Review

Contribution of Intestinal Cytochrome P450-Mediated Metabolism to Drug-Drug Inhibition and Induction Interactions

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Summary: Current drug-drug interaction (DDI) prediction models incorporate intestinal interaction as the ratio of the intestinal availability in the presence and absence of an inhibitor/inducer (F_G and F_G, respectively). The incorporation of the gut is commonly associated with a reduced number of false negative predictions; however, in some instances a trend for over-prediction is apparent. This differential success is highly dependent on the initial model assumptions and parameter estimates used (often inconsistent between the datasets) and cannot be associated exclusively with the incorporation of the intestine. The current review provides an assessment of the contribution of intestinal inhibition and induction in conjunction with different perpetrator and victim drug-related properties, focusing in particular on victim drugs with high intestinal first-pass extraction (>75%). Recommendations are given in order to avoid significant over-estimation of true positives and increased number of false positive predictions. This review discusses advantages and limitations of different in vitro and in vivo methods for assessing intestinal availability and associated inter-individual variability, due to the sensitivity of the DDI prediction models to the F_G.

Keywords: CYP3A4; drug-drug interactions; intestinal first-pass; inhibition; induction

Metabolic enzymes and transporters in the human small intestine

Human small intestine expresses a range of oxidation and conjugation metabolic enzymes.1–13) Cytochrome P450 (CYP) enzymes have been characterised in most detail, with CYP3A accounting for approximately 80% of total P450s in the gut (59–96%), followed by CYP2C9 (~14%). A number of other P450 enzymes (CYP2C19, CYP2J2 and CYP2D6) are expressed, but expression levels are <2% of total P450s (Fig. 1); these estimates are based on the analysis of CYP abundance data from 31 individuals.1) The total amount of CYP3A expressed in the human small intestine (65.7–70.5 nmol) represents approximately 1% of the estimated hepatic levels.14–16) Although intestinal CYP3A4 content was reported to be highly variable (17-fold), variability in total CYP3A (18–151 pmol/mg protein) was on average comparable to variability observed for other CYP enzymes in the gut (7- to 9-fold for CYP2C19, CYP2D6 and CYP2C9).1) A number of different UDP-glucuronosyltransferase (UGT) conjugation enzymes are also expressed to some extent in the human small intestine, including UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9 and UGT1A10 as well as UGT2B4 and UGT2B6;9,10,17) UGT1A8 and UGT1A10 expression is exclusive to the small intestine.18) Extensive conjugation by intestinal UGTs has been suggested to contribute to the low bioavailability of raloxifene19,20) and troglitazone.21,22) In addition to UGTs, intestine also expresses a number of sulfotransferases (SULTs); in contrast to CYPs and UGTs, these conjugation enzymes are not located in the endoplasmic reticulum but in the cytosol.13,23,24) Recent analysis of the expression levels of the major human SULTs in a range of human tissues has shown that the small intestine contains the largest overall amount of SULT. Of the 5 principal human SULTs investigated, SULT1B1 and SULT1A3 were predominantly expressed.

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(36 and 31% of total SULTs, respectively), whereas SULTs 1A1, 1E1 and 2A1 represent minor forms (6–19% of total).

The expression of metabolic enzymes along the small intestine is not uniform, with the highest levels of P450 and UGT protein found in the proximal region of the intestine, declining distally. Consistent with these findings is the declining activity of CYP3A from the duodenum to the ileum, with the maximum activity shown in the upper jejunum for erythromycin N-demethylation and 1\(^-\)midazolam hydroxylation. This heterogeneous expression of metabolic enzymes is also observed within the small intestinal villi, where the highest levels are found in mature enterocytes lining the villus tips. The distribution of UGTs along the small intestine is regarded as being analogous to the regional distribution of CYPs. A recent study by Cao et al. indicated a 3-fold greater UGT gene expression relative to CYP3A4 in the human duodenum, but it is questionable whether this estimate will reflect the UGT:P450 protein abundance ratio along the whole length of the gut.

