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

Brian J. Kirby and Jashvant D. Unadkat

Department of Pharmaceutics, University of Washington, Seattle, Washington

Received May 27, 2010; accepted August 18, 2010

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 administrated 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 \( f_{\text{m, CVN}} \), 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 \( \geq 30\% \) 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 (\( \lambda/K_{\infty} \) or \( \lambda/K_{\text{ind}} \)) \( \geq 1.3 \) or fold induction \( \geq 2.1 \); for intestinal contribution the boundaries are 0.37 and 1.5, respectively. To accurately predict the intravenous AUC ratio, intestinal contribution, or \( f_{\text{m, CVN}} \), 1 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 (\( \geq 0.3 \)), 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 >0.3) to high (EH \( \geq 0.7 \)) 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 (Holbeckner 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 (\( f_{\text{m, CVN}} \)) (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

ABBREVIATIONS: AUC, area under the concentration-time curve; EH, hepatic extraction ratio; DDI, drug-drug interaction; IV, intravenous; \( f_{\text{m, CVN}} \), fractional clearance due to a specific enzymatic pathway.
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 \( f_{\text{m,CYP}} \)) and the severity of the DDI
(degree of inhibition or induction). Therefore, we determined bound-
aries outside which the simplification of ignoring the EH of the victim
drug has a significant effect (\( \geq 30\% \)) on the predicted AUC ratio,
estimated \( f_{\text{m,CYP}} \), 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 \( \geq 30\% \) error under moderate
enzyme inhibition (\( K_i \) or \( k_{\text{deg}} = 1–10 \)) or induction (\( \geq 3\)-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 DDIIs, 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 Intravenously Administered Drugs.** The AUC ratio
(\( \text{AUC}_{\text{IV}}/\text{AUC}_{\text{cl}} \)) of a victim drug in the presence and absence of a perpetrator
drug can be described by eq. 1, assuming that only hepatic clearance (\( \text{Cl}_{\text{hep}} \)) of the
victim drug is affected but hepatic blood flow (\( Q \)), unbound fraction (\( f_u \)),
and the renal clearance (\( \text{Cl}_{\text{renal}} \)) are not.

\[
\frac{\text{AUC}_{\text{IV}}}{\text{AUC}_{\text{cl}}} = \frac{\text{Cl}_{\text{IV}}}{\text{Cl}_{\text{cl}}} = \frac{\text{Cl}_{\text{hep}} + \text{Cl}_{\text{renal}}}{\text{Cl}_{\text{hep}} + \text{Cl}_{\text{renal}}} = \frac{1 - f_u}{f_u} \cdot \text{Cl}_{\text{hep}}
\]

\( \text{Cl}_{\text{hep}} \) and \( \text{Cl}_{\text{renal}} \) are the hepatic clearance (determined by the well stirred
model) in the absence and the presence of the perpetrator, respectively, and \( f_u \)
is the fraction of \( \text{Cl}_{\text{IV}} \) that is subject to the hepatic blood flow limitation.
The ratio of \( \text{Cl}_{\text{IV}}/\text{Cl}_{\text{cl}} \) can be simplified to eq. 2, where \( Q \) is liver blood flow
and is assumed not to be affected by the DDI, \( f_u \) is the unbound fraction of
the victim drug in blood and \( \text{Cl}_{\text{m}} \) and \( \text{Cl}_{\text{int}} \) are the intrinsic clearance of the victim
drug in the presence and absence of the perpetrator.

\[
\frac{\text{Cl}_{\text{hep}}}{\text{Cl}_{\text{renal}}} = \frac{Q \cdot f_u \cdot \text{Cl}_{\text{int}}}{Q + f_u \cdot \text{Cl}_{\text{m}}} = \frac{(Q / f_u) \cdot \text{Cl}_{\text{int}} + 1}{(Q / f_u) \cdot \text{Cl}_{\text{m}} + 1} = \frac{1}{\frac{1}{\text{EF}} - 1 - \frac{1}{1 + \text{f}_{\text{m,CYP}}}}
\]

