Impact of Ignoring Extraction Ratio When Predicting Drug-Drug Interactions, Fraction Metabolized, and Intestinal First-Pass Contribution

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ABSTRACT:

Many mathematical models for in vitro to in vivo prediction of drug-drug interactions (DDIs) of orally administered victim drugs have been developed. However, to date, none of these models have been applicable to all intravenously administered victim drugs. We derived and conducted a sensitivity/error analysis of a modification to the existing multiple mode interaction prediction model such that it is applicable to all intravenously administered victim drugs. Using this model we showed that ignoring the hepatic extraction ratio (EH) (as low as 0.3) of intravenously administered victim drugs can result in 1) substantial underestimation of $f_{m,CVN}$ (the fraction of hepatic clearance of the victim drug via a given enzymatic pathway) and 2) error in dissecting the contribution of intestinal and hepatic components of DDIs for orally administered drugs. Using this model we describe DDI boundaries (degree of inhibition or induction) at which ignoring the EH of commonly used victim drugs results in $\geq30\%$ error in the predicted area under the concentration-time curve (AUC) ratio or contribution of intestinal interaction to a DDI (CYP3A probes only). For the most widely used victim drug midazolam, these boundaries for AUC ratio are net inhibition ($\Delta/k_i$ or $\Delta/k_{ind}$) $\geq1.3$ or fold induction $\geq2.1$; for intestinal contribution the boundaries are 0.37 and 1.5, respectively. To accurately predict the intravenous AUC ratio, intestinal contribution, or $f_{m, CVN}$ for all induction DDIs irrespective of EH of the victim drug and 2) for modest to potent inhibition DDIs even when the EH is moderate ($\geq0.3$), we propose that our model be used.

Introduction

The concept of hepatic clearance has been described by either a well stirred or a parallel tube model (Rowland et al., 1973; Pang and Rowland, 1977). Through many iterations (Ito et al., 1998; Mayhew et al., 2000; Ernest et al., 2005; Venkatakrishnan and Obach, 2005; Obach et al., 2006; Shou et al., 2008), this concept has been applied to predict the change in clearance and, therefore, AUC of a victim drug in the presence and absence (AUC ratio, AUC/AUC) of a perpetrator drug operating via enzyme inhibition, inactivation, and/or induction. The culmination of these iterations has resulted in a combined model that accounts for all three of these mechanisms in both the intestine and the liver (Fahmi et al., 2008). All of these models have been derived for oral administration of the victim drug, for which the AUC ratio is directly proportional to the change in intrinsic clearance. This type of model can be applied to the intravenous administration of a victim drug without error if the victim drug can be assumed to have a very low hepatic extraction ratio (EH). For moderate to high EH drugs, either predictions have been made with the assumption that the drug is a low EH drug (Ito et al., 1998; Ernest et al., 2005; Fahmi et al., 2008; Shou et al., 2008) or a ratio of the full well stirred model has been used, necessitating an estimate of the true hepatic intrinsic clearance of the victim drug (Ibrahim et al., 2003). This second approach does account for the blood flow limitations of the well stirred model but requires more laborious calculations and generation of a unique relationship between change in intrinsic clearance and AUC ratio for each victim drug (Ibrahim et al., 2003). Many of the victim drugs used in drug-drug interaction (DDI) studies are moderate (EH $\geq0.3$) to high (EH $\geq0.7$) extraction ratio drugs: midazolam, 0.44 (Thummel et al., 1996); alfentanil, 0.28 (Kharasch et al., 2004); desipramine, 0.56 (Ciraulo et al., 1985, 1988); and metoprolol, 0.69 (Patrick, 2002). Therefore, we set out to develop a more generally applicable prediction model that does not assume a low EH, but retains the full well stirred model of hepatic clearance and is therefore applicable to the intravenous administration of a victim drug in the presence of a perpetrator drug.

Intravenous administration of victim drugs is used for a multitude of reasons including 1) determining the contribution of hepatic and/or intestinal enzyme inhibition or induction by a precipitant drug in a DDI study (Holtbecker et al., 1996; Kharasch et al., 2004; Galetin et al., 2010) and 2) determining the fraction of hepatic metabolism of a victim drug by a specific enzyme ($f_{m, CYP}$) (Shou et al., 2008). In these situations, if the EH of the victim drug is not accounted for, the inherent error described above is propagated to the parameters gleaned from these investigations. Therefore, using our generally
applicable model we were able to investigate the impact that this simplification has on the parameters determined. The magnitude of this error will vary based on the specific pharmacokinetic parameters of the victim drug (e.g., EH and $f_{Clint/CYP}$) and the severity of the DDI (degree of inhibition or induction). Therefore, we determined boundaries outside which the simplification of ignoring the EH of the victim drug has a significant effect (≥30%) on the predicted AUC ratio, estimated $f_{m/CYP}$, and the DDI contribution of intestinal enzyme alteration for some of the most common intravenously administered victim drugs. We found that for most of the commonly used probe drugs, ignoring the EH will incorporate ≥30% error under moderate enzyme inhibition ($K_I$ or $k_{deg} = 1–10$) or induction (~3-fold). This mathematical error can be eliminated by using the slightly more complex yet easily manageable model proposed here.

In this report, we first present a comprehensive model that can be used to predict multiple mode DDIs, irrespective of the EH of the victim drug. Then, we present a sensitivity analysis of the model as well as practical guidelines on when this comprehensive model should be used.

