INTRODUCTION

The utility of kinetic data 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
- Incubation buffer composition
- Pooling of HLM
- Use of full V_{max} / K_{m} kinetics vs. single point CL_{int}
- Effect of cytochrome b5 (b5)

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.

RESULTS

- 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_{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 CYP2C9 system (± b5) using Equation 1B.

CONCLUSIONS

- Reproducible CYP2C9 ISEFs can be determined using single point CL_{int} data from b5 containing CYP 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 CYP2C9 activity to differences in buffer composition.
- For consistency in relative activity across substrates, use of a b5 expressing recombinant enzyme system is recommended.

REFERENCES