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

\[
\frac{\text{Cl}_{\text{hep}}}{\text{Cl}_{\text{renal}}} = \frac{(Q / f_u) \cdot \text{Cl}_{\text{int}} + 1}{(Q / f_u) \cdot \text{Cl}_{\text{m}} + 1} = \frac{(Q / f_u) \cdot \text{Cl}_{\text{int}} + 1}{(Q / f_u) \cdot \text{Cl}_{\text{m}} + 1} = \frac{1}{\frac{1}{\text{EF}} - \frac{1}{1 + \text{f}_{\text{m,CYP}}}}
\]

\( \text{f}_{\text{m,CYP}} \) is the product of the fraction of intrinsic clearance remaining as a result of
inactivation (\( f_{\text{inactivation}} \)), induction (\( f_{\text{induction}} \)), and inhibition
(\( f_{\text{inhibition}} \), eq. 6), which are analogous to the A, B, and C terms, respectively,
described previously (Fahmi et al., 2008).

\[
f_{\text{inactivation}} = \frac{1}{1 + i \cdot k_d} = \frac{1}{1 + i \cdot k_d} \frac{k_{\text{deg}}(1 + k_i)}{1 + k_{\text{deg}}(1 + k_i)}
\]

\( \text{f}_{\text{induction}} = 1 - f_{\text{inhibition}} = \frac{1}{1 + i \cdot k_d} \frac{k_{\text{deg}}(1 + k_i)}{1 + k_{\text{deg}}(1 + k_i)}
\]

\( \text{f}_{\text{inhibition}} = \frac{1}{1 + I / K_i}
\]

where \( \lambda \) is the predicted inactivation rate of the enzyme, \( i \) 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), \( K_{\text{deg}} \) is the maximum inactivation rate
constant, \( K_i \) is the concentration that produces half-maximum inactivation, \( k_{\text{deg}} \)
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, \( \text{Ind}_{\text{max}} \) is the maximum
fold induction of mRNA of the affected enzyme, \( \text{EC}_{50} \) 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 (\( f_{\text{m,CYP}} = 1 \)).

\[
\frac{\text{AUC}_{\text{IV}}}{\text{AUC}_{\text{cl}}} = \frac{\text{Cl}_{\text{hep}}}{\text{Cl}_{\text{hep}}} = \frac{1}{1 + \text{f}_{\text{m,CYP}}} = \frac{1}{1 + \text{f}_{\text{m,CYP}}}
\]

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

\[
\frac{\text{AUC}_{\text{IV}}}{\text{AUC}_{\text{cl}}} = \frac{\text{Cl}_{\text{hep}}}{\text{Cl}_{\text{hep}}} = \frac{1}{1 + \text{f}_{\text{m,CYP}}} = \frac{1}{1 + \text{f}_{\text{m,CYP}}}
\]

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 \( f_{\text{m,CYP}} \) 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: \( f_{\text{m,CYP}} \) (the fraction of IV clearance that is hepatic elimination),
\( f_{\text{m,CYP}} \) (the fraction of hepatic clearance that is mediated by the affected enzyme),
\( f_{\text{inhib}} \) (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 - \( f_{\text{m,CYP}} \)).
The contribution of some of these parameters such as \( f_{\text{m,CYP}} \), other clearance
pathways, and enzyme degradation half-life (implicit to inactivation) to the AUC
ratio have been evaluated and will not be addressed further (Venkataraman 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 predic-
tions. Therefore, we conducted a sensitivity/error analysis to evaluate the contri-
bution of \( f_{\text{m,CYP}} \), EH, and net change in intrinsic clearance (\( f_{\text{inhib}} \)) to the error
in predicted AUC ratio using a No EH model (where EH was set to a very low
number 1 × 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.
% error = \left( \frac{\text{AUCRatio}_{\text{No EH Model}} - \text{AUCRatio}_{\text{EH model}}}{\text{AUCRatio}_{\text{No EH Model}}} \right) \cdot 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 intestinal clearance is the product of the inactivation (\(f_{\text{Clint, intraduodenum}}\)) fold induction (\(f_{\text{Clind, intestinal}}\)) and reversible inhibition (\(f_{\text{Clinb, basel}}\)) terms. For example, if a precipitant has \(f_{\text{Clinb, basel}}\) of 0.2 (1 + \(K_{\text{I}}/K_{\text{F}}\) = 5), \(f_{\text{Clind, intestinal}}\) of 5 (5-fold induction), and \(f_{\text{Clint, intraduodenum}}\) of 0.2 (1 + \(K_{\text{I}}/K_{\text{F}}\) = 5), the net result is that the intestinal clearance will decrease to one-fifth (\(f_{\text{Clint, intraduodenum}}\) of 0.2 · 5 · 0.2 = 0.2) of the basal intrinsic clearance. Historically the potencies of inactivation, induction, and inhibition have been quantified by \(K_{\text{I}}\) and deg, fold increase in enzyme expression, and 1 + \(K_{\text{I}}/K_{\text{F}}\), respectively. Because these terms are grouped, we conducted the sensitivity/error analysis with respect to the value of the product (\(f_{\text{Clint, intraduodenum}}\)) 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 (Gailet, 2007; Gailet 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 (Gailet 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 \(f_{\text{Clint, intraduodenum}}\) term is the net effect on intestinal intrinsic clearance, similar to the \(f_{\text{Clint, ileum}}\) term for hepatic intrinsic clearance), namely, eq. 10 for \(f_{\text{Clint, ileum}}\) as shown in eq. 11.