Materials and Methods

Modified Model for Intronously Administered Drugs. The AUC ratio ($AUC_{IV}/AUC_{IV}$) of a victim drug in the presence and absence of a perpetrator drug can be described by eq. 1, assuming that only hepatic clearance ($C_{h}$) of the victim drug is affected but hepatic blood flow ($Q$), unbound fraction ($f_u$), and the renal clearance ($C_{renal}$) are not.

$$\frac{AUC_{IV}}{AUC_{IV}} = \frac{Cl_{h} + Cl_{renal}}{Cl_{h} + Cl_{renal}} = \frac{1 - f_{Clint}}{f_{Clint}} \cdot \frac{Cl_{hep}}{Cl_{h} + Cl_{renal}} = \frac{1}{f_{Clint}} \cdot \frac{Cl_{hep}}{Cl_{h} + Cl_{renal}}$$

where $f_{Clint}$ is the fraction of intrinsic clearance remaining as a result of the net effect of the perpetrator drug ($Cl_{h}$ is $f_{Clint}$, $Cl_{hep}$ is the fraction of hepatic clearance that is mediated by the affected enzyme, $Cl_{renal}$ is the intrinsic clearance of the victim drug in the presence and absence of the perpetrator).

$$\frac{Cl_{hep}}{Cl_{h} + Cl_{renal}} = \frac{Q \cdot f_{Clint} \cdot Cl_{IV}}{Q + f_{Clint} \cdot Cl_{IV}} = \frac{(Q f_{Clint} + 1)}{(Q f_{Clint} + 1)} \cdot \frac{Cl_{hep}}{Cl_{h} + Cl_{renal}}$$

An equation similar to the first half of eq. 2 has been described previously (Shou et al., 2008) but was simplified to either assume very low EH ($Cl_{hep} = Cl_{renal}$) or high EH ($Cl_{hep} = Q$). A rearrangement of the well stirred model $Cl_{hep} = Q \cdot EH = [(Q f_{Clint} + 1)/Q f_{Clint} + 1]$ reveals that $(Q f_{Clint} + 1)$, which can be substituted into eq. 2 to give eq. 3 in which $f_{Clint}^{\text{hep}}$ is the fraction of hepatic intrinsic clearance remaining as a result of the net effect of the perpetrator drug ($Cl_{hep} = f_{Clint}^{\text{hep}}$, $Cl_{renal}$).

$$\frac{Cl_{hep}}{Cl_{h} + Cl_{renal}} = \frac{(Q f_{Clint} + 1)}{(Q f_{Clint} + 1)} \cdot \frac{Cl_{hep}}{Cl_{h} + Cl_{renal}} = \frac{Cl_{hep}}{Cl_{h} + Cl_{renal}}$$

where $f_{Clint}^{\text{hep}}$ is the product of the fraction of intrinsic clearance remaining as a result of inactivation ($f_{\text{inactivation}}^{\text{hep}}$, eq. 4), induction ($f_{\text{induction}}^{\text{hep}}$, eq. 5), and inhibition ($f_{\text{inhibition}}^{\text{hep}}$, eq. 6), which are analogous to the A, B, and C terms, respectively, described previously (Fahmi et al., 2008).

$$f_{\text{inactivation}}^{\text{hep}} = \frac{1}{1 + \lambda K_{I}} = \frac{1}{1 + \frac{i}{k_{deg}(I + K_{I})}}$$

(4)

$$f_{\text{induction}}^{\text{hep}} = \frac{1}{1 + \frac{d \cdot \text{Indmax} \cdot f_{u}}{I_{u} + EC_{50}}}$$

(5)

$$f_{\text{inhibition}}^{\text{hep}} = \frac{1}{1 + f_{u}/K_{i}}$$

(6)

where $\lambda$ is the predicted inactivation rate of the enzyme, $I_u$ is the unbound perpetrator drug concentration for which the plasma unbound concentration (maximum, average, or maximum portal vein) has been used as a surrogate (Ito et al., 2004; Obach et al., 2006), $k_{deg}$ is the maximum inactivation rate constant, $K_i$ is the concentration that produces half-maximum inhibition, $f_{u}$ is the normal degradation rate constant of the affected enzyme in the liver, $d$ is the in vitro to in vivo scaling factor for induction, $\text{Indmax}$ is the maximum fold induction of mRNA of the affected enzyme, $EC_{50}$ is the concentration that results in half-maximal induction, and $K_i$ is the inhibition constant. Equation 3 can then be substituted back into eq. 1 to arrive at eq. 7, which describes the AUC ratio of a victim drug administered intravenously in which the entire hepatic clearance is a result of one enzymatic process ($f_{m/CYP} = 1$).

$$\frac{AUC_{IV}}{AUC_{IV}} = \frac{1}{f_{Clint}} \cdot \frac{Cl_{hep}}{Cl_{h} + Cl_{renal}} = \frac{1}{f_{Clint}} \cdot \frac{1}{f_{u} \cdot Cl_{IV}(I_{u} + K_{I}) + 1}$$

(7)

To describe the hepatic clearance of a victim drug that is a result of multiple enzymatic processes ($\rho$), the inclusion of an $f_{m/CYP}$ term, which is the fraction of the hepatic clearance of the victim drug that is a result of the $m$th enzyme is required and results in eq. 8.

$$\frac{AUC_{IV}}{AUC_{IV}} = \frac{1}{f_{Clint}} \cdot \frac{Cl_{hep}}{Cl_{h} + Cl_{renal}} = \frac{1}{f_{Clint}} \cdot \frac{1}{f_{u} \cdot Cl_{IV}(I_{u} + K_{I}) + 1}$$

(8)

When the EH in eq. 8 is set to a very small value (very low extraction ratio drug) the equation will collapse to an equation similar to that described by Fahmi et al. (2008) with the inclusion of the $f_{m/CYP}$ term and devoid of the term addressing the change in intestinal intrinsic clearance.

