In Vivo Clearance Prediction for the UGT2B7 Substrates Carbamazepine, Diclofenac, Gemfibrozil and Zidovudine Using a Mechanistic Population-Based Pharmacokinetic Model

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BACKGROUND

- There is a tendency for under-prediction of *in vivo* clearance of UGT substrates using intrinsic clearance ($CL_{int,u}$) obtained from human liver microsomes (HLM) *in vitro.*
- There is increasing awareness of the importance of UGT2B7 as a major hepatic drug-metabolising enzyme, as it is one of the few UGTs with known probe substrates (Figure 1).

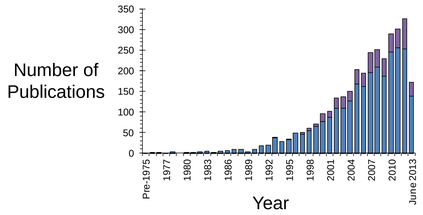


Figure 1. Trend of number of publications investigating glucuronidation (■) and specifically UGT2B7 (■) over time

- Recombinant human UGT microsomes (rUGT) are useful for assessment of UGT isoform specificity *in vitro,* particularly, to inform drug-drug interaction (DDI) studies involving UGTs.

AIMS

To use rUGT2B7 $CL_{int,u}$ data and a tissue scalar approach to:

- Predict *in vivo* clearance of carbamazepine (CBZ), diclofenac (DCF), gemfibrozil (GFZ) and zidovudine (AZT) and associated variability.
- Consider kidney and intestinal metabolism in addition to hepatic UGT $CL_{int,u}$.
- Estimate the contribution of UGT2B7 metabolism to total systemic clearance.

METHODS

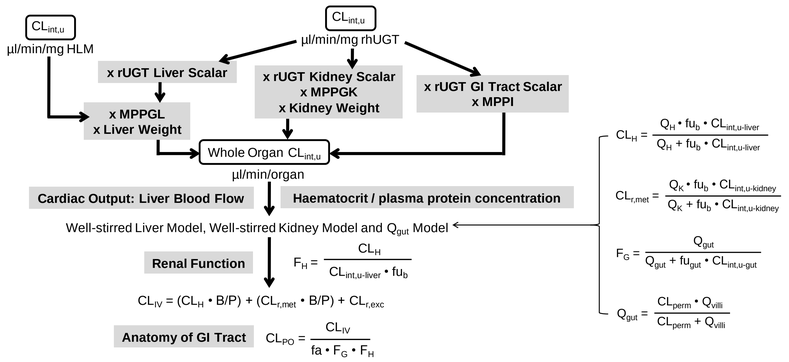


Figure 2. Incorporation of Population-Specific Variability into the prediction of *in vivo* oral clearance indicated by grey boxes.

MPPGL , MPPGK and MPPI = microsomal protein per gram liver, per gram kidney and per GI Tract, respectively. CL_H = total hepatic blood clearance. $CL_{r,met}$ = total kidney blood metabolic clearance. $CL_{r,exc}$ = kidney excretory plasma clearance. Q_H, Q_K and Q_{villi} = hepatic, kidney and villus blood flow, respectively. CL_{perm} = permeability clearance. fu_b and fu_{gut} = fraction unbound in blood or enterocyte, respectively. B/P = ratio of concentration of drug in blood to plasma. fa = fraction of drug absorbed. F_G and F_H = fraction of drug escaping intestinal and hepatic metabolism, respectively.

- Metabolic clearance, renal clearance, permeability, protein binding and physicochemical data were obtained from the literature and incorporated into the pharmacokinetic model within the Simcyp Simulator (Version 12 release 2).
- rUGT tissue scalars, calculated as the ratio of microsomal $CL_{int,u}$ (from human liver, intestinal or kidney microsomes) to rUGT2B7 $CL_{int,u}$, were incorporated into the prediction of *in vivo* oral clearance (Figure 2).

RESULTS

Scaling of rUGT2B7 in vitro $CL_{int,u}$

- *In vitro* $CL_{int,u}$ values obtained using rUGT2B7 ranged from 0.03 to 1145 µl/min/mg for CBZ and DCF, respectively (Table 1).

