PREDICTION OF THE ORAL CLEARANCE OF MIDAZOLAM USING IN VITRO DATA FROM RECOMBINANTLY EXPRESSED ENZYMES AND INTER SYSTEM EXTRAPOLATION FACTORS

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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 allows for correction of such differences (Proctor *et al.,* 2004); an ISEF > 1 indicates greater activity in HLM than rCYP and an ISEF of < 1 indicates greater activity in rCYP than HLM.
- Therefore, the aim of this study was to assess the impact of rCYP system specific ISEFs on the accuracy of prediction of oral clearance (CL_{po}) of the CYP3A probe substrate midazolam

MATERIALS & METHODS

- Values of V_{max} and K_m for midazolam 1 (1-OH) and 4 (4-OH) hydroxylation determined using 3 different rCYP 3A4 and 3A5 systems with varying expression of cytochrome b5 [no expression (-b5), co-expression (+b5-co) and incubate supplementation (+b5-sup)] and HLM were obtained from the literature.
- Midazolam intrinsic clearance (CL_{int}) was calculated for each system from the *in vitro* V_{max} / K_m data (Equation 1). Reporting of CYP3A4 and CYP3A5 kinetic parameters in the rCYP studies allowed determination of the % contribution of the individual enzymes to overall midazolam CL_{int}.
- Initially, ISEFs were calculated (Proctor *et al.,* 2004) using literature average values of HLM CYP3A4 and 3A5 abundance without accounting for possible differences between rCYP3A systems (Equations 1 & 2).
- Alternatively, ISEF values were calculated for each rCYP system individually and incorporated in Equation 2.
- Predicted median values of midazolam CL_{po} determined with and without application of ISEFs (Equation 2) using the Simcyp® Population Based ADME Simulator Version 6.10 (www.simcyp.com) were compared to observed (*in vivo*) CL_{po} values.

$$ISEF_{ij} = \frac{CL_{intji}\ (HLM)\ (\mu L/min/mg)}{CL_{inti}\ (rCYP_j) \times CYP_j\ abundance\ (HLM)\ (\mu L/min/pmol\ CYP \times pmol\ CYP/mg)}$$

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

Equation 1: Calculation of ISEF

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

(CYP_j abundance; *j* CYP isoforms; *i* metabolic pathways; amount of microsomal protein per gram of liver)

Equation 2: *In vitro – in vivo* extrapolation of rCYP determined CL_{int}

Hepatic scaling factors and frequency of CYP3A5 expression in the population of 100 virtual patients used in simulations of clearance (Simcyp® ADME Simulator Version 6.10):

CYP3A4 abundance: $Mean_{geo}$ 108 pmol/mg; range 11 – 547 pmol/mg
CYP3A5 abundance: $Mean_{geo}$ 48pmol/mg; range 9 – 112 pmol/mg
CYP3A5 poor metaboliser frequency: 0.83
MPPGL: $Mean_{geo}$ 29 mg/g; range 17 –58 mg/g
Liver weight: $Mean_{geo}$ 1611 g; range 1066 –2433 g

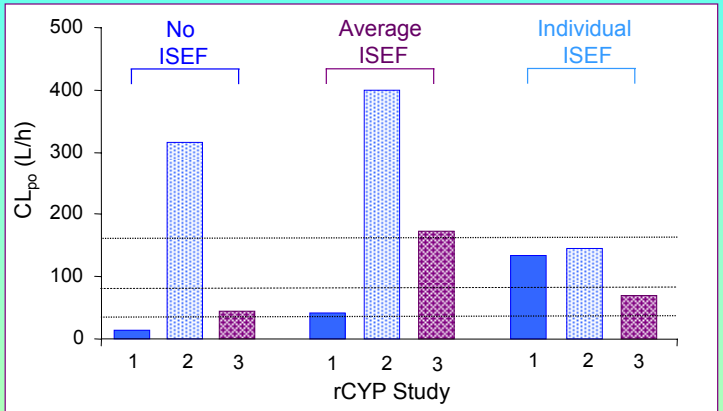

Figure 1: Predicted median *p.o.* clearance of midazolam using CL_{int} values from 3 literature sources and application of No ISEF, an average CYP3A ISEF or individual ISEFs calculated for each study. The median (·····) and 2-fold either side of the median (——) observed *in vivo* midazolam CL_{po} are indicated.