Sensitivity and Error Analysis of Key Prediction Parameters. The main determinants of the AUC ratio as calculated in eq. 8 can be separated into five major variables: $f_{Clint}$ (the fraction of IV clearance that is hepatic elimination), $f_{m/CYP}$ (the fraction of hepatic clearance that is mediated by the affected enzyme), $f_{\text{inactivation}}^{\text{hep}}$ (the combined effect of inactivation, induction, and inhibition or net change in intrinsic clearance), EH (hepatic extraction ratio of the victim drug), and other nonhepatic clearance mechanisms such as renal clearance denoted by ($1 - f_{\text{hep}}$). The contribution of some of these parameters such as $f_{m/CYP}$, other clearance pathways, and enzyme degradation half-life (implicit to inactivation) to the AUC ratio have been evaluated and will not be addressed further (Venkatakrishnan and Obach, 2005; Galetin et al., 2006). A major focus of this article is to describe the impact of not accounting for victim drug hepatic extraction ratio on AUC predictions. Therefore, we conducted a sensitivity/error analysis to evaluate the contribution of $f_{\text{inactivation}}^{\text{hep}}$, $f_{m/CYP}$, EH, and net change in intrinsic clearance ($f_{\text{inhibition}}^{\text{hep}}$) to the error in predicted AUC ratio using a No EH model (where EH was set to a very low number $1 \times 10^{-10}$) and the EH model in which the EH was accounted for. The percent error in the predicted AUC ratio as a result of using the No EH model was calculated by eq. 9.

$$\frac{\text{percent error}}{\text{error}} = \frac{1}{f_{Clint}} \cdot \frac{\text{predicted AUC ratio}}{\text{true AUC ratio}} = \frac{1}{f_{Clint}} \cdot \frac{1}{f_{u} \cdot Cl_{IV}(I_{u} + K_{I}) + 1} \cdot \left(1 - f_{\text{hep}}\right)$$

(9)
% error = \left(\frac{(\text{AUCRatio}_{\text{No EH Model}}) - (1 - F_{\text{EH}})}{(\text{AUCRatio}_{\text{No EH Model}})}\right) \times 100\% \tag{9}

Because this model accounts for inactivation, induction, and inhibition, it is important to note that these three terms are combined in a multiplicative fashion as justified previously (Fahmi et al., 2008). Therefore, if a precipitant elicits interactions through all three mechanisms, the overall effect on intrinsic clearance is the product of the inactivation ($f_{\text{Clint}}^\text{Hep}$), fold induction ($f_{\text{Clint}}^\text{Hep}$), and reversible inhibition ($f_{\text{Clint}}^\text{Hep}$) terms. For example, if a precipitant has $f_{\text{Clint}}^\text{Hep}$ of 0.2 (1 + $L/K_{c}$ = 5), $f_{\text{Clint}}^\text{Hep}$ of 5 (5-fold induction), and $f_{\text{Clint}}^\text{Hep}$ of 0.2 (1 + $L/K_{c}$ = 5), the net result is that the intrinsic clearance will decrease to one-fifth ($f_{\text{Clint}}^\text{Hep}$ = 0.2 · 5 · 0.2 = 0.2) of the basal intrinsic clearance. Historically the potencies of inactivation, induction, and inhibition have been quantified by $L/K_{c}$ fold increase in enzyme expression, and 1 + $L/K_{c}$, respectively. Because these terms are grouped, we conducted the sensitivity/error analysis with respect to the value of the product ($f_{\text{Clint}}^\text{Hep}$), which can be easily thought of as the fold change in intrinsic clearance or fraction of intrinsic clearance remaining as a result of the interaction. A value >1.0 implies a net induction, and a value <1.0 implies a net inhibition or inactivation.

Impact of EH on Estimating Intestinal Contribution to DDIs. A more thorough understanding of intestinal metabolism and its contribution to DDIs has increased predictability of DDIs (Galetin, 2007; Galetin et al., 2007). One method that has been used to dissect the contribution of intestinal drug-metabolizing enzymes to DDIs, i.e., the intravenous and oral administration of a victim drug, was recently reviewed (Galetin et al., 2010). The degree of interaction observed with the intravenously administered victim drug is dissected from the interaction observed after oral administration of the object drug to yield the magnitude of interaction in the intestine. This can be done by solving a modified version of the model of Fahmi et al. (2008) (in which the $f_{\text{Clint}}^\text{GI}$ term is the net effect on intestinal intrinsic clearance, similar to the $f_{\text{Clint}}^\text{Hep}$ term for hepatic intrinsic clearance), namely, eq. 10 for $f_{\text{Clint}}^\text{GI}$ as shown in eq. 11.

$$\frac{\text{AUC}_{\text{PO}}}{\text{AUC}_{\text{PO}}} = \left(\frac{1}{f_{\text{Clint}}^\text{GI} \times f_{\text{m}} + (1 - f_{\text{m}})}\right) \times \left(\frac{1}{f_{\text{Clint}}^\text{Hep} \times (1 - F_{\text{EH}}) + F_{\text{EH}}}\right) \tag{10}$$

$$\frac{\text{AUC}_{\text{PO}}}{\text{AUC}_{\text{GI}}} \cdot \frac{f_{\text{Clint}}^\text{GI} \cdot f_{\text{m}} - f_{\text{m}} - f_{\text{m}} \cdot (1 - f_{\text{m}})}{f_{\text{m}} - f_{\text{m}} \cdot (1 - f_{\text{m}})} \tag{11}$$

The piece of information needed from the intravenously administered interaction is the fold change in hepatic intrinsic clearance ($f_{\text{Clint}}^\text{Hep}$). We determined the impact that incorrectly determining the fold change in hepatic intrinsic clearance using the No EH model has on the estimate of the intestinal interaction. To do this, we generated intravenous and oral AUC ratios using eqs. 8 and 10, respectively, for a victim drug with $f_{\text{m}}$ = 1.0, $f_{\text{m}}$ = 0.95, and $F_{\text{EH}}$ = 0.5 (values similar to those of midazolam, a widely used CYP3A victim drug) with EH ranging from 0.1 to 0.9 by setting the fold change in hepatic ($f_{\text{Clint}}^\text{Hep}$) and intestinal ($f_{\text{Clint}}^\text{GI}$) intrinsic clearance equal and varying the values from 25 to 0.0001 (25-fold induction to 99.99% inhibition). The intravenous AUC ratios were then used to estimate the fold change in hepatic intrinsic clearance using a rearrangement of the No EH model (eq. 12).

