A SIGNIFICANT DRUG-METABOLIZING ROLE FOR CYP3A5?

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ABSTRACT:

Recent research on CYP3A5 in vitro and in humans has provided discordant information on whether CYP3A5 plays a significant role in the metabolism of CYP3A substrates in vivo. For example, six separate studies have reported CYP3A5 to contribute between 2 and 60% of the total hepatic CYP3A. Suggested explanations for the reported differences in hepatic CYP3A5 levels are evaluated in this article. Furthermore, a sensitivity analysis of the contribution of CYP3A5 (in addition to CYP3A4) to the metabolism of a “midazolam”-type substrate based on recently published in vitro and clinical data is compared with the results of two in vivo studies that investigated the influence of CYP3A5 genotype on midazolam pharmacokinetics. The sensitivity analysis predicts an approximately 3-fold lower AUC\text{\textsubscript{0-\infty}} for midazolam for those expressing the highest hepatic and intestinal levels of CYP3A5 (e.g., possessing CYP3A5*1 alleles) compared with those individuals who express insignificant amounts of CYP3A5, assuming CYP3A4 levels are the same in both groups and that CYP3A5 levels do not exceed those of CYP3A4 in CYP3A5*1 homozygotes. In contrast, the two in vivo studies show no statistically significant influence of CYP3A5 genotype on midazolam pharmacokinetics. The discordance between the prediction and the results from the two in vivo studies is discussed.

Perspectives on the Predictive Value of In Vitro Data

Although there appear to be differences between CYP3A4 and CYP3A5 in the in vitro clearance of CYP3A substrates (Williams et al., 2002), there are no apparent differences in substrate specificity (Williams et al., 2002). Differentiation of the contributions of CYP3A4 or CYP3A5 to clearance of CYP3A substrates in vivo can be divided into three categories: regulation of expression, patterns and levels of expression, and comparative metabolic capabilities. These are reviewed in the following paragraphs.

Regulation of Expression. In contrast to regulation of CYP3A4 expression, which is mainly controlled by nuclear hormone receptors (Kliever et al., 2002), possession of single nucleotide polymorphisms that cause alternative splicing and protein truncation have the greatest influence on regulation of CYP3A5 (Kolars et al., 1994; Kuehl et al., 2001; Lin et al., 2002). The CYP3A5*1 allele is associated with the highest levels of CYP3A5 expression, whereas the CYP3A5*3 and CYP3A5*6 alleles result in lower amounts of CYP3A5 enzyme in the liver (Kuehl et al., 2001) and intestine (Lin et al., 2002) [for the splice variant CYP3A5*3, there is a premature stop codon in exon 3B that results in a protein that is truncated after amino acid 102 (Kuehl et al., 2001)]. Aside from the major influence of genotype on CYP3A5 expression levels, recent evidence suggests that regulation of the CYP3A5 gene is controlled via the glucocorticoid receptor in a lung-derived cell line (Hukkanen et al., 2003), and by the pregnant X receptor in HepG2 cells (Landes et al., 2003).

Patterns and levels of expression. CYP3A4 and CYP3A5 are expressed in the liver (Kuehl et al., 2001) and intestine (Lin et al., 2002). CYP3A5 is also expressed in the lung (Anttila et al., 1997), kidney (Haehner et al., 1996), and breast (Williams and Phillips 2000).

Accurate quantitative determination of expression levels of CYP3A enzymes in the intestine and liver is a critical component for the successful differentiation of CYP3A4 and CYP3A5 in vivo with regard to metabolism of CYP3A substrates. Two separate studies show CYP3A5 mRNA to account for, on average, 2% (Koch et al., 2002, n = 63 livers from white subjects) or 3.9% (Westlund-Johnsson et al., 2003, n = 45 livers from white subjects) of the total hepatic CYP3A mRNA transcripts. In the study by Koch et al. (2002), 14% of livers exhibited an increased share of CYP3A5 mRNA transcripts (range 4–20% of the total CYP3A mRNA), which were either heterozygous for the CYP3A5*1 allele or expressed very low levels of CYP3A4 mRNA (Koch et al., 2002). Relative translation rates and efficiencies of the respective CYP3A mRNA transcripts into active CYP3A enzymes are not known.