\[
\frac{\text{AUC}_{\text{IV}}}{\text{AUC}_{\text{PO}}} = \left( \frac{1}{f_{\text{ClPO}} \times f_{m} + (1 - f_{m})} \right) \times \left( \frac{1}{f_{\text{ClIV}} \times (1 - F_{G}) + F_{G}} \right) \tag{10}
\]

\[
\frac{\text{AUC}_{\text{IV}}}{\text{AUC}_{\text{PO}}} = \frac{1}{f_{\text{ClIV}} \cdot f_{\text{ClPO}} \cdot f_{\text{Clcyt}} \cdot f_{\text{Clvict}} + (1 - f_{\text{Clvict}})} - F_{G} \tag{11}
\]

The piece of information needed from the intravenously administered interaction is the fold change in intestinal intrinsic clearance (\(f_{\text{Clint, intraduodenum}}\)). We determined the impact that incorrectly determining the fold change in intestinal 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 \(f_{\text{ClPO}} = 1.0, f_{\text{Clcyt}} = 0.95,\) and \(F_{G} = 0.5\) (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 (\(f_{\text{ClHEP}}\)) and intestinal (\(f_{\text{Clint}}\)) 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).

\[
f_{\text{ClHEP}}^{\text{IV}} = \frac{\frac{\text{AUC}_{\text{IV}}}{\text{AUC}_{\text{PO}}} - (1 - f_{\text{ClPO}})}{f_{\text{ClPO}} - (1 - f_{\text{Clvict}})} \tag{12}
\]

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 (\(f_{\text{Clint, ileum}}^{\text{IV}}\)) and the \(F_{G}\) ratio (eq. 13).

\[
F_{G}' = \frac{1}{f_{\text{ClHEP}}^{\text{IV}}(1 - F_{G}) + F_{G}} \tag{13}
\]

These calculated values were compared with the parameter values, e.g., \(F_{G}\) 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 \(F_{G}\) on this error by varying \(F_{G}\) 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 \(F_{G}\) 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 \(f_{\text{ClPO}}\) and \(f_{\text{ClHEP}}\). One method historically used to estimate \(f_{\text{ClPO}}\) and \(f_{\text{ClHEP}}\) (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, \(f_{\text{ClPO}}\), is calculated using eq. 14.

\[
\frac{\text{AUC}_{\text{IV}}}{\text{AUC}_{\text{PO}}} = \frac{1}{1 - f_{\text{ClPO}}} \tag{14}
\]

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

\[
f_{\text{ClPO}} = \frac{1}{1/EH - 1} \tag{15}
\]