The most common method of assessing the relative importance of intestinal metabolism is based on the comparison of hepatic and intestinal activity (expressed per mg of microsomal protein); differential catalytic activity in comparison to the liver has been reported across studies. Comparison of intestinal and hepatic catalytic activities normalised for the mean population relative abundance of P450 enzymes in specific organs results in a very good agreement in the metabolic activities between liver and intestine and across the range of different P450s. However, normalisation for the enzyme abundance is challenging in the case of UGTs, as the absolute UGT expression levels are currently not available in the liver and in the small intestine. In order to allow valid comparison between hepatic and intestinal glucuronidation, Cubitt et al. have expressed glucuronidation clearance data per gram of tissue. Scaling of intestinal and hepatic glucuronidation CL\(_{int}\) was performed using the intestinal microsomal recovery of 20.6 mg/g intestine. This weighted scaling factor was estimated from mucosal microsomal protein and mucosal CYP3A4 in each of the intestinal sections from the original data reported by Paine et al. Hepatic data were scaled using standard hepatic microsomal recovery of 40 mg protein/g liver. The age of the donors has been shown to affect the estimate of hepatic microsomal recovery with a maximum value (40 mg protein/g liver) obtained in approximately 28-year-old donors, followed by a gradual decrease in older age. In contrast, the impact of age on intestinal recovery is currently unknown. In addition, it should be noted that intestinal microsomal recovery was estimated from samples obtained using the mucosal scraping method for the preparation of intestinal microsomes. The application of this method has been reported to give lower metabolic activity in intestinal microsomes than the enterocyte elution method; however, it is as yet unknown whether the choice of enterocyte preparation method will affect the microsomal protein yield.

In addition to human in vitro systems, a number of animal models have been used to assess intestinal metabolic activity. Komura and co-workers have noted differential intestinal metabolic activities for midazolam and nisoldipine with a rank order of monkey > human > rat. Despite different CYP3A expression levels in the intestine between monkey and human and differential parameter estimates for established CYP3A probe midazolam, the authors have proposed the use of monkey as an appropriate animal model for evaluating small intestinal first-pass metabolism of CYP3A substrates; although this has been questioned. Recent stu-
dies have also used transgenic Cyp3a/- mice expressing human CYP3A4 in either intestine or liver in order to determine the relative importance of intestinal versus hepatic CYP3A. Studies with tissue-specific CYP3A4 transgenic mice revealed that intestinal CYP3A4 has a major impact on oral triazolam exposure, with minimal contribution of the hepatic CYP3A4. The same authors investigated further the utility of knockout and CYP3A4 transgenic mice for drug-drug interaction assessment in the liver and intestine using triazolam interactions with ketoconazole and gefitinib as examples. Based on these preliminary studies, the authors have proposed the use of humanised mouse models as useful animal models in early drug development. However, the extrapolation to human has to be cautious (as with all animal models mentioned above), as illustrated by the study in Cyp3a knockout mice using common CYP3A4 probe midazolam.

In contrast to expected, the study has shown marked midazolam metabolism in Cyp3a knockout mice due to up-regulation of CYP2C enzymes.

The presence of efflux transporters, in particular P-glycoprotein (P-gp), on the apical membrane of enterocytes can modulate intestinal CYP3A first-pass metabolism by decreasing the intracellular concentration of drugs and metabolites via active efflux. This proposed interplay between the two proteins allows recirculation of the drug and in conjunction with the variable abundance of CYP3A4 and P-gp contributes to the inter-individual variability in drug absorption and bioavailability. A number of studies have suggested that the expression of P-gp increases progressively from the stomach, whereas other studies report that the efflux transporters have a similar distribution pattern to CYP3A, with higher expression levels in jejunum in comparison to ileum and colon. In addition to P-gp, MRP1 and MRP2 transporters are located in the intestine at the basolateral and apical membrane, respectively, and are primarily involved in the efflux of conjugative metabolites. In contrast to efflux transporters, uptake transporters and their potential interplay with metabolic enzymes and efflux transporters have received less attention. Recent studies have reported mRNA levels of a number of organic anion transporting polypeptide (OATP) uptake transporters on the apical membrane of enterocytes, including OATP1A2, OATP2B1, OATP1B3 and OATP1B1, the latter two were previously thought to be liver-specific. Considering that only mRNA levels of OATP1B1 and OATP1B3 were detectable in intestine, their role in intestinal uptake is most likely negligible.

