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A Simple Methodology to Differentiate Changes in Bioavailability from Changes in Clearance Following Oral Dosing of Metabolized Drugs

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Abstract (245 of 250 words):

Accurately discriminating changes in clearance (CL) from changes in bioavailability (F) following an oral drug-drug interaction is difficult without carrying out an IV interaction study. This may be true for drugs that are clinically-significant transporter substrates, however, for interactions that are strictly metabolic it has been recognized that volume of distribution remains unchanged between both phases of the interaction study. With the understanding that changes in volume of distribution will be minimal for metabolized drugs, the inverse of the change in apparent volume of distribution (V_{ss}/F) can provide adequate estimates of the change in bioavailability alone. Utilization of this estimate of F change in tandem with the observed apparent clearance (CL/F) change in an oral drug-drug interaction can provide an estimate of the change in clearance alone. Here, we examine drug-drug interactions involving 5 known inhibitors and inducers of CYP3A4 on victim drugs midazolam and apixaban for which the interaction was carried out both orally and intravenously, allowing for evaluation of this methodology. Predictions of CL and F changes based on oral data were reasonably close to observed changes based on intravenous studies, demonstrating that this simple yet powerful methodology can reasonably differentiate changes in F from changes in CL for oral metabolic drug interactions when only oral data are available. Utilization of this relatively simple methodology to evaluate DDIs for orally dosed drugs will have a significant impact on how DDIs are interpreted from a drug development and regulatory perspective.

Introduction:

Anticipation of extent of change in clearance (CL) of victim drugs in drug-drug interaction (DDI) studies is critical in recognizing potential drug combinations that may result in loss of efficacy or a safety finding due to alterations in drug exposure (AUC; area under the curve), as changes in clearance are inversely related to exposure changes (Eq. 1).

$$AUC = \frac{F \cdot Dose}{CL} \quad (1)$$

Following oral dosing, however, changes in bioavailability (F) must also be considered since changes in extent of absorption or first pass extraction due to a DDI may also result in AUC changes. As evident in Eq. 1, knowledge of dose and the readily measurable AUC results in a ratio of CL to F, two parameters that are difficult to distinguish from one another after oral dosing. Oral bioavailability can be estimated if the drug is also dosed intravenously (IV) via examination of the dose-normalized AUC ratio from oral to IV administration. However, most orally approved drugs have not been studied under IV dosing conditions and therefore these clearance determinations are confounded by bioavailability.

Changes in half-life and mean residence time (MRT) are not related to F, therefore for primarily metabolized drugs one may attempt to differentiate changes in CL versus F in a DDI by examining the magnitude of change in half-life and MRT compared to AUC and C_{max}, as we have recently reviewed[1]. If a drug were to follow simple one compartment disposition kinetics, the change in half-life would reflect the change in CL, and knowing the change in AUC for an orally dosed drug with a metabolic DDI, the change in F could be determined using Eq. 1. However, for

drugs only dosed orally this would not be known. Alternatively, low extraction ratio drugs will have minimal first pass elimination, therefore changes in CL/F can be primarily attributed to a change in CL rather than F. However, extraction ratio cannot be determined if only oral data are available.

It is important to recognize that MRT and half-life ($t_{1/2}$) are a function of both clearance and volume of distribution as given in Equation 2[2]

$$MRT = \frac{V_{ss}/F}{CL/F} \quad (2)$$

where V_{ss} is the volume of distribution at steady-state. And, it has been recognized that when transporters are involved in drug disposition, significant transporter drug interactions may result in volume of distribution changes in addition to potential changes in clearance[3]. Due to the dependence of MRT and terminal half-life on both clearance and volume of distribution, attempts to predict changes in drug concentration-time curves following DDI or pharmacogenomic variance studies may prove challenging if changes in volume of distribution are not considered. It is possible that interactions can alter V_{ss} differently than CL, even resulting in half-life changes that are counterintuitive to the direction of change in clearance (i.e. an interaction with a decrease in clearance can also display a decrease in half-life due to large decreases in volume of distribution). Recently, our laboratory has critically analyzed[4] and summarized[5] such changes in apparent clearance (CL/F), apparent volume of distribution at steady state (V_{ss}/F), MRT and terminal half-life for orally dosed transporter substrates (atorvastatin[6], glyburide[7] and rosuvastatin[8]) in clinical DDI studies with concomitant IV rifampin

(an OATP1B1 and BCRP inhibitor). In all of these DDIs, a decrease in CL/F was associated with a decrease in terminal half-life (rather than a prolonged half-life) due to a significant decrease in V_{ss}/F.

