UTILITY OF DATA FROM RECOMBINANT EXPRESSED GENETIC VARIANTS OF CYPs IN PREDICTING THE INFLUENCE OF GENOTYPE ON *IN VIVO* DRUG KINETICS: THE CASE FOR S-WARFARIN

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BACKGROUND

- Limited availability of human liver microsomes (HLM) or hepatocytes (HHP) from individuals with less common genetic variants of CYPs can hinder the prediction of associated *in vivo* drug kinetics from *in vitro* data.
- However, this is not an issue using readily available recombinant forms of the enzyme variants.

OBJECTIVE

- We therefore set out to assess the prediction of genotype specific *in vivo* kinetics of S-warfarin using data from *in vitro* rCYP2C9 allelic variants.

METHODS

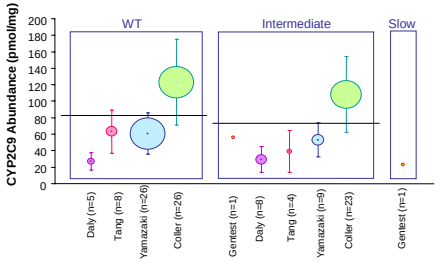
- Mean values of the frequency and abundance of each genotype (*2 and *3) were weighted for study size[1].
- S-warfarin intrinsic clearances (CL_{int}) in different *in vitro* systems were combined after application of inter system extrapolation factors[2] (ISEF). The free fraction in microsomal incubations (fu_{mic}) in each study was also noted.
- Percentage decreases in intrinsic clearance (CL_{int}) with respect to wild type (*1/*1) enzyme were calculated assuming that the *in vitro* activity of heterologously expressed variant enzymes represented the respective homozygous genotype. Values of CL_{int} in heterozygous genotypes were assumed to be the average of those for homozygotes.
- The CL_{int} derived above and associated fu_{mic} were used in conjunction with Caucasian CYP2C9 genotype frequencies and genotype specific abundances (which were obtained following a meta-analysis[1]) to simulate the *in vivo* CL_{po} of S-warfarin for each genotype using Simcyp Software (Version 6.0).
- All available *in vivo* data describing the CL_{po} of S-warfarin in different CYP2C9 genotypes were combined (weighted for study size) to give reference values for assessment of the predictions.

Table 1 Meta-analysis of CYP2C9 genotype frequencies in European Caucasians[1]

	Genotype Frequency (%)					
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
Weighted Mean %	*67.2*	*18.6*	*11.1*	*1.1*	*1.7*	*0.3*
Total n	*2297*	*629*	*376*	*37*	*59*	*10*

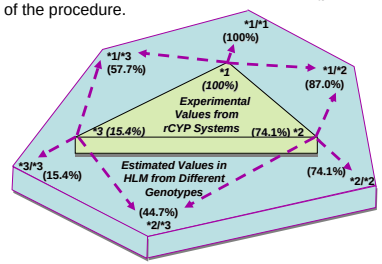
Based on 14 studies (details presented in Reference 1)

Figure 1 Meta-analysis of CYP2C9 abundances for WT (*1/*1), intermediate (*1/*2, *1/*3, *2/*2, *2/*3) and slow (*3/*3) genotypes. Data are expressed as mean ± s.d. The size of circles reflect the number of observations. —— indicates the weighted means derived from the meta-analysis[1].



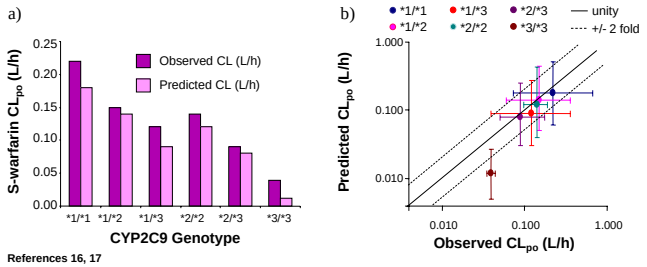
References 1, 3-7

Figure 2 The % CL_{int} of s-warfarin relative to *1/*1 was calculated assuming the *in vitro* activity of rCYPs represented the respective homozygote genotype in HLM. Values for heterozygotes were the average of homozygote CL_{ints}. The pictures is schematic representation of the procedure.



References 8-15

Figure 3 a) Predicted and observed CL_{po} of S-warfarin in different genotypes and b) comparison of predicted and observed values and their associated variability. Data are expressed as medians ± 5th and 95th percentiles



References 16, 17

RESULTS

- Genotype frequencies (Table 1) and genotype specific enzyme abundance data (Figure 1) were compiled in meta-analyses[1].
- Based on data from 9 studies, the relative percentage decreases in CL_{int} (Figure 2) for*1/*2, *1/*3, *2/*2, *2/*3 and *3/*3 were 13.0, 42.3, 25.9, 55.3, and 84.6%, respectively[8-15].
- Combined median observed CL_{po} values for S-warfarin were 0.22, 0.15, 0.12, 0.14, 0.09 and 0.04 for *1/*1 (n=201), *1/*2 (n=43), *1/*3 (n=36), *2/*2 (n=2), *2/*3 (n=4) and *3/*3 (n=2), respectively[16, 17].
- A significant correlation was found between the predicted and experimentally observed (*in vivo*) values of the CL_{po} of S-warfarin in the various genotypes (r^2=0.96, p<0.001; Figure 3A).
- Predicted values of CL_{po} were consistent with observed values (1.1 to 1.3 fold difference) with the exception of the value for the very rare *3/*3 genotype (3.3 fold difference ; Figure 3B).

CONCLUSIONS

- Although many investigators prefer to use HLM or HHP for prediction of CL, these data show combination of *in vitro* rCYP kinetic data with genetic and demographic information allows accurate prediction of the CL_{po} of S-warfarin in individuals with different CYP2C9 genotypes.
- This may be particularly useful for practical reasons as tissue availability from individuals with the rarer enzyme variants is very limited.

REFERENCES

[1]Almond et al., 2006 Drug Metab Rev, **38** Suppl 1: 92-93
[2]Proctor et al., 2004; *Xenobiotica*, **34**: 151-178
[3]Coller *et al.*, 2002; *Br J Clin Pharmacol*, **54**: 157-167
[4]Daly *et al.*, 2005, *Personal Communication*
[5]Tang *et al.*, 2001; *Pharmacogenetics*, **11**: 223-235
[6]Yamazaki *et al.*, 2005, *Personal Communication*
[7]Gentest (*www.gentest.com*)
[8]Crespi and Miller, 1997; *Pharmacogenetics*, **7**: 203-210
[9]Dickman *et al.*, 2001; *Mol Pharmacol*, **60**: 382-387
[10]Haining *et al.*, 1996; *Arch Biochem Biophys*, **333**: 447-458
[11]Sullivan-Klose *et al.*, 1996; *Pharmacogenetics*, **6**: 341-349
[12]Takahashi *et al.*, 1998; *Pharmacogenetics*, **8**: 365-373
[13]Takanashi *et al.*, 2000; *Pharmacogenetics*, **10**: 95-104
[14]Yamazaki *et al.*, 1998; *Biochem Pharmacol*, **56**: 243-251
[15]Hanatani *et al.*, 2003; *Eur J Clin Pharmacol*, **59**; 233-235
[16]Loebstein *et al.*, 2001; *Clin Pharmacol Ther*, **70**:159-164
[17]Scordo *et al.*, 2002; *Clin Pharmacol Ther*, **72**: 702-710

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