Clinical evidence of intestinal metabolism

CYP3A4 is susceptible to a number of inhibition and induction metabolic drug-drug interactions (DDI) due to its high abundance in both liver and intestine. In addition, the relatively low blood flow to the intestinal mucosa means that the outflow of the drugs from enterocytes is not as high as that from hepatocytes and hence lower abundance of CYP3A can act several fold more efficiently than in the liver. A number of clinical studies have indicated a more pronounced inhibition and induction of intestinal CYP3A in comparison to the liver. For mutual P-gp and CYP3A4 substrates (e.g., cyclosporine, tacrolimus) the interaction is complex and a result of interactive nature and differing inter-relationship of these two proteins in the intestine and liver. However, certain studies suggest that for high permeability substrates (e.g., verapamil) the role of P-gp in the in vivo pharmacokinetics after oral administration and its contribution to the overall inhibition effect may have been overestimated.

Numerous drug-drug interaction studies where victim drugs were administered both i.v. and orally in the absence and presence of an inhibitor/inducer provide indirect clinical evidence for the contribution of the intestine to the magnitude of DDI, although this interpretation has been challenged. In all the cases shown in Table 1 and discussed throughout this review, the term intestine refers to intestinal CYP3A, as equivalent information on any UGT-mediated DDI is very limited. Table 1 shows up to 2.8-fold greater increase in the AUC ratio observed after oral dose of the victim drug in comparison to the i.v. administration, in the presence of either reversible or time-dependent inhibitors, suggesting a contribution of first-pass metabolism in the small intestine. In studies where grapefruit juice (GFJ) was used as a perpetrator, selective inhibition of intestinal CYP3A4 metabolism was observed, allowing the delineation of the importance of the small intestine; this approach is discussed in more detail below. Differential change in the AUC and potential contribution of the small intestine was also shown in the case of induction studies, resulting in up to 8.5-fold greater changes in the AUC after oral administration of nifedipine in the presence of rifampicin (Table 1).

Prediction of metabolic drug-drug interactions

In recent years numerous efforts have been made to predict the magnitude of in vivo DDI from in vitro data, with varying degrees of success. The accurate determination of perpetrator concentration in vivo is problematic because a direct measurement is not possible and there is no generally accepted consensus for the extrapolation of inhibitor/inducer concentration in plasma to that at the enzyme site. DDI prediction models have been refined over the years to incorporate the contribution of multiple inhibitors (or metabolites) and/or the consequence of multiple inhibition mechanisms. Additionally, enzyme properties and characteristics of the victim drugs, namely incorporation
of parallel elimination pathways,\textsuperscript{63,65,103,104} in vitro CYP3A4 kinetic complexities,\textsuperscript{62,105} contribution of the intestinal inhibition and recently also induction have been considered.\textsuperscript{61,63–65,91,100,106–108} There is an interest in the potential impact of metabolic intestinal interactions and this is increasingly becoming a part of the prediction strategy. This review will discuss the ability and limitations of the current approaches to estimate the extent of intestinal inhibition and induction DDI, focusing in particular on the most abundant intestinal P450, CYP3A4. Considering the multifactorial nature of the prediction models, the assessment will consider different perpetrator and victim drug-related properties, focusing in particular on the extent of intestinal first-pass extraction of victim drugs.

**Drug-drug interaction prediction models:** The most common in vivo metric used to assess DDIs is the change in area under the plasma concentration time curve (AUC) of the victim drug following multiple dosing of a second interacting drug relative to the control state.\textsuperscript{60,109–114} Under the assumption that the change in P450 mediated clearance is not associated with any effect on the intestinal absorption or plasma protein binding of the victim drug, comparison of AUC under control conditions and in the presence of the inhibitor/inducer depends on both hepatic and intestinal events, as illustrated in Eq. (1):

\[
\frac{AUC'}{AUC} = \frac{F_G'}{F_G} \cdot \frac{1}{\sum_i \frac{f_{\text{mCYP}_i}}{\text{CL}_{\text{int}}/\text{CL}_{\text{int}}} + \left(1 - \sum_i f_{\text{mCYP}_i}\right)}
\]

where $\text{CL}_{\text{int}}'$ and $\text{CL}_{\text{int}}$ represent intestinal intrinsic clearance in the presence and absence of the perpetrator drug, $f_{\text{mCYP}_i}$ denotes the fraction of the victim drug clearance attributed to the particular P450 subject to the inhibition/induction effect, and the term $i$ indicates the existence of multiple enzymes (n).\textsuperscript{104,115} The $1 - \sum f_{\text{mCYP}_i}$ term in the denominator accounts for other clearance pathways of the victim drug (either other metabolic enzymes, biliary or renal excretion), assuming that the other pathways are not subject to inhibition.\textsuperscript{62,103,104,116}