However, for a metabolic drug interaction (no transporter involvement) it is expected that volume of distribution would remain unchanged. In Table 1 we summarize V_{ss} changes for clinical DDI studies involving IV administration of the primarily metabolized drugs caffeine, midazolam and theophylline[9-12]. The magnitude of change in exposure ranged from 1.3 – 3.2 in these DDI studies, however V_{ss} remains unchanged (0.92 – 1.1). Current efforts of our laboratory involve a comprehensive analysis of V_{ss} changes for CYP index substrates in clinical IV DDI and pharmacogenomics variance studies, but here we present the methodology to distinguish CL and F for DDIs that only involve metabolism.

With knowledge that V_{ss} does not change for metabolic drug interactions, the inverse of the change in V_{ss}/F in the interaction versus control phase for oral metabolic interactions can provide an estimate of change in F in the interaction versus control phase as given in Eq. 3

$$\frac{V_{SS}/F^{treated}}{V_{SS}/F^{control}} = \frac{V_{SS}^{treated}}{V_{SS}^{control}} \cdot \frac{F^{control}}{F^{treated}} \cong 1 \cdot \frac{F^{control}}{F^{treated}} = \frac{1}{F^{treated}/F^{control}} \qquad (3)$$

In other words, the change in V_{ss}/F is approximately equal to the inverse of the change in bioavailability in the interaction versus control phase for metabolic interactions. By accounting for the estimated change in bioavailability (result of Eq. 3) in the observed ratio of change in

available clearance (CL/F), it is possible to calculate the change in clearance alone, as outlined in Eq. 4.

$$\frac{CL^{treated}}{CL^{control}} = \frac{CL/F^{treated}}{CL/F^{control}} \cdot \frac{1}{F^{treated}/F^{control}} \cong \frac{CL/F^{treated}}{CL/F^{control}} \div \frac{V_{SS}/F^{treated}}{V_{SS}/F^{control}} \quad (4)$$

This methodology is quite simple yet powerful, as it can provide reasonable estimates of how changes in *F* can be differentiated from changes in *CL* for oral metabolic drug interactions when only oral data are available.

Methods:

The CYP3A4 *in vivo* index substrate midazolam was selected as a model metabolized drug for evaluation of the proposed methodology. Drug interaction studies were identified for which midazolam was dosed both orally and IV as the victim drug and the perpetrator was a clinically recommended CYP3A4 inhibitor or inducer based on a recent compilation of clinical index substrates and inhibitors[13]. Apixaban was also selected as an additional drug to further evaluate this methodology.

Changes in exposure (AUC), clearance (CL), apparent clearance (CL/F), volume of distribution at steady state (V_{ss}), apparent volume of distribution (V_{ss}/F), bioavailability (F), and percent extrapolation of AUC were examined and reported as ratios of interaction/control. The published pharmacokinetic values reported by the original investigators were utilized in priority, however all clinical studies investigated here did not report V_{ss}/F, therefore it was necessary to utilize the published pharmacokinetic profiles to estimate this ratio and supplement any other

parameters not reported. This was achieved by digitization of victim drug mean plasma-concentration time profiles that were subsequently analyzed by noncompartmental analysis using WinNonlin® Professional Edition Version 2.1 (Pharsight, Mountain View, CA). All pharmacokinetic ratios calculated from digitization of published pharmacokinetic profiles are specifically indicated in Tables 2-5 as a footnote for clarity. Digitized AUC values were compared to reported AUC values and differences were found to be less than 20%, indicating that the reported average concentration-time profiles investigated here reasonably represented the study population. The percent of AUC extrapolations are listed in Tables 2-5 following both IV and oral drug administration as an indication of the potential confidence in the derived pharmacokinetic parameters.

Mean absorption time (MAT) was estimated, as we previously described[6], as the reciprocal of the first-order absorption rate constant after the oral concentration-time data were fit to a 2-compartment model with absorption from the gut compartment using WinNonlin®. Mean residence time (MRT) was calculated as the ratio of the area under the first moment curve ($AUMC_{0-\infty}$) divided by $AUC_{0-\infty}$ for intravenous interactions. However, for oral interactions calculation of MRT requires that MAT must be subtracted from the ratio of $AUMC / AUC$. Equation 2 was utilized to calculate V_{ss} or V_{ss}/F.

Prediction of extent of change of F and CL following oral dosing was calculated using Eqs. 3 and 4, respectively. In each DDI presented, the comparison of the change in terminal half-life following IV and oral dosing is also reported in footnotes of Tables 2-5. Assuming the change in half-life following oral dosing accurately reflected the change in CL, it is possible to then predict the change in F using Eq. 1.