To show the magnitude of underestimation of \(f_{\text{ClPO}}\) by eq. 14, intravenous AUC ratios were first simulated using eq. 8 for victim drugs with varying \(f_{\text{ClPO}}\) values, across a range of extraction ratios from 0.001 to 1.0, assuming complete inhibition (\(f_{\text{ClHEP}}^{\text{IV}} = 0\)), and \(f_{\text{ClPO}}\) was assumed to be 1.0 (negligible nonhepatic elimination). These simulated AUC ratios were then used to estimate the \(f_{\text{ClPO}}\) values using eq. 14 (the No EH model). The impact of the error in \(f_{\text{ClPO}}\), 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 \(f_{\text{ClHEP}}^{\text{IV}} = 0\)) calculated using eq. 10 with the \(f_{\text{ClPO}}\) value estimated by the No EH model (eq. 14) and the \(f_{\text{ClPO}}\) value used for the simulation. For simplicity no intestinal 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 \(f_{\text{ClHEP}}\) was not readily available, a value of 1.0 was assigned. Then, using the modified model, we determined the DDI boundaries, degree of inhibition or induction, 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 \(f_{\text{ClHEP}}\), \(f_{\text{ClPO}}\), \(EH\), and the degree of the interaction (inhibition or induction). The minor nonhepatic clearance mechanisms (\(f_{\text{ClHEP}}\) > 0.9, which is the case for many victim drugs) (Table 1), have a measurable 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.
TABLE 1

DDI boundaries (magnitude of inhibition or induction) at which the error in ignoring EH for intravenous administration of commonly used victim drugs exceeds 30% for predicted AUC ratio and intestinal contribution to a DDI and the error in estimated \( f_{\text{Clint}} \), GI Error when EH is ignored for intravenously administered victim drugs.

<table>
<thead>
<tr>
<th>Victim Drug</th>
<th>EH</th>
<th>( f_{\text{Clint}} ), GI Error</th>
<th>F_G</th>
<th>Inhibition of ( F_{\text{Clint}} ), GI Error</th>
<th>% Error in Maximum Oral AUC Ratio of Victim Drug, EH Ignored</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midazolam</td>
<td>0.1</td>
<td>0.44</td>
<td>0.83</td>
<td>Not possible</td>
<td>0.83</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>0.5</td>
<td>0.45</td>
<td>0.80</td>
<td>Not possible</td>
<td>N.A.</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>0.5</td>
<td>0.84</td>
<td>0.37</td>
<td>Not possible</td>
<td>N.A.</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.5</td>
<td>0.62</td>
<td>0.37</td>
<td>Not possible</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

Data from Thummel et al. (1996).

Data from Miners et al. (1982).

Data from Ito et al. (2005).

Data from Abernethy et al. (1984).

Data from Leemann et al. (1993).

Data from Feely et al. (1981).

N.A. = not applicable.

N/A = not available.  

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 (\( f_{\text{hep}} = 1.0, f_{\text{int}} = 0.95 \), and \( F_G = 0.5 \)), we used the simulation approach described above to determine the EH and fraction of hepatic intrinsic clearance remaining (\( f_{\text{Clint}}^{\text{hep}} \) or \( f_{\text{Clint}}^{\text{int}} \)) 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 \( f_{\text{Clint}}^{\text{hep}} \) is 0.1 will completely mask (\( F_G^{\text{int}} = 1.0 \) or no change in \( F_G \)) a 2-fold induction in the intestine (\( f_{\text{Clint}}^{\text{int}} = 2 \)). 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 ($f_{Cl_{int}}/f_{Cl_{int}} > 1$) 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 $f_{m,CYPi}$** Estimated fractions of hepatic clearance due to a specific enzymatic pathway ($f_{m,CYPi}$) 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 $f_{m,CYPi}$ values of 0.5 to 0.95. As expected, as the EH of a victim drug increases, the estimated $f_{m,CYPi}$ calculated by the No EH model decreases. To better illustrate the impact of the underprediction of $f_{m,CYPi}$, 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 $f_{m,CYPi}$ values and shows a linear relationship dependent on EH. Therefore, the magnitude of this error can be directly calculated as percent error = $f_{m,CYPi} \cdot EH \cdot 100$. The incorrect $f_{m,CYPi}$ 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 $f_{m,CYPi}$. 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%, respec-

