Using Physiologically Based Pharmacokinetic Modeling for *in vitro* – *in vivo* Extrapolation to Predict Chemical Exposure

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PURPOSE

Mechanistic absorption and physiologically based pharmacokinetic (MA/PBPK) models are useful tools in risk assessment. These models incorporate complex processes related to a compound's disposition, such as dissolution, absorption, metabolism, and protein binding in plasma and tissues, and also offer the possibility of predicting target tissue concentrations. In this study, the MA/PBPK model in GastroPlus™ version 9.0 was applied to predict the exposure of eighteen chemicals with reported exposure in human after oral administration. The *in vivo* metabolism was estimated from either *in vitro* metabolic clearance measured in human hepatocytes (Wetmore et al. *Toxicol Sci*, 125(2012) and 148(2015)) or *in silico* predictions (ADMET Predictor™ version 7.2) .

METHODS

- The physicochemical and biopharmaceutical properties of all compounds were predicted from structure using ADMET Predictor version 7.2.
- The effects of physiological pH and bile salt concentrations on solubility/dissolution/precipitation, as well as effects of physiological pH and surface areas on passive absorption rates, were included in the model to predict the fraction absorbed.
- The distribution in systemic circulation was based on physiological tissue sizes of human physiology combined with tissue/plasma partition coefficients calculated from tissue compositions and physicochemical properties using the default Lukacova method in GastroPlus 9.0.
- Kidney filtration was estimated by the product of the fraction unbound in plasma and glomerular filtration rate.
- *In vivo* metabolic clearance was predicted using *in vitro* measurements that incorporated the fraction unbound in hepatocytes (fu_{hep}) calculated by Austin's equation (Austin et al., *Drug Metab Dispos*, 33(2005)).

$$rate/[tissue] = rate/[one\ million\ cells] \div fu{\downarrow}hep \times [millions\ of\ cells]/[g\ of\ tissue] \times [g\ of\ tissue]$$

- The *in silico* predictions (ADMET Predictor version 7.2) of Vmax and Km for 5 CYP enzymes were incorporated along with physiological expression levels of these enzymes in intestine and liver to calculate *in vivo* metabolism.

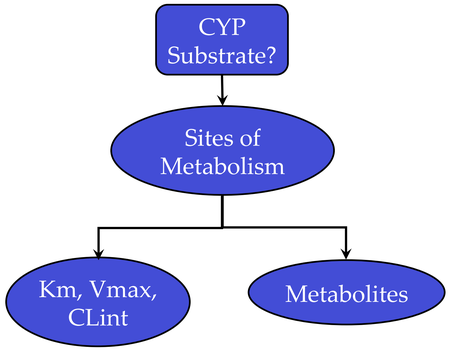


Figure 1: CYP kinetic models for 5 CYP isozymes: 1A2, 2C9, 2C19, 2D6, and 3A4. First, the model predicts whether a molecule is a substrate for each CYP isoform. Next, sites of metabolism are predicted for compounds that are predicted as substrate. Finally, kinetic parameters are predicted and metabolites are depicted.

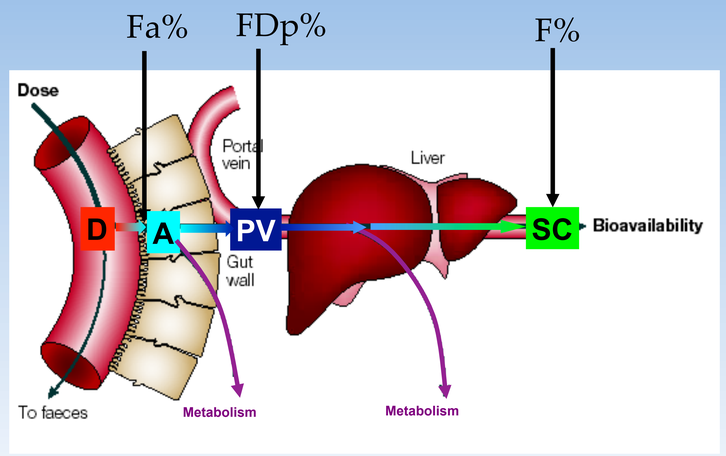


Figure 2: Schematic representation of processes that a drug undergoes after oral administration. Fa% (fraction absorbed) is the fraction of the dose that is absorbed into the apical membrane of the gut epithelium. FDp% is the fraction of the dose that reaches the portal vein. F% (oral bioavailability) is the fraction of the dose that enters systemic circulation. Modified from van de Waterbeemd and Gifford (*Nat Rev Drug Disc*, 2(2003)).

RESULTS

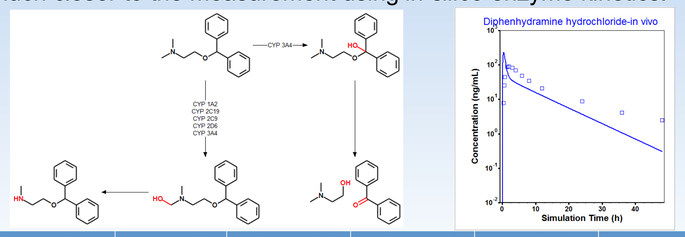
Experimentally measured plasma protein binding (fu_p) and *in vitro* metabolic clearance measured in human hepatocytes ($CL_{in,vitro}$) were obtained from Wetmore et al. (*Toxicol Sci*, 125(2012)and 148(2015)).

Table 1: Comparison of predicted and observed exposure. Of the 18 chemicals, the IVIVE predicted $AUC_{0\text{-}inf}$ of 7 and 11 compounds were within 2- and 5–fold of the $AUC_{0\text{-}inf}$ that were derived from published human *in vivo* PK data.