Quantitation of CYP3A enzymes has more commonly been determined using Western blot analysis of microsomes. Two studies using recombinant CYP3A5 as a source of enzyme for a “standard curve” report significantly higher levels (50% or 60%) of CYP3A5 in the liver (Kuehl et al., 2001, n = 47 livers, 50% of total hepatic CYP3A in some individuals; and Tateishi et al., 1999, n = 15 livers, 60% of total CYP3A in those expressing CYP3A5) than reports using CYP3A5 purified from human liver microsomes (2%, 3%, 22%, or 23%) as a source of enzyme for a standard curve. Using CYP3A5 enzyme purified from human liver microsomes, Wrighton and others have reported CYP3A5 to contribute, on average, 23% (Wrighton et al., 1989, n = 7 livers) and 22% (Wrighton et al.1990, n = 7 livers) of the total CYP3A. Hirota et al. (2001) reported the mean value to be
3% in 9 of 10 livers that expressed CYP3A5. Most recently, Westlind-
Johnsson et al. (2003) indicated that CYP3A5 protein accounted for
only 2% of the overall total hepatic CYP3A enzyme content.

In summary, the six studies report CYP3A5 to contribute between
2 and 60% of the total hepatic CYP3A. Which is the true value? It
could be argued, regardless of the source, that either recombinant
CYP3A5 or the human liver microsomal enzyme should suffice for
the purposes of quantitation. However, the 25- to 30-fold difference
in CYP3A5 levels between the lowest (2% of total CYP3A; Westlind-
Johnsson et al., 2003) and the highest estimation (50–60%; Tateishi
et al., 1999; Kuehl et al., 2001) suggests that further work is neces-
sary. A side by side validation of the two enzyme sources comparing
levels quantified by immunoblot analysis against absorbance levels at
450 nm in the presence of the cytochrome P450 (P450) ligand carbon
monoxide should help to understand these reported differences and
to develop the optimal system for quantification of CYP3A5 in human
microsomes from the liver, intestine, and other organs.

**Comparative Metabolic Capabilities.** An accurate assessment of
the comparative metabolic capabilities of CYP3A4 and CYP3A5 is
another key component for optimizing predictive value for the in vivo
situation. Early studies comparing the metabolic capabilities of
CYP3A4 and CYP3A5 forms used only a very limited range of
substrates and usually at only one concentration (Gorski et al., 1994;
Gillam et al., 1997; Maenpaa et al., 1998). Furthermore, these studies
did not utilize common assay conditions for CYP3A4 and CYP3A5,
and thus, it is not appropriate to make comparisons between the
results. By establishing consistent and relatively simple incubation
conditions with regard to P450/NADPH reductase/cytochrome b5
ratios, evidence was provided (Williams et al., 2002) for equal or
reduced metabolic capability of CYP3A5 compared with CYP3A4,
dependent on substrate. However, it could be argued that even this
system is not optimal, since additions of exogenous NADPH reduct-
ase and cytochrome b5 to CYP3A5 (and CYP3A7) were necessary to
obtain a consistent P450/NADPH reductase/cytochrome b5 ratio.
These additions might introduce an unwanted source of variability
between measured kinetic parameters for the CYP3A5 enzymes (Hirot a
et al., 2001). Perhaps an optimal in vitro system for comparison of
CYP3A4 with CYP3A5 would be one that did not require exogenous
addition of coenzymes. Hypothetically, this would require two insect
cell lines transfected with CYP3A4 or CYP3A5 cDNA that expressed
consistent ratios of CYP3A enzyme/NADPH reductase/cytochrome
b5 in the endoplasmic reticulum. Identification of a potent and selec-
tive CYP3A5 inhibitor is a significant challenge (Ekins et al., 2003)
but would be very useful for differentiation of the contributions of
CYP3A4 and CYP3A5 to the in vivo clearance of CYP3A substrates.

