1 allele.27,51 Similarly, alfentanil metabolite ratios (noralfentanil:N-phenylpropionamide) were higher in livers with greater CYP3A5 content. The influence of CYP3A5 on ketoconazole inhibition of liver microsomal metabolism was also similar for midazolam and alfentanil. Ketoconazole was a more potent inhibitor of expressed CYP3A4 than CYP3A5-catalyzed midazolam hydroxylation, and human liver microsomes containing CYP3A5 showed less ketoconazole inhibition of midazolam metabolism.36 Similarly, ketoconazole was a more potent inhibitor of expressed CYP3A4 than CYP3A5-catalyzed alfentanil metabolism,35 and human liver microsomes containing CYP3A5 showed less ketoconazole inhibition of alfentanil metabolism.

For most CYP3A substrates, however, such as verapamil, alprazolam, triazolam, nifedipine, haloperidol, and eplerenone, in vitro intrinsic clearance or Vmax values for CYP3A4 were greater than for CYP3A5,19–23 in contrast to alfentanil and midazolam. The contribution of CYP3A5 to hepatic metabolism of these other substrates is less apparent. For example, although the specific activities of expressed CYPs 3A4 and 3A5 for testosterone 6β-hydroxylation were nearly identical, the contribution of CYP3A5 protein to human liver microsomal testosterone 6β-hydroxylation was considered limited, because neither the CYP3A5 polymorphism nor incorporation of CYP3A5 improved a correlation comparing metabolism and CYP3A content.52

Although the current results clearly indicate CYP3A5
participation in liver microsomal alfentanil metabolism in vitro, the role of CYP3A5 protein and the influence of CYP3A5 polymorphisms on alfentanil metabolism and clearance in vivo remains unknown. Indeed, the role of CYP3A5 on drug clearance in general remains disputed. Although there is generally good concordance between expressed and microsomal CYP3A in the turnover of CYP3A5 substrates, there is less concordance in the relation between in vitro and clinical studies regarding the role of CYP3A5, as well as some discordance between studies. Plasma concentrations and areas under the curve of midazolam and 1'-hydroxymidazolam after oral dosing were not different in homozygous CYP3A5*3/*3 subjects, there was no difference between CYP3A5 heterozygotes or homozygotes in the clearance of either oral or intravenous midazolam and no difference in the oral midazolam area under the curve between CYP3A5*1/*3 and CYP3A5*3/*3 subjects. In contrast, clearances of intravenous midazolam in patients with at least one CYP3A5*1 allele (CYP3A5*1/*1 and CYP3A5*1/*3) were significantly (30%) greater than CYP3A5*3 homozygotes, and the clearance of intravenous or oral midazolam was significantly (1.7 times) higher in CYP3A5*1/*3 compared with CYP3A5*3/*3 subjects. Nevertheless, these differences are small, particularly compared with in vitro microsomal results showing a major effect of the CYP3A5 polymorphism, and not clinically significant. The disposition of nifedipine, which is a poor CYP3A5 substrate, was not affected by CYP3A5 genotype (CYP3A5*1/*1 vs. CYP3A5*3/*3), as expected. In contrast, there is a significant relation between the CYP3A5 polymorphism and the disposition of the calcineurin inhibitors cyclosporine and tacrolimus. Typically in these investigations, dose-adjusted trough concentrations are substituted for formal clearance measurements. Cyclosporine dose-adjusted trough concentrations were significantly higher in CYP3A5*3/*3 patients than in CYP3A5*1/*3 patients. Tacrolimus dose-adjusted trough concentrations were significantly different (92, 61, and 44 ng/ml per mg/kg, respectively) in CYP3A5*3/*3, CYP3A5*1/*3, and CYP3A5*1/*1 patients. They were also significantly higher in CYP3A5*3/*3 patients than in *1/*3 or *1/*1 patients (median 94 vs. 61 ng/ml per mg/kg), and the former group required lower doses to reach target concentrations. Several other investigations have also shown a CYP3A5 pharmacogenetic influence of CYP3A5 on the tacrolimus concentration: dose ratio and greater dose requirements in patients carrying at least one CYP3A5*1 allele. The CYP3A5 polymorphism explained up to 45% of the variability in the tacrolimus dose requirement, indicating the clinical significance of this polymorphism. Clearly, however, the influence of the CYP3A5 polymorphism is substrate dependent. A clinical investigation is required to assess the influence of the CYP3A5 polymorphism on alfentanil metabolism and clearance. In summary, this investigation demonstrates that hepatic microsomal CYP3A5 does metabolize alfentanil to the two major in vitro metabolites, noralfentanil and N-phenylpropionamide, in addition to CYP3A4, and that pharmacogenetic variability in CYP3A5 expression does significantly influence human liver microsomal alfentanil metabolism. Further investigation is warranted to assess whether CYP3A5 pharmacogenetics is a factor in the interindividual variability of alfentanil metabolism and clearance in vivo.

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