Prediction of the oral clearance of tolbutamide in individuals with different CYP2C9 genotypes using in vitro enzyme kinetic data

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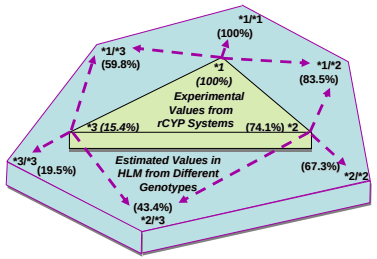
BACKGROUND

- We have previously shown that the oral clearance (CL_{po}) of S-warfarin and its associated variability can be predicted successfully using genotype specific CYP2C9 enzyme activity and liver abundances.[1] We have now applied this approach to tolbutamide, a drug that is metabolised mainly by CYP2C9 but also by CYP2C19.
- For CYP2C19 there are 2 phenotypes, poor metaboliser (PM) and extensive metaboliser (EM), governed by the presence or absence of a null allele. However, a PM phenotype for CYP2C9 is not as easily defined, as polymorphisms are associated with decreased rather than absent activity.
- The aim of this study was to evaluate and combine published data on CYP2C19 PM frequencies and CYP2C9 genotype specific tolbutamide kinetic data for use in the prediction of CL_{po} values.

METHODS

- Mean values of CYP2C19 PM frequency, CYP2C9 genotype frequencies and liver abundances were weighted for study size.
- Tolbutamide intrinsic clearances (CL_{int}) obtained from 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 (Figure 1).
- All available *in vivo* data describing the CL_{po} of tolbutamide in different CYP2C9 genotypes were combined (weighted for study size) to give reference values for assessment of the predictions.

Figure 1 A schematic representation of procedure used to calculate genotype specific CL_{int}s. Each % CL_{int} of tolbutamide 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_{int}s



METHODS (CONT.)

- The derived values of CYP2C19 PM frequency and tolbutamide CL_{int}s (with associated fu_{mic}) for each genotype were then used in conjunction with Caucasian CYP2C9 genotype frequencies and genotype specific abundances (Meta-analysis[1]) to simulate the *in vivo* CL_{po} of tolbutamide for each genotype using Simcyp Software (Version 6.0).

RESULTS

- Based on 9 independent studies[3-11] in European Caucasians (5138 subjects), the CYP2C19 PM frequency was 2.4% (Table 1).
- Genotype frequencies (Table 2) and genotype specific enzyme abundance data (Figure 2) were compiled in meta-analyses[1].

Table 1 Meta-analysis of CYP2C19 PM phenotype frequencies in European Caucasians

	CYP2C19 Meta-analysis			
	Method	Total n	PM n	% PM
Allabi *et al.*, 2003	Genotyping	121		1.6
Drohse *et al.*, 1989	Phenotyping	358	9	2.5
Jacqz *et al.*, 1988	Phenotyping	132	8	6.1
Kupfer & Preisig, 1984	Phenotyping	221	12	5.4
Marandi *et al.*, 1997	Phenotyping	218	5	2.3
Roddam *et al.*, 2000	Genotyping	952	28	2.9
Sanz *et al.*, 1989	Phenotyping	253	7	2.8
Tamminga *et al.*, 1999	Phenotyping	2607	47	1.8
Zackrisson *et al.*, 2004	Genotyping	276	5	1.8
Weighted Mean %				*2.4*

PM, poor metaboliser.

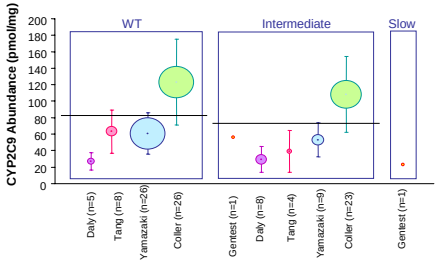
References 3-11

Table 2 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 2 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].