**A Sensitivity Analysis for Midazolam Exposure**

Sufficient data from recent studies have been made available to
attempt a simulation of the influence of CYP3A5 genotype on the
pharmacokinetics of a “midazolam”-type substrate (Fig. 1). Midazo-
lam is a CYP3A substrate with a moderate hepatic extraction ratio
(Thummler et al., 1996) well characterized for its intestinal and hepatic
metabolism (Paine et al., 1996; Thummler et al., 1996), and appears
not to be a substrate of P-glycoprotein (Kim et al., 1999).

Importantly, there is a precedent for successful prediction of midazo-
lam clearance in humans using data generated from incubation of mida-
zolam with human hepatic microsomes (Thummler et al., 1994). In
addition, other reports successfully predict the in vivo performance of
other benzodiazepine CYP3A substrates. For example, alprazolam clear-
ance in humans was well predicted from experiments using recombinant
enzymes and human liver microsomes (Hirota et al., 2001). The simulation
in the current study is aimed at differentiating the roles of contribu-
tions of CYP3A4 and CYP3A5 in vivo. With the current sensitivity
analysis, CYP3A5*1/*1 homozygotes (predicted to express high
CYP3A5 levels) are compared with those predicted to express insignif-
ificant levels of CYP3A5. CYP3A4 levels are assumed not to be affected
by genetic variability in CYP3A5 (Kuehl et al., 2001).

The sensitivity analysis is based on the following assumptions: 1)
work showing that CYP3A5 contributes up to, but not more than, 50% of
the total hepatic CYP3A in some individuals (Kuehl et al., 2001),
particularly those possessing the CYP3A5*1 allele, whereas it con-
tributes a minor or undetectable proportion of the total CYP3A in
those possessing CYP3A5*3 or CYP3A5*6 alleles (Kuehl et al., 2001); 2)
the investigation of the effects of CYP3A5*1 alleles on intestinal
CYP3A enzymes, which indicated that for those with CYP3A5*1 alleles,
CYP3A5 can contribute up to 50% of the total intestinal CYP3A content (Lin et al., 2002); and 3) the comparison of metabolic
capabilities of CYP3A4 and CYP3A5 (and CYP3A7) in vitro for 10
substrates using consistent ratios of P450/NADPH reductase/cyto-
chrome b5 (Williams et al., 2002), which, on an equimolar basis,
predicted that CYP3A5 plays an equivalent role to CYP3A4 in the in
vivo clearance of midazolam. This finding (Williams et al., 2002)
confirmed previous observations in human liver microsomes that
CYP3A5 appears to play a significant role in the turnover of mida-
zolam in human liver microsomes expressing CYP3A4 and CYP3A5
(Gorski et al., 1994) and that midazolam turnover is significantly
higher in liver microsomes in those predicted to have relatively high
levels of CYP3A5 due to possession of at least one CYP3A5*1 allele
(Kuehl et al., 2001).

The sensitivity analysis evaluates the effect of 1) hepatic intrinsic
clearance (CLint), 2) gut extraction (Egut), and 3) ratio of CYP3A5 to
CYP3A4 (Ratio) in individuals expressing both on expected oral AUC
values (AUCpo). It is assumed that the Ratio in the intestine and liver is
identical. The data are simulated from the following sets of equations:

\[
CL_{H} = \frac{(1 + \text{Ratio}) \cdot CL_{int} \cdot Q_{H}}{(1 + \text{Ratio}) \cdot CL_{int} + Q_{H}}
\]

\[
E_{\text{gut}} = 1 - \frac{1}{1 + \frac{1}{(1 + \text{Ratio}) \cdot Q_{\text{gut,baseline}} - 1}}
\]

\[
F_{\text{gut}} = 1 - E_{\text{gut}}
\]

\[
F_{H} = \frac{Q_{H}}{(1 + \text{Ratio}) \cdot CL_{int} + Q_{H}}
\]