Results:

We identified clinical DDIs in the literature where the effects of widely-used metabolic inhibitors or inducers were examined following both IV and oral dosing of the primarily metabolized victim drug midazolam, as well as for an additional drug apixaban to further evaluate this methodology. Sufficient data and concentration-time curves were available in the publications for us to demonstrate the utility and potential reliability of this methodology. Midazolam was dosed orally and IV with and without the inhibitors clarithromycin[14], fluconazole[15], itraconazole[11], and ritonavir[16], and both midazolam and apixaban were dosed orally and IV with and without multiple dosing of the inducer rifampin[16,17]. In each of these six metabolic interactions, no significant change in V_{ss} was observed following IV dosing of the victim drug, with V_{ss} ratios ranging from 0.87-1.19.

Table 2 displays the ratios of change in IV and oral midazolam pharmacokinetic parameters in the perpetrator versus control phase for the clarithromycin[14], fluconazole[15], and ritonavir[16] interaction studies. In the clarithromycin study, clarithromycin (500 mg BID; 7 days) caused a 63% decrease in midazolam IV clearance[14]. Assuming that this decrease in clearance would also occur following oral dosing, the investigators estimated clarithromycin increased oral bioavailability by 2.42-fold. Using the methodology proposed here to predict changes in CL and F for the oral data only, with the assumption that V_{ss} is unchanged for this metabolic interaction, the predicted change in F was a 2.94-fold increase and that CL had decreased 59%. In the fluconazole study, concomitant fluconazole administration (200 mg; single dose) resulted in a 32% decrease in midazolam IV clearance (predicted 40% decrease from oral study), and a 2.33-fold increase in oral bioavailability (predicted 2.38 increase from oral study)[15]. In the ritonavir

interaction, multiple dosing of ritonavir (800 mg; 14 days) resulted in a 71% decrease in midazolam IV clearance (predicted 72% decrease from oral only study) and a 2.55-fold increase in bioavailability (predicted 2.78 increase from oral only study [16].

Changes in midazolam pharmacokinetic parameters in the interaction with itraconazole (200 mg; 4 days (IV); 6 days (oral)) are listed in Table 3[11]. Administration of itraconazole for 4 days resulted in a 69% decrease in IV midazolam clearance. The oral interaction between itraconazole and midazolam was studied on day 6, and with the assumption that alteration in midazolam clearance is similar between day 4 (IV DDI) and day 6 (oral DDI), the resulting increase in bioavailability is 2.46-fold. The methodology predicted a 2.00-fold increase in bioavailability and a 70% reduction in clearance.

Table 4 shows the changes in oral and IV midazolam pharmacokinetic parameters due to multiple doses of rifampin (600 mg QD; 14 days), which resulted in a 2.16-fold increase in midazolam IV clearance and 81% decrease in bioavailability[16]. The oral midazolam interaction data results in an 11.7-fold increase in available clearance (CL/F), but by utilizing the methodology presented here, it is possible to predict that the large change in CL/F is a result of an approximate 2.93-fold increase in clearance and a 75% reduction in oral bioavailability.

Table 5 shows that multiple doses of rifampin caused a 1.64-fold increase in apixaban IV clearance and a 24% decrease in oral bioavailability[17]. Using the methodology proposed here for the oral data only predicts that CL had increased 1.50-fold and that F decreased by 30%.

Discussion:

Utilization of this relatively simple methodology to evaluate DDIs for orally dosed drugs will have a significant impact on how DDIs are interpreted from a drug development and regulatory perspective. For metabolic interactions, this methodology can reasonably differentiate the extent of change in F from changes in CL when IV dosing data are unavailable. Here we demonstrate the utility of this methodology for the primarily metabolized drug midazolam, a commonly-used *in vivo* index substrate of CYP3A4, and for one study with apixaban, for which both oral and IV interaction data were available in the same subjects.