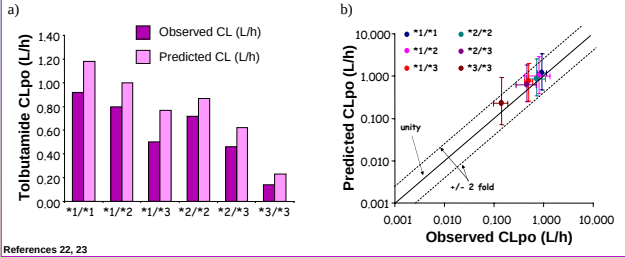


References 1, 12-16

RESULTS (CONT.)

- Percentage decreases in CL_{int} for CYP2C9*1/*2, *1/*3, *2/*2, *2/*3 and *3/*3 were 16.3, 40.2, 32.7, 56.6, and 80.5%, respectively (6 independent studies[17-21]).
- Combined median observed CL_{po} values for tolbutamide were 0.92, 0.80, 0.50, 0.72, 0.46 and 0.14 for *1/*1 (n=11), *1/*2 (n=9), *1/*3 (n=9), *2/*2 (n=3), *2/*3 (n=3) and *3/*3 (n=3), respectively.[22,23]
- A significant correlation was found between the predicted and experimentally observed (*in vivo*) values of the CL_{po} of tolbutamide in the various CYP2C9 genotypes ($r^2 = 0.97$; $p < 0.001$)
- Despite the paucity of *in vivo* data for some of the rare genotypes (n < 6 subjects), the rank order of predicted and observed CL_{po} values in the different genotypes was the same (Figure 3a).
- Predicted values of CL_{po} were consistent with observed values (1.2 - 1.6-fold) for all CYP2C9 genotypes (Figure 3b). Generally, predicted variability was greater than that observed but it is important to note the small sample size available for observed data.

Figure 3 a) Predicted and observed CL_{po} of tolbutamide 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 22, 23

CONCLUSIONS

- The combination of *in vitro* rCYP kinetic data with genetic and demographic information allows accurate prediction of tolbutamide CL_{po} values in individuals with different CYP2C9 genotypes.

REFERENCES

[1]Almond *et al.*, 2006 *Drug Metab Rev*, **38** Suppl 1: 92-93
[2]Proctor et al., 2004; *Xenobiotica*, **34**: 151-178
[3]Allabi *et al.*, 2003; *Br J Clin Pharmacol*, **56**: 653-657
[4]Drohse *et al.*, 1989; *Br J Clin Pharmacol*, **27**: 620-625
[5]Jacqz *et al.*, 1988; *Eur J Clin Pharmacol*, **35**: 161-171
[6]Kupfer & Preisig, 1984; *Eur J Clin Pharmacol*, **26**: 753-759
[7]Marandi *et al.*, 1997; *Eur J Clin Pharmacol*, **53**: 257-260
[8]Roddam *et al.*, 2000; *Pharmacogenet*, **10**: 605-615
[9]Sanz *et al.*, 1989; *Clin Pharmacol Ther*, **45**: 495-499
[10]Tamminga *et al.*, 1999; *Eur J Clin Pharmacol*, **55**: 177-184
[11]Zackrisson *et al.*, 2004; *Eur J Clin Pharmacol*, **60**:547-522
[12]Coller *et al.*, 2002; *Br J Clin Pharmacol*, **54**: 157-167
[13]Daly *et al.*, 2005, *Personal Communication*
[14]Tang *et al.*, 2001; *Pharmacogenet*, **11**: 223-235
[15]Yamazaki *et al.*, 2005, *Personal Communication*
[16]Gentest (*www.gentest.com*)
[17]Sullivan-Klose *et al.*, 1996; *Pharmacogenet*, **6**: 341-349
[18]Takanashi *et al.*, 2000; *Pharmacogenet*, **10**: 95-104
[19]Guo *et al.*, 1996; *Xenobiotica*, **35**: 853-861
[20]Veronese *et al.*, 1993; *Biochem J*, **289**: 533-538
[21]Rettie *et al.*, 1994; *Pharmacogenet*, **4**: 39-42
[22]Kirchheiner *et al.*, 2002; *Pharmacogenet*, **12**; 101-109
[23]Lee *et al.*, 2002; *Clin Pharmacol Ther*, **72**: 562-271

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