

Role of CYP3A5 and CYP3A7 in Drug-Drug Interactions

David M. Stresser, Stephanie D. Turner, Thuy Ho and Charles L. Crespi
BD Biosciences Discovery Labware, Woburn, MA

Abstract

CYP3A5 is present in significant quantities in 20-60% of human livers, whereas CYP3A7 is most abundant in fetal liver. The role of these enzymes in drug interactions is not well understood. A panel of 16 compounds was tested for potential to inhibit dealkylation of 7-benzyloxy-4-trifluoromethylcoumarin (BFC) catalyzed by cDNA-expressed CYP3A5, CYP3A7, CYP3A4 and human liver microsomes (HLM). CYP1A2 was used as a non-CYP3A comparator. Three of 16 compounds exhibited 50% inhibition of CYP1A2 activity (IC_{50}) whereas at least 13 of 16 compounds exhibited 50% inhibition of CYP3A4, CYP3A5, CYP3A7 and HLM activity. α -Naphthoflavone inhibited CYP1A2, but activated all CYP3A enzymes as well as HLM catalysis. The IC_{50} values for all CYP3A enzymes and HLM correlated well with each other ($r > 0.77$). Relative mean IC_{50} values for CYP3A4, CYP3A5, CYP3A7 and HLM were 0.12, 1.00, 2.12 and 0.76, respectively. We show that inhibitor specificity for CYP3A5 and CYP3A7 is similar to CYP3A4 but inhibitor potency for these enzymes is on average, 45-fold less than with CYP3A4. Since HLM is a mixture, expect intermediate potency when using HLM as an enzyme source in inhibition assays. Indeed, this was observed. Because of similarities in inhibitor specificity between CYP3A4 and CYP3A7, CYP3A7-mediated drug-drug interactions and/or drug-endobiotic interactions in fetus may exhibit a specificity profile similar to that of CYP3A4 in adults, however, inhibition potency appears to be an order of magnitude less with CYP3A7.

Introduction

The potential of CYP3A5 and CYP3A7 as mediators of metabolism-based drug interactions in adult and fetus, respectively, and the effects of those enzymes on responses observed within HLM is not well understood.

CYP3A5 is present in significant quantities in 20-60% of human livers and is reported to be at least 50% of total CYP3A in those individuals^{1,2}. It is the most abundant CYP3A form in kidney³. CYP3A5 catalyzes reactions similar to that of CYP3A4, but is generally found less active. An exception is midazolam 1'-hydroxylation⁴. Additionally, under certain assay conditions, activity of CYP3A5 has been found comparable to CYP3A4 with several other substrates⁵.

CYP3A7 comprises ~ 50% of the total P450 in embryonic (< 60 days of gestation), fetal and neonate liver, declines rapidly in the first week of life, and is not readily detectable in adults⁶. CYP3A7 catalyzes reactions similar to that of CYP3A4 and CYP3A5, however, studies with this enzyme have not been extensive⁷. Important reactions catalyzed preferentially by CYP3A7 include the 16 α -hydroxylation of dehydroepiandrosterone 3-sulfate⁸ and the 4-hydroxylation of all-trans-retinoic acid⁹. Regulation of these endogenous metabolic pathways are suspected crucial to normal growth and development of the fetus. These pathways may be susceptible to inhibition by drug entering the fetus.

Using the fluorometric substrate probe, 7-benzyloxy-4-trifluoromethylcoumarin (BFC), we compared inhibition specificity and potency of 16 xenobiotics known to modulate CYP3A4 in vitro metabolism against cDNA-expressed CYP3A5, CYP3A7 as well as CYP3A4. CYP1A2 was used as a negative control. In addition, inhibition of liver microsomal metabolism of BFC, which is selective for CYP3A, was also examined.

Objectives:

- 1) Determine inhibition potency of 16 CYP3A4 modulators with cDNA-expressed CYP3A5, CYP3A7 and HLM using the CYP3A-selective fluorometric probe BFC.
- 2) Assess the potential role of CYP3A5 and CYP3A7 as mediators of drug-drug and drug-endobiotic interactions.

Methods

Enzyme sources: Baculovirus/insect cells cDNA-expressed CYP1A2+OR, CYP3A4+OR+b5, CYP3A5+OR, CYP3A7+OR+b5 and control microsomes (BD Gentest™ Supersomes™) and pooled or single donor human liver microsomes (HLM) were from BD Biosciences Discovery Labware, Woburn, MA.

Probe Substrate: 7-benzyloxy-4-trifluoromethylcoumarin (BFC) (BD Biosciences Discovery Labware, Woburn, MA).