$$f_{\text{Clint}}^\text{Hep} = \frac{\left(\frac{\text{AUC}_{\text{GI}}}{\text{AUC}_{\text{PO}}} - (1 - f_{\text{EH}})\right)}{f_{\text{m}} - f_{\text{m}} \cdot (1 - f_{\text{m}})} \tag{12}$$

The No EH model determined fold change in hepatic intrinsic clearance values along with the generated oral AUC ratios were used in eq. 11 to estimate the fold change in intestinal intrinsic clearance ($f_{\text{Clint}}^\text{GI}$) and the $F_{\text{GI}}$ ratio (eq. 13).

$$\frac{F_{\text{GI}}}{F_{\text{H}}} = \frac{1}{f_{\text{Clint}}^\text{GI} \times (1 - F_{\text{EH}}) + F_{\text{EH}}} \tag{13}$$

These calculated values were compared with the parameter values, e.g., $F_{\text{H}}$ values that were used to generate the AUC ratios. Percent error was calculated for the value determined using the No EH model relative to the true value. A similar analysis was done to show the impact of $F_{\text{GI}}$ on this error by varying $F_{\text{EH}}$ from 0.1 to 0.75 using a fixed EH of 0.25. This low EH of 0.25 (at the lower boundary of having a significant impact on the intravenous AUC ratio) was chosen to highlight the fact that a significant impact on $F_{\text{GI}}$ can be observed when there is minimal impact on intravenous AUC ratio. Mixed interactions (net induction in the liver and net inhibition in the intestine or vice versa) were evaluated using the simulation approach listed above to determine the boundaries at which net induction or inhibition in the intestine could be masked by net inhibition or induction in the liver, respectively.

Impact of EH on Estimating $f_{\text{m}}$, CYPi. One method historically used to estimate $f_{\text{m}}$, CYPi (the fraction of the victim drug metabolized by a specific enzyme) is to administer the victim drug intravenously in the presence and absence of a very potent inhibitor (Shou et al., 2008). Based on the maximum observed AUC ratio, $f_{\text{m}}$, CYPi, is calculated using eq. 14.

$$\frac{\text{AUC}_{\text{IV}}}{\text{AUC}_{\text{H}}} = \frac{1}{1 - f_{\text{m}} \cdot \text{CYPi}} - 1 \tag{14}$$

Because this equation does not account for the blood flow-limited nature of hepatic clearance or nonhepatic elimination of the probe drug, the true $f_{\text{m}}$, CYPi is underestimated. To account for these shortcomings of eq. 14, eq. 8 was rearranged to eq. 15 by assuming that $f_{\text{Clint}}^\text{Hep}$ was negligible (complete inhibition) and solving for $f_{\text{m}}$, CYPi.

$$f_{\text{m}} \cdot \text{CYPi} = 1 \tag{15}$$

To show the magnitude of underestimation of $f_{\text{m}}$, CYPi, by eq. 14, intravenous AUC ratios were first simulated using eq. 8 for victim drugs with varying $f_{\text{m}}$, CYPi values, across a range of extraction ratios from 0.001 to 1.0, assuming complete inhibition ($f_{\text{Clint}}^\text{Hep}$ = 0), and $f_{\text{m}}$ was assumed to be 1.0 (negligible nonhepatic elimination). These simulated AUC ratios were then used to estimate the $f_{\text{m}}$, CYPi values using eq. 14 (the No EH model). The impact of the error in $f_{\text{m}}$, CYPi determined by the No EH model would be greatest when the victim drug was given orally in the presence of complete inhibition. Therefore, we determined the percent error in the predicted maximum AUC ratio (assuming $f_{\text{Clint}}^\text{Hep}$ = 0) calculated using eq. 10 with the $f_{\text{m}}$, CYPi, estimated by the No EH model (eq. 14) and the $f_{\text{m}}$, CYPi value used for the simulation. For simplicity no nonhepatic extraction was assumed for the simulation.

DDI Boundaries Where the Impact of EH Is Greatest. Because of the multiple parameters that contribute to the impact of ignoring EH, it is difficult to immediately discern benchmarks at which ignoring the EH has a significant impact on accurately predicting DDIs. Therefore, we compiled the necessary parameters for some commonly used victim drugs across a range of EH (Table 1). For those drugs for which $f_{\text{m}}$ was not readily available, a value of 1.0 was assigned. Then, using the modified model, we determined the DDI boundaries, degree of induction or inhibition, that would result in ≥30% error in the predicted AUC ratio (for all victim drugs) or the contribution of intestinal enzymes to the DDI (CYP3A drugs only, following the same assumptions as in the section Impact of EH on Estimating Intestinal Contribution to DDIs).

Results

Sensitivity/Error Analysis. A concise summary of the sensitivity/error analysis is provided here (for a comprehensive analysis, the reader is referred to the supplemental data). In brief, the impact of ignoring EH on the predicted AUC ratio of an intravenously administered victim drug is dependent on $f_{\text{m}}$, CYPi, EH, and the degree of the interaction (inhibition or induction). The minor nonhepatic clearance mechanisms ($f_{\text{m}}$, CYPi > 0.9, which is the case for many victim drugs) (Table 1), have a measureable impact (≥30% error) on the predicted intravenous AUC ratio and should be taken into account when this ratio is predicted in the presence of potent inhibition.
Impact of EH on Estimating Intestinal Contribution to DDIs.

