The Use of Recombinantly Expressed Standards for Immuno-quantification of Hepatic CYP3A5 Can Result in an Underprediction of CYP3A5 Abundance

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INTRODUCTION

- The mean apparent abundance of hepatic CYP3A5 reported in the literature for individuals with the *1/*3 genotype ranges from 3 to 109 pmol/mg microsomal protein.
- The mean literature value of CYP3A5 abundance determined using Supersomes® as the calibration standard (18 pmol/mg microsomal protein) is significantly lower ($p < 0.01$) than that determined using characterised human liver microsomes and purified CYP3A5 (83 pmol/mg microsomal protein), (Table 1).
- Owing to the high variability in CYP3A4 (Wilson *et al*., 2005) and CYP3A5 abundance, the formation rates of 6β-hydroxytestosterone per unit CYP3A also show high variability between studies. The values range from 10 to 55 pmol/min/pmol CYP3A, (Figure 2).

AIM & OBJECTIVES

- To investigate if variability in reported CYP3A5 abundance is related to the use of different standards with different ratios of holo:apo CYP3A5 protein.
- To compare the immuno-detection of CYP3A5 by Western Blot, using two recombinantly expressed (rCYP) standards, Supersomes® (Gentest) and Bactosomes® (Cypex), against human liver microsomes characterised for total CYP3A5 apoprotein (HLMSTD).

METHODS

- Samples of CYP3A5 Supersomes® and Bactosomes® were to diluted to equal concentrations of holoprotein (determined by the supplier using carbon monoxide (CO) difference spectroscopy).
- Total CYP3A5 apoprotein (holo + non-holoprotein) was measured by Western Blot and compared to that measured in human liver microsomes characterised for total apoprotein (HLMSTD).
- CYP3A5 band intensity was measured using Kodak Digital Science 1D software. Values of maximal intensity and CYP3A5 concentration at 50% maximal intensity (fmol) were determined for each standard by non-linear regression (Grafit Erithicis software).
- The ratio of CYP3A5 concentration (fmol) required to achieve 50% maximal intensity was determined for each standard relative to HLMSTD and used to correct values of enzyme abundance reported in the literature.
- The significance of differences between CYP3A5 abundance determined from studies using rCYP standards and those using HLMSTD or purified enzyme were assessed by Student's t-test.

RESULTS

- The amount of CYP3A5 in HLMSTD needed to achieve half maximal blot intensity was 7.7 and 12.3 fold higher than that for spectrally measured CYP in Supersomes® and Bactosomes®, respectively (Figure 1).
- Using these correction factors the mean values of CYP3A5 abundance reported using rCYP as standard increased from 18 to 151 pmol mg/microsomal protein, values which are not significantly different from the 83 pmol mg/microsomal protein obtained using HLMSTD and purified enzyme (Table 1).
- The correction factors for CYP3A5 and CYP3A4 (Wilson *et al*., 2005) were applied to formation rates of 6β-hydroxytestosterone (pmol/min/pmol CYP3A) reported using rCYP for immuno-quantification of liver samples. The mean rate decreased from 50 to 17 pmol/min/pmol 3A. This was similar to the value of 12 pmol/min/pmol 3A obtained using HLMSTD and purified enzyme for immuno-quantification of CYP3A (Figure 2).

DISCUSSION

- The results suggest that the high variability observed in values of CYP3A5 abundance reported in various studies is at least partly due to the use of different standards with different ratios of holo:apo CYP3A5 protein.
- CO difference spectroscopy, used to quantify the CYP3A5 content in these standards, detects only holoprotein. In contrast, immuno-quantification methods detect both holo and non-holoprotein. Since rCYP standards appear to express a significant proportion of non-holoprotein, the amount of CYP3A5 measured using immunodetection is greater than that using HLMSTD or purified enzyme as calibration standard. This results in an underprediction of CYP3A5 abundance.

Figure 1: Western blot intensity profiles of Supersomes®, Bactosomes® and HLMSTD

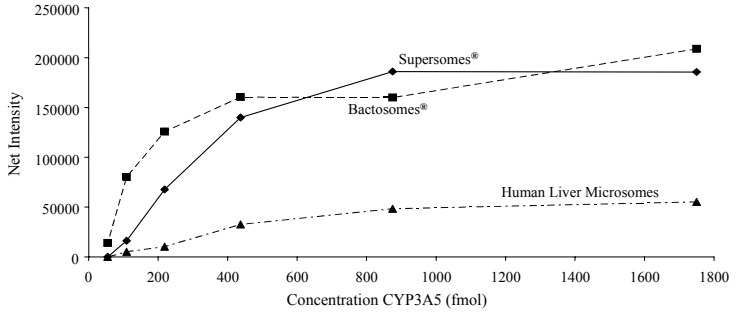
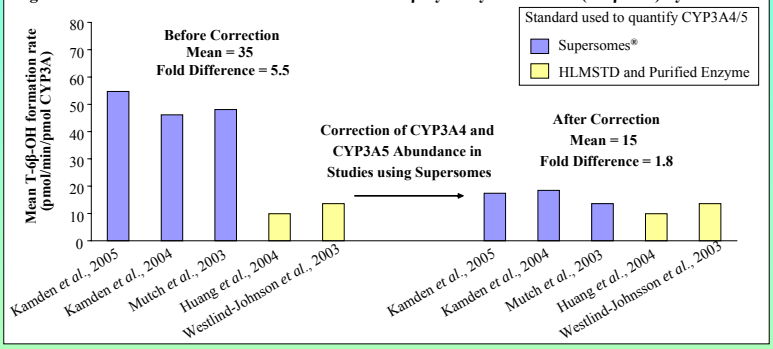


Table 1: Literature values of CYP3A5 abundance

*Significant difference ($p < 0.01$); † No significant difference

Study	n	CYP3A5 (pmol/mg)	Standard	Correction Factor	Corrected CYP3A5 (pmol/mg)
Wang *et al* ., 2005	2	3	SUP	7.7	19
Von Richter *et al* ., 2004	2	3	SUP	7.7	21
Tateishi *et al* ., 1999	6	30	SUP	7.7	229
King *et al* ., 2002	5	42	SUP	7.7	320
Kamdem *et al* ., 2004	5	13	BAC	12.3	165
Mean		**18** *			**151** †
Westlind-Johnson *et al* ., 2003	3	44	HLMSTD	1.0	44
Perrett *et al* ., unpub	6	99	HLMSTD	1.0	99
Lin *et al* ., 2002	13	78	PUR	1.0	78
Kuehl *et al* ., 2001	9	109	PUR	1.0	109
Mean		**83** *			**83** †
Total Weighted Mean		**61**			**130**

Figure 2: Literature values of the formation rate of 6β-hydroxytestosterone (T-6β-OH) by HLM



- Correction factors reported previously for CYP3A4 were 2.4 and 3.2 for Supersomes® and Bactosomes®, respectively (Wilson *et al*., 2005), indicating a greater discrepancy between measurement methods for CYP3A5 than CYP3A4.

CONCLUSION

- When using rCYP standards for immuno-quantification of CYP3A5/4, caution should be exercised in equating levels of spectrally measured CYP (holoprotein) with those of total CYP (apo & holoprotein).
- Specific correction factors may apply to other CYPs.

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