EXPERIMENTAL CONSIDERATIONS IN DETERMINING INTER SYSTEM EXTRAPOLATION FACTORS REQUIRED FOR PREDICTION OF HUMAN DRUG CLEARANCE FROM RECOMBINANTLY EXPRESSED ENZYMES

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INTRODUCTION

- The utility of kinetic data derived from recombinantly expressed cytochrome P450 enzymes (rCYP) for prediction of human metabolic clearance may be compromised by differences in intrinsic activity per unit enzyme between the recombinant system and human liver microsomes (HLM).
- Application of Inter System Extrapolation Factors (ISEFs) to rCYP data (Equation 1A) allows correction for such differences [1].
- Previously, ISEFs have been calculated from literature values of probe substrate intrinsic clearance (CL_{int}) [1,2]. Such values may be compromised by the use of HLM and rCYP data from different sources, poorly designed protocols and the use of small numbers of liver samples.
- A strategy for the experimental determination of key IVIVE scalars is required.
- The aim of this study was to determine an ISEF for CYP2C9 including an investigation of the following factors:
  - Effect of probe substrate
  - Pooling of HLM
  - Effect of cytochrome b5 (b5)
  - Incubation buffer composition
  - Use of full V_{max} / K_m kinetics *vs.* single point CL_{int}

MATERIALS & METHODS

- Microsomes were prepared as described previously [3] from 50 Caucasian livers held within the liver bank at the Academic Unit of Clinical Pharmacology, University of Sheffield.
- Individual HLMs were combined such that the contribution of each liver to the pool was equal in terms of mg microsomal protein. This approach differs from that commonly employed commercially where HLMs are pooled on the basis of relatively equal activity.
- rCYP2C9 + P450 reductase with and without b5 Supersomes™ were provided by BD Gentest (Woburn, MA).
- Warfarin and tolbutamide were selected as probe substrates for CYP2C9 [4].
- For kinetic studies, incubation times were 20 minutes and protein concentrations were 0.2 mg/ml for HLM and 10-40 pmol/ml for rCYP2C9 ± b5.

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

(CYP_j abundance in target population; j^{th} CYP isoforms; i^{th} metabolic pathways; microsomal protein per gram of liver)

Equation 1A: *In vitro – in vivo* extrapolation of rCYP determined CL_{int}

$$ISEF_{ij} = \frac{CL_{int\,ji}\ (HLM)\ /\ CYP_j \text{ abundance (HLM)}}{CL_{int\,i}\ (rCYP_j)}$$

(numerator: µL/min/mg ÷ pmol CYP/mg; denominator: µL/min/pmol CYP)

Where there are *i* metabolic pathways for each of *j* CYPs and $CL_{int} = \frac{V_{max}}{K_m}$

Equation 1B: Calculation of ISEF using data from HLM and rCYP incubations

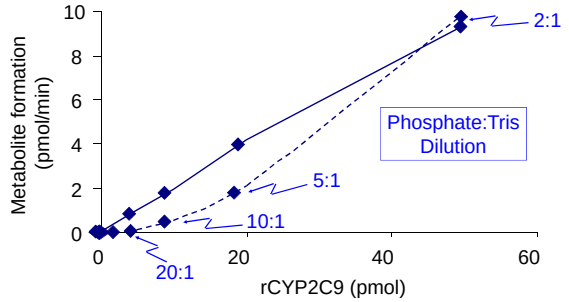

Figure 1: Effect of incubate buffer composition on enzyme activity.

Fixed phosphate:tris ratio (2:1 v/v) ----- Varying phosphate:tris ratio

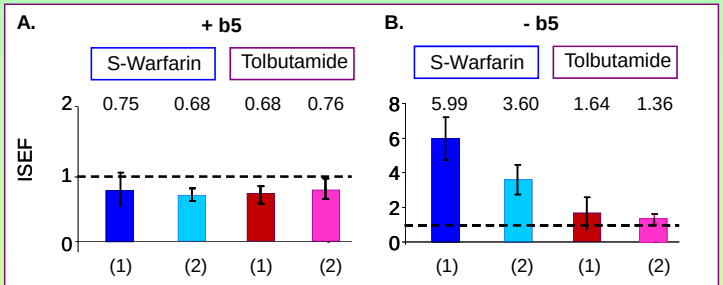

Figure 2: ISEF values for rCYP2C9 (Mean ± SD) using 2 different methods of determining CLu_{int} – An ISEF of 1 (- - -) means activity of each unit of CYP2C9 is the same in HLM and rCYP samples; n = 3 for each probe substrate (warfarin and tolbutamide). **A.** with b5 rCYP2C9, **B.** without b5 rCYP2C9

- Values of V_{max} and K_m were obtained using non linear regression (Prism 5, Graphpad Software, San Diego, CA).
- Single point CL_{int} values were determined at a concentration well below K_m.
- Correction of K_m values for non specific microsomal binding was made using the Prediction Toolbox within the Simcyp Population–Based ADME Simulator V7.10.
- HLM CL_{intu} values were converted to a rate per pmol CYP2C9 using an average CYP2C9 HLM content of 73 pmol/mg [5].
- ISEFs were calculated for each rCYP2C9 system (± b5) using Equation 1B.

RESULTS

- Diluting the phosphate:tris composition of incubates resulted in a non linear relationship between rCYP2C9 content and enzyme activity (Figure 1).
- Activity in rCYP microsomes without b5 was substantially lower than that observed in those containing b5. This resulted in higher ISEFs (Figure 2A and B).
- The impact of the method of determining the ISEF was substrate dependent when using the same rCYP without b5 (Figure 2B). Consistent ISEFs were obtained independent of method when rCYP with b5 was used (Figure 2A).

CONCLUSIONS

- Reproducible CYP2C9 ISEFs can be determined using single point CL_{intu} data from b5 containing rCYP Supersomes (BD Gentest) and pooled HLM. Full V_{max} / K_m data were not required.
- These data highlight the importance of experimental protocol, particularly in light of the sensitivity of rCYP2C9 activity to differences in buffer composition.
- For consistency in relative activity across substrates, use of a b5 expressing recombinant enzyme system is recommended.

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