In silico predictions of the hepatic metabolic clearance in humans for 10 drugs with highly variable *in vitro* pharmacokinetics

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ABSTRACT

Challenges/problems for *in vitro* methodologies for prediction of human clinical pharmacokinetics include inter- and intra-laboratory variability, and common occurance of high limits of quantification, low recovery, low parameter validity and low reproducibility. In this study, 10 drugs with substantial differences in human hepatocyte intrinsic metabolic clearance (CL_{int}) and fraction unbound in plasma (f_u) between laboratories were selected. The average and maximum ratios between highest and lowest reported predicted *in vivo* hepatic metabolic clearance (CL_H) for the drugs were 529- and 2436-fold, respectively. The *in vivo* CL_H was predicted using *in vitro* CL_{int} and f_u data from the various highly sources and using our *in silico* methodology. The main aim was to compare the predictive accuracies for the *in vitro* and *in silico* methodologies. Prediction errors for *in vitro* methodology ranged from 1.1- to 578-fold, with an average of 150-fold for lowest predicted estimates and 16-fold for highest predicted estimates. The *in vitro* based predictions produced 36- to 38-fold higher average and maximum prediction errors than the *in silico* methodology, respectively. Mean and maximum *in silico* prediction errors were 4.2- and 15-fold, respectively, which is consistent with earlier results. In contrast to the *in vitro* methodology the *in silico* models did not predict high hepatic extraction ratio for drugs with low CL_H. Overall, the *in silico* method clearly outperformed *in vitro* data for prediction of CL_H in man for 10 drugs with large interlaboratory variability.

INTRODUCTION

Challenges/problems for *in vitro* methodologies for prediction of human clinical pharmacokinetics (PK) include inter- and intra-laboratory variability, and common occurance of high limits of quantification (such as for conventional hepatocyte and microsome assays), low recovery (such as for the Caco-2 assay), low parameter validity (such as for aqueous solubility (S) *vs in vivo* dissolution potential) and low reproducibility.[1-8]

Ten physico-chemically and pharmacokinetically different drugs with large interlaboratory variability of intrinsic metabolic hepatic clearance (CL_{int}) and unbound fraction (f_u) are shown in Table 1 - atenolol, cimetidine, desipramine, diazepam, diclofenac, diltiazem, gemfibrozil, midazolam, naloxone and naproxen.[1-10] The average and maximum ratios between highest and lowest reported predicted *in vivo* CL_{int} for these are 43- and 138-fold, respectively. Corresponding ratios for measured f_u are 13- and 50-fold, respectively. For gemfibrozil, diclofenac and naproxen, $CL_{int} \bullet f_u$ differs 3442-, 2842- and 1654-fold depending of choice of sources for CL_{int} and f_u, respectively.

For both CL_{int} and f_u the ratio between highest and lowest *in vitro* values tend to increase with number of measurements/sources (R^2-values of 0.36 and 0.18 for n *vs* log CL_{int} and f_u, respectively).[1,3] Based on these correlations, the ratio between highest and lowest CL_{int} for a compound increases by 10-fold when increasing the number of measurements from 1 to 10. A 3.1-fold increase is expected for f_u. Thus, for every additional source there is a higher chance to find a deviating (but not necessarily less accurate) predicted estimate. Such variability/uncertainty is not an issue with *in silico* prediction methods.

We have developed and validated *in silico* methodology for prediction of prediction of human clinical PK, including the ANDROMEDA by Prosilico software.[3,4,7,8,11-13] In a major benchmarking study ANDROMEDA outperformed laboratory methods in predictive accuracy and range.[8] Our *in silico* methodology has also outperformed animal models for prediction of volume of distribution and oral bioavailability and hepatocytes for prediction of CL_{int}.[4,11,13] PK-predictions from ANDROMEDA have also been approved by the German authority BfArM for use and main preclinical PK-source in clinical trial applications.

The main objective of this study was to compare the accuracy of our previously developed *in silico* methodology and the laboratory data-based methodology to predict the human CL_H of atenolol, cimetidine, desipramine, diazepam, diclofenac, diltiazem, gemfibrozil, midazolam, naloxone and naproxen.

