A META-ANALYSIS OF CYP3A4 ABUNDANCE IN HUMAN LIVER: USE OF DIFFERENT STANDARDS CONTRIBUTES TO APPARENT HETEROGENEITY IN REPORTED VALUES

ZE Barter[1,2], K Rowland-Yeo[1], G Tucker[1,2] and A Rostami-Hodjegan[1,2]

Correspondence: k.r.yeo@simcyp.com

[1]Academic Unit of Clinical Pharmacology, Royal Hallamshire Hospital, University of Sheffield, UK

[2] Simcyp Limited, Blades Enterprise Centre, John St, Sheffield, UK

BACKGROUND

- Recombinant systems expressing human cytochrome P450 (rCYP) isoforms are used in conjunction with abundances of the respective enzymes in human liver to extrapolate *in vitro* data to CYP related metabolic clearance *in vivo.*
- We previously reported a meta-analysis for CYP3A4 in human liver; mean values for individual studies ranged from 37 to 248 pmol.mg^{-1} microsomal protein (mmp; weighted mean – 114; Table 1).
- Heterogeneity was observed (p<0.0002) and it was postulated that the variability may be due to the use of different standards with varying ratios of holo:apo CYP3A4 protein.

OBJECTIVES

- This study aimed to investigate the influence of calibration standard on the quantification of CYP3A4 by comparing levels of immunodetectable CYP3A4 in two rCYP systems with that in human liver microsomes (HLM) characterised for total CYP3A4 apoprotein (HLMSTD).

MATERIALS & METHODS

- Three sources of CYP3A4 were used as standards: baculovirus-insect cells (Supersomes–Gentest®) (SUP), human lymphoblastoid cells (Gentest®) (LYMPH) and a sample of HLM quantified for total CYP3A4 protein (HLMSTD) (Westlind-Johnsson *et al., 2003*).
- Standard CYP3A4 contents were provided by the suppliers. Levels of CYP3A4 in rCYP standards were determined by CO difference spectroscopy (holoprotein), and the CYP3A4 content of the HLMSTD was determined by immunological methods (non-holo & holoprotein).
- Standards were diluted to give approximately equal concentrations of CYP3A4 (as stated by the supplier). A competitive ELISA and non linear fitting (Grafit Erithicus Software) were used to determine the maximum immunodetectable signal (IMD) and CYP3A4 content producing 50% IMD (Figure 1). The effect of the different standards on the estimation of HLM CYP3A4 abundance was then compared (Figure 2).

Table 1: Literature values of CYP3A4 abundance. Purified enzyme (PUR), Baculovirus-insect cells (Supersomes–Gentest®) (SUP), human lymphoblastoid cells (Gentest®) (LYMPH) and a sample of HLM quantified for total CYP3A4 protein (HLMSTD) (Westlind-Johnsson *et al*, 2003).

Study	n	CYP3A4 (pmol.mg^{-1})	Calibration Standard	Correction Factor	Corrected CYP3A4 (pmol.mg^{-1})
Guengerich & Turvy, 1991	36	248	PUR	1.0	248
Shimada *et al.,* 1994	28	106	PUR	1.0	106
Wandel *et al.,* 1998	14	68	PUR	1.0	68
Lin *et al.,* 2002	60	81	PUR	1.0	81
Westlind-Johnson *et al.,* 2003	29	168	HLMSTD	1.0	168
Lipscomb *et al.,* 2003	20	91	BAC	2.4	218
Wolbold *et al.,* 2003	39	56	LYMPH	2.0	139
Galetin *et al.,* 2004	12	73	BAC	2.4	176
King *et al.,* 2003	22	36	BAC	2.4	86
Wang *et al.,* 2005	5	37	BAC	2.4	90
Total	235				
Weighted Mean		114			142

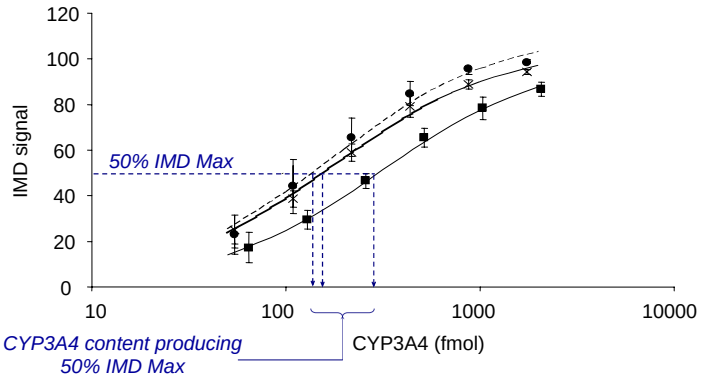

Figure 1: Mean (± standard deviation; n=4) % Competition profiles following ELISA of HLMSTD (■), SUP (●) and LYMPH (×). The amount of CYP3A4 required to produce 50% maximal IMD by each of the 3 standards is highlighted.

RESULTS

- The amount of CYP3A4 in HLMSTD needed to achieve half maximal blot intensity was 2.4 and 2.0 fold higher than that for spectrally measured CYP in SUP and LYMPH systems, respectively.
- Application of these correction factors to CYP3A4 abundances obtained using rCYP standards led to mean values for individual studies that were less variable (68 to 248 pmol.mg^{-1} mmp; weighted mean – 142; Table 1).
- Corrected abundance values showed no statistically significant heterogeneity within reports (p=0.08; Figure 3).

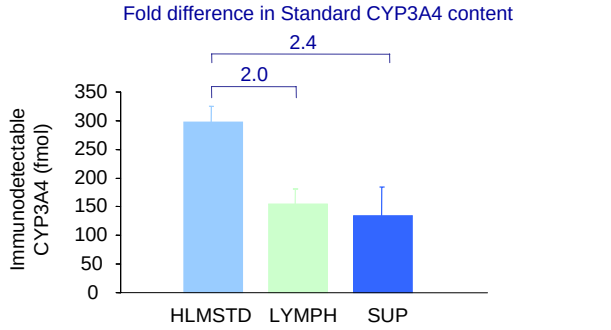

Figure 2: Fold difference in levels of IMD CYP3A4 producing 50% maximal IMD signal for 3 different calibration standards

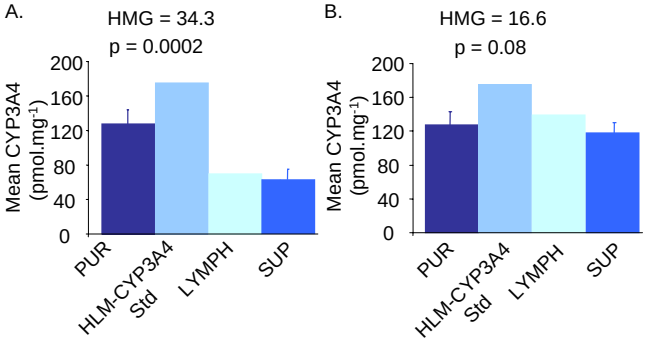

Figure 3: Weighted mean values (± 95% confidence intervals) of literature CYP3A4 abundance categorised by calibration standard.
A. Without correction for calibration standard resulting in significant heterogeneity between studies. B. Following correction for calibration standard resulting in no significant heterogeneity between studies

CONCLUSIONS

The findings of the current study suggest that some of the discrepancy in values of CYP3A4 abundance obtained between studies is due to differences in the standard used for immunoquantification.

Correction for such disparities is essential for accurate prediction of population means and inter-individual variability in metabolic clearance.

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