Table 2 outlines the results of the clarithromycin[14], fluconazole[15], and ritonavir[16] drug interaction studies. In the clarithromycin-midazolam interaction study[14] significant differences in exposure change (AUC ratios) were observed when comparing the IV and oral DDI studies (2.66- and 7.0-fold, respectively), indicating that a significant change in both oral bioavailability and clearance occurred as a result of the interaction. The methodology presented here adequately distinguished the contribution of change in clearance from bioavailability in the oral DDI; the estimated change in F differed by 21% from the observed change (2.94 estimated vs. 2.42 observed), while the estimated change in CL only differed by 11% from the observed change with IV dosing (0.41 estimated vs. 0.37 observed). In the midazolam-fluconazole interaction study[15], the predicted changes in F and CL were quite close to observed changes calculated with IV dosing data, with only a 2% difference in F (2.38 estimated vs. 2.33 observed) and a 12% difference in CL (0.60 estimated versus 0.68 observed). In the ritonavir-midazolam DDI[16], a 9% difference in F and only a 3% difference in CL was observed between predicted and actual values. For all three of these interactions, assuming that changes in oral terminal half-life accurately reflected the

change in CL and using Eq. 1 would also have given reasonable estimates of CL and F (as noted in footnote b-d of Table 2).

In Table 3 for the itraconazole-midazolam DDI[11], the observed changes in CL were remarkably close to predictions based on oral data only (3% difference in CL) accompanied by a 19% difference in F. Utilizing changes in oral terminal half-life to predict CL changes and Eq. 1 to estimate the changes in F would not have been as accurate, with prediction errors of 25% for both parameters.

The induction effect of multiple dosing of rifampin on midazolam was examined[16] (Table 4); the estimated change in F differed by 32% and the estimated change in CL differed by 35% from observed values. Although a prediction error of 30% may be considered to be quite high, it should be noted that the 12.3-fold decrease in exposure as a result of the rifampin-midazolam oral DDI was significantly larger in magnitude than other midazolam DDIs investigated, which ranged from 3.9[15] to 8.3[16]. Of note, the estimated change in F and CL based on oral terminal half-life changes and Eq. 1 resulted in much less accurate predictions, with errors in F and CL of 63% and 78%, respectively.

In contrast to the midazolam-rifampin DDI, estimates for the apixaban-rifampin interaction study[17] were much closer to observed values with both F and CL differing by only 9% (although AUC only changed approximately 2-fold). As noted in footnote b of Table 5, the estimated change in F and CL using oral terminal half-life and Eq. 1 resulted in markedly poorer predictions, with errors in F and CL of 40% and 41%, respectively. Of note, apixaban V_{ss} following IV dosing indicates minimal change with a ratio of 0.87, suggesting that transporters inhibited by rifampin are not involved in apixaban disposition. The success of the methodology in

discriminating F and CL further supports this observation since it relies on the assumption that V_{ss} is unchanged. These findings are contrary to the apixaban FDA label, which proposes that the efflux transporters BCRP and P-gp may play a clinically significant role, and further demonstrates the utility of this simple methodology in recognizing transporter versus metabolism drug interactions.

It is important to recognize the assumptions and limitations of this methodology to appropriately guide its use and prevent misinterpretations of interaction data. Calculation of V_{ss}/F using Equation 2 relies on measurements of CL/F and MRT, two parameters that are derived from AUC, which highlights the importance of accurate determination of AUC for the success of this methodology. Adequate plasma sampling describing the terminal slope of the concentration-time profiles is crucial since AUC must be extrapolated from the final time-point to infinity. Therefore, it is imperative to inspect the percentage of AUC that has been extrapolated after the final sampling time point to ensure that data estimates can be reliably interpreted. In our analysis, we point out the percentage of total AUC that was extrapolated in each phase of the DDIs to highlight the degree of AUC estimation; low extrapolation percentages indicate lower probability of error in AUC determination, however, the converse is not necessarily true. Higher percent extrapolations may or may not indicate inaccuracies in AUC determination; if the terminal phase of the concentration-time profile is accurate, then the degree of extrapolation does not introduce error. The degree of extrapolation in AUC determinations is magnified in calculations of the area under the moment-time curve ($AUMC$), further affecting calculations of MRT following IV dosing (which is calculated by the ratio of $AUMC/AUC$). Following oral dosing, the ratio of $AUMC/AUC$ results in the sum of MRT and

mean absorption time (MAT). We proposed that MAT may be reasonably approximated by estimating the oral absorption rate constant (k_a) from pharmacokinetic profiles ($MAT = 1/k_a$) by fitting the data to a compartmental model that assumes first order absorption from a single compartment absorption site[6]. Certainly, all drug absorption will not follow first order kinetics from a one compartment absorption site, but the objective here is not to calculate MAT in each phase, but rather how MAT changes under conditions where a perpetrator is present versus in its absence. The high relative accuracy of our predictions in Tables 2-5 suggests that our assumption is reasonable. In three of the six interactions presented in Tables 2-5, attempts to use changes in terminal half-life and Eq. 1 to predict the changes of CL and F would not have been as accurate as the methodology proposed here. Since, when only oral DDI data is available, it is not possible to know if estimates using Eq. 1 may be accurate, we recommend that the procedure here always be preferred.