Table 1. *In vitro* V_{max}, $K_{m,u}$ and $CL_{int,u}$ values for CBZ, DCF, GFZ and AZT taken from published literature sources

	V_{max} (pmol/min/mg)	$K_{m,u}$ (µM)	$CL_{int,u}$ (µl/min/mg rUGT2B7)
CBZ [1]	0.79	127	0.03 [a]
DCF [2]	2800	12	1145 [a]
GFZ [3]	353	2.1	166
AZT [4]	3100	320	10 [b]

[a] 5-fold correction factor applied to account for inhibition of UGT2B7 by fatty acids [5]

[b] *In vitro* assay used albumin to saturate inhibitory fatty acids

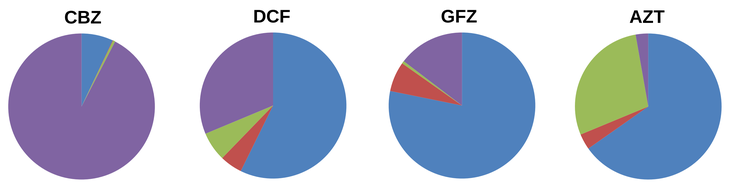
- Different baculovirus rUGT tissue scalars were obtained for GFZ and AZT. This may reflect different *in vitro* assay conditions or substrate differences; total maximum liver $CL_{int,u}$ was 1000 L/h for GFZ and 123 L/h for AZT (Table 2).

Table 2. Hepatic, intestinal and renal rUGT tissue scalars for CBZ, DCF, GFZ and AZT

	rUGT system	Liver	Intestine	Kidney
CBZ	V79 cells	5.29	0.63	0.85
DCF	HEK293 cells	1.28	0.00	0.24
GFZ	Baculovirus	1.33	0.13	1.27
AZT	Baculovirus	3.12	0.13	1.64

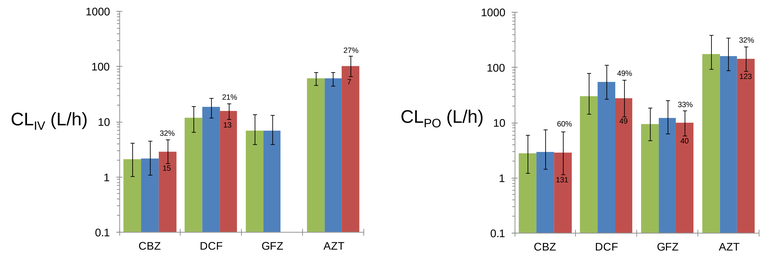
- Although CBZ, DCF, GFZ and AZT are glucuronidated mainly by UGT2B7, other non-UGT routes contribute to their elimination as well.
- The fraction metabolised by UGT2B7 (fmUGT2B7) was 7%, 62%, 85% and 69%, respectively (Figure 3).

Figure 3. Contribution of UGT2B7 metabolism to total *in vivo* systemic clearance (CL_{IV})



Prediction of in vivo clearance

Figure 4. Prediction of *in vivo* clearance



Predicted *in vivo* clearance using HLM UGT (■) or rUGT2B7 (■) *in vitro* $CL_{int,u}$ and observed *in vivo* clearance (■). Data points are geometric mean, error bars are 90% confidence intervals. Predictions are based on 100 simulated individuals. Numbers above and below error bars are the CV for the observed *in vivo* clearance (above) and the number of individuals from which the observed *in vivo* clearance values were obtained (below).

Accounting for all UGT and non-UGT elimination routes, the accuracy of geometric mean predicted *in vivo* clearance (using rUGT2B7 $CL_{int,u}$) was:

- within 45% of observed CL_{IV} for CBZ, DCF and AZT
- within 25% of observed CL_{PO} for CBZ, GFZ and AZT
- 98% higher than observed CL_{PO} for DCF
- improved in comparison to predictions using HLM UGT $CL_{int,u}$ for prediction of CL_{IV} for CBZ and DCF
- comparable to predictions using HLM UGT $CL_{int,u}$ for prediction of CL_{PO} for CBZ, GFZ and AZT
- reduced in comparison to prediction using HLM UGT $CL_{int,u}$ for DCF CL_{PO}

Variability associated with *in vivo* clearance (CV) was well-predicted (within 50% of observed CV for CL_{PO}) using either method (HLM or rUGT in vitro $CL_{int,u}$)

CONCLUSION

- rUGT tissue scalars in combination with rUGT $CL_{int,u}$ data can be used to estimate the fraction metabolised via specific UGTs and incorporate extra-hepatic metabolism into predictions of *in vivo* clearance.
- Overall, prediction accuracy using rUGT2B7 $CL_{int,u}$ was improved in comparison to predictions using HLM UGT $CL_{int,u}$.
- More investigation into the impact of experimental conditions on rUGT tissue scalars is required

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Clinical data from meta-analysis of >20 clinical studies