METHODOLOGY

Compound and data selection

Atenolol, cimetidine, desipramine, diazepam, diclofenac, diltiazem, gemfibrozil, midazolam, naloxone and naproxen were selected as compounds as these have demonstrated large variability of predicted *in vivo* CL_{int} and measured *in vitro* f_u between laboratories/sources (Tables 1 and 2).[1-3,9,10]

In vitro to *in vivo* predictions

In vivo CL_H in humans was predicted using lowest and highest predicted *in vivo* CL_{int} and measured *in vitro* f_u for each drug and the well-stirred liver model ($CL_H = (CL_{int} \bullet f_u \bullet Q_H) / (CL_{int} \bullet f_u + Q_H)$, where Q_H is the liver blood flow). Predicted and observed *in vivo* CL_H were compared.

In silico to *in vivo* predictions

The *in silico* methodology used in papers 3, 4 and 13 was used for *in silico* predictions of CL_{int}, f_u and CL_H. None of the drugs in the study was included in the training sets for the *in silico* models. Thus, every prediction was a forward-looking prediction where each compound was unknown to the models. Predicted and observed *in vivo* CL_H were compared.

RESULTS & DISCUSSION

In vitro based predictions

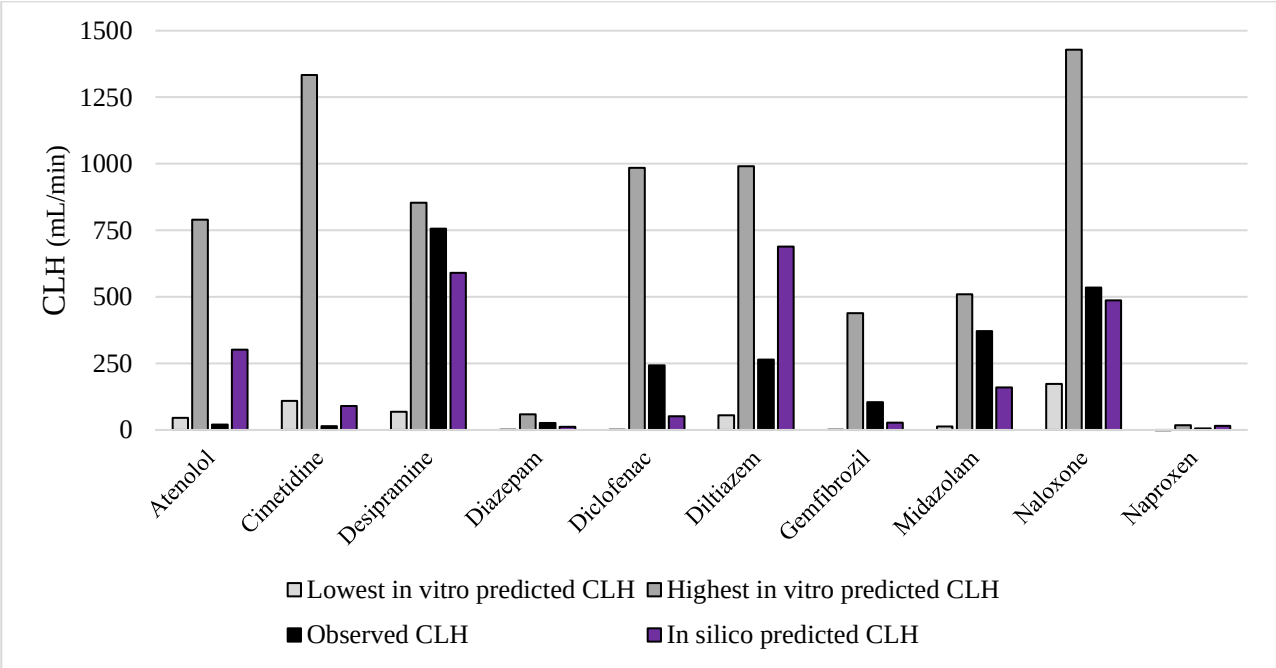
CL_H predicted from *in vitro* data differed up to 2436-fold (for gemfibrozil) depending on source of data (Table 2, Figure 1). For diclofenac, diazepam, naproxen and midazolam, ratios between highest and lowest predicted CL_H were 976-, 144-, 1634- and 39-fold, respectively (Table 2, Figure 1). Prediction errors ranged from 1.1- to 578-fold, with an average of 150-fold for lowest predicted estimates and 16-fold for highest predicted estimates. 70 % of the CL_H-predictions had an error of ≤28-fold (n=20).