The methodology is only applicable to interactions where V_{ss} is unchanged, hence its appropriate application to strictly metabolic drug-drug interactions. Another scenario where it is possible that V_{ss} may change (even for purely metabolic interactions) is if a perpetrator drug alters protein binding of the victim drug by displacing it from plasma or tissue proteins, resulting in increased fraction unbound of victim drug. We believe that a protein binding interaction can be adequately predicted based on *in vitro* analysis as detailed in Figure 1. Perpetrator drugs could potentially alter blood flow that may result in increased or decreased clearance of victim drugs, however changes in V_{ss} are not anticipated with changes in blood flow. Therefore, the impact of such perpetrators is not expected to affect the utility of this methodology.

Finally, although the pharmacokinetic values reported by the original authors were utilized in priority, the data analyzed here are partially based on average reported concentration-time profiles since digitization was required to estimate the unreported V_{ss}/F for all oral interactions. When available, it may be more appropriate to utilize individual PK profiles to make predictions of changes in CL and F for each subject based on this methodology. The limitation of utilizing average pharmacokinetic concentration-time profiles is that in many cases average profiles do not accurately represent changes within a particular individual in the drug interaction study. Utilizing the average drug concentrations of each subject at each time point results in pharmacokinetic profiles that do not necessarily represent a single subject within the study. Individual patient pharmacokinetic data are very rarely published, and further, drug interaction studies for which a victim drug is administered both orally and IV in the same patients are quite uncommon (we do not have such drug interaction data in our clinical archive), therefore it was impossible to identify such data in the literature for utilization here. Thus, we propose that utilization of this methodology be carried out for each subject in the DDI study. Efforts are underway towards establishing collaborations with laboratories that may have access to such data for further evaluation of the methodology.

For well-studied marketed drugs such as midazolam, it is often known whether or not transporters are significantly involved in drug disposition due to the availability of well-designed IV or oral interaction studies utilizing clinically-demonstrated transporter inhibitors. And for most investigational drugs, there is good evidence of the pathways governing drug disposition before drug-drug interaction studies are undertaken. However, if such data are not available for a particular drug-of-interest, we suggest the use of the Biopharmaceutics Drug Disposition

Classification System (BDDCS) to anticipate which drugs may be susceptible to transporters *in vivo*[18]. The unfavorable membrane permeability of BDDCS Class 3 and 4 compounds implies their reliance on xenobiotic transporters to cross biological membranes *in vivo*, and this theory is supported by the observation that Class 3 and 4 drugs are primarily eliminated by transporter-dependent processes (i.e. renal or biliary excretion of unchanged drug). BDDCS Class 1 and 2 drugs have favorable permeability characteristics that allow passage across biological membranes via passive processes, which is supported by the observation that these drugs are primarily metabolized. It is theorized that the rapid membrane permeability combined with the high solubility of BDDCS Class 1 drugs allows these drugs to rapidly cross membranes at concentrations high enough to saturate active transport, or alternatively the active transport amounts are small compared to the passive permeability amounts, overcoming any potential transporter effects *in vivo*, even if shown to be a transporter substrate *in vitro*[18]. BDDCS Class 2 drugs also display high permeability, but due to their low solubility it is thought that the resulting lower soluble concentrations available for passive diffusion may be incapable of saturating transporters, or passive transport may not be much greater than the contribution of active transport. Therefore, involvement of uptake or efflux transporters cannot be ruled out in the absorption and disposition of BDDCS Class 2 drugs despite their status as being primarily metabolized. However, the *in vitro* transporter interaction studies proposed in our guide to appropriate use of the methodology (Figure 1) will assist in making this decision. In summary, the proposed methodology is appropriate for BDDCS class 1 drugs, not recommended for BDDCS class 3 and 4, and should be used with caution for BDDCS class 2 drugs with recognition that transporter involvement may or may not be clinically relevant. Evaluation of the association of

BDDCS class with the extent of change in V_{ss} in IV interactions is an ongoing effort in our laboratory to validate this hypothesis.

In addition to utilization of BDDCS to inform the appropriate use of our methodology, we have outlined additional *in vitro* studies that may be helpful in identifying strictly metabolic interactions (Figure 1). The recommendations outlined in Figure 1 will be helpful for investigational compounds that inherently are less well-characterized than marketed drugs, as there is increased likelihood of clinical evidence regarding the potential involvement of transporters versus enzymes with known index inhibitors.

