Prediction of the oral clearance of S-warfarin in CYP2C9 genotypes from *in vitro* enzyme kinetic data

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INTRODUCTION

- *In vitro* studies have indicated that the 2 main allelic variants of CYP2C9 prevalent in Caucasians (*2 and *3) show reduced catalytic activity compared to wild type (*1).
- The aim of this study was to evaluate and combine published data on the frequencies, liver enzyme abundances and *in vitro* kinetic data for specific CYP2C9 genotypes, in order to predict corresponding *in vivo* oral clearances (CL_{po}) of S-warfarin.

METHODS

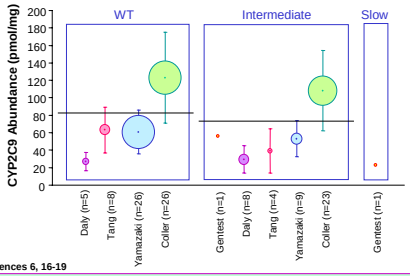
- An extensive search of the available literature was carried out; each study was evaluated and data from independent sources combined.
- In combining the data, genotype frequencies and CYP2C9 liver abundances were weighted for study size (inclusion and exclusion criteria are available on request).
- Owing to a paucity of CYP2C9 genotype specific abundance values, data were combined to give mean enzyme abundances for fast (*1/*1), intermediate (*1/*2, *1/*3, *2/*2, *2/*3) and slow (*3/*3) metaboliser genotypes.
- S-warfarin intrinsic clearances (CL_{int}) in different *in vitro* systems were combined after application of inter system extrapolation factors[1] (ISEF). The free fraction in microsomal incubations (fu_{mic}) in each study was also noted.
- Genotype specific CL_{int} values with respect to *1/*1 enzyme were calculated, assuming that the *in vitro* activity of rCYP variant enzymes represented the respective homozygous genotype. Values of CL_{int} in heterozygous genotypes were assumed to be the average of those for homozygotes.
- 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.
- The derived values (genotype frequencies, abundances and S-warfarin CL_{int}s with associated fu_{mic} values) were used to simulate the CL_{po} of S-warfarin for each genotype using Simcyp Software (Version 6.0).

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

	Genotype Frequency (%)					
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
Aithal *et al.*, 2000	60.0	20.0	17.0	0.0	2.0	1.0
Allabi *et al.*, 2003	67.0	18.2	11.6	0.0	1.6	0.8
Brockmoller *et al.*, 2005	66.2	15.8	13.0	0.0	2.9	0.7
Burian *et al.*, 2002	63.5	25.4	9.3	0.85	0.85	0.0
Coller *et al.*, 2002	54.3	17.4	19.6	2.2	6.5	0.0
Gaikovitch *et al.*, 2003	67.9	18.3	11.4	0.7	1.4	0.3
Jetter *et al.*, 2004	57.7	26.9	11.5	3.8	0.0	0.0
Pederson *et al.*, 2004	68.8	19.2	8.3	1.4	2.2	0.0
Stubbins *et al.*, 1996				3.0		1.0
Taube *et al.*, 2000	69.9	19.1	9.4	0.5	1.1	0.0
van der Weide *et al.*, 2001	61.7	15.0	15.0	5.0	3.3	0.0
Yang *et al.*, 2003	62.3	19.9	10.6	2.6	4.0	0.7
Yasar *et al.*, 1999	66.7	18.6	11.6	0.5	1.9	0.7
Yasar *et al.*, 2001	68.1	17.8	11.1	1.2	1.5	0.3
Weighted Mean %	***67.2***	***18.6***	***11.1***	***1.1***	***1.7***	***0.3***
Total n	***2297***	***629***	***376***	***37***	***59***	***10***

References 2-15

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



References 6, 16-19

Table 2 CL_{int} of S-warfarin in CYP2C9 Genotypes

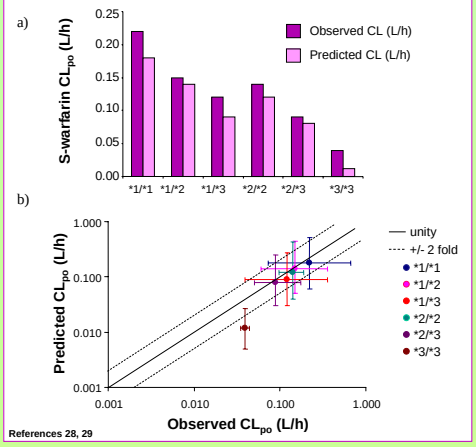
	rCYP Allele	n	Genotype	% Decrease	CL_{int} (µl/min/pmol)	
1	*1	19	*1/*1		0.111	4
	*2	7	*2/*2	25.9	0.082	
	*3	17	*3/*3	84.6	0.017	
			*1/*2	13.0	0.097	
			*1/*3	42.3	0.064	
			*2/*3	55.3	0.050	

2 3

1 Meta-analysis: % decrease relative to *1 (weighted means)
2 Assumption that rCYP allele = homozygous genotype
3 % decreases for heterozygotes derived
4 % decreases applied to the weighted mean *1/*1 CL_{int}

References 20-27

Figure 2 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 28, 29

RESULTS

- Based on 14 independent studies, the frequencies of *1/*1, *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3 genotypes were estimated to be 67.2, 18.6, 11.1, 1.1, 1.7 and 0.3%, respectively (Table 1).
- Mean enzyme abundances for fast (*1/*1), intermediate (*1/*2, *1/*3, *2/*2, *2/*3) and slow (*3/*3) metaboliser genotypes were 83.4, 75.8 and 23.0 pmol/mg of liver microsomal protein, respectively (5 sources; Figure 1). All studies used rCYP standards to quantify protein concentrations.
- The percentage decreases in CL_{int} relative to *1/*1 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 (9 independent studies; Table 2).
- 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[28, 29].
- There was concordance in the rank order of predicted and observed values, despite the few *in vivo* data available for some of the rare genotypes (Figure 2a).
- A significant correlation was found between the predicted and observed (*in vivo*) values of the CL_{po} of S-warfarin in the various genotypes (r^2 = 0.96, $p < 0.001$). Predicted values of CL_{po} were consistent with observed values (1.1-1.3-fold; Figure 2b) with the exception of the value for the very rare *3/*3 genotype (3.3-fold).

CONCLUSIONS

- These data indicate that the combination of *in vitro* rCYP kinetic data with genetic and demographic information allows accurate prediction of the $CL_{po}$of S-warfarin in different genotypes, although further data are required for the rare *3/*3 genotype.

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