The first term in the equation comprises of the $F_G'/F_G$ ratio (intestinal availability in the presence and absence of the perpetrator drug, respectively), which allows incorporation of the changes in intestinal metabolism; the remaining part of the equation relates to changes in

### Table 1. Increase in AUC of a range of victim drugs administered i.v. and orally in the presence and absence of CYP3A4 inhibitors and inducers

<table>
<thead>
<tr>
<th>Victim drug</th>
<th>Inhibitor</th>
<th>Fold increase in AUC (i.v.)</th>
<th>Fold increase in AUC (oral)</th>
<th>Fold difference (oral/i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfentanil</td>
<td>Grapefruit juice\textsuperscript{88}</td>
<td>1.1</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Alfentanil</td>
<td>Troleandomycin\textsuperscript{88}</td>
<td>7.3</td>
<td>19</td>
<td>2.6</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Grapefruit juice\textsuperscript{82}</td>
<td>1.1</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Ketoconazole\textsuperscript{174}</td>
<td>1.9</td>
<td>5.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Felodipine</td>
<td>Grapefruit juice\textsuperscript{88}</td>
<td>0.9</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Clarithromycin\textsuperscript{174}</td>
<td>2.7</td>
<td>7.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Grapefruit juice\textsuperscript{84}</td>
<td>1.3</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Itraconazole\textsuperscript{173}</td>
<td>3.2</td>
<td>6.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Flucytosine\textsuperscript{179}</td>
<td>2.0</td>
<td>3.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Erythromycin\textsuperscript{170}</td>
<td>2.2</td>
<td>4.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Saquinavir\textsuperscript{78}</td>
<td>2.5</td>
<td>5.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Voriconazole\textsuperscript{71}</td>
<td>3.5</td>
<td>9.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Ketoconazole\textsuperscript{72}</td>
<td>5.0</td>
<td>14</td>
<td>2.7</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Diltiazem\textsuperscript{100}</td>
<td>1.7</td>
<td>4.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Grapefruit juice\textsuperscript{177}</td>
<td>1.1</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>Grapefruit juice\textsuperscript{178}</td>
<td>0.9</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Ketoconazole\textsuperscript{139}</td>
<td>1.3</td>
<td>2.9</td>
<td>2.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Fold decrease in AUC (i.v.)</th>
<th>Fold decrease in AUC (oral)</th>
<th>Fold difference (oral/i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfentanil</td>
<td>Rifampicin\textsuperscript{86}</td>
<td>2.7</td>
<td>22</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Rifampicin\textsuperscript{139}</td>
<td>1.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Rifampicin\textsuperscript{78}</td>
<td>2.2</td>
<td>9.7</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Rifampicin\textsuperscript{130}</td>
<td>1.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Rifampicin\textsuperscript{78}</td>
<td>1.4</td>
<td>12</td>
</tr>
</tbody>
</table>
hepatic metabolism. Interaction with intestinal CYP3A enzymes is accommodated in an analogous way to the liver and the $F_G'/F_G$ ratio can be expanded as shown in Eq. (2). This approach is applicable for both inhibition (reversible and irreversible) and induction interactions, as shown in Table 2.

$$
\frac{F_G'}{F_G} = \frac{1}{F_G + (1 - F_G) \frac{CL_{int}'}{CL_{int}' + EC_{50j}}}
$$

(2)

Reversible, time-dependent inhibition and induction mechanisms can all be defined by a specific mechanistic equation for the $CL_{int}$ ratio which can be substituted within the generic equations (Eqs. (1) and (2)) for the hepatic and intestinal components (Table 2). In these equations several surrogate concentration terms are used for the inhibitor/inducer concentration at the enzyme/effect site ([I])—the average systemic plasma concentration after repeated oral administration or the maximum hepatic input concentration for the liver and the estimated inhibitor concentration in the intestine during the absorption phase. In contrast to the most commonly used time-averaged value, certain simulation programmes (e.g., Simcyp®) can incorporate the time course of the inhibitor concentration.

