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SHORT COMMUNICATION

Strategies for Determining Correct Cytochrome P450 Contributions in Hepatic Clearance Predictions: In Vitro–In Vivo Extrapolation as Modelling Approach and Tramadol as Proof-of Concept Compound

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Abstract

Background and Objective Although the measurement of cytochrome P450 (CYP) contributions in metabolism assays is straightforward, determination of actual in vivo contributions might be challenging. How representative are in vitro for in vivo CYP contributions? This article proposes an improved strategy for the determination of in vivo CYP enzyme-specific metabolic contributions, based on in vitro data, using an in vitro–in vivo extrapolation (IVIVE) approach. Approaches are exemplified using tramadol as model compound, and CYP2D6 and CYP3A4 as involved enzymes.

Methods Metabolism data for tramadol and for the probe substrates midazolam (CYP3A4) and dextromethorphan (CYP2D6) were gathered in human liver microsomes (HLM) and recombinant human enzyme systems (rhCYP). From these probe substrates, an activity-adjustment factor (AAF) was calculated per CYP enzyme, for the determination of correct hepatic clearance contributions. As a reference, tramadol CYP contributions were scaled-back from in vivo data (retrograde approach) and were compared with the ones derived in vitro. In this view, the AAF is an enzyme-specific factor, calculated from reference probe activity measurements in vitro and in vivo, that allows appropriate scaling of a test drug's in vitro activity to the 'healthy volunteer' population level. Calculation of an AAF, thus accounts for any 'experimental' or 'batch-specific' activity difference between in vitro HLM and in vivo derived activity.

Results In this specific HLM batch, for CYP3A4 and CYP2D6, an AAF of 0.91 and 1.97 was calculated, respectively. This implies that, in this batch, the in vitro CYP3A4 activity is 1.10-fold higher and the CYP2D6 activity 1.97-fold lower, compared to in vivo derived CYP activities.

Conclusion This study shows that, in cases where the HLM pool does not represent the typical mean population CYP activities, AAF correction of in vitro metabolism data, optimizes CYP contributions in the prediction of hepatic clearance. Therefore, in vitro parameters for any test compound, obtained in a particular batch, should be corrected with the AAF for the respective enzymes. In the current study, especially the CYP2D6 contribution was found, to better reflect the average in vivo situation. It is recommended that this novel approach is further evaluated using a broader range of compounds.

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Key Points

Pooled HLM CYP contributions do not necessarily represent in vivo CYP contributions for the average population individual.

The calculation of an AAF corrects for this potential discrepancy.

1 Introduction

In setting up a physiologically relevant in vitro–in vivo extrapolated physiologically-based pharmacokinetic (IVIVE-PBPK) model, not only must the total clearance be captured well, also the relevant cytochrome P450 (CYP) contributions used in the model should be representative of the in vivo situation [1, 2]. This IVIVE approach assumes that the CYP contributions measured in vitro [pooled human liver microsomes (HLM)] are the same as the ones observed in vivo. However, in vitro activities might not represent healthy adult activity due to, e.g. the source of the liver tissue (often diseased patients) [3] or binding competition of the compound under study with free fatty acids [4]. Therefore, methods are needed that ensure this predictability. In the current work, the use of an activity-adjustment factor (AAF) is presented as an alternative method and evaluated against the conventional (uncorrected) approach. The conventional approach consists of IVIVE from HLM and human recombinant (rhCYP) enzyme kinetic data, including determination of the preferred inter-system extrapolation factor (ISEF) [5]. Tramadol is used as a proof-of concept compound since it is metabolized by different and clinically important CYP enzymes (i.e., CYP3A4, CYP2D6 and CYP2B6) [6]. Besides, clinical data is available concerning the effect of (1) CYP2D6 polymorphisms, and (2) rifampicin induction on tramadol's clearance. In the current work, the main focus is on the CYP2D6–CYP3A4 interplay, and less on CYP2B6. This is because the initial focus of this project was on the most relevant CYP enzymes and because CYP2B6 plays a minor role in tramadol metabolism, as indicated in the "Discussion" section.