Chemical	$AUC_{0\text{-}inf}$ (μg-h/ml)		Ratio	Predicted F %	Reference
	In vivo	Predicted			
Erythromycin	8.43	7.48	0.9	37	Kroboth et al., *Antimocrob Agents Ch*, 21 (1982)
Acetaminophen	91.23	40.17	0.4	79	Critchley et al., *J Clin Pharm Ther*, 30 (2005)
6-Propyl-2-thiouracil	21.06	27.13	1.3	90	Kabanda et al., *J Pharm Pharmcol*, 48 (1996)
Candoxatril	0.9	5.74	6.4	58	Kaye et al., *Xenobiotica*, 27 (1997)
Flutamide	5.98	8.94	1.5	52	Anjum et al., *Br J Clin Pharmacol*, 47 (1999)
Triamcinolone	0.64	0.55	0.9	76	Hochhaus et al., *Pharmaceut Res*, 7 (1990)
Rifampicin	40.79	33.77	0.8	50	Rafiq et al., *Int J Agric Biol*, 12 (2010)
Sulfasalazine	49.76	450.8	9	56	Gu et al., *J Chromatogr B*, 879 (2011)
5,5-Diphenylhydantoin	135.56	67.25	0.5	94	Brien et al., *Europ J Clin Pharmacol*, 9 (1975)
Coumarin	0.007	0.183	25.7	64	Lamiable et al., *J Chromatogr*, 620 (1993)
Diphenhydramine hydrochloride	0.94	16.42	17.5	100	Toothaker et al., *Biopharm Drug Dispos*, 21 (2000)
Lovastatin	0.065	7.1	109	93	Kothare et al., *Int J Clin Pharm Th*, 45 (2007)
Carbaryl	0.15	0.51	3.4	37	May et al., *J Pharmacol Exp Ther*, 262 (1992)
Triabendazole	17.07	46.75	2.7	91	Bapiro et al., *Eur J Clin Pharmacol*, 61 (2005)
2,4-D	423.25	1209.5	2.9	100	Sauerhoff et al., *Toxicology*, 8 (1977)
Oxytetracycline dihydrate	14.29	97.2	6.8	50	Green et al., *Europ J Clin Pharmacol*, 10 (1976)
Picloram	0.97	166.66	171	98	Nolan et al., *Toxicol Appl Pharm*, 76 (1984)
Triclosan	1.41	0.76	0.5	96	Sandborgh-Englund et al., *J Toxicol Environ Health A*, 69 (2006)

Reasons for compounds that have poor PBPK predictions:
1). Active excretion by the kidney, e.g., Nolan et al. (*Toxicol Appl Pharm*, 76 (1984) showed the renal clearance of picloram was close to the total renal blood flow.
2). Excretion in bile, e.g., lovastatin, Butterfield et al. (*Pharmacol Res*, 64 (2011) reported that 83% of the orally administered dose is excreted in feces.

For diphenhydramine hydrochloride, the *in vitro* measured CL is 0, but the *in silico* prediction shows it is substrate of CYP enzymes, which is consistent with the results from Akutsu et al. (DMD, 35 (2007)). The predicted PK profile is much closer to the measurement using *in silico* enzyme kinetics.



CYP Isoform	1A2 (80%)	2C19 (78%)	2C9 (55%)	2D6 (81%)	3A4
Vmax (μg/s/mg-enz)	0.4	1.3	0.39	0.15	0.8
Km(mg/L)	6.66	15.9	19.88	0.5	51.8

Figure 3: Metabolite predictions for diphenhydramine. It is predicted to be a substrate of all 5 major CYP isoforms with confidence estimates shown in parenthesis. The graph shows the simulated C_p time curve (solid line) using *in silico* enzyme kinetics compared to *in vivo* data (squares).

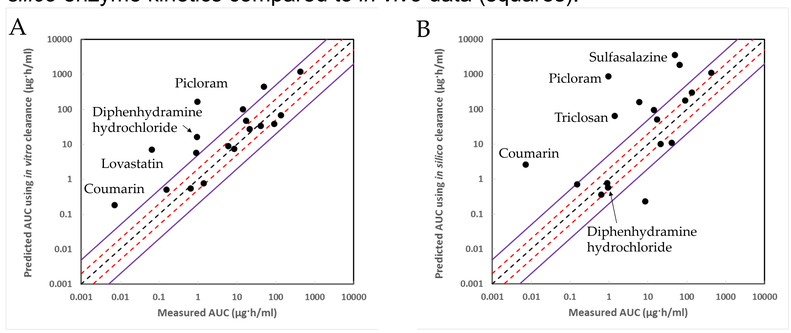


Figure 4: Observed vs. predicted $AUC_{0\text{-}inf}$ for 18 compounds. (A). *In vitro* metabolic clearance. The predicted AUC of 7 and 11 compounds are within 2- and 5-fold of the observed data. (B). *In silico* prediction of Vmax and Km for 5 CYP enzymes. The predicted AUC of 5 and 10 compounds are within 2- and 5-fold of the observed data. The dashed black line is the line of unity, dashed red line and solid purple lines show boundaries for 2- and 5-fold prediction errors.

CONCLUSIONS

PBPK modelling using *in vitro* measured clearance in hepatocytes provides a reliable method to predict the human exposure of chemicals. Nonhepatic clearances and elimination via bile are the main reasons for the mismatch between the predicted and measured AUCs. Predicted enzyme kinetics provided a useful validation method for the compound with undetectable *in vitro* clearance in hepatocytes. PBPK modelling using *in silico* predicted metabolism is another useful method for exposure prediction.

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