\[
F = F_{\lambda} \cdot F_{\text{gut}} \cdot F_{H} \cdot F_{L}
\]

\[
AUC_{po} = \text{Dose}/CL_{H}/F
\]

where CL_{H} is the observed hepatic plasma clearance; Q_{H} is the
hepatic plasma flow; E_{\text{gut,baseline}} is gut extraction in individuals only
expressing CYP3A4 (see the Appendix for derivation of gut equa-
tion); F_{\lambda} is the fraction absorbed; F_{\text{gut}} and F_{H} are the fractions
absorbed, surviving metabolism (for nontransporter efflux compounds)
in the intestine, liver, and lung, respectively; and F is the
overall bioavailability. In the simulations, F_{\lambda}, F_{\text{gut}} and F_{H} are assumed
to be 1: the lung is assumed not to contribute significantly to systemic
clearance of midazolam. When not being varied during a simulation,
CL_{int} is given a value of 499 ml/min/70 kg (data recalculated from
Thummler et al., 1996, assuming a hematocrit of 0.47 for men and 0.42
for women); E_{\text{gut}} is given a value of 0.43 (Thummler et al., 1996);
Ratio is given a value of 1 (Kuehl et al., 2001); and Q_H a value of 839 ml/min/70 kg (Thummel et al., 1996). When calculating data for individuals with CYP3A4 only, Ratio is set to 0. To compare results of the simulation to the only study in healthy volunteers that has characterized both individual subject’s CYP3A5 genotype and in vivo midazolam pharmacokinetics (Shih and Huang, 2002), a dose of 7.86 mg/70 kg is used. Simulations are run where CL_int varies between 100 and 900 ml/min/kg, E_gut varies between 0 and 1, and Ratio varies between 0 and 1. Results are shown in Fig. 1.

Under the assumptions detailed above (Fig. 1), the sensitivity analysis predicts a 2.86-fold lower exposure (AUC_oral) for those individuals expressing the highest levels of CYP3A5 in the liver and gut (where [CYP3A4] = [CYP3A5]) compared with those expressing the low or insignificant levels in these tissues. The AUC_oral ([CYP3A4]/[CYP3A4 + CYP3A5/CYP3A4]) ratio is predicted to be independent of hepatic intrinsic clearance (Fig. 1) in the range chosen (100–900 ml/min/kg).

Figure 1 (middle row, left panel) shows that as gut extraction ratio (E_gut) increases, systemic exposure (AUC_oral) is predicted to decrease more steeply for those expressing CYP3A4 and CYP3A5.
compared with those expressing CYP3A4 only. Another observation (Fig. 1, bottom row, right panel) is that the line representing the predicted AUC ratio based on the CYP3A5/CYP3A4 ratio intersects with the observed AUC ratio based on the results of Shih and Huang (2002) at a CYP3A5/CYP3A4 ratio of 0.05. If the various assumptions of the sensitivity analysis are correct, the interpretation would be that CYP3A5 contributes significantly less to the total hepatic and intestinal CYP3A than the reported values of 60% (Tateishi et al., 1999), 50% (Kuehl et al., 2001), or 23 and 23% (Wrighton et al., 1989, 1990) and would be closer to the 3% and 2% values reported, respectively, by Hirota (2001) and Westlind-Johnsson (2003).