Figure 1, A through C, shows the impact of ignoring the hepatic extraction ratio of an intravenously administered victim drug on the estimation of the intestinal interaction when an intravenous and oral dose of the victim drug ($F_G = 0.5$) is used to dissect the intestinal and hepatic contribution to a DDI. The percent error in the estimated fraction of intestinal clearance remaining ($f_{	ext{Clint}}^{\text{net}}$) is shown to be greatly overpredicted (Fig. 1A), by as much as $\sim 140\%$ for a net induction of 5-fold with a victim drug EH of 0.3. In contrast, when the DDI is a net inhibition interaction ($f_{	ext{Clint}}^{\text{net}}$ or $f_{	ext{Hep}}^{\text{net}} < 1$), the predicted intestinal interaction is greatly underestimated ($\sim 280\%$ for $f_{	ext{Clint}}^{\text{net}} = 0.1$ with a victim drug EH of 0.3). This error in predicted fold change in fraction of intestinal clearance remaining is also shown as percent error in the predicted change in intestinal bioavailability, or $F_G/F_G$ ratio (Fig. 1B). Expressed as the $F_G/F_G$ ratio, the net induction interactions are underpredicted and the net inhibition interactions are overpredicted.

The degree of under- or overprediction is dependent on EH and fold induction or inhibition, respectively. For net inhibition interactions, the predicted $F_G$ is always higher than the true $F_G$ and as the degree of inhibition and the EH of the victim drug increase, this difference is larger. For example, the predicted $F_G$ will erroneously increase to a value greater than 1.0, which is not possible. The predicted $F_G$ becomes overpredicted by $\sim 30\%$ ($F_G > 1.3$) when net inhibition is $\geq 90\%$ for a victim drug with EH $\geq 0.35$ (Fig. 1C). When the DDI is a net induction interaction, intestinal induction is overpredicted, resulting in predicted $F_G$ values substantially less than the true value.

The percent error in predicted $f_{	ext{Clint}}^{\text{net}}$ for victim drugs with varying $F_G$ values assuming a fixed EH of 0.25 (typically thought of as low EH) is shown in Fig. 2A and is heavily dependent on $F_G$, but the percent error in the predicted $F_G/F_G$ ratio is not dependent on the value of $F_G$ (Fig. 2B). Likewise, the degree of inhibition at which the predicted $F_G$ value will exceed 30% of the theoretical maximum (1.0) is largely independent of $F_G$.

The results above show that when the EH of the victim drug is not accounted for, net induction in the liver can result in a perceived greater induction in the intestine and net inhibition in the liver can result in a perceived greater degree of inhibition in the intestine. Therefore, it is possible, if the EH of the victim drug is ignored, that induction in the liver could mask inhibition in the intestine and inhibition in the liver could mask induction in the intestine. With the hypothetical victim drug described above ($f_{\text{Hep}} = 1.0, f_{\text{iso}}^{\text{CYP}} = 0.95$, and $F_G = 0.5$), we used the simulation approach described above to determine the EH and fraction of hepatic intrinsic clearance remaining ($f_{\text{Clint}}^{\text{net}}$), values that would be necessary to mask either a 2-fold induction or 100-fold inhibition in the intestine.

Ignoring the EH of a victim drug when it is 0.35 and $f_{\text{Hep}}^{\text{CYP}}$ is 0.1 will completely mask ($F_G = 1.0$ or no change in $f_{\text{Clint}}^{\text{net}}$) a 2-fold induction in the intestine ($f_{\text{Clint}}^{\text{net}} = 2$). As the EH of the victim drug increases, the degree of hepatic inhibition necessary to mask intestinal induction will decrease. A dependence on $F_G$ was also observed. As $F_G$ increased above 0.5 (less intestinal extraction), a lower value of EH and less potent inhibition in the liver was necessary for this
masking to occur. If $F_G$ was smaller than 0.5 (more intestinal extraction), a higher EH and more potent inhibition in the liver was necessary to produce this masking.

To completely mask a 100-fold inhibition in the intestine ($f_{Cl,int}^{G} = 0.01$) by ignoring the hepatic EH, the hypothetical victim drug described above must have an EH of 0.25 or greater and hepatic net induction of 5-fold or greater. As the EH of the victim drug increases, the fold induction in the liver necessary to mask the intestinal inhibition will decrease. In addition, as $F_G$ was increased above 0.5, a lesser degree of hepatic induction was necessary to mask the 100-fold inhibition in the intestine. As $F_G$ was decreased (greater intestinal extraction), a greater degree of hepatic induction was necessary to produce this masking.

**Impact of EH on Estimating $f_{in,CYPi}$**

Estimated fractions of hepatic clearance due to a specific enzymatic pathway ($f_{in,CYPi}$) calculated using eq. 14 (No EH model) are shown in Fig. 3A across an EH range of 0.01 to 0.9 for simulated victim drugs with true $f_{in,CYPi}$ values of 0.5 to 0.95. As expected, as the EH of a victim drug increases, the estimated $f_{in,CYPi}$ calculated by the No EH model decreases. To better illustrate the impact of the underprediction of $f_{in,CYPi}$, we calculated the percent error in the predicted maximum AUC ratio when the victim drug is given orally, assuming no intestinal extraction (Fig. 3B). The slope of this curve is larger for the victim drugs with higher $f_{in,CYPi}$ values and shows a linear relationship dependent on EH. Therefore, the magnitude of this error can be directly calculated as percent error $= -f_{in,CYPi} \cdot EH \cdot 100$. The incorrect $f_{in,CYPi}$ and percent error in the maximum predicted oral AUC ratio for commonly used victim drugs are listed in Table 1. For low EH drugs, this error is minimal, but for high EH drugs, the error is also dependent on the magnitude of $f_{in,CYPi}$. For example, the error in the maximum predicted oral AUC ratio for the high EH drugs metoprolol (0.84) and imipramine (0.70) is −70 and −32%, respect-
intensively. This result shows that the impact of ignoring EH when one is determining $f_{m,CYP}$ is dependent not only on the EH of the victim drug but also on the magnitude of $f_{m,CYP}$.