**Table 2. Mechanistic equations describing changes in hepatic and intestinal $CL_{int}$**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Hepatic $CL_{int}/CL_{int}'$</th>
<th>Intestinal $CL_{int}G/CL_{int}'G$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversible inhibition$^a$</td>
<td>$1 + \sum_j [I_j]/K_{ij}$</td>
<td>$1 + \sum_j [I_{Gj}]/K_{ij}$</td>
</tr>
<tr>
<td>Irreversible inhibition$^b$</td>
<td>$1 + \sum_j \frac{k_{\text{inact}, j} [I_j]}{k_{\text{deg}, H} (K_{ij} + [I_j])}$</td>
<td>$1 + \sum_j \frac{k_{\text{inact}, j} [I_j]}{k_{\text{deg}, G} (K_{ij} + [I_j])}$</td>
</tr>
<tr>
<td>Induction$^c$</td>
<td>$1 + \sum_j \frac{E_{\text{max}} [I_j]}{[I_j] + EC_{50j}}$</td>
<td>$1 + \sum_j \frac{E_{\text{max}} [I_j]}{[I_j] + EC_{50j}}$</td>
</tr>
</tbody>
</table>

$CL_{int}'$ and $CL_{int}G$ represent intestinal intrinsic clearance in the presence and absence of the perpetrator drug.

$[I]$ is the inhibitor concentration available to the enzyme and $K_i$ is the inhibition constant. $k_{\text{deg}, H}$ and $k_{\text{deg}, G}$ represent the endogenous degradation rate constants in the enzyme and intestine, respectively.

$E_{\text{max}}$ is the maximum induction effect and $EC_{50i}$ the concentration at 50% of $E_{\text{max}}$. In each case the possibility of $m$ inhibitory/induction species is accounted for by the subscript $j$.

Limitations and accuracy of different methods to estimate $F_G'/F_G$ ratio: The $F_G'/F_G$ ratio can be estimated in three different ways, as outlined below; in each case the $F_G$ control values can be obtained from either in vivo or in vitro data (discussed in detail in section “In vivo and in vitro methods for estimation of $F_G$”).

1) In vivo $F_G'/F_G$ ratio—obtained from i.v. and oral data in the presence of an inhibitor or inducer (limited availability of such datasets). The assumption behind this approach is that the perpetrator drug does not affect the fraction absorbed ($F_A$) or the plasma protein binding of a victim drug and does not alter hepatic blood flow.

2) Maximum $F_G'/F_G$ ratio—assuming the ‘worst case’ scenario, i.e., maximum inhibition of intestinal CYP3A4 enzymes is accommodated in an analogous way to the liver and the $F_G'/F_G$ ratio can be expanded as shown in Eq. (2). This approach is applicable for both inhibition (reversible and irreversible) and induction interactions, as shown in Table 2.

$$
\frac{F_G'}{F_G} = \frac{1}{F_G + (1 - F_G) \frac{CL_{int}'}{CL_{int}' + EC_{50j}}}
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(2)

Reversible, time-dependent inhibition and induction mechanisms can all be defined by a specific mechanistic equation for the $CL_{int}$ ratio which can be substituted within the generic equations (Eqs. (1) and (2)) for the hepatic and intestinal components (Table 2). In these equations several surrogate concentration terms are used for the inhibitor/inducer concentration at the enzyme/effect site ([I])—the average systemic plasma concentration after repeated oral administration or the maximum hepatic input concentration for the liver and the estimated inhibitor concentration in the intestine during the absorption phase. In contrast to the most commonly used time-averaged value, certain simulation programmes (e.g., Simcyp®) can incorporate the time course of the inhibitor concentration. Prediction principles in the static and time course (dynamic) models are the same, but the time course can be important in the assessment of reversible inhibition DDIs and in particular for inhibitors with active metabolites contributing to the inhibitory effect (e.g., itraconazole). The time course should be less critical for time-dependent inhibition or induction where the altered state of activity is relatively stable during the dosing interval in line with the recovery half-life of the affected enzyme. However, it can provide useful information on the potential variation in the magnitude of induction, depending on the changes in the inducer concentration relative to $EC_{50}$ associated with the time course. In contrast, the static model assumes a constant extent of induction. The generic equations can be adapted to simultaneously accommodate different inhibition mechanisms (reversible and time-dependent) or even inhibition and induction mechanisms for one DDI (Eq. (3)), where the term $k$ indicates the existence of multiple mechanisms $p$.

$$
\frac{AUC'}{AUC} = \frac{1}{\sum_p \frac{fm_{\text{CYP}}}{CL_{int}/CL_{int}'}}
$$

(3)

The time course should be less critical for time-dependent inhibition or induction where the altered state of activity is relatively stable during the dosing interval in line with the recovery half-life of the affected enzyme. However, it can provide useful information on the potential variation in the magnitude of induction, depending on the changes in the inducer concentration relative to $EC_{50}$ associated with the time course. In contrast, the static model assumes a constant extent of induction. The generic equations can be adapted to simultaneously accommodate different inhibition mechanisms (reversible and time-dependent) or even inhibition and induction mechanisms for one DDI (Eq. (3)), where the term $k$ indicates the existence of multiple mechanisms $p$.