2 Materials and Methods

2.1 Chemicals and Materials

All chemicals and reagents used were of the highest available grade: Na_2HPO_4, KH_2PO_4, KCl, $MgCl_2$, NADP, HCl (Merck, Darmstadt, Germany), glucose-6-phosphate, glucose-6-phosphate dehydrogenase (Roche Diagnostics GmbH, Mannheim, Germany), midazolam, dextromethorphan, 1-OH midazolam, dextrorphan, deuterated 1-OH midazolam (TRC inc, Toronto, Canada), chlorpropamide (Sigma Aldrich, St. Louis, USA). Human liver microsomal pool (BD Biosciences, Woburn, USA) consisted of 50 adult donors (mixed gender).

2.2 Incubations in HLM and rhCYP of Midazolam, Dextromethorphan, and Tramadol

For midazolam/dextromethorphan, the incubation mixture consisted of 120 µL diluted microsomes, 100 µL cofactor mix for NADPH regeneration system, and 5 µL test compound (0.5 % MeOH in final incubation mixture) for both HLM as well as rhCYP systems. After a preincubation period of 5 min at 37 °C and 100 oscillations/min, NADP was added to the preincubation mixture to a final volume of 250 µL to initiate the reaction. Midazolam was incubated in the range of 0.1–16 µM at 0.15 mg protein/mL (HLM) and 10 pmol CYP3A4/mL (rhCYP). The reaction was stopped after 10 min with 250 µL DMSO containing deuterated 1-OH midazolam as the internal standard (0.1 µg/mL). Dextromethorphan was incubated in the range of 0.5–16 µM at 0.3 mg protein/mL (HLM) and 4 pmol CYP2D6*1/mL (rhCYP). The reaction was stopped after 10 min with 250 µL DMSO containing chlorpropamide as the internal standard (0.22 µg/mL). Although these conditions differ from the ones used in the Walsky and Obach paper [7], linearity as a function of time and protein concentration for these probe substrates was demonstrated (data on file). Samples were centrifuged for 10 min at 1711*g* and the supernatant introduced to the UPLC-MS method. The intrinsic clearance was calculated in the Enzyme Kinetics module of Sigma Plot. For tramadol linearity experiments, incubations, and phenotyping experiments in pooled HLM and rhCYP systems, we refer to our previous publication [8]. The unbound fraction in the incubates for probe substrates midazolam and dextromethorpan was calculated from the reported literature values [9, 10]. Details on the determination of these fu values are provided in the Electronic Supplementary Material.

2.3 Bioanalysis

The midazolam metabolite, 1-OH midazolam, was analyzed using a Waters Acquity UPLC system coupled to a Thermo LTQ mass spectrometer (Thermo Fisher Scientific, San Jose, USA) in APCI+. The column was an Acquity UPLC BEH C18 (1.7 µm) 50 × 2.1 mm held at 60 °C with mobile phase constituents 0.1 % HCOOH in ULC water and 0.1 % HCOOH in CH_3CN in a linear gradient. Run time was 3 min and flow rate 0.6 mL/min. Mass

transitions for 1-OH midazolam and deuterated 1-OH midazolam (internal standard) using a collision energy (CE) of 30 eV were 342 > 325, and 346 > 328, respectively. Calibration curves were always made in the same microsomal matrix as the incubates use at least 8 calibrator levels and 3 QC levels for the calibration curve. The dextromethorphan metabolite, dextrorphan, was analyzed using a Waters Acquity UPLC system coupled to a Micromass Quattro Ultima triple quadrupole, operating in ESI+. The column was an Acquity UPLC BEH C18 (1.7 µm) 50 × 2.1 mm at 35 °C with mobile phase constituents 0.1 % HCOOH in ULC water and 0.1 % HCOOH in CH_3CN in a linear gradient. Run time was 5.25 min and flow rate 0.4 mL/min. Mass transitions for dextrorphan and chlorpropamide using a CE of 28 and 25 eV were 258 > 157, and 277 > 275. Details about the dextromethorphan and tramadol bioanalysis methods can be found in De Bock et al. [11] and T'jollyn et al. [8], respectively.