**DDI Boundaries Where the Impact of EH Is Greatest.** Table 1 shows the empirically determined DDI boundaries at which there is a $\geq 30\%$ error in the AUC ratio or contribution of intestinal interaction to a DDI as a result of ignoring EH of the victim drug when administered intravenously. For the widely used CYP3A victim drug midazolam, a net inhibition interaction with $IIK_i$ of $\geq 1.3$ or fold induction $\geq 2.1$ will give rise to greater than $30\%$ error in the predicted intravenous AUC ratio if the modified model described here is not used. The boundaries for $\geq 30\%$ error in the contribution of intestinal DDIs for midazolam are lower than those of the AUC ratio, 0.37 and 1.5, for inhibition and induction, respectively. These boundaries are determined in a large part by $f_{m,CYP}$, $F_G$, and EH with a much smaller contribution from $f_{hep}$. Varying $f_{hep}$ from 1.0 to 0.95 resulted in only minor changes ($<10\%$ increase) in the DDI inhibition boundary for the victim drugs with high ($>0.80$) $f_{m,CYP}$ and $\sim 20\%$ increase for the drugs with low $f_{m,CYP}$ (imipramine and metoprolol). The DDI boundaries for induction were not sensitive ($<5\%$ change) to changing $f_{hep}$ from 1.0 to 0.95. In general, except for the two very low EH drugs (tolbutamide and warfarin) or the low $f_{m,CYP}$ drug propranolol, ignoring the EH of the victim drug will produce an error of $\geq 30\%$ in the presence of moderate inhibition or modest induction.

**Discussion**

Mathematical models used to predict the AUC ratio of a victim drug as a result of a DDI have evolved to include multiple modes of interaction (enzyme inactivation, induction, and inhibition). These models have been derived for the oral administration of a victim drug, which is the most common route of drug administration. These models have been used, based on a simplifying assumption (low EH) for the
intravenous administration of victim drugs even though the victim
drug is in fact not a low EH drug. We developed a comprehensive, yet
easily manageable model that does not rely on the assumption of the
victim drug having a low EH. Using this model, we evaluated the
error that is introduced when one is 1) predicting the AUC ratio,
2) estimating $f_{m, \text{CYPi}}$, and 3) estimating the contribution of intestinal
enzymes to a DDI, by making the assumption that the victim drug is
low EH when in fact it is not.

In our sensitivity analysis section (supplemental data), we described
the impact that ignoring EH of the victim drug has on the predicted
AUC ratio of that drug as a result of a DDI. We showed that the
magnitude of error is dependent on the EH, $f_{m, \text{CYPi}}, f_{\text{hep}},$ and mag-
nitude of the DDI. We also set boundaries of DDI magnitudes (degree
of inhibition or induction) for which the impact of ignoring the victim
drug EH is minimal for commonly used victim drugs (Table 1). These
boundaries indicate that for most victim drugs (except those of very
low EH, e.g., tolbutamide and warfarin, or low $f_{m, \text{CYPi}},$ e.g., propran-
olol) inhibition interactions with $I/K_i$ between 0.5 and 9 and induction
interactions of $<3$-fold will be susceptible to $\geq 30\%$ error in the AUC
ratio by ignoring EH of the victim drug.

With respect to the determination of the $f_{m, \text{CYPi}}$ of a victim drug
administered intravenously, a concept that is not readily apparent from
our analyses is the fact that the contribution of the EH cannot be
overcome in the presence of "complete metabolic inhibition," which must

![Fig. 3. Impact of EH on estimating $f_{m, \text{CYPi}}$. A, when the $f_{m, \text{CYPi}}$ of
a victim drug is determined by intravenous administration of the
drug in the presence and absence of potent inhibition, ignoring the
EH of the victim drug affects the estimated $f_{m, \text{CYPi}},$ and the error in
this parameter is a function of both EH and $f_{m, \text{CYPi}}.$ B, the impact
of this error is most apparent in the percent error in the maximum
oral AUC ratio. This error is linear with respect to EH with a slope
equal to $-f_{m, \text{CYPi}} \cdot 100$ and therefore can be directly calculated as
percent error $= -f_{m, \text{CYPi}} \cdot \text{EH} \cdot 100.$]
be assumed to estimate \( f_{m, 	ext{CYP}} \). This implies that if the \( f_{m, 	ext{CYP}} \) is to be determined by intravenous administration of the victim drug in the presence of a potent inhibitor, the EH of the victim drug must be considered. Consider the situation in which the EH of the victim drug is 0.44, \( f_{m, 	ext{CYP}} = 1.0 \), and \( f_{m, 	ext{CYP}} = 0.93 \) (a situation similar to that of midazolam). If we assume that hepatic flow is 1.5 l/min, the observed hepatic clearance (\( C_{\text{hep}} \)) using the well stirred model is 0.66 l/min, but the total hepatic intrinsic clearance is 1.18 l/min. Of this intrinsic clearance, 93% (1.097 l/min) is via the inhibited pathway (e.g., CYP3A) and 7% (0.083 l/min) is via the unaffected pathway. When CYP3A is completely inhibited, the remaining \( C_{\text{hep}} = 0.079 \) l/min (using the well stirred model with \( C_{\text{hep}} = 0.083 \) l/min, the unaffected pathway). This would result in an observed AUC ratio of 8.4. The incorrect \( f_{m, 	ext{CYP}} \) calculated using this AUC ratio would be 0.88 using a rearrangement of eq. 14. If the equation accounting for the EH of the victim drug were used (eq. 15), the resulting estimated \( f_{m, 	ext{CYP}} \) would be the true value of 0.93. Even though the error in the estimated \( f_{m, 	ext{CYP}} \) (0.88 versus 0.93) seems to be minor, it would equate to an observed maximum AUC ratio when the drug was orally administered in the presence of complete inhibition (assuming no intestinal involvement) of 8.4 versus 14.3 or \( \approx 41\% \). Our analysis showed that this underestimation can be directly calculated as \( 1 - f_{m, 	ext{CYP}} \cdot \text{EH} \cdot 100 \). Moreover, a comparison of the observed maximum intravenous AUC ratio (8.4) with the observed maximum oral AUC ratio (14.3) would imply contribution of intestinal extraction of \( \approx 40\% \) when none was assumed in this example. We have calculated this error for the victim drugs listed in Table 1. For the CYP2D6 drugs desipramine and metoprolol, an incorrect intestinal extraction of \( \approx 40 \) to 70% may be implied if EH is ignored in an inhibition DDI study.