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resulting in $F_G' = 1$, and therefore the maximum ratio as $1/F_G$. However, there is no equivalent scenario suitable for induction DDI.

3) Model predicted $F_G'/F_G$ ratio—obtained from the change in the intestinal intrinsic clearance in the presence of an inhibitor/inducer ($C_{I\text{act}}'$) using the in vitro obtained parameters (e.g., $K_i$ in the case of reversible inhibition) and the estimated inhibitor concentration in the intestinal wall during the absorption phase ($I_G$) (Eq. (4)):

$$I_G = \frac{Dk_IF_i}{Q\text{ent}}$$  

where $F_i$ is the fraction absorbed, $k_i$ the absorption rate constant, $D$ the dose, and $Q\text{ent}$ the enterocytic blood flow (average 0.3 L/min). Decrease in the intestinal intrinsic clearance of the victim drug in the presence of time-dependent inhibitor is obtained from corresponding in vitro and enzyme parameters ($k_{\text{inact}}, K_i$ and $k_{\text{deg}, i}$) and $I_G$,\textsuperscript{46,107} the case of induction, corresponding in vitro parameters ($E_{\text{max}}, EC_{50}$) are used to estimate the extent of change in the intestinal clearance (Table 2).

Intestinal CYP3A4 $k_{\text{deg}, i}$ has been estimated from the reported changes in the AUC of a victim drug at different times after ingestion of grapefruit juice. The rationale behind this approach is selective and irreversible inhibition of intestinal CYP3A4 by components of grapefruit juice, with no effect on hepatic enzymes.\textsuperscript{87,123} The CYP3A4 recovery $t_{1/2}$ estimated by this method ranges from 13 to 33 h across different studies (mean value of 23 ± 10 h), resulting in the corresponding intestinal $k_{\text{deg}}$ of $5 \times 10^{-4}$ min$^{-1}$.\textsuperscript{64,86,101} The estimated intestinal CYP3A4 $t_{1/2\text{deg}}$ is shorter in comparison to hepatic estimates\textsuperscript{63,124} probably reflecting the turnover of intestinal mucosal cells.\textsuperscript{5,125} The accurate estimation of this enzyme property is very important for the assessment of the contribution of the intestinal time-dependent inhibition and induction interactions. Impact of inter-individual variability in hepatic CYP3A4 $t_{1/2\text{deg}}$ (20–146 h, $k_{\text{deg}, H}$ of 0.8–5 $\times 10^{-4}$ min$^{-1}$) on the assessment of time-dependent inhibition potential and DDI prediction accuracy was investigated previously.\textsuperscript{63} The study recommended the use of average hepatic CYP3A4 $t_{1/2\text{deg}}$ of 3 days (i.e., $k_{\text{deg}, H}$ of 1.6 $\times 10^{-4}$ min$^{-1}$) in the assessment of time-dependent interaction potential and this approach yielded a high prediction success rate (defined by 2-fold of observed in vivo values criteria).