Although our methodology relies on the assumption that V_{ss} changes in transporter drug-drug interactions, our laboratory has previously summarized how volume of distribution was observed to change based on localization of the transporter (in the liver versus kidney) and if the transporter affected is an uptake versus efflux transporter[3]. In general, large decreases in volume of distribution are observed for hepatic uptake transporters, whereas renal uptake transporter interactions do not result in volume of distribution changes, although there were exceptions observed. Inhibition of hepatic efflux transporters generally leads to a decrease in volume of distribution while renal tubule efflux transporter inhibition results in increased volume of distribution. In analysis of transporter interactions, further consideration of the inhibitory specificity of perpetrator drugs is necessary, as currently there are a limited number of well-characterized and specific clinical transporter inhibitors[13]. Therefore, there may be specific transporter interactions where V_{ss} does not change significantly and this methodology may appropriately discriminate CL from F changes. However, further validation is warranted prior to

applying this methodology to transporter interactions and is an ongoing effort of our laboratory, and therefore we do not recommend its use for transporter interactions at this time.

For decades, the field has believed that changes in clearance could not be accurately discriminated from changes in bioavailability for oral drug interaction studies without performing an IV interaction study to confirm the extent of clearance changes. This has led to challenges in understanding the contribution of bioavailability change in oral DDI studies, often resulting in an overprediction of clearance change and an underestimation of the impact bioavailability changes can have on observed exposure. The ingenuity of this relatively simple methodology leverages the understanding that volume of distribution appears to remain unchanged where disposition is limited to metabolism, therefore calculation of changes in oral volume of distribution can reliably provide estimation of bioavailability versus clearance changes. We recommend that this methodology be routinely utilized in the evaluation of clinical drug-drug interaction studies.

Study Highlights: (144 of 150 words)

What is the current knowledge on the topic? Accurately discriminating clearance changes from bioavailability changes following oral dosing has been considered difficult without also conducting an IV interaction study.

What question did this study address? This study assesses the possibility of discriminating changes in clearance from bioavailability for orally dosed metabolized drugs without IV drug interaction data.

What does this study add to our knowledge? By leveraging the fact that volume of distribution remains unchanged in metabolic drug interactions, the change in apparent volume of distribution can provide estimates of bioavailability changes for drugs that are not clinically significant transporter substrates, which can be further utilized to estimate clearance changes. For metabolic interactions, this methodology can reasonably differentiate the extent of change in *F* from changes in *CL* when IV dosing data are unavailable.

How might this change clinical pharmacology or translational science?

Utilization of this relatively simple methodology to evaluate DDIs for orally dosed drugs will have a significant impact on how DDIs are interpreted from a drug development and regulatory perspective.

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Author Contributions:

J.K.S. and L.Z.B. wrote the manuscript, designed the research, performed the research, analyzed the data and contributed new analytical tools.

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Figure Legend:

Figure 1: Methodology scheme to guide appropriate use of the clearance and bioavailability discrimination methodology for strictly metabolic interactions. Abbreviations: ADME, Absorption, Distribution, Metabolism, Excretion; BDDCS, Biopharmaceutical Drug Disposition Classification System; *CL*, clearance; CYP, Cytochrome P450; *F*, bioavailability; V_{ss}, volume of distribution at steady state

Table 1: Changes in Exposure (*AUC*), Clearance (*CL*) and Volume of Distribution at Steady State (V_{ss}) (Expressed as Ratios of Interaction/Control) in Metabolic Drug-Drug Interactions for Primarily Metabolized Drugs Dosed Intravenously

Victim Drug	Primary Enzyme	Perpetrator Drug	Inhibition Target	$\frac{AUC^{DDI}}{AUC^{Con}}$	$\frac{CL^{DDI}}{CL^{Con}}$	$\frac{V_{ss}^{DDI}}{V_{ss}^{Con}}$	Reference
Caffeine	CYP1A2	Terbinafine	CYP2D6 CYP1A2	1.3	0.79	1.1	[9]
Midazolam	CYP3A4	Erythromycin	CYP3A4 P-gp	1.5	0.66	0.93	[10]
Midazolam	CYP3A4	Fluconazole	CYP3A4, CYP2C9, CYP2C19	2.0	0.49	0.92	[11]
Midazolam	CYP3A4	Itraconazole	CYP3A4, CYP2J2, P-gp	3.2	0.31	1.1	[11]
Theophylline	CYP1A2	Cimetidine	CYP1A2, OCT2	1.6	0.60	1.1	[12]
Theophylline	CYP1A2	Ciprofloxacin	CYP1A2, CYP3A4	1.4	0.69	1.0	[12]
Theophylline	CYP1A2	Cimetidine + Ciprofloxacin	CYP1A2, CYP3A4, OCT2	1.8	0.55	1.1	[12]