Test chemicals: The test chemicals and their supplier were as follows: cisapride, (Janssen Biotech, Flanders, NJ); nifedipine, cyclosporin A, erythromycin, terfenadine, clotrimazole, (+/-)-verapamil, testosterone, troleandomycin,

α -naphthoflavone, nicardipine, astemizole (Sigma-Aldrich, St. Louis, MO); midazolam, ketoconazole, (Ultrafine Chemicals, Manchester, UK), nimodipine, (ICN Biochemicals, Inc., Aurora, OH) and mibefradil (a generous gift of Dr. Rudolfo Gasser, Roche Pharmaceuticals, Basel, Switzerland).

Fluorometric Enzyme Inhibition assays: Incubations were conducted in a 200 μ l volume in 96-well microplates (BD Falcon™) based on the method described on the BD Biosciences website (www.gentest.com) and is described below. Serial dilutions were performed using a Multiprobe II liquid handling station (Packard Instruments, Downers Grove, IL). A cofactor/serial dilution (C/SD) buffer was prepared in 50 mM potassium phosphate, pH 7.4. This buffer contained 2.6 mM NADP⁺, 6.6 mM glucose-6-phosphate, 0.8 U glucose-6-phosphate dehydrogenase/mL and 0.1 mg/mL microsomal protein prepared from wild type baculovirus infected insect cells. To the first well in each row, 100 μ l of C/SD buffer was added that contained twice the upper concentration of inhibitor. In the second well, 50 μ l of that solution was added. In the second well and all remaining wells, 100 μ l of C/SD buffer that contained the solvent vehicle but lacked test compound was added. Fifty microliters of the inhibitor solution from the second well in each row was then transferred into the third well and serially diluted 1:3 through the eighth well. Wells 9 and 10 contained no inhibitor and wells 11 and 12 were used as controls for background fluorescence (enzyme and substrate were added after the reaction was terminated). The final concentration of the inhibitors in the first well varied between 1 μ M and 200 μ M, depending on the solubility characteristics or potency of the inhibitor. All inhibitors were dissolved in acetonitrile for addition to the incubations. The plate was then pre-warmed at 37°C for 10 minutes, and the reaction initiated by the addition of 100 μ l of pre-warmed enzyme/substrate (E/S) mix. The E/S mix contained 0.35 M potassium phosphate buffer (pH 7.4), cDNA-expressed P450 in insect cell microsomes or HLM. Wild type baculovirus insect cell microsomes was added to standardized protein to 0.25 mg/mL except for HLM incubations. Reactions were terminated after various times (see Table 1) by addition of 75 μ l of a 4:1, acetonitrile: 0.5 M Tris base solution. Fluorescence in each well was measured using a BMG LabTechnologies, Inc. FLUOstar model 403 fluorescence plate reader (Durham, NC). The BFC metabolite 7-hydroxy-4-trifluoromethylcoumarin was measured using an excitation wavelength of 410 nm and emission wavelength of 538 nm. Production of the product was proportional with time and protein concentration. Data were exported and analyzed using an Excel spreadsheet. The IC_{50} values were calculated by linear interpolation.

1 Assay Parameters

Enzyme	CYP1A2	CYP3A4	CYP3A5	CYP3A7	HLM
Apparent K_m	18 μ M	99 μ M	102 μ M	73 μ M	39 μ M
Substrate Concentration	50 μ M	50 μ M	50 μ M	50 μ M	50 μ M
Enzyme Concentration	10 nM	1 nM	40 nM	25 nM	25 μ g/mL
Incubation Time	30 min	30 min	30 min	45 min	15 min

Substrate concentration chosen to be within 3-fold of apparent K_m

Protein concentration chosen to yield similar and low amount of BFC metabolism, thus minimizing inhibitor depletion

2 Mean IC_{50} values for 16 Compounds (n=2 experiments in duplicate on independent days). All values are expressed in units of micromolar

Test substance	CYP1A2	CYP3A4	CYP3A5	CYP3A7	HLM
Astemizole	> 200	0.59	15	27	10
Cisapride	> 50	0.038	5.4	16.5	0.74
Clotrimazole	> 5	0.002	0.042	0.046	0.011
Cyclosporin A	>100	0.46	35	42	0.30
Erythromycin	> 7	1.8	> 7	> 7	> 7
Ketoconazole	> 5	0.016	0.10	0.42	0.004
Mibefradil	> 20	0.006	0.23	0.44	0.015
Midazolam	99	0.46	5.6	24	53.5
α -Naphthoflavone	0.34	activation	activation	activation	activation
Nicardipine	> 20	0.008	0.14	0.35	0.10
Nifedipine	7.7	6.6	12	14	11
Nimodipine	> 20	0.62	3.0	3.6	1.5
Terfenadine	> 100	1.4	11	29	6.8
Testosterone	> 200	activation	activation	activation	activation
Troleandomycin	> 100	0.36	1.52	8.9	5.6
Verapamil	> 100	0.12	2.2	9.1	7.0

CYP3A4 gave lowest IC_{50} values except HLM-ketoconazole and HLM-Cyclosporin A

Inhibition response tracks among the CYP3A isoforms and HLM, but not with CYP1A2. All CYP3As and HLM were subject to activation. These findings are consistent with structural similarities in the active site of the enzyme(s) catalyzing the reaction.

