IMPLICATIONS OF THE DISPARITY IN RELATIVE HOLO:APO-PROTEIN CONTENTS OF DIFFERENT STANDARDS USED FOR IMMUNO-QUANTIFICATION OF HEPATIC P450 3A4 (CYP3A4)

Z.E. Wilson[1,2], S.W.Ellis[1], R.J.Edwards[3], G.T.Tucker[1,2] and A. Rostami-Hodjegan[1,2]

Correspondence: z.wilson@simcyp.com

1- Academic Unit of Clinical Pharmacology, Royal Hallamshire Hospital, University of Sheffield,
2- Simcyp Limited, Blades Enterprise Centre, John St, Sheffield, UK,
3- Experimental Medicine and Toxicology, Division of Medicine, Imperial College, Hammersmith Hospital, London, UK

INTRODUCTION

Reported values of hepatic CYP3A4 abundance vary widely, with mean study values ranging from 37 – 248 pmol P450/mg microsomal protein (Table 1). A possible source of variability is the protein standard used in immuno-quantification. Standards used include purified CYP450 isoforms (PUR), human liver microsomes (HLM) and recombinantly expressed enzymes (rCYP). Although suppliers advise that rCYP preparations may contain a high proportion of non-holoprotein, relative differences in holo:non-holoprotein contents between standards are often ignored (Fig 1). The aim of this study was to evaluate different protein standards for the estimation of CYP3A4 abundance in HLM.

METHODS

Four sources of CYP3A4 were used as standards: baculovirus-insect cells (Supersomes–Gentest®) (SUP), *E.Coli* (Bactosomes-Cypex®) (BAC), human lymphoblastoid cells (Gentest®) (LYMPH) and a sample of HLM quantified for total CYP3A4 protein (HLMSTD) (Westlind-Johnsson *et al*).

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 (Fig 2) (cross validated using Western Blotting: $r^2 = 0.62$, $p < 0.001$) and non linear fitting (Grafit Erithicus Software) were used to generate values of % Comp max and $Comp_{C50}$ 3A4 (fmol) (Fig 3). The effect of the different standards on the estimation of HLM CYP3A4 abundance was then compared (Fig 4).

Table 1: Literature values of CYP3A* and CYP3A4 abundance

Study	n	CYP3A4 /3A (pmol/mg)	Standard	Correction Factor	Corrected CYP3A4 /3A (pmol/mg)
Guengerich & Turvy, 1991*	36	248	PUR	-	248
Shimada *et al.*, 1994*	30	121	PUR	-	121
Wandel *et al.*, 1998	14	64	PUR	-	64
Lipscomb *et al.*, 2003*	20	91	SUP	2.4	218
Westlind-Johnsson *et al.*, 2003	32	175	HLMSTD	1.0	175
Wolbold *et al.*, 2003	94	70	LYMPH	2.0	140
Galetin *et al.*, 2004	12	73	SUP	2.4	175
Wang *et al.*, 2005	5	37	SUP	2.4	89
Wilson *et al.*, unpub	53	110	HLMSTD	1.0	110
Total	296				
Weighted Mean		**114**			**150**