Table 2: Utilization of Proposed Methodology to Discriminate Clearance (CL) from Bioavailability (F) Changes for Orally Dosed Midazolam (Victim) and the Perpetrators: Clarithromycin (500 mg BID, 7 Days) from the Study of Gorski et al.[14]; Fluconazole (200 mg, Single Dose) from the Study of Kharasch et al.[15]; Ritonavir (800 mg QD, 14 Days) from the Study of Kirby et al.[16]

Victim	Perpetrator	$\frac{AUC^{DDI}}{AUC^{Control}}$	Percent AUC Extrapolation (DDI/Control)	$\frac{V_{ss}/F^{DDI}}{V_{ss}/F^{Control}}$	$\frac{V_{ss}{}^{DDI}}{V_{ss}{}^{Control}}$	$\frac{F^{DDI}}{F^{Control}}$	$\frac{CL/F^{DDI}}{CL/F^{Control}}$	$\frac{CL^{DDI}}{CL^{Control}}$
Gorski et al., 1998[14]								
Midazolam (IV)	Clarithromycin	Observed: 2.66	Observed: 38% / 12%[a]	–	Observed: 1.05[a]	Observed: 2.42	–	Observed: 0.37
Midazolam (Oral)	Clarithromycin	Observed: 7.00	Observed: 34% / 22%[a]	Observed: 0.34[a]	Assumed: 1	Estimated: 2.94[b]	Observed: 0.14	Estimated: 0.41[b]
Kharasch et al., 2005[15]								
Midazolam (IV)	Fluconazole (200 mg)	Observed: 1.4	Observed: 17% / 7%	–	Observed: 1.10[a]	Observed: 2.33	–	Observed: 0.68
Midazolam (Oral)	Fluconazole (200 mg)	Observed: 3.9	Observed: 19% / 8%	Observed: 0.42[a]	Assumed: 1	Estimated: 2.38[b]	Observed: 0.25	Estimated: 0.60[c]
Kirby et al., 2011[16]								
Midazolam (IV)	Ritonavir	Observed: 3.31	Observed: 21% / 3%[a]	–	Observed: 1.04[a]	Observed: 2.55	–	Observed: 0.29
Midazolam (Oral)	Ritonavir	Observed: 8.28	Observed: 25% / 5%[a]	Observed: 0.36[a]	Assumed: 1	Estimated: 2.78[b]	Observed: 0.10	Estimated: 0.28[d]

Pharmacokinetic values reported in the table are based on published average values, unless otherwise noted

Abbreviations: *AUC*, area under the curve; *CL*, clearance; DDI, drug-drug interaction; *F*, bioavailability; V_{ss}, volume of distribution at steady state

[a]Ratios are calculated by digitization of published average plasma concentration-time profiles and performing non-compartmental analysis

[b]Terminal half-life increased 2.7-fold following IV dosing and 2.6-fold following oral dosing. Therefore, similar estimates of the change in F and CL could have been made by using the change in oral terminal half-life and Eq. 1

[c]Terminal half-life increased 1.2-fold following IV dosing and 1.5-fold following oral dosing. Therefore, similar estimates of the change in F and CL could have been made by using the change in oral terminal half-life and Eq. 1

[d]Terminal half-life increased 3.0-fold following IV dosing and 2.9-fold following oral dosing. Therefore, similar estimates of the change in F and CL could have been made by using the change in oral terminal half-life and Eq. 1

Table 3: Utilization of Proposed Methodology to Discriminate Clearance (CL) from Bioavailability (F) Changes for Orally Dosed Midazolam (Victim) and Itraconazole (Perpetrator; 200 mg QD, 4 or 6 Days) from the Study of Olkkola et al.[11]

Victim	Perpetrator	$\frac{AUC^{DDI}}{AUC^{Control}}$	Percent AUC Extrapolation (DDI/Control)	$\frac{V_{ss}/F^{DDI}}{V_{ss}/F^{Control}}$	$\frac{V_{ss}{}^{DDI}}{V_{ss}{}^{Control}}$	$\frac{F^{DDI}}{F^{Control}}$	$\frac{CL/F^{DDI}}{CL/F^{Control}}$	$\frac{CL^{DDI}}{CL^{Control}}$
Midazolam (IV) (Day 4)	Itraconazole (Day 4)	Observed: 3.22[b]	Observed: 16% / 1%[a]	–	Observed: 1.08	Observed: 2.46	–	Observed: 0.31
Midazolam (Oral) (Day 6)	Itraconazole (Day 6)	Observed: 6.64	Observed: 22% / 0%[a]	Observed: 0.50[a]	Assumed: 1	Estimated: 2.00[c]	Observed: 0.15[b]	Estimated: 0.30[c]