2.4 Calculation of ISEF and Activity-Adjustment Factors Using Probe Substrates

ISEF values were calculated for CYP3A4 (midazolam) and CYP2D6 (dextromethorphan) using the formula below (Eq. 1).

$$\mathrm{ISEF} = \frac{\mathrm{CLint_{u,\ HLM}}}{\mathrm{CLint_{u,\ rhCYP}} \times [\mathrm{CYP}]_{\mathrm{HLM}}} \quad (1)$$

$\mathrm{CLint_{u,HLM}}$ and $\mathrm{CLint_{u,rhCYP}}$ are the unbound intrinsic clearances (determined via metabolite formation) of a specific probe substrate in HLM and rhCYP systems, respectively. $[\mathrm{CYP}]_{\mathrm{HLM}}$ represents the typical enzyme abundance values of 137 and 8 pmol CYP/mg used for CYP3A4 and CYP2D6, respectively [5, 12].

The activity-adjustment factor (AAF) is calculated as the ratio of the (unbound) in vivo back-calculated hepatic CLint and the unbound in vitro HLM CLint for a specific enzyme using the specific probe substrate (Eq. 2; Table 1).

$$\mathrm{AAF_{CYP}} = \frac{\mathrm{CLint_{u,invivo,CYP}}}{\mathrm{CLint_{u,HLM,CYP}}} \quad (2)$$

Then, the AAF, which is calculated for each CYP isozyme, is multiplied with the relevant parameter involved in the IVIVE (i.e., either $\mathrm{CLint_{u,HLM,CYP}}$ or $\mathrm{ISEF_{CYP}}$. This yields the $\mathrm{CLint_{aa,u,HLM,CYP}}$ for HLM data and $\mathrm{ISEF_{aa,CYP}}$, for rhCYP data (Eq. 3; Table S1 in Electronic Supplementary Material).

$$\begin{aligned} \mathrm{CLint_{aa,u,HLM,CYP}} &= \mathrm{CLint_{u,HLM,CYP}} \times \mathrm{AAF_{CYP}} \\ \mathrm{ISEF_{aa,CYP}} &= \mathrm{ISEF_{CYP}} \times \mathrm{AAF_{CYP}} \end{aligned} \quad (3)$$

2.5 IVIVE-PBPK Model Development

The unbound intrinsic clearance calculated per in vitro system with/without correction (see previous section), is used in the well-stirred liver approach in Simcyp® (v12.1, Certara, Sheffield, UK) to come up with an in vivo hepatic clearance. The AAF-corrected hepatic clearance predictions are compared to their uncorrected counterparts, as published in T'jollyn et al. [8]. In the HLM and $\mathrm{HLM_{aa}}$ models, CLint values (Table S1 in Electronic Supplementary Material, columns 1 and 2) were provided in the enzyme kinetics tab of the Simcyp Simulation platform, whereas in the rhCYP and $\mathrm{rhCYP_{aa}}$ models, both CLint and ISEF values (Table S1, columns 3 and 4) were provided. In the retrograde model, CLint values were calculated from in vivo data, as described below (Table S1, column 5).

2.6 Retrograde Method: Individual CYP Contributions from Tramadol In Vivo Data

The retrograde-scaled approach calculates a hepatic intrinsic clearance per CYP enzyme based on in vivo

Table 1 Overview of probe substrate data

Parameter	CYP3A4 (midazolam)	CYP2D6 (dextromethorphan)
$\mathrm{CLint_{u,in\ vivo,CYP}}$ (µL/min/mg)	336[a]	58.9[b]
$\mathrm{CLint_{u,HLM,CYP}}$ (µL/min/mg)	369	29.9
AAF	0.91	1.97
ISEF	0.23	0.45

These values were obtained for the probe substrates midazolam (CYP3A4) and dextromethorphan (CYP2D6). AAF was calculated from the ratio of the CLint values in this table as described in the "Materials and methods" section. ISEF was calculated per probe substrate from the ratio of the CLints obtained in HLM and rhCYP systems

CLint intrinsic clearance, $CLint_{u,in\ vivo,CYP}$ the unbound in vivo CLint for a specific CYP enzyme, $CLint_{u,HLM,CYP}$ the unbound HLM CLint for a specific CYP enzyme, *AAF* activity-adjustment factor, *ISEF* inter-system extrapolation factor

[a] [15]

