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

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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) was used to estimate HLM CYP3A4 abundance. The effect of the different standards on the estimation of HLM CYP3A4 abundance was then compared (Fig 4).

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.

REFERENCES

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