In vitro - in vivo extrapolation of the clearance of drugs by glucuronidation:

Scaling factors for recombinant UGT1A1 data

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Background

Increasing numbers of drugs have been shown to be metabolised directly by UDP glucuronosyltransferases (UGT), prompting the implementation of screens for UGT activity in drug development. These screens primarily utilise recombinantly expressed enzymes owing to a lack of specific inhibitors for use with liver microsomes or hepatocytes. The focus is on polymorphic UGT1A1, which exhibits a high degree of inter-individual variability with respect to hepatic and intestinal expression, and which metabolises important drugs such as irinotecan. Indeed, the US Food and Drug Administration (FDA) recently revised the labelling of irinotecan with respect to individuals homozygous for the UGT1A1*28 allele.

Quantitative *in vitro-in vivo* extrapolation (IVIVE) using enzyme kinetic data and relevant scaling factors has been applied effectively to drugs metabolised by cytochromes P450. However, in the case of UGTs, lack of information on their abundance in human liver and relative enzyme activity in recombinant systems hampers accurate quantitative prediction of clearance by glucuronidation *in vivo*.

Aim

The aim of this study was to derive a scaling factor for UGT1A1 that accounts for differences in intrinsic activity per unit enzyme in human liver microsomes (HLM) and recombinant systems (rhUGT).

Methods

Values of V_{max} (pmol/min/mg microsomal protein) and Km (µM) (corrected for non specific microsomal binding) for bilirubin, oestradiol and etoposide determined using both HLM (n = 10 – 15 livers) and rhUGT1A1 Supersomes™ (BD Biosciences) were collated from the literature *via* Pubmed (www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed) and from reports in *Drug Metabolism and Disposition*.

Activity per unit rhUGT enzyme (mg microsomal protein) is known to vary between recombinant systems. Thus, the scalar determined will be **system** specific (Supersomes) as well as **tissue** specific (Liver). Only studies reporting kinetic variables from assays with HLM using alamethicin as activating agent were identified from the literature.

Scalars for each substrate were calculated from the ratio of intrinsic clearance by HLM relative to rhUGT1A1.

Results

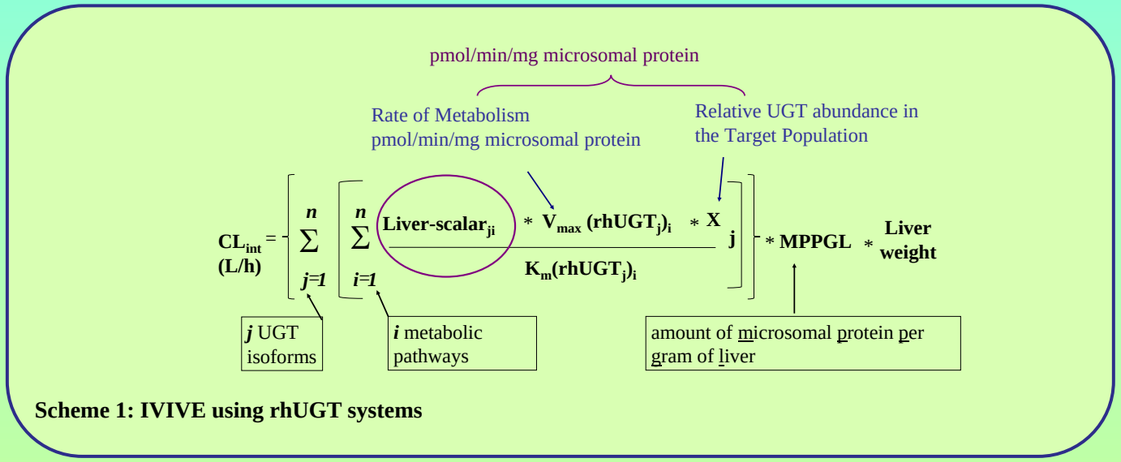
The values of the scaling factor were 1.05 for bilirubin (Patten *et al*., 2001), 1.03 for oestradiol (Patten *et al*., 2001) and 0.79 for etoposide (Watanabe *et al*., 2003; Wen *et al*., 2007) giving an overall mean value of 0.92 ± 0.29 SD (Table 1).

Table 1: Values of Km and V_{max} for UGT1A1 substrates obtained using rhUGT1A1 (Baculovirus Supersomes, wild type *1/*1) and human liver microsomes (fu = free fraction in incubate)

Compound	System	Km [µM]	Km x fu [µM]	V_{max} [pmol/min/mg]	CLu_{int} [µL/min/mg]	Scalar (HLM / rhUGT)	Reference
Bilirubin	HLM	0.8	0.281	1313	4672.6	1.05	Patten et al., 2001
Bilirubin	rhUGT1A1	1.1	0.386	1722	4461.1		
Etoposide	HLM	530	491.3	110	0.224	0.48	Wen et al., 2007
Etoposide	rhUGT1A1	285	264.2	124	0.469		
Etoposide	HLM	439.6	422.9	255.6	0.604	1.10	Watanabe et al., 2003
Etoposide	rhUGT1A1	503.2	484.1	266.5	0.551		
Estradiol (E2-3G)	HLM	25	11.58	1125	97.15	1.03	Patten et al., 2001
Estradiol (E2-3G)	rhUGT1A1	23	10.65	1003	94.18		

Conclusion

The consistency of the scaling factor for the 3 UGT1A1 substrates suggests that the mean value of 0.92 may be used to extrapolate *in vitro* data obtained using rhUGT1A1 Supersomes to estimate the *in vivo* clearance of other UGT1A1 substrates (Scheme 1).



Scheme 1: IVIVE using rhUGT systems

References

• Patten *et al*., (2001) Analysis of UGT enzyme levels in human liver microsomes using specific anti-peptide antibodies, probe substrate activities and recombinant UGT enzymes. BD Biosciences (www.bd.com).
• Watanabe *et al*., (2003) Glucuronidation of etoposide in human liver microsomes is specifically catalyzed by UDP-glucuronosyltransferases 1A1. *Drug Metabolism and Disposition* 31: 589-595.
• Wen *et al*., (2007) UDP-Glucuronosyltransferase 1A1 is the principal enzyme responsible for etoposide glucuronidation in human liver and intestinal microsomes: structural characterization of phenolic and alcoholic glucuronides of etoposide and estimation of enzymes kinetics. *Drug Metabolism and Disposition* 35: 371-380.