[b] [16]

hepatic clearance values and apparent in vivo CYP contributions in the total metabolism. Two different approaches were used to quantitatively define CYP2D6, CYP2B6 and CYP3A4 involvement using tramadol in vivo data. For details about the CYP2D6 contribution, we refer to T'jollyn et al. [8]. The CYP2B6-CYP3A4 contribution was assessed by performing a clinical trial simulation in which a tramadol-rifampicin drug–drug interaction (DDI) was considered. To this end, the study population and study design as described by Saarikoski et al. [13] was matched in a PBPK modelling environment. The rifampicin drug-specific parameters that were used in the PBPK model and describe its PK and induction effects on CYP2B6 and CYP3A4, are described elsewhere [14]. They were proven to be capable to describe rifampicin's DDI potential. While keeping the CYP2D6 contribution in the tramadol retrograde (RG) model fixed, the CYP2B6 contribution was varied between 0 and 30 % at the expense of the CYP3A4 contribution. Simulation results were expressed as the geometric mean ratio of the $AUC_{control}/AUC_{induced}$ for 100 simulated trials and compared to the observed geometric mean ratio from the actual in vivo DDI study between tramadol and rifampicin [13].

3 Results

For the calculation of the AAF, the back-calculation of the CYP3A4 $CLint_{u,invivo}$ (probe substrate midazolam) involved a well-stirred liver model and its value is based on 31 investigational midazolam PK studies [15]. This CYP3A4-specific contribution was assessed by accounting for the conversion midazolam→1-OH midazolam, representing 74 % of the in vivo derived hepatic CLint (Simcyp midazolam compound file, v12.1) (see Electronic Supplementary Material). Next, the AAF_{3A4} was calculated and had a value of 0.91 (Table 1). To calculate the AAF_{2D6}, the CYP2D6 $CLint_{u,invivo}$ (probe substrate dextromethorphan) was collected from one study [16], using a parallel tube model. In addition, a factor of 1.58 accounts for dextromethorphan's accumulation in the hepatocyte's cytosol [17] (see Electronic Supplementary Material). Next, the AAF_{2D6} was calculated and had a value of 1.97 (Table 1). Final metabolism parameters used in the IVIVE are displayed in Table S1 (Electronic Supplementary Material). In addition, Table 1 reports ISEF values per CYP enzyme. Based on midazolam and dextromethorphan metabolism data, an ISEF of 0.23 and 0.45 could be calculated for CYP3A4 and CYP2D6, respectively.

The different hepatic clearance models were evaluated based on prediction errors (calculated as $(CL_{obs} - CL_{pred})/CL_{obs} \times 100$ %) and the different CYP enzyme contributions (Table 2). Tramadol's in vitro CYP contributions (with and without AAF correction) were compared with the ones calculated from the RG method. Tramadol's CYP contributions from activity-adjusted models (HLM_{aa} and $rhCYP_{aa}$ model; Table 2) agree well with those from the tramadol retrograde-scaled clearance approach (RG model, Table 2), although the absolute values of total clearance display some prediction bias (indicated by the prediction error). The CYP2D6 contribution (HLM_{aa} 45 %; $rhCYP_{aa}$ 44.6 %; RG 45.6 %) corresponds very well between the AAF models and is more accurate as compared to the HLM and rhCYP models without AAF. The contributions of CYP3A4 (HLM_{aa} 39.2 %; $rhCYP_{aa}$ 39.9 %; RG 45.1 %) and CYP2B6 (HLM_{aa} 15.8 %; $rhCYP_{aa}$ 15.5 %; RG 9.3 %) differ by maximum 5 % with the RG approach.

In the tramadol RG model, the CYP2D6 contribution was estimated using the dataset from [18], by determining which percentage of the hepatic clearance (48 %, Table S1) is required to increase it 1.74-fold between poor and extensive metabolizers. Next, the CYP2B6-CYP3A4 involvement was estimated by comparing geometric mean AUC ratios from a DDI clinical trial simulation approach. The resulting geometric mean AUC ratio with 90 % confidence intervals was compared to the observed geometric mean AUC ratio (Fig. 1). Only with a CYP2B6 contribution of less than 10 % and a CYP3A4 contribution of more than 42 % (Table S1), the observed geometric mean AUC ratio fell within the 90 % confidence interval of the trial simulations (Fig. 1). The main driver of this rifampicin-tramadol DDI is CYP3A4, in view of its important role in tramadol's metabolism. The steady-state rifampicin induction increases CYP2B6's contribution with only 2 %, whereas CYP3A4's contribution is increased with 31 % (data not shown). The CYP2B6 contribution was calculated to be maximally 10 % of tramadol's hepatic intrinsic clearance (Table S1).