The previous example shows how intestinal extraction may be implied when in fact it is not present. If intestinal extraction is present, which is the case for midazolam and many of the other CYP3A drugs, the contribution of intestinal enzymes to the DDI can be greatly misestimated if the EH of the victim drug is not accounted for. For a net hepatic induction interaction, if the EH of the victim drug is not accounted for, even for low EH drugs, the fold induction in the liver is underestimated, and this error is propagated to the intestine, resulting in an overestimation of the intestinal induction (Fig. 1A). In a DDI study between nifedipine and rifampin (Holtbecker et al., 1996), a selective inhibition of warfarin metabolism by dietizam in humans. J Pharmacol Exp Ther 287:411–415.


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Sensitivity/Error Analysis Results:

Figure S1 and S2 show the results of the sensitivity and error analysis. Notice the X axes (fraction of clearance remaining) in all Figures are on a logarithmic scale. The analysis presented below is conducted over the range of parameters (EH {0.1-0.9}, f_{hep} {0.5-1.0} and f_{m,CYPi} {0.5-1.0}) that are observed for many of the commonly used victim drugs such as midazolam, alfentanil and nifedipine for CYP3A and metoprolol and desipramine for CYP2D6. Most of these drugs have f_{hep} and f_{m,CYPi} values close to or above 0.9 and EH values from 0.3 to 0.7 (see Table 1 for values). Therefore, these commonly observed ranges were used to highlight the sensitivity/error analysis.

The analysis below evaluates the sensitivity of EH, f_{hep} and f_{m,CYPi} individually.

EH (Hepatic Extraction Ratio)

The true novelty of our model (Eq. 8) is that it affords the opportunity to quantify the error introduced into an AUC ratio prediction solely as a result of assuming that a drug is a low extraction ratio drug when in fact it is not. For all our simulations we assumed constant hepatic blood flow (Q =1.5 L/min), recognizing that variability in blood flow will change EH. The magnitude of change in EH will be most pronounced for highly extracted drugs and will affect the impact of ignoring EH.

Figure S1 shows that the error is dependent on the magnitude of the DDI-induced fold-change in intrinsic clearance and f_{m,CYPi}. For a drug with an f_{hep} = 0.95 and f_{m,CYPi} = 1.0, when the fold-change in intrinsic clearance (f_{Hep C/int}^Hep) is in the range of 1 to 0.1 (0 to 90% inhibition), an error of as much as 20% is introduced into the predicted AUC ratio as a result of ignoring the EH of the victim drug even when the true EH is as low as 0.25 (a typical scenario). For a drug with EH of 0.9 ignoring the EH can result in an error that is as much as 300%. Interestingly, as the fraction of clearance remaining becomes very small (f_{Hep C/int}^Hep <0.01, greater degree of inhibition), the error is significantly diminished as the AUC ratio is ultimately determined by non-hepatic clearance. This is because the inhibited enzyme is solely responsible for the hepatic clearance (f_{m,CYPi} =1) and when that pathway is almost completely inhibited, the liver is no longer a clearing organ, and the resulting clearance is solely
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determined by non-hepatic clearance. As a result, there is no effect of ignoring EH and the % error approaches 0%.

**Figure S2: Impact of EH When $f_{m,CYP}$=0.95**

In contrast, for a drug with $f_{hep}$ = 0.95 and $f_{m,CYP}$ = 0.95 (Figure S2, above), as the fraction of clearance remaining becomes smaller ($f^{C_{int}}_{C_{hep}} <0.01$), the % error in the AUC ratio plateaus at a constant value and this value depends on the EH. This is a result of the un-inhibited remaining fraction (0.05) of hepatic elimination constituting a larger fraction of the observed hepatic clearance than it did when the inhibited pathway was present. For example, a drug with an EH of 0.5 (total intrinsic clearance is equal to hepatic blood flow 1.5 L/min, but observed hepatic clearance is half of hepatic blood flow or 750 ml/min) if the drug has an $f_{m,CYP}$ of 0.95, then 37.5 ml/min of observed hepatic clearance will be due to the minor pathway, which has an intrinsic clearance of 75 ml/min (1500ml/min * 0.05). When the major pathway is inhibited, the remaining observed hepatic clearance will be ~75 ml/min rather than 37.5 ml/min (750 ml/min *0.05) when not accounting for EH dampening of hepatic clearance. In figure S1 panel B, the point at which the maximum % error is achieved is shifted to the right for net inhibition ($f^{C_{hep}}_{C_{int}}$<1) as the EH of the victim drug increases. This implies that as the victim drug EH increases, more potent inhibition is necessary to completely inhibit the clearance pathway. This phenomenon can be described by a hypothetical situation in which the clearance of two drugs (EH=0.1 and 0.9) are inhibited 99% ($f^{C_{hep}}_{C_{int}}=0.01$). In this situation the hepatic clearance of the low EH drug would be 0.11% of blood flow whereas for the high EH drug it would be 9.0 % of blood flow. 99.99% inhibition ($f^{C_{hep}}_{C_{int}}=0.0001$or 100 times higher concentration of the inhibitor) would be necessary to inhibit the clearance of the high EH drug to 0.1% of blood flow.