Although the simulation in which CYP3A5 levels contribute 50% of the total CYP3A levels predicts a significant influence of CYP3A5 genotype on AUC\textsubscript{oral} for midazolam, two studies in humans show no influence of CYP3A5 genotype on midazolam pharmacokinetics. Specifically, AUC\textsubscript{oral} 0-\infinity was 235 ± 37.8 ng · min/mL/kg (mean ± standard error of the mean) in CYP3A5*3 homozygotes (predicted to express lower or insignificant levels of CYP3A5) versus 215 ± 41.9 ng · min/mL/kg in heterozygous CYP3A5*1/*3 subjects (predicted to express significant levels of hepatic and intestinal CYP3A5), respectively (Shih and Huang, 2002). This represents a 1.1-fold reduction in exposure for those predicted to be CYP3A5 expressors compared with those predicted not to express CYP3A5. The midazolam concentration-time profiles appear to be identical for all subjects in that study (Fig. 1, in Shih and Huang, 2002). Another study (Goh et al., 2002) in cancer patients (n = 25) showed no statistically significant difference in mean midazolam clearance values among CYP3A5*1 homozygotes (385.0 ± 118.9 mL/min), CYP3A5*1/*3 heterozygotes (490.0 ± 116.4 mL/min), and CYP3A5*3 homozygotes (510.5 ± 281.4 mL/min), although midazolam clearance was lower (Student’s t test, P = 0.03) in those possessing at least one CYP3A5*1 allele compared with CYP3A5*3 homozygotes.

Suggested Explanations for Discordance between Predicted and Observed Data

Why should the predicted influence of CYP3A5 genotype on midazolam pharmacokinetics be so far removed from the actual in vivo data? Admittedly, there are only two in vivo studies using midazolam on which to base a comparison, and one (Goh et al., 2002) is in cancer patients and not healthy volunteers. A meta-analysis of multiple repeated studies with a large study population would be necessary to confidently confirm that CYP3A5 genotype truly has no influence on midazolam pharmacokinetics. One possible contributor to the absence of an in vivo/in vitro correlation is that, in vivo, CYP3A4 levels increase in CYP3A5*3 homozygotes to compensate for the low CYP3A5 levels. This was shown in the jejunum in one study (Lin et al., 2002), but is inconsistent with the finding that hepatic CYP3A4 levels are not influenced by CYP3A5 genotype (Kuehl et al., 2001), unless the control of regulation of CYP3A5 expression differs between the liver and the intestine. As previously mentioned, it is possible that the levels of CYP3A5 in the intestine and liver could have been overestimated in some studies (Wrighton et al., 1989, 1990; Tateishi et al., 1999; Kuehl et al., 2001) relative to the true amount. The assessment of the relative roles of CYP3A4 and CYP3A5 using an in vitro system (Williams et al., 2002), far removed from the in vivo situation, may have overestimated the role of CYP3A5 in midazolam metabolism. Finally, the AUC values reported by Shih and Huang (2002) are notably different from those that would be expected by previous midazolam studies (Smith et al., 1981; Heizmann et al., 1983; Balson et al., 1996; Thummel et al., 1996).

Influence of CYP3A5 Genotype on Cyclosporine Pharmacokinetics or Tacrolimus Dosage Requirements

Three other published studies have assessed the influence of CYP3A5 genotype on the disposition of the CYP3A substrates cyclosporine (one study) and tacrolimus (two studies). Unlike midazolam, cyclosporine and tacrolimus are substrates of P-glycoprotein, which may confound any reported association between CYP3A5 genotype and phenotype. Another possible confounding factor is that polymorphisms in MDR1, the gene encoding P-glycoprotein, appear to influence CYP3A4 expression (Goto et al., 2002).

A pilot study (n = 17) investigating the influence of CYP3A5 genotype on another CYP3A substrate (Yates et al., 2002) showed that oral clearance of cyclosporine was 31% higher in those individuals predicted by CYP3A5 genotype (CYP3A5*3/*3) to be poor metabolizers compared with those predicted to be extensive metabolizers (CYP3A5*1/*1, CYP3A5*1/*3 or CYP3A5*1/*6). These unexpected results showed that CYP3A5 genotype was correlated with cyclosporine pharmacokinetics, but the prediction is opposite to that expected from in vitro data: a comparison of cyclosporine metabolism in vaccinia virus-expressed CYP3A4 and CYP3A5 showed multiple metabolites formed at relatively high rates by CYP3A4 compared with a single metabolite formed at a much lower rate by CYP3A5 (Aoyama et al., 1989).