4 Discussion

Whenever pooled HLM activities are measured, the assumption is that every enzyme represents the average population activity. If this is not the case, the activity-adjustment factor (AAF) provides a way to correct for the difference between in vitro and in vivo activities. The AAF (Eq. 2; Electronic Supplementary Material) is an enzyme-specific factor, calculated from reference probe activity measurements in vitro and in vivo that allows appropriate scaling of a test drug's in vitro activity to the 'healthy volunteer' population level. The AAF for CYP3A4 in this study is 0.91 (Table 1) (1 represents no activity adjustment is needed). This implies that the CYP3A4 activity in the pooled HLM batch at hand is nearly identical to that derived from the midazolam CLint, which was back-

Table 2 Model performance and characteristics presented per clearance model

Model	Prediction error (%)	% hepatic clearance	% CYP2D6 in hep CL	% CYP3A4 in hep CL	% CYP2B6 in hep CL
HLM model	−27	56.9	29.1	51.9	19.0
HLM_{aa} model	−19	61.3	45.0	39.2	15.8
rhCYP model	+22	75.9	29.2	51.8	19.0
$rhCY_{aa}$ model	+39	78.7	44.6	39.9	15.5
Retrograde model	+2	70.5	45.6	45.1	9.3

The HLM and rhCYP models are built-up from in vitro data in HLM and rhCYP systems, respectively. The HLM_{aa} and $rhCYP_{aa}$ models are corrected with the AAF. The Retrograde model can be considered the reference model in terms of total clearance and CYP contributions in the hepatic clearance

HLM human liver microsomes, *rhCYP* human recombinant CYP enzymes, *AAF* activity-adjustment factor, HLM_{aa} the activity-adjusted HLM model, $rhCYP_{aa}$ the activity-adjusted rhCYP model