**Fig. 1.** Impact of EH on estimating intestinal contribution to DDIs. The effect of ignoring the EH of an intravenously administered victim drug in estimation of the effect of a DDI on intestinal enzymes shows the following. A, the percent error in estimated fraction of intestinal intrinsic clearance remaining ($f_{Clint}^{G'/F}$) rapidly increases as fold induction ($f_{Clf}^{G'/F}$ and $f_{Clf}^{G}$ increase above 1) and EH increase. Net inhibition ($f_{Clf}^{G'/F}$ and $f_{Clf}^{G}$ < 1) is greatly underpredicted as EH and degree of inhibition increase. B, the percent error in the predicted $F_{G'/F_{G}}$ ratio (or fold change in intestinal bioavailability) shows that for net inhibition ($f_{Clf}^{G'/F}$ and $f_{Clf}^{G}$ < 1), $F_{G'/F_{G}}$ is overpredicted when EH is moderate to high (≥0.35) and underpredicted for net induction ($f_{Clf}^{G'/F}$ and $f_{Clf}^{G}$ > 1) even when EH is low (≤0.3). C, the estimated $F_{G'/F_{G}}$ is shown to increase beyond the maximum theoretical value of 1.0 by greater than 30% ($F_{G'} ≥ 1.3$) when the EH is ≥0.35 and net inhibition is ≥90% ($f_{Clf}^{G'}$ and $f_{Clf}^{G} ≥ 0.1$). Note: the fraction of hepatic ($f_{Clf}^{G'/F}$) and intestinal ($f_{Clf}^{G}$) clearance remaining was set equal and varied between 25 and 0.0001; hepatic extraction ratio was varied between 0.1 and 0.9. The fraction of hepatic ($f_{Clf}^{G'/F}$) and intestinal ($f_{Clf}^{G}$) intrinsic clearance remaining axes are on a logarithmic scale.
tively. This result shows that the impact of ignoring EH when one is determining \( f_{m, \text{CYPi}} \) is dependent not only on the EH of the victim drug but also on the magnitude of \( f_{m, \text{CYPi}} \).

**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 \( \frac{I}{K_i} \) 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 \( f_{m, \text{CYPi}}, F_G \), and EH with a much smaller contribution from \( f_{\text{hep}} \). Varying \( f_{\text{hep}} \) from 1.0 to 0.95 resulted in only minor changes (\(<10\% \) increase) in the DDI inhibition boundary for the victim drugs with high \((>0.80)\) \( f_{m, \text{CYPi}} \) and \~20\% increase for the drugs with low \( f_{m, \text{CYPi}} \) (imipramine and metoprolol). The DDI boundaries for induction were not sensitive (\(<5\% \) change) to changing \( f_{\text{hep}} \) from 1.0 to 0.95. In general, except for the two very low EH drugs (tolbutamide and warfarin) or the low \( f_{m, \text{CYPi}} \) 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 $f_{m,CYPi}$, 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, $f_{m,CYPi}$, $f_{hep}$ and magnitude 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 $f_{m,CYPi}$, e.g., propranolol) inhibition interactions with $I/K_i$ between 0.5 and 9 and induction interactions of <3-fold will be susceptible to ≥30% error in the AUC ratio by ignoring EH of the victim drug.

With respect to the determination of the $f_{m,CYPi}$ 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
be assumed to estimate $f_{m,CYP}$. This implies that if the $f_{m,CYP}$ 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, $F_{int} = 1.0$, and $f_{m,CYP} = 0.93$ (a situation similar to that of midazolam). If we assume that hepatic blood flow is 1.5 l/min, the observed hepatic clearance ($CL_{H}$) 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 $CL_{m,CYP}$ is 0.079 l/min (using the well stirred model with $CL_{m,CYP} = 0.083$ l/min, the unaffected pathway). This would result in an observed AUC ratio of 8.4. The incorrect $f_{m,CYP}$ 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 was used (eq. 15), the resulting estimated $f_{m,CYP}$ would be the true value of 0.93. Even though the error in the estimated $f_{m,CYP}$ (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 $\sim 41\%$. Our analysis showed that this underestimation can be directly calculated as $\frac{f_{m,CYP}}{f_{m,CYP}} \cdot EH \cdot 100$. 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 $\sim 40\%$ 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 victim drugs is to 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 nonsignificant intravenous AUC ratio of 0.70 was observed and an study between nifedipine and rifampin (Holtbecker et al., 1996), a ing in an overestimation of the intestinal induction (Fig. 1A). In a DDI