Study 1: Sup –b5, baculovirus insect cell expressed CYP3A4 and CYP3A5 (Supersomes) with co-expressed NADPH CYP450 reductase (NCR) (Galetin *et al.,* 2004)
Study 2: Sup +b5 sup, CYP3A4 and CYP3A5 Supersomes supplemented with NCR and b5 (Williams *et al.,* 2002)
Study 3: Sup +b5 co, CYP3A4 Supersomes with co-expressed NCR and b5, CYP3A5 Supersomes supplemented with NCR and b5 (Huang *et al.,* 2005)

RESULTS

- The predicted CL_{po} values were 0.18, 3.88 and 0.56 fold of the observed *in vivo* CL_{po} for –b5, +b5-co and +b5-sup systems, respectively (Figure 1).
- Application of an average CYP3A ISEF failed to improve prediction accuracy (fold difference 0.42, 10.04 and 1.33 for -b5, +b5 co and +b5 sup systems respectively (Figure 1).
- However, application of individual ISEFs of 5.5, 0.6 and 1.3 (Figure 2) reduced differences to within 2 fold (1.7, 1.8 and 0.9 fold for -b5, +b5 co and +b5 sup systems respectively) of the observed *in vivo* CL_{po} for all 3 systems (Figure 1).
- Remaining differences in CL_{po} between studies were due to differences in the contribution of the polymorphically expressed enzyme, CYP3A5, to the overall *in vitro* CL_{int} (Figure 3) between studies.

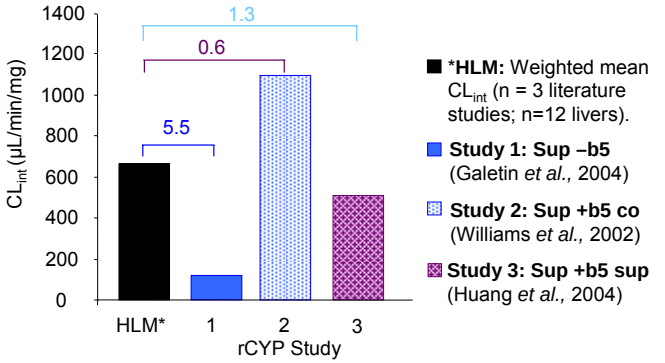

Figure 2: Determination of ISEFs from rCYP and HLM *in vitro* intrinsic clearance (CL_{int}).data

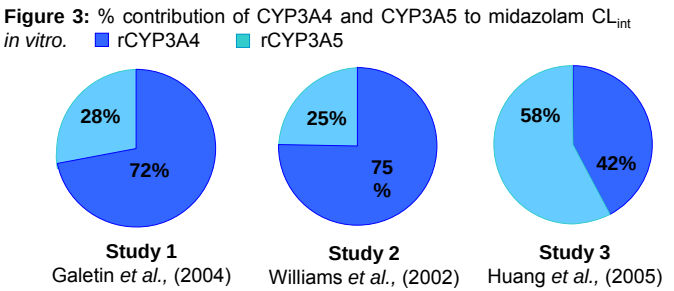

Figure 3: % contribution of CYP3A4 and CYP3A5 to midazolam CL_{int} *in vitro.* rCYP3A4 rCYP3A5

CONCLUSIONS

When performing *in vitro-in vivo* extrapolations using rCYP kinetic data, reasonable clearance predictions can be obtained if a system specific ISEF is applied. The contribution of individual enzymes, in particular those expressed polymorphically, to overall metabolism should be considered.

REFERENCES

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Galetin *et al.,* (2004) *DMD* **32**: 1411
Williams *et al.,* (2002) *DMD* **30:** 883
Huang *et al.,* (2005) *DMD* **32**: 1434