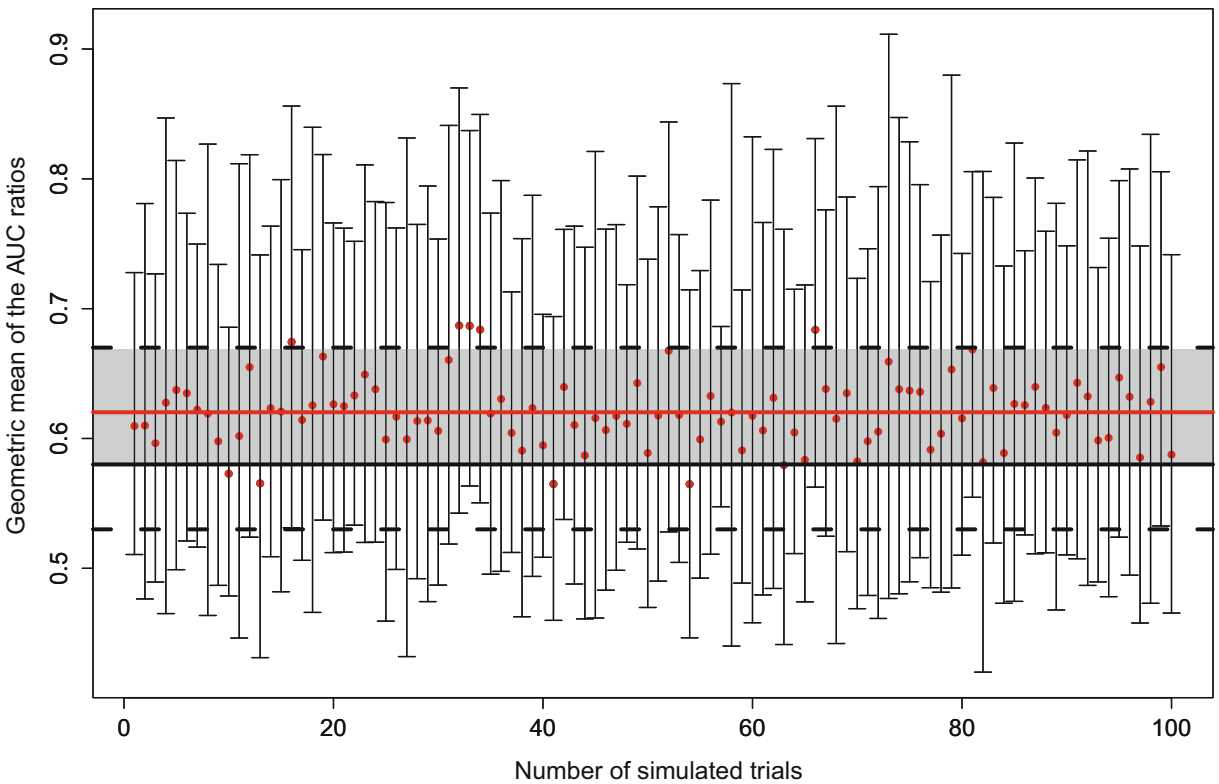

Fig. 1 DDI clinical trial simulation of tramadol and rifampicin to assess the contribution of CYP2B6 and CYP3A4. The figure depicts the results of 100 trial simulations mimicking the original trial, when CYP2B6 and CYP3A4 contributions are assumed to be 10 and 42 %, respectively. *Black horizontal lines* represent the in vivo observed ratio of AUC geometric means (*solid*) and 90 % confidence limits (*dashed*). The *red dots* represent the geometric means of each trial simulation (and *error bars* represent the 90 confidence interval). The *solid red line* represents the average ratio of simulated AUC geometric means. The *solid black line* is in the 95 % confidence region (*greyed area*) only if the CYP2B6 contribution is less than or equal to 10 %. *DDI* drug–drug interaction, *AUC* area under the curve

calculated from in vivo clearance values. Consequently, CYP3A4 activity measurements in this batch will be representative for the average population CYP3A4 activity. However, for CYP2D6, the AAF was computed at 1.97 (Table 1). This indicates that the CYP2D6 in vitro activity in this batch is about twofold below the typical 'healthy volunteer' population CYP2D6 activity. Calculation of an AAF thus accounts for any 'experimental' or 'batch-specific' activity difference between in vitro HLM and in vivo derived activity. The importance of using the AAF concept is illustrated by integrating it in the IVIVE of tramadol metabolism to its two primary metabolites *O*-

desmethyl tramadol (ODT) and *N*-desmethyl tramadol (NDT). The metabolism parameters used in the IVIVE are displayed in Table S1 (Electronic Supplementary Material).

Although the retrograde-scaled approach is an attractive way to calculate CYP contributions based on in vivo data, it also depends on the quality of these in vivo data. The 10 % involvement threshold for CYP2B6 in the retrograde model should be viewed as an approximate value for two reasons: (1) for CYP2B6, an AAF could not be calculated since CYP2B6 in vitro probe data were not available (see Table 2), and (2) the design of the rifampicin-tramadol in vivo DDI study allowed only a partial differentiation of the CYP2B6 and CYP34 contributions. In essence, since for CYPD6 the different data elements were all available, the CYP2D6 approach could serve as proof-of concept for the proposed methodology. The activity-adjusted CYP2D6 contribution turned out to be very similar to the one estimated from in vivo clearance data (RG approach), and shows a large improvement versus its contribution calculated in non-activity adjusted models (Table 2). This finding underscores the method's potential benefit.

5 Conclusion

Taken together, the AAF (i) provides compound-independent information about specific enzyme activities that should be incorporated whenever in vitro HLM activities are measured for a test drug, and (ii) allows to accurately calculate CYP contributions in vivo, even before clinical data of the test drug is available. In view of the increasing role of IVIVE-PBPK in drug development programs, it is important to determine the actual enzyme contributions in the hepatic clearance as early as possible. This way, clinical trial designs and clinically important drug–drug interactions may be better anticipated. In the current work, tramadol was used as a proof-of-concept compound, but more work is needed to extensively validate the proposed approach across a broader range of compounds.

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Compliance with Ethical Standards

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Conflict of interest JS, AVP, GM and AV own stock and/or stock options from Johnson and Johnson. HT, JVB, LDB, PA, KA and KB have no conflicts of interest to declare.