The magnitude of percent error with respect to net induction ($f^{C_{hep}}_{C_{int}}$>1) is not as prominent because it is artificially “capped” at -100% as a result of the AUC ratios being less than 1.0 and the method of calculation of % error. For a victim drug with EH = 0.25, $f_{hep}$ = 0.95, $f_{m,CYP}$ = 0.95 and modest induction of 2.5-fold, the % error is ~25% or AUC ratios of 0.57 and 0.42 for EH and No EH model respectively (Figure S2). For victim drugs with higher EH, the error approaches -100% at much lower net induction ($f^{C_{hep}}_{C_{int}}$>1). This shows that for
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an IV administered victim drug in the presence of induction, the observed AUC ratio will be substantially higher (which will appear as less potent induction) than that predicted from a No EH model. This is a result of increasing the extraction ratio of the drug and thereby causing increased dampening on the decrease of the AUC ratio. As the EH of a drug increases, a larger degree of induction is necessary to have a comparable decrease in AUC ratio.

Looking at the vertical lines in Figure S2 (fixed degree of inhibition or induction) it is apparent that as the EH of the victim drug increases the % error in the AUC ratio increases. Therefore, for the same perpetrator drug the % error in the AUC ratio is dependent on the EH of the victim drug used and the error will increase as EH increases.

The surface in Figure S2 shows that the maximum difference between the predicted AUC ratios is seen when the fraction of clearance remaining is ~0.1 to 0.01, beyond which this value decreases somewhat and the % error stabilizes at a slightly lower plateau. This phenomenon is highlighted in Figure S3 in which the traces of predicted AUC ratio for a drug with f_{hep} of 0.95, f_{m,CYPi} of 0.95 and EH of 0.25 or assuming the No EH model. A plot of the % error in AUC ratio is overlaid on the secondary y axis.

Figure S3 shows that the maximum % error is achieved not upon complete inhibition, but rather when ~90 % inhibition is seen (fraction of clearance remaining of 0.1). The % error after more potent inhibition plateaus at a slightly lower but constant value. The early maximum is a result of the change in the shape of the inhibition curve when the EH is accounted for and, arises as a result of evaluating the % change and not the absolute AUC ratio. The largest absolute difference in predicted AUC ratios is observed at maximum inhibition.

f_{hep} (the fraction of total clearance that is hepatic elimination)

To evaluate the contribution of f_{hep} (Figure S4) to AUC ratio prediction error we used a situation in which the EH is set at 0.5 and f_{m,CYPi} is 0.95 and varied the f_{hep}. 

![Figure S3: Impact of EH When f_{m,CYPi}=0.95](image-url)
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Figure S4: Impact of $f_{\text{hep}}$

As $f_{\text{hep}}$ is increased from 0.5 to 0.99, the % error curves and maximum % error are shifted up and to the right. The rightward shift is similar to that described in the EH evaluation above and the magnitude of the upward shift is proportional to $f_{\text{hep}}$. This upward shift, or a greater % error as $f_{\text{hep}}$ increases, is a result of the unaltered hepatic clearance pathway representing a larger fraction of the total clearance and that the contribution of this pathway is not adequately characterized by the No EH model as described in the EH evaluation above. A substantial $f_{\text{hep}}$ ($\geq 0.90$) is required for this effect to contribute more than 30% error in the AUC ratio.

$f_{\text{m,CYPi}}$ (the fraction of hepatic clearance by the affected pathway)

The contribution of $f_{\text{m,CYPi}}$ (Figure S5) was generated by setting the victim drug EH at 0.5 and $f_{\text{hep}}$ at 0.95 while varying the value of $f_{\text{m,CYPi}}$ from 0.5 to 1.0.

Figure S5 shows that the % error curves are slightly shifted vertically as $f_{\text{m,CYPi}}$ is increased showing that as $f_{\text{m,CYPi}}$ increases, the % error in the predicted AUC ratio increases when EH is not accounted for. Interestingly though, the shape of this surface changes as $f_{\text{m,CYPi}}$ is increased towards 1.0. In this plot, as the fraction of clearance remaining ($f_{\text{Cl, int}}^{\text{Hep}}$) decreases below 0.1 (>90% inhibition), and $f_{\text{m,CYPi}}$ increases above 0.9, the % error begins to decrease until at $f_{\text{m,CYPi}} = 1.0$ there is no difference between the two models at very high inhibition ($f_{\text{Cl, int}}^{\text{Hep}} \sim 0.0001$). This again highlights the importance of hepatic blood flow dampening of hepatic clearance via the unaltered pathway which can contribute as much as 30% for a victim drug with EH $\geq 0.35$ and $f_{\text{m,CYPi}}$ of $\geq 0.9$. 
Summary of Sensitivity/Error Analysis

The impact of ignoring EH on the predicted AUC ratio of an IV administered victim drug is dependent on \( f_{\text{hep}}, f_{\text{m,CYPi}}, \) EH and the degree of the interaction (inhibition or induction). The minor non hepatic clearance mechanisms (\( f_{\text{hep}} > 0.9 \), which is the case for many victim drugs, See Table 1), have a measurable impact (\( \geq 30\% \) error), and should be taken into account when predicting IV AUC ratios especially for potent inhibition interactions (\( f_{\text{Clint}}^{\text{Hep}} < 0.1, >90\% \) inhibition). Likewise, ignoring the hepatic blood flow dampening of the unaffected hepatic clearance pathways (\( 1-f_{\text{m,CYPi}} \leq 0.1 \)) for drugs with \( \text{EH} \geq 0.35 \) for potent inhibition interactions (\( f_{\text{Clint}}^{\text{Hep}} < 0.1, >90\% \) inhibition) will contribute greater than 30% error to the predicted IV AUC ratio. For net induction interactions, the effect of EH is substantial (-25% error for \( \text{EH} = 0.25 \) and modest 2.5-fold induction), and should always be taken into consideration. For a specific inhibitor/inducer, as EH of the victim drug increases, the % error in the AUC ratio increases irrespective of whether or not the victim drug is moderate or high EH.