3 Relative Mean IC_{50} values of 13 compounds for CYP3A4, 3A5, 3A7 and HLM and ratiometric comparison with CYP3A4

Test substance	CYP3A4	CYP3A5	CYP3A7	HLM	3A5/3A4	3A7/3A4	HLM/3A4
Astemizole	0.04	1.12	2.05	0.79	28	51	20
Cisapride	0.01	0.94	2.92	0.13	94	292	13
Clotrimazole	0.08	1.66	1.83	0.43	21	23	5.4
Cyclosporin A	0.02	1.80	2.16	0.02	90	108	1.0
Ketoconazole	0.12	0.77	3.09	0.03	6.4	26	0.3
Mibefradil	0.03	1.35	2.53	0.09	45	84	3.0
Midazolam	0.02	0.27	1.15	2.56	14	58	128
Nicardipine	0.05	0.94	2.32	0.69	19	46	14
Nifedipine	0.61	1.07	1.27	1.04	1.8	2.1	1.7
Nimodipine	0.28	1.37	1.66	0.69	4.9	5.9	2.5
Terfenadine	0.12	0.88	2.43	0.57	7.3	20	4.8
Troleandomycin	0.09	0.37	2.17	1.37	4.1	24	15
Verapamil	0.03	0.48	1.98	1.51	16	66	50
Overall mean	0.12	1.00	2.12	0.76	27	62	20
Median	0.05	0.94	2.16	0.69	16	46	5.4

On average, inhibitor potency for CYP3A5 was 27-fold less, and CYP3A7 62-fold less compared to CYP3A4. IC_{50} s with HLM as enzyme source were 20-fold less than 3A4, however the median response was ~ 5-fold less.

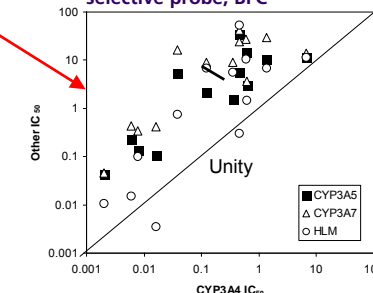
4 Correlation analysis of the log transformed IC_{50} values

Correlation analysis	CYP3A5	CYP3A7	HLM
CYP3A4	0.87	0.84	0.82
CYP3A5		0.96	0.77
CYP3A7			0.82

There was a strong correlation of inhibition potency between and among the cDNA-expressed enzymes and HLM. 7

However, IC_{50} values for CYP3A5, CYP3A7 and HLM were clearly shifted to the left of unity when compared to CYP3A4 in a correlation analysis

5 Comparison of CYP3A4 IC_{50} values to CYP3A5, CYP3A7, and those from HLM using the CYP3A selective probe, BFC



IC_{50} values determined using HLM from a donor with elevated CYP3A5 content (H066) gave 1.5-6.6 fold higher IC_{50} values than those obtained with a donor expressing negligible CYP3A5 (H003). Content of other enzymes that can metabolize BFC, albeit much more slowly (e.g. 2C19, 1A2) were comparable.

6 Effect of CYP3A5, content in single donor liver microsomes on IC_{50} values

Test Substance	IC_{50} ratio (H066/H003)
Astemizole	1.7
Cisapride	6.6
Cyclosporin A	1.5
Terfenadine	2.2

	H066	H003
CYP3A* content (pmol/mg)	103	95
CYP3A5 content (pmol/mg)	18	0.9

* - Predominantly CYP3A4.

Discussion

Role of CYP3A5 in drug interactions

It was recently reported that abundance of CYP3A5 in human liver is greater than thought previously, especially in African Americans². Additionally, CYP3A5 is believed to have a similar substrate specificity as CYP3A4, which can metabolize 50% of all drugs. Therefore, the role of CYP3A5 in drug metabolism may be underestimated. We sought to determine whether CYP3A5 is differentially susceptible to enzyme inhibition and therefore drug-drug interactions. Gibbs et al¹⁰ found ketoconazole and fluconazole to be 4- and 9-fold less potent with CYP3A5 compared to CYP3A4, respectively.