References

1. Johnson TN, Rostami-Hodjegan A. Resurgence in the use of physiologically based pharmacokinetic models in pediatric clinical pharmacology: parallel shift in incorporating the knowledge of biological elements and increased applicability to drug development and clinical practice. Pediatr Anesth. 2010;21(3):291–301.
2. Salem F, Johnson TN, Barter ZE, Leeder JS, Rostami-Hodjegan A. Age related changes in fractional elimination pathways for drugs: assessing the impact of variable ontogeny on metabolic drug–drug interactions. J Clin Pharmacol. 2013;53(8):857–65.
3. Donato MT, Lahoz A, Jimenez N, Perez G, Serralta A, Mir J, et al. Potential impact of steatosis on cytochrome P450 enzymes of human hepatocytes isolated from fatty liver grafts. Drug Metab Dispos. 2006;34(9):1556–62.
4. Rowland A, Knights KM, Mackenzie PI, Miners JO. The "albumin effect" and drug glucuronidation: bovine serum albumin and fatty acid-free human serum albumin enhance the glucuronidation of UDP-glucuronosyltransferase (UGT) 1A9 substrates but not UGT1A1 and UGT1A6 activities. Drug Metab Dispos. 2008;36(6):1056–62.
5. Chen Y, Liu L, Nguyen K, Fretland AJ. Utility of intersystem extrapolation factors in early reaction phenotyping and the quantitative extrapolation of human liver microsomal intrinsic clearance using recombinant cytochromes P450. Drug Metab Dispos. 2011;39(3):373–82.
6. Subrahmanyam V, Renwick AB, Walters DG, Young PJ, Price RJ, Tonelli AP, et al. Identification of cytochrome P-450 isoforms responsible for cis-tramadol metabolism in human liver microsomes. Drug Metab Dispos. 2001;29(8):1146–55.
7. Walsky RL, Obach RS. Validated assays for human cytochrome P450 activities. Drug Metab Dispos. 2004;32(6):647–60.
8. T'Jollyn H, Snoeys J, Colin P, Van Bocxlaer J, Annaert P, Cuyckens F, et al. Physiology-based IVIVE predictions of tramadol from in vitro metabolism data. Pharm Res. 2015;32(1):260–74.
9. Lu C, Li P, Gallegos R, Uttamsingh V, Xia CQ, Miwa GT, et al. Comparison of intrinsic clearance in liver microsomes and hepatocytes from rats and humans: evaluation of free fraction and uptake in hepatocytes. Drug Metab Dispos. 2006;34(9):1600–5.
10. Witherow LE, Houston JB. Sigmoidal kinetics of CYP3A substrates: an approach for scaling dextromethorphan metabolism in hepatic microsomes and isolated hepatocytes to predict in vivo clearance in rat. J Pharmacol Exp Ther. 1999;290(1):58–65.
11. De Bock L, Boussery K, Colin P, De Smet J, T'Jollyn H, Van Bocxlaer J. Development and validation of a fast and sensitive UPLC-MS/MS method for the quantification of six probe metabolites for the in vitro determination of cytochrome P450 activity. Talanta. 2012;30(89):209–16.
12. Yeo KR. Abundance of cytochromes P450 in human liver: a meta-analysis. Br J Clin Pharmacol. 2004;57(5):687–8.
13. Saarikoski T, Saari T, Hagelberg N, Neuvonen M, Neuvonen P, Scheinin M, et al. Rifampicin markedly decreases the exposure to oral and intravenous tramadol. Eur J Clin Pharmacol. 2013;69(6):1293–301.
14. Rekic D, Roshammar D, Mukonzo J, Ashton M. In silico prediction of efavirenz and rifampicin drug–drug interaction considering weight and CYP2B6 phenotype. Br J Clin Pharmacol. 2011;71(4):536–43.
15. Gertz M, Harrison A, Houston JB, Galetin A. Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. Drug Metab Dispos. 2010;38(7):1147–58.

16. Ito T, Kato M, Chiba K, Okazaki O, Sugiyama Y. Estimation of the interindividual variability of cytochrome 2D6 activity from urinary metabolic ratios in the literature. Drug Metab Pharmacokinet. 2010;25(3):243–53.
17. Hallifax D, Houston JB. Saturable uptake of lipophilic amine drugs into isolated hepatocytes: mechanisms and consequences for quantitative clearance prediction. Drug Metab Dispos. 2007;35(8):1325–32.
18. Pedersen RS, Damkier P, Brosen K. Enantioselective pharmacokinetics of tramadol in CYP2D6 extensive and poor metabolizers. Eur J Clin Pharmacol. 2006;62(7):513–21.