COMPARISON OF THE CATALYTIC ACTIVITY PER UNIT ENZYME OF RECOMBINANTLY EXPRESSED AND HUMAN LIVER MICROSOMAL CYTOCHROME P450 2C9: DETERMINATION OF INTER SYSTEM EXTRAPOLATION FACTORS

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INTRODUCTION

- The utility of kinetic data derived from recombinantly expressed cytochrome P450 enzymes (rhCYP) for the prediction of the extent and variability of drug–drug interactions has been recognised [1], However, their use may be compromised if the differences in intrinsic activity per unit enzyme between the recombinant system and human liver microsomes (HLM) are not accounted for.
- Application of Inter System Extrapolation Factors (ISEFs) to rhCYP data (Equation 1A) allows correction for such differences [2].

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

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 rhCYP determined CL_{int}

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

(µL/min/~~mg~~ ; pmol CYP/~~mg~~)

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 rhCYP incubations

- ISEF values obtained from diverse literature sources are not ideal [2]; a strategy for their experimental determination is preferable.
- The aim of this study was to determine an ISEF for CYP2C9 including evaluation of the following experimental variables: probe substrate, effect of cytochrome b5 and method of intrinsic clearance (CLu_{int}) determination.

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.
- rhCYP2C9 + P450 reductase with and without b5 Supersomes™ were kindly provided by BD Gentest (Woburn, MA).

Table 1: Kinetic parameters for three 2C9 substrates in each enzyme system. Values are means of 3 incubations ± SD. HLM CLu_{int} values were converted to a rate per pmol CYP2C9 using an average CYP2C9 HLM content of 73 pmol/mg [4]

Substrate	SYSTEM	$K_{m,u}$ (µM)	V_{max} (pmol/min/pmol)	CLu_{int} (µl/min/pmol)
DIC	HLM	16.7 ± 2.80	17.1 ± 2.01	1.03 ± 0.07
	rhCYP2C9+b5	6.13 ± 1.67	12.7 ± 1.32	2.14 ± 0.37
	rhCYP2C9-b5	8.77 ± 0.74	11.8 ± 0.87	1.34 ± 0.04
S-WARF	HLM	4.87 ± 0.75	0.12 ± 0.004	0.02 ± 0.01
	rhCYP2C9+b5	5.49 ± 1.48	0.15 ± 0.01	0.03 ± 0.01
	rhCYP2C9-b5	20.2 ± 4.75	0.06 ± 0.01	0.003 ± 0.0005
TOL	HLM	72.4 ± 20.5	2.04 ± 0.10	0.03 ± 0.008
	rhCYP2C9+b5	70.0 ± 5.7	2.92 ± 0.44	0.04 ± 0.01
	rhCYP2C9-b5	161 ± 58.7	3.13 ± 0.24	0.02 ± 0.01

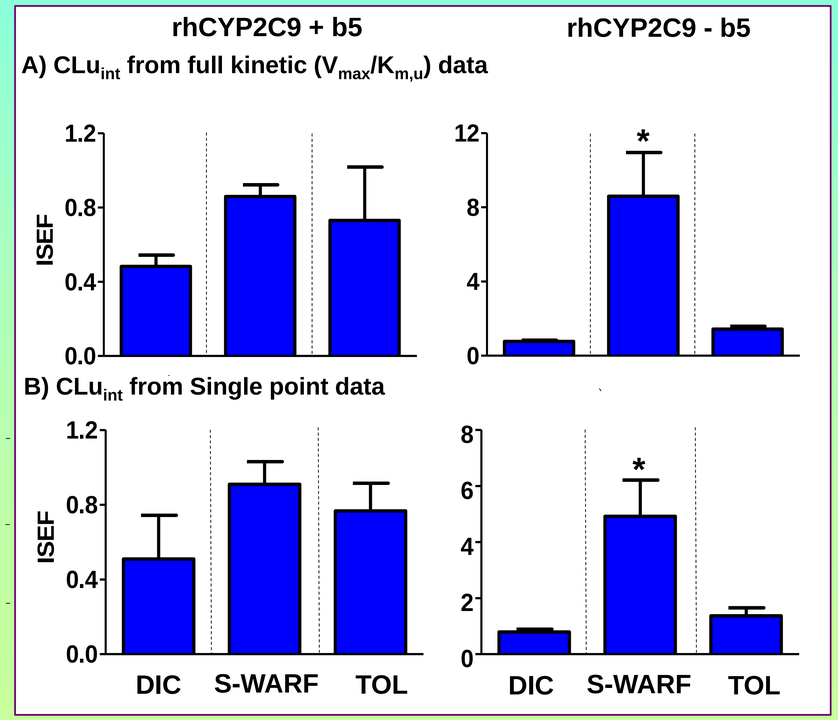

Figure 1: ISEF values for CYP2C9 ± b5 (mean ± SD) obtained from (A) full kinetic data and (B) single point data at [S]<<K_m. * Indicates that ISEF values for S-WARF generated using rhCYP2C9 –b5 were significantly different to those determined using DIC and TOL (p<0.05 ANOVA, post hoc Tukeys B)

- S-Warfarin (S-WARF), tolbutamide (TOL) and diclofenac (DIC) were selected as probe substrates for CYP2C9 [4].
- Values of CL_{int} were obtained from full kinetic studies (as V_{max} and K_m, obtained using non linear regression; Prism 5, Graphpad Software, San Diego, CA) and using the rate of metabolite formation at a single substrate concentration well below the K_m ('single point').
- 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 CLu_{int} 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 rhCYP2C9 system (± b5) using Equation 1B
- Differences in ISEF between methods of CLu_{int} determination and probes were assessed using the paired t test or One-way ANOVA (SPSS v12, Chicago, IL, USA).

RESULTS

- Kinetic parameters (V_{max}, $K_{m,u}$ and CLu_{int}), obtained for the three substrates in each enzyme system are shown in Table 1.
- Using rhCYP2C9 with b5 the ISEF values determined from full kinetics were, 0.86, 0.73 and 0.50 for S-WARF, TOL and DIC respectively (Figure 1A), compared to 0.91, 0.77 and 0.51 when determined from single point CLu_{int} (Figure 1B).
- No significant difference in values of ISEF was observed between probe substrates or method of CLu_{int} determination.
- Using rhCYP2C9 without b5, values of CLu_{int} were lower and ISEF values correspondingly higher (Figure 1). In the absence of b5, ISEF values for the three substrates differed significantly (p=0.01; 1-way ANOVA) with S-WARF being most susceptible to the lack of b5 (Figure 1).

CONCLUSIONS

- We conclude that full kinetic data are not required to establish accurate ISEF values for rhCYP2C9 Supersomes™, and that the inclusion of b5 affords more consistent values across substrates.

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