In this set of 16 compounds known to be CYP3A4 inhibitors, we found that inhibition potency was closely correlated with CYP3A4, however, on average, was nearly 30-fold less for 13 compounds that inhibited both enzymes. Lower potency was also observed with 4 compounds in single donor HLM with similar levels of CYP3A4, but large differences in CYP3A5. Our data show that the potency differential between CYP3A4 and CYP3A5 is pervasive and usually large. Unless CYP3A5 is identified as a major isoform involved in drug clearance, above and well-beyond CYP3A4, it is unlikely that CYP3A5 is a target for metabolism-based drug-drug interactions. Disruption of renal CYP3A5 metabolism of endobiotics may be of concern. CYP3A4 inhibition studies with HLM are likely to yield higher and different values as CYP3A5 and other enzymes are present.

Role of CYP3A7 in drug interactions

CYP3A7 is the most abundant P450 isoform in fetal liver and is suspected as a major contributor in fetal drug elimination. However, there is little direct evidence to support this. Pharmacokinetic models are complex and contributions of placental and maternal metabolism are often difficult to disassociate.¹¹ Physiology differences in the fetus including blood flow and oxygenation patterns need also be considered. Nevertheless, our data demonstrate that CYP3A7 exhibits a striking qualitative similarity in inhibition response suggesting potent CYP3A4 inhibitors are likely to impact CYP3A7 catalyzed endo- and xenobiotic metabolism in fetus. CYP3A7 inhibition potency was 60-fold less than CYP3A4 in our data set. This suggests drug-drug interactions that may affect the fetus may be likely to originate via CYP3A4 maternally, rather than at the level of CYP3A7 metabolism. In the absence of maternal drug interactions, drug-endobiotic reactions in fetus may be more of a concern where CYP3A7 metabolism of steroids and other modulators of human development are likely to be precisely regulated.

Conclusions

1. Inhibitor potency with CYP3A5 and CYP3A7 among 16 compounds was well correlated with CYP3A4, but on average was much less (>20-fold) for both enzymes. There was no correlation with CYP1A2 as expected.
2. CYP3A5 content in HLM is expected to elevate IC_{50} values obtained using "CYP3A4" probe substrates. Therefore, the use of HLM (single donor or pools) with substantial 3A5 content may underpredict the extent of an interaction for the majority of individuals who do not have substantial levels of hepatic 3A5.
3. Since CYP3A4 inhibitors are CYP3A7 inhibitors (albeit less potent), drug interaction risk in adult may be a reasonable predictor for that in fetus. Drug-endobiotic interactions may be more of a concern.

References

1. Wrighton SA, Brian WR, Sari M-A, et al. (1990) Studies on the expression and metabolic capabilities of human liver cytochrome P450III_{A5} (HLP3). Mol. Pharmacol. **38**:207-213.
2. Kuehl P, Zhang J, Lin Y, et al (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat. Genet. **27**:383-391.
3. Schuetz EG, Schuetz JD, Grogan WM, et al (1992) Expression of cytochrome P450 3A in amphibian, rat and human kidney. Arch. Biochem. Biophys. **294**:206-214.
4. Gorski JC, Hall SD, Jones DR et al (1994) Regioselective biotransformation of midazolam by members of the human cytochrome P450 3A (CYP3A) subfamily. Biochem. Pharmacol. **47**:1643-1653.
5. Gillam EMJ, Guo Z, Uegn Y-F, et al (1995) Expression of cytochrome P450 3A5 in Escherichia coli: effects of 5' modification, purification, spectral characterization, reconstitution conditions, and catalytic activities. Arch. Biochem. Biophys. **317**:374-384.
6. De Wildt SN, Kearns LG, Leeder JS and van den Anker JN (1999) Cytochrome P450 3A: ontogeny and drug disposition. Clin. Pharmacokinet. **37**:485-505.
7. Kitada M, Kamataki T, Itahashi K et al (1987) Significance of cytochrome P-450 (P-450 HFLA) of human fetal livers in the steroid and drug oxidations. Biochem. Pharmacol. **36**:453-456
8. Kitada M, Kamataki T, Yasumori T et al (1987) P-450 HFLA, a form of cytochrome P-450 purified from human fetal livers, is the 16 α -hydroxylase of dehydroepiandrosterone 3-sulfate. J. Biol. Chem. **262**:13534-13537.
9. Chen H, Fantel AG and Juchau MR (2000) Catalysis of the 4-hydroxylation of retinoic acids by CYP3A7 in human fetal hepatic tissues. Drug Metab. Dispos. **28**:1051-1057.
10. Gibbs MA, Thummel KE, Shen DD and Kunze KL (1999) Inhibition of cytochrome P-450 3A (CYP3A) in human intestinal and liver microsomes: Comparison of Ki values and impact of CYP3A5 expression. Drug Metab. Dispos. **27**:180-187
11. Ring JA, Ghabrial H, Ching MS et al (1999) Fetal hepatic drug elimination. Pharmacol. Ther. **84**:429-445.