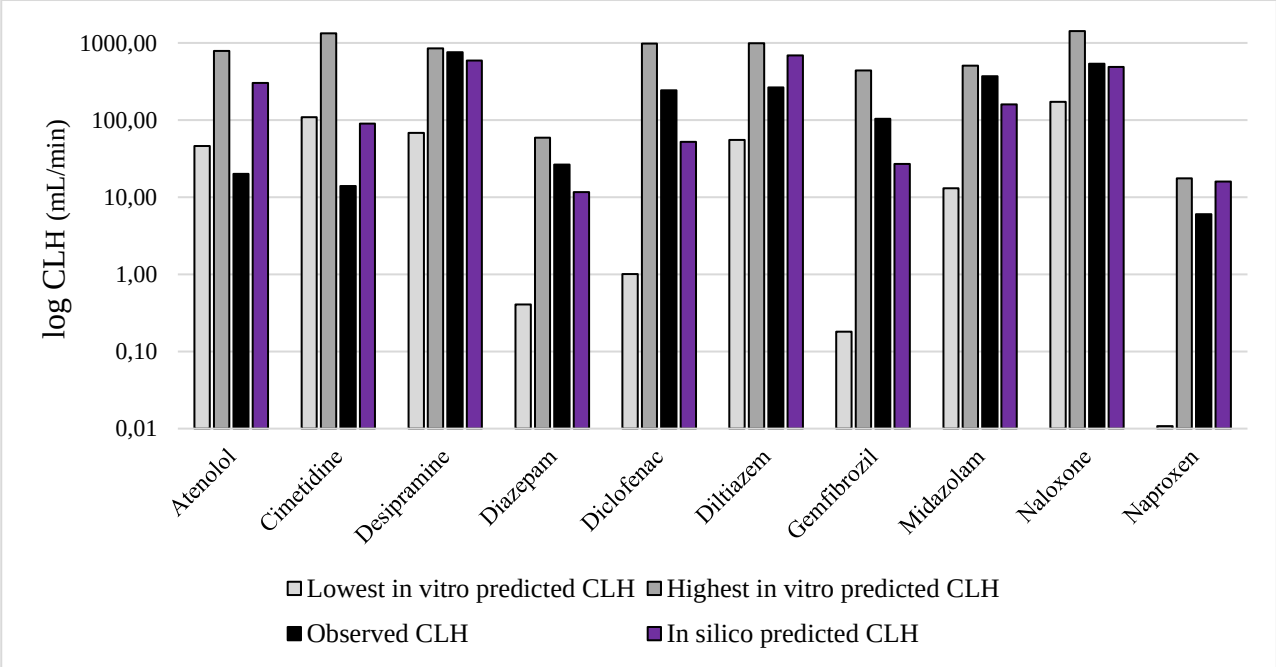
For cimetidine, diclofenac, diltiazem and naloxone, predicted hepatic extraction ratios ranged from low to high depending of sources of CL_{int} and f_u, and for cimetidine and naloxone, maximum predicted CL_H is incompatible with adequate/desirable PK-profile (too low predicted oral bioavailability). Minimum predicted CL_H of <1 mL/min for diazepam, gemfibrozil and naproxen (and potentially very long half-life) are also in line with risk of withdrawal during the preclinical phase.

Observed *in vivo* CL_H for all compounds except for atenolol and cimetidine were within the ranges for *in vitro* predicted CL_H (Figure 1).

Variability and reproducibility of these sizes could not only jeopardize compound drug selection and development, but also human safety in early clinical trials.

Figure 1 *In vitro* and *in silico* predicted and observed *in vivo* CL_H for the 10 drugs.
a) linear CL_H-scale, b) logarithmic CL_H-scale.





For atenolol and diclofenac, maximum prediction errors for CL_H obtained using *in vitro* data were 39- and 240-fold, respectively.

Atenolol (moderate permeability and gastrointestinal uptake, and efflux) has also shown highly variable Caco-2 permeability values (65-fold interlaboratory range and up to 16-fold intralaboratory

variability) and efflux ratios (21-fold difference between laboratories and 4.4-fold intralaboratory variability, and 1/3 of measurements showing no efflux).[2,5,6]

Diclofenac (high permeability and complete gastrointestinal uptake) has reported S ranging between 2 (low) to 9000 (high) mg/L (7-30000 µM) and its half-life has been underpredicted by 5-fold and daily oral dose overpredicted by 70-fold based on laboratory data.[5,14]

In silico based predictions

Mean, median and maximum prediction errors for *in silico* predictions of CL_H were 4.2-, 2.6- and 15-fold, respectively (Table 3, Figure 1). This is in line with results in other studies with our *in silico* prediction methodology.[3,4,8,12] 70 % of CL_H-predictions had maximum 4.7-fold prediction error. For both CL_{int} and f_u an approximate 5-fold 70 % confidence interval was predicted (70 % of observations/measurements anticipated to lie within ±5-fold errors; ANDROMEDA-based predictions).