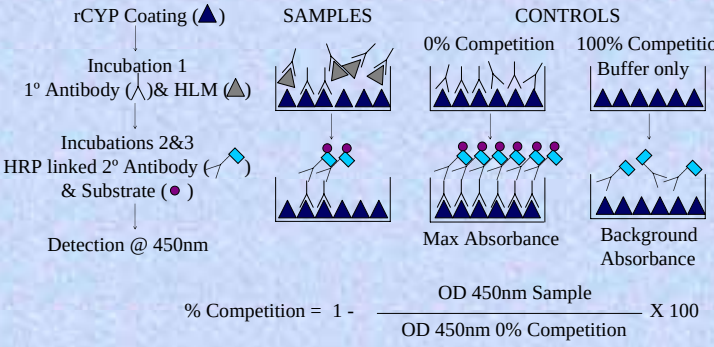

***Figure 2*: Competitive ELISA methodology**

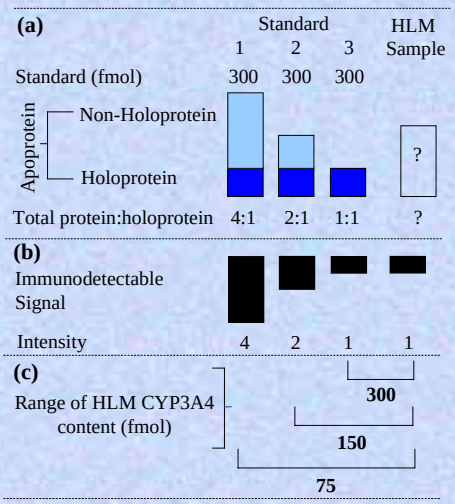

***Figure 1*: Implications of the disparity in relative holo:non-holoprotein contents of different CYP3A4 standards used in the immuno-quantification of HLM CYP3A4.**

(a) Equal amounts of CYP3A4 holoprotein are chosen for each standard and an immunodetectable signal is produced by ELISA. The signal intensities (b) are used to calculate the CYP3A4 content of an uncharacterised HLM sample (c), with standard 3 indicating an HLM CYP3A4 abundance of 300 fmol (due to equal intensities of sample and standard), and standards 2 and 3 indicating abundances of 150 and 75 fmol (due to the intensity of the sample being 50% and 25% the intensity of standards 2 & 3, respectively).

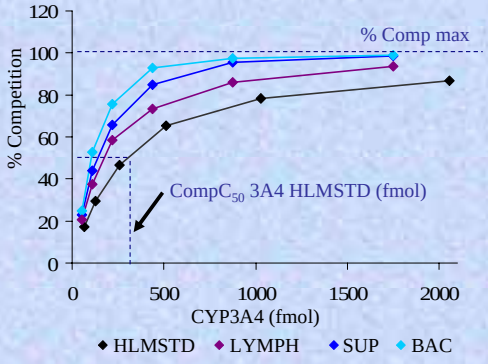

***Figure 3*: % Competition profiles**

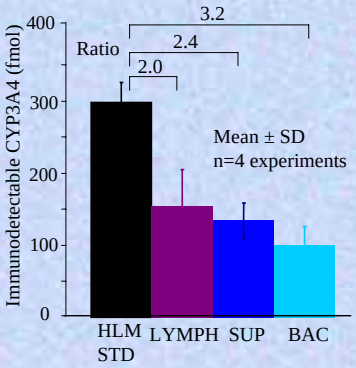

***Figure 4*: Effect of different CYP3A4 standards on values of HLM CYP3A4 abundance**

RESULTS & DISCUSSION

Equal amounts of CYP3A4 produced relative immunodetectable signal intensities of 1.5, 1.8 and 2.4 in LYMPH, SUP and BAC systems compared to HLMSTD. When used to calculate the CYP3A4 abundance of an uncharacterised HLM sample these differences translated to abundance values of 150, 125 and 94 fmol when using LYMPH, SUP and BAC systems as standards compared to 300 fmol when using the HLMSTD (3.2:2.4:2.0) (Fig 4). Differences in immunodetectable signal and resulting values of abundance may be due to different ratios of holo:non holoprotein between standards (Fig 1).

Application of these ratios to reported HLM CYP3A4 abundance in the literature (Table 1), where authors used rCYP as the standard for immuno-quantification, increased the weighted mean abundance from 116 to 152 pmol/mg and reduced the fold difference in mean study values from 6.7 to 3.9.

The choice of protein standard can have a significant effect on values of HLM CYP3A4 abundance. This should be considered, particularly when selecting values for use in the scaling of *in vitro* drug metabolism data to predict hepatic clearance *in vivo*.

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