References


Address correspondence to: Brian J. Kirby, Department of Pharmaceutics, University of Washington, Box 357610, Seattle, WA 98195. E-mail: bkirby98@u.washington.edu
The Impact of Ignoring Extraction Ratio When Predicting drug-drug Interactions, Fraction Metabolized and Intestinal First-Pass Contribution.
Brian J. Kirby and Jashvant D. Unadkat
Drug Metabolism and Disposition

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}, \( f_{\text{hep}} \) {0.5-1.0} and \( f_{\text{m,CYPi}} \) {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 \( f_{\text{hep}} \) and \( f_{\text{m,CYPi}} \) 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, \( f_{\text{hep}} \) and \( f_{\text{m,CYPi}} \) 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 \( f_{\text{m,CYPi}} \). For a drug with an \( f_{\text{hep}} = 0.95 \) and \( f_{\text{m,CYPi}} = 1.0 \), when the fold-change in intrinsic clearance (\( f_{\text{Cint}}^{\text{Hep}} \)) 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 typically 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 (\( f_{\text{Cint}}^{\text{Hep}} < 0.01 \), 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 \( (f_{\text{m,CYPi}} = 1) \) and when that pathway is almost completely inhibited, the liver is no longer a clearing organ, and the resulting clearance is solely...
The Impact of Ignoring Extraction Ratio When Predicting drug-drug Interactions, Fraction Metabolized and Intestinal First-Pass Contribution.
Brian J. Kirby and Jashvant D. Unadkat
Drug Metabolism and Disposition

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 fm,CYPi=0.95**

In contrast, for a drug with fhep = 0.95 and fm,CYPi = 0.95 (Figure S2, above), as the fraction of clearance remaining becomes smaller (f_Clin<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 fm,CYPi 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_Clin<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_Clin=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_Clin = 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_Clin>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, fhep = 0.95, fm,CYPi = 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_Clin>1). This shows that for
The Impact of Ignoring Extraction Ratio When Predicting drug-drug Interactions, Fraction Metabolized and Intestinal First-Pass Contribution.
Brian J. Kirby and Jashvant D. Unadkat
Drug Metabolism and Disposition

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 \( f_{\text{hep}} \) 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.

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

To evaluate the contribution of \( f_{\text{hep}} \) (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 \( f_{\text{hep}} \).
The Impact of Ignoring Extraction Ratio When Predicting drug-drug Interactions, Fraction Metabolized and Intestinal First-Pass Contribution.
Brian J. Kirby and Jashvant D. Unadkat
Drug Metabolism and Disposition

Figure S4: Impact of f_{hep}

As f_{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_{hep}. This upward shift, or a greater % error as f_{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_{hep} (≥ 0.90) is required for this effect to contribute more than 30% error in the AUC ratio.

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

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

Figure S5 shows that the % error curves are slightly shifted vertically as f_{m,CYPi} is increased showing that as f_{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_{m,CYPi} is increased towards 1.0. In this plot, as the fraction of clearance remaining (f_{Hep}^{C/int}) decreases below 0.1 (>90% inhibition), and f_{m,CYPi} increases above 0.9, the % error begins to decrease until at f_{m,CYPi} = 1.0 there is no difference between the two models at very high inhibition (f_{C/int}^{Hep} ~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 ≥0.35 and f_{m,CYPi} of ≥ 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 $f_{\text{hep}}$, $f_{m,CYPI}$, 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 measureable impact ($\geq 30\%$ error), and should be taken into account when predicting IV AUC ratios especially for potent inhibition interactions ($f_{C_{\text{int}}}^{\text{Hep}} < 0.1$, >90% inhibition).

Likewise, ignoring the hepatic blood flow dampening of the unaffected hepatic clearance pathways ($1-f_{m,CYPI} \leq 0.1$) for drugs with EH $\geq 0.35$ for potent inhibition interactions ($f_{C_{\text{int}}}^{\text{Hep}} < 0.1$, >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 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.