There was no *in silico* prediction with high hepatic extraction ratio and corresponding low estimate (as found for *in vitro*-based predictions).

In vitro-based mean and maximum prediction errors for CL_H were 36- and 38-fold higher than corresponding *in silico* estimates, respectively.

Our *in silico* prediction methodology has previously been shown to be useful for accurate predictions of PK-parameters that have not been possible to quantify and predict using *in vitro* methods.[15] With the new results it has also been successful for prediction of human PK for compounds with highly and uncertain *in vitro* PK.

Conclusion

In conclusion, 36- to 38-fold higher average and maximum prediction errors of human CL_H were found for *in vitro* methodology compared to the *in silico* methodology. Mean and maximum *in silico* prediction errors were 4.2- and 15-fold, respectively, which is consistent with earlier results. The maximum prediction error found for the *in vitro* methodology was 578-fold. In contrast to the *in vitro* methodology the *in silico* models did not predict high hepatic extraction ratio for drugs with low CL_H.

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TABLES

Table 1 Predicted *in vivo* CL_{int}, measured f_u and ratios between upper and lower estimates for the 10 selected drugs.

	In vitro data and predictions					
Drug	Low CL_{int} mL/min	High CL_{int} mL/min	Ratio -fold	Low f_u	High f_u	Ratio
Atenolol	*<200*	1719	>8,6	0,49	0,97	2,0
Cimetidine	227	13850	61	0,52	0,87	1,7
Desipramine	770	12390	16	0,093	0,16	1,7
Diazepam	51	1701	33	0,0080	0,0360	4,5
Diclofenac	505	28700	57	0,0020	0,100	50
Diltiazem	2345	11690	5,0	0,0244	0,25	10
Gemfibrozil	90	12390	138	0,0020	0,050	25
Midazolam	777	10710	14	0,0170	0,0720	4,2
Naloxone	1114	41720	37	0,175	0,722	4,1
Naproxen	16	987	62	0,0007	0,0180	27
		Minimum	5,0			1,7
		Median	35			4,4
		Mean	43			13
		Maximum	138			50

Table 2 Predicted and observed/measured *in vivo* CL_H, ratios between upper and lower estimates and estimated minimum and maximum prediction errors for the 10 selected drugs.

	In vitro data and predictions					*In vivo* data
Drug	Low CL_H mL/min	High CL_H mL/min	Ratio -fold	CL_H pred low error	CL_H pred high error	*In vivo* CL_H mL/min
Atenolol	<92	790	>8,6	<4,6	39	20
Cimetidine	109	1334	12	7,8	95	14
Desipramine	68	854	13	11	1,1	756
Diazepam	0,41	59	144	65	2,2	27
Diclofenac	1,01	985	976	240	4,1	243
Diltiazem	55	991	18	4,8	3,8	264
Gemfibrozil	0,18	438	2436	578	4,2	104*
Midazolam	13	509	39	28	1,4	371
Naloxone	173	1429	8,3	3,1	2,7	535*
Naproxen	0,011	18	1634	558	2,9	6*
		Minimum	8,3	3,1	1,1	
		Median	28	20	3,3	
		Mean	529	150	16	
		Maximum	2436	578	95	

*Extrahepatic and renal elimination was taken into consideration when estimating *in vivo* CL_H.

Table 3 *In silico* predicted *in vivo* CL_H for the 10 selected drugs.

	In silico predictions	*In vivo* data	Error
Drug	CL_H mL/min	CL_H mL/min	-fold
Atenolol	302	20	15
Cimetidine	90	14	6,4
Desipramine	591	756	1,3
Diazepam	12	27	2,2
Diclofenac	52	243	4,7
Diltiazem	689	264	2,6
Gemfibrozil	27	104*	3,8
Midazolam	160	371	2,3
Naloxone	487	535*	1,1
Naproxen	16	6*	2,7
		Minimum	1,1
		Median	2,6
		Mean	4,2
		Maximum	15

*Extrahepatic and renal elimination was taken into consideration when estimating *in vivo* CL_H.