**Estimation of intestinal concentration of the perpetrator drug.** Analogous to the liver, a direct estimate of the concentration of the perpetrator drug at the enzyme site in the intestine is not possible. The current approach to estimate $I_G$ (Eq. (4)) accounts for some of the drug/formulation dependent ($F_i, k_i$, and $D$) and physiological properties ($Q\text{ent}$). In a recent analysis of 36 reversible and time-dependent inhibitors,\textsuperscript{106} estimated inhibitor concentrations during the absorption phase span three orders of magnitude, with the concentrations of digoxin and azithromycin among the lowest ($<0.1 \mu M$) and fluconazole and gemfibrozil among the highest ($>100 \mu M$). Although informative, this approach has certain limitations. Drug solubility, i.e., aqueous at pH 7.4 or in biorelevant media (e.g., fasted-state simulated intestinal fluid, FaSSIF),\textsuperscript{126,127} is not taken into consideration when interpreting $I_G$ estimates. One of the assumptions in this approach is that the inhibitor/inducer is not subject to extensive first-pass metabolism itself. This may not be correct for certain inhibitors for which extensive metabolism has been reported, for example diltiazem and itraconazole.\textsuperscript{98,100,128} A recent study in rats\textsuperscript{129} suggests that hydroxy-itraconazole formed by intestinal CYP3A controls the time course of hepatic CYP3A inhibition and is mainly responsible for the observed increase in hepatic availability of itraconazole. In addition, currently there is no agreement on the estimate of any potential binding within the enterocytes and hence this can lead to potential over-estimation of $I_G$. Physiological variability in enteric blood flow (0.1–0.5 L/min, 2–10% cardiac output)\textsuperscript{130,131} results in a range of $I_G$ after a standard dose (e.g., 8–42 $\mu M$ ketoconazole after 200 mg dose and 23–117 $\mu M$ rifampicin after 600 mg dose),\textsuperscript{106} contributing potentially to the inter-individual differences in the magnitude of induction/inhibition effect in conjunction with other factors (dose of the perpetrator drug, intake of food, CYP3A4/3A5 intestinal abundance and activity). Current FDA regulations for the classification of potential P-gp inhibitors\textsuperscript{132} propose estimation of the intestinal lumen concentration by dividing the dose by a volume of 250 mL.\textsuperscript{132,133} The concentrations estimated by this approach are generally two orders of magnitude higher compared to estimates based on $F_i$, $k_i$, $D$ and $Q\text{ent}$.

**Impact of incorporation of intestinal interaction in the prediction strategy**

**Intestinal inhibition:** High concentrations of the putative inhibitor/inducer achieved during the absorption phase imply a high potential for DDI at the level of the intestine. The potential for an interaction in the intestine is determined by the ratio of intestinal concentration of the inhibitor/inducer to its estimated potency, i.e., $I_G/K_i$ or $I_G/EC_{50}$ for reversible inhibition and induction interactions, respectively. In addition, significant intestinal first-pass metabolism of the victim drug may contribute to the inter-individual variability in the magnitude of DDI, as seen for a number of CYP3A4 substrates.\textsuperscript{72,74,85,134,135} The impact of both inhibitor- ($I_G$, $K_i$) and substrate-related ($F_G$) characteristics on the $F_G$ ratio was addressed recently over the $I_G/K_i$ range from 0.01–100 and for victim drugs with differential levels of intestinal extraction, from low to high.\textsuperscript{106} A relative $I_G/K_i$ ratio of > 10 indicates a potential for interaction in the intestine. Similarly, in the case of time-dependent inhibitors, tight binders
The incorporation of the intestinal interaction into the DDI prediction strategy in the form of the $F_G'/F_G$ ratio has resulted in variable prediction success.\(^{51,64,65,89,91,106,107}\) This differential success cannot be associated exclusively with the incorporation of the intestine, as other parameters were not consistent between the datasets, e.g., value of $f_{mCYP3A4}$, use of different inhibitor concentrations in the prediction model, different enzyme degradation rate constants and number of victim drugs investigated.

Table 3. List of bioavailability and intestinal availability values for 25 CYP3A4 substrates. The $F_G$ values were estimated either from i.v. and oral data\(^{80}\) or from grapefruit juice interaction data.\(^{80}\) In case of multiple studies, numbers in brackets represent 95% confidence interval.

