

# What is the Level of Expression of CYP2D6 in Human Liver? Influence of Different Calibration Standards on the Estimation of CYP2D6 Abundance



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## INTRODUCTION

- Human hepatic CYP abundance is required for the scaling of *in vitro* data from recombinant human CYP systems (rhCYP) to *in vivo* metabolic drug clearance (Equation 1). Within this process, estimates of CYP expression are also used to determine Intersystem Extrapolation Factors (ISEFs), measures of the relative activity (per unit CYP) of the enzymes in human liver and rhCYP microsomes (Equation 2) (Proctor *et al.*, 2004).
- CYP enzymes are present in liver as apoprotein (non-catalytic) and holoprotein (haem containing, catalytically active form). When rhCYP systems are used as standards for CYP quantification, the holo:apoprotein ratio is assumed to be similar to that in liver microsomes (HLM).

## AIMS

- To assess the variability in reported CYP2D6 abundance and determine if this is related to the use of different standards with different ratios of holo:apo CYP2D6 protein.
- Standard specific values of CYP2D6 abundance will be assigned to an uncharacterised pool of human liver microsomes (HLM) in order to investigate the effect of standard on values of ISEF.

## METHODS

- CYP2D6 abundance values were collated by searching two electronic databases ("MEDLINE" and "Web of Knowledge") and the studies were grouped according to the calibration standard used; BD baculovirus Supersomes<sup>TM</sup> (BD SUP, n=2), CYP2D6 purified from human liver microsomes (HLM PUR, n=2), lymphoblastoid (LYMPH, n=2) and yeast (YEAST, n=3). Weighted mean abundances were calculated for each group and the heterogeneity of the abundance values was assessed (Perrett *et al.*, 2007).
- ISEFs were calculated using the mean CYP2D6 abundance from each standard type and mean *in vitro* kinetic data for the CYP2D6 probe substrates debrisoquine, bufuralol and dextromethorphan (Simcyp internal data, unpublished), Equation 2.

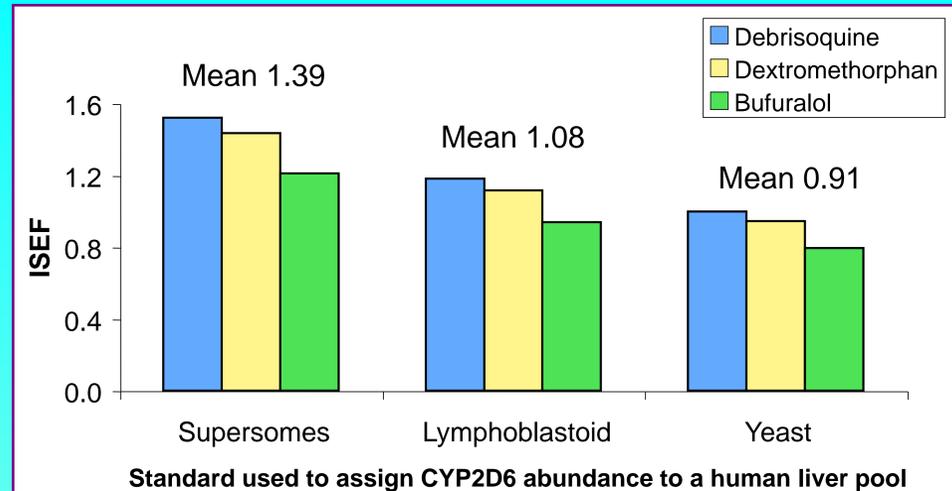


Figure 1: Effect of standard and probe substrate on values of CYP2D6 ISEF

Table 1: Literature values of CYP2D6 abundance

Study	n	Mean CYP2D6 abundance (pmol/mg)	Standard
Nakajima <i>et al.</i> , 1999	6	6	BD SUP
Olesen <i>et al.</i> , 2001	10	8	BD SUP
<b>Mean</b>		<b>7.3</b>	
Zanger <i>et al.</i> , 2001	71	8	HLM PUR
Coller <i>et al.</i> , 2002	50	8	HLM PUR
<b>Mean</b>		<b>8.0</b>	
Shimada <i>et al.</i> , 1994	30	9	LYMPH
Venkatakrishnan <i>et al.</i> , 2000	11	10	LYMPH
<b>Mean</b>		<b>9.4</b>	
Perrett <i>et al.</i> (unpub)	58	10	YEAST
Imaoka <i>et al.</i> , 1996	16	14	YEAST
Isobe <i>et al.</i> , 2005	3	18	YEAST
<b>Mean</b>		<b>11.1</b>	
<b>Total Weighted Mean</b>		<b>9.1</b>	

$$CL_{int}(L/h) = \left[ \sum_{j=1}^n \left( \frac{\sum_{i=1}^n ISEF_{ji} \times V_{max}(rhCYP)_i \times CYP_j \text{abundance}}{K_m(rhCYP)_i} \right) \right] \times MPPGL \times \text{Liver weight}$$

Equation 1: *In vitro*-*in vivo* extrapolation of rhCYP determined  $CL_{int}$

$$ISEF_{ij} = \frac{CL_{intji}(HLM) \text{ (ml/min/mg)}}{CL_{inti}(rhCYP)_j \text{ (ml/min/pmol CYP)}} \times \frac{CYP_j \text{abundance(HLM) (pmol CYP/mg)}}{CYP_j \text{abundance(HLM) (pmol CYP/mg)}}$$

Equation 2: Calculation of ISEF using data from HLM and rhCYP incubations. Where the *i*<sup>th</sup> pathway is metabolised by the *j*<sup>th</sup> CYP isoform, MPPGL = Microsomal Protein per gram of Liver

## RESULTS

- Mean values of apparent liver microsomal abundance of CYP2D6 ranged from 6 to 18 pmol/mg protein (Table 1). Values obtained using BD SUP as a standard gave similar results (7.3 pmol/mg) to HLM PUR (8 pmol/mg). Using HLM PUR as the standard gave values which appeared lower than those obtained using LYMPH (9.4 pmol/mg) and YEAST (11.1 pmol/mg) standards. However, no significant heterogeneity was found between studies.
- Application of different standard specific CYP2D6 abundances translated into ISEF values ranging from 0.91 (YEAST) to 1.39 (BD SUP) (Figure 1), a 1.5-fold difference. The mean ISEF for the probe substrates were 0.98 (bufuralol), 1.16 (dextromethorphan) and 1.23 (debrisoquine), a 1.2-fold difference.

## DISCUSSION

- A trend for rhCYP2D6 standards to produce similar or higher values of abundance compared to those estimated using standards based on purified enzyme contrasts to our previous finding with respect to CYP3A4/5 systems (Wilson *et al.*, 2005, Perrett *et al.*, 2006).
- We have recently shown that CYP2D6 holoprotein is present at lower levels in preparations purified from human liver than in *e.coli* rhCYP systems (Perrett *et al.*, 2007). However, holoprotein levels for other rhCYP2D6 systems, including the ones shown here, have yet to be determined.
- The choice of CYP2D6 standard used to quantify CYP2D6 abundance in a HLM pool has more influence on ISEF values than the choice of CYP2D6 probe substrate.

## REFERENCES

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