Pharmacokinetic values reported in the table are based on published average values, unless otherwise noted

Abbreviations: *AUC*, area under the curve; *CL*, clearance; DDI, drug-drug interaction; *F*, bioavailability; V_{ss}, volume of distribution at steady state

[a]Ratios are calculated by digitization of published average plasma concentration-time profiles and performing non-compartmental analysis

[b]AUC or CL was calculated with the equation $AUC = dose / CL$ using known dose and reported values of CL or AUC

[c]Terminal half-life increased 2.4-fold following IV dosing and 3.6-fold following oral dosing. Estimates of the changes in F and CL would not have been accurate by using the change in oral terminal half-life and Eq. 1

Table 4: Utilization of Proposed Methodology to Discriminate Clearance (CL) from Bioavailability (F) Changes for Orally Dosed Midazolam (Victim) and Multiple Dosed Rifampin (Perpetrator; 600 mg QD, 14 Days) from the Study of Kirby et al.[16]

Victim	Perpetrator	$\frac{AUC^{DDI}}{AUC^{Control}}$	Percent AUC Extrapolation (DDI/Control)	$\frac{V_{ss}/F^{DDI}}{V_{ss}/F^{Control}}$	$\frac{V_{ss}{}^{DDI}}{V_{ss}{}^{Control}}$	$\frac{F^{DDI}}{F^{Control}}$	$\frac{CL/F^{DDI}}{CL/F^{Control}}$	$\frac{CL^{DDI}}{CL^{Control}}$
Midazolam (IV)	Rifampin	Observed: 0.44	Observed: 4% / 3%[a]	–	Observed: 1.19[a]	Observed: 0.19	–	Observed: 2.16
Midazolam (Oral)	Rifampin	Observed: 0.081	Observed: 6% / 5%[a]	Observed: 3.93[a]	Assumed: 1	Estimated: 0.25[b]	Observed: 11.7	Estimated: 2.93[b]

Pharmacokinetic values reported in the table are based on published average values, unless otherwise noted
Abbreviations: *AUC*, area under the curve; *CL*, clearance; DDI, drug-drug interaction; *F*, bioavailability; V_{ss}, volume of distribution at steady state
[a]Ratios are calculated by digitization of published average plasma concentration-time profiles and performing non-compartmental analysis
[b]Terminal half-life decreased by 39% following IV dosing and by 74% following oral dosing. Estimates of the changes in F and CL would be significantly poorer and inaccurate using Eq. 1

Table 5: Utilization of Proposed Methodology to Discriminate Clearance (CL) from Bioavailability (F) Changes for Orally Dosed Apixaban (Victim) and Rifampin (Perpetrator) from the Study of Vakkalagadda et al.[17]

Victim	Perpetrator	$\frac{AUC^{DDI}}{AUC^{Control}}$	Percent AUC Extrapolation (DDI/Control)	$\frac{V_{ss}/F^{DDI}}{V_{ss}/F^{Control}}$	$\frac{V_{ss}{}^{DDI}}{V_{ss}{}^{Control}}$	$\frac{F^{DDI}}{F^{Control}}$	$\frac{CL/F^{DDI}}{CL/F^{Control}}$	$\frac{CL^{DDI}}{CL^{Control}}$
Apixaban (IV)	Rifampin (Multiple Dose)	Observed: 0.61	Observed: 1% / 2%	–	Observed: 0.87	Observed: 0.76	–	Observed: 1.64
Apixaban (Oral)	Rifampin (Multiple Dose)	Observed: 0.48	Observed: 10% / 9%	Observed: 1.42[a]	Assumed: 1	Estimated: 0.70[b]	Observed: 2.14	Estimated: 1.50[b]

Pharmacokinetic values reported in the table are based on published average values, unless otherwise noted
Abbreviations: *AUC*, area under the curve; *CL*, clearance; DDI, drug-drug interaction; *F*, bioavailability; V_{ss}, volume of distribution at steady state
[a]Ratios are calculated by digitization of published average plasma concentration-time profiles and performing non-compartmental analysis
[b]Terminal half-life decreased by 49% following IV dosing but slightly increased 1.03-fold following oral dosing. Estimates of the changes in F and CL would be significantly poorer and inaccurate using Eq. 1.