Two published reports are concordant with the predictions of the simulated data (Macphee et al., 2002; Zheng et al., 2003). In the first investigation (Macphee et al., 2002, n = 187 patients), possession of the A/G \textsuperscript{44} polymorphism in the CYP3A41 pseudogene, linked to high hepatic expression levels of CYP3A5 (Kuehl et al., 2001), was associated with a high dosage requirement for the CYP3A substrate tacrolimus compared with those not possessing that genotype. Ethnicity (white versus African-American) had no influence on dose requirement in this study (Macphee et al., 2002).

In another study (n = 54) in heart transplant patients at 3, 6, and 12 months post-transplantation, a significant difference in tacrolimus blood level per dose per kilogram per day was found between the CYP3A5 *1/*3 (CYP3A5 expressor) versus *3/*3 (nonexpressor) genotypes with the *1/*3 patients requiring a larger tacrolimus dose to maintain the same blood concentration (Zheng et al., 2003). The results of these studies are somewhat confounded, however, by the potential influence of at least one polymorphism in gene MDR1 (tacrolimus is a P-glycoprotein substrate) on CYP3A4 expression levels (Goto et al., 2002).

Future Perspectives and Differentiation of CYP3A4 and CYP3A5 in Vivo

Further clarity is required from in vitro studies on enzyme activities and genotype/phenotype analysis to provide definitive data on the clinical relevance of CYP3A5. Suggestions for experimental strategies to address these issues are included in this article. It will be a very significant challenge to differentiate the relative contributions of CYP3A4 and CYP3A5 in vivo, particularly in the absence of a form-specific substrate for CYP3A5, since both enzymes are expressed in the majority of individuals (Wrighton et al., 1989, 1990; Tateishi et al., 1999; Hirota et al., 2001; Kuehl et al., 2001; Westlind-Johnson et al., 2003). Identification of a potent and selective CYP3A5 inhibitor poses a significant challenge (Ekins et al., 2003). However, there may be some individuals (1/40; Aoyama et al., 1989) who only express CYP3A5 in the liver,
suggestion that human liver microsomes may be available to study CYP3A5 in an in vitro system closer to the in vivo situation than by using recombinant enzymes.

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Appendix: Derivation of E\textsubscript{gut} Equation

Let the following equation represent fraction absorbed through the gut (F\textsubscript{gut}):

\[
F_{\text{gut}} = \frac{Q_{\text{gut}}}{(1 + \text{Ratio}) \cdot CL_{\text{int,gut}} + Q_{\text{gut}}}
\]

where CL\textsubscript{gut} and CL\textsubscript{int,gut} are the observed and intrinsic gut clearance and Q\textsubscript{gut} is flow through the gut. This can be rewritten by dividing the numerator and denominator by Q\textsubscript{gut}:

\[
F_{\text{gut}} = \frac{1}{(1 + \text{Ratio}) \cdot CL_{\text{int,gut}} / Q_{\text{gut}} + 1}
\]

Since F\textsubscript{gut} = 1 – gut extraction (E\textsubscript{gut}), the above equation can be rewritten in terms of E\textsubscript{gut}:

\[
E_{\text{gut}} = 1 \frac{1}{(1 + \text{Ratio}) \cdot CL_{\text{int,gut}} / Q_{\text{gut}} + 1}
\]

Let E\textsubscript{gut,baseline} equal the amount of gut extraction when Ratio = 0

\[
E_{\text{gut,baseline}} = 1 \frac{1}{CL_{\text{int,gut}} / Q_{\text{gut}} + 1}
\]

Solving for CL\textsubscript{int,gut}/Q\textsubscript{gut} yields:

\[
CL_{\text{int,gut}} = \frac{1}{E_{\text{gut,baseline}} - 1}
\]

Substitution into the E\textsubscript{gut} equation yields:

\[
E_{\text{gut}} = 1 \frac{1}{(1 + \text{Ratio}) \cdot \left( \frac{1}{E_{\text{gut,baseline}} - 1} \right) - 1} + 1
\]

References


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