<table>
<thead>
<tr>
<th>Drug</th>
<th>$F$ ($F_G$ (i.v/oral))</th>
<th>$F_G$ (oral)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfentanil</td>
<td>0.42</td>
<td>0.61</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>0.84</td>
<td>0.94</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>0.14</td>
<td>0.24</td>
</tr>
<tr>
<td>Buspirone</td>
<td>0.05</td>
<td>0.21</td>
</tr>
<tr>
<td>Cisapride</td>
<td>0.4–0.5</td>
<td>—</td>
</tr>
<tr>
<td>Cyclosporine (healthy)</td>
<td>0.22–0.36</td>
<td>0.44 (0.35, 0.54)</td>
</tr>
<tr>
<td>Felodipine</td>
<td>0.14</td>
<td>0.45</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>≤0.05</td>
<td>—</td>
</tr>
<tr>
<td>Maraviroc</td>
<td>0.23</td>
<td>0.75–0.93</td>
</tr>
<tr>
<td>Methadone</td>
<td>0.92</td>
<td>0.78</td>
</tr>
<tr>
<td>Midazolam</td>
<td>0.24–0.41</td>
<td>0.51 (0.47, 0.56)</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>0.41</td>
<td>0.78</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>0.05–0.08</td>
<td>0.11</td>
</tr>
<tr>
<td>Quinidine</td>
<td>0.78</td>
<td>0.9</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>0.56</td>
<td>—</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>0.2</td>
<td>0.21</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>&lt;0.04</td>
<td>0.18</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>0.38</td>
<td>0.54</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>&lt;0.05</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>&lt;0.01</td>
<td>—</td>
</tr>
<tr>
<td>Trazodone</td>
<td>0.77</td>
<td>0.83</td>
</tr>
<tr>
<td>Triazolam</td>
<td>0.55</td>
<td>0.75</td>
</tr>
<tr>
<td>Verapamil</td>
<td>0.22</td>
<td>0.59</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>0.72</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*Not recommended as GFJ interaction study changed buspirone t\(_{1/2}\) suggesting inhibition of hepatic metabolism.*

(K\(_r\) < 1 \(\mu M\)) or fast inactivating compounds (k\(_{\text{inact}}\) > 0.01 min\(^{-1}\)) have a potential for interaction in the intestine when I\(_C\) > 1 \(\mu M\). The greatest sensitivity is observed for interactions involving victim drugs with moderate to high intestinal extraction (F\(_G\) < 0.5). In contrast, the effect is minimal (maximum 2-fold F\(_G\) ratio) for drugs with an intestinal extraction ≤ 50% (e.g., midazolam, triazolam), irrespective of the potency of the inhibitor.\(^{106}\) Comparison of the relative ratios of the plasma and intestinal concentrations of the perpetrator drug to its potency could rationalise the contribution of each organ to the magnitude of the DDI. For example, low plasma concentrations (for example, if fu < 0.01) can lead to low I/K\(_r\) ratios and negligible interaction potential at the level of the liver. At the same time, the analogous ratio for the intestine could be high, indicating a more pronounced effect on the level of intestine.
the magnitude of interactions was over-predicted by >4.5-fold (Fig. 2A). These findings were in agreement with over-predictions of reversible CYP3A4-mediated DDIs of atorvastatin and tacrolimus, reported previously. However, incorporation of the intestinal interaction improved the prediction success of DDIs with midazolam, triazolam and nifedipine as victim drugs. This might be explained by the greater confidence in FG estimates for the latter three drugs in comparison to atorvastatin and lovastatin (Table 3), as it will be addressed in section “In vivo and in vitro methods for estimation of FG.”

Table 5 shows the DDI prediction accuracy for the scenarios investigated (+/- intestinal component) in relation to the FG of the victim drugs in the dataset. For victim drugs with moderate to low intestinal extraction (FG > 0.5), highly accurate DDI predictions were observed, as ≥ 90% studies were predicted within 2-fold regardless of whether intestinal component was included in the model or not (Table 5, Fig. 3). This subset included a representative range of DDIs, as the observed magnitude spanned from 1.2- to 5.1-fold increase in AUC for quinidine-erythromycin and triazolam-clarithromycin studies, respectively. The largest discrepancy in predic-
Fig. 3. Prediction accuracy of 50 time-dependent inhibition and 36 induction DDIs in the absence and presence of the intestinal contribution.

Drug-drug interactions are classified into groups $F_G < 0.5$ or $F_G > 0.5$ according to the extent of intestinal extraction of the victim drug involved. First bar in each case represents the prediction without the intestinal interaction incorporated in the Eq. (1), whereas second bar refers to the combined hepatic and intestinal model. When incorporated, intestinal interaction is obtained as the predicted $F_G$ ratio. The box-and-whisker plots illustrate the distribution in the prediction success; the upper and lower whisker represent highest and lowest value that is not an outlier (represented by •). The black line represents the median value.

Intestinal induction: In contrast to inhibition, the number of studies investigating prediction of induction DDIs has been small. In developing prediction strategies, advantage has been taken of experiences with inhibition and modelling approaches that integrate both induction and inhibition are available. Recently, a comprehensive set of 103 DDI studies involving 6 inducers of CYP3A4 and 38 victim drugs ($fm_{\text{CYP3A4}}$ ranging from 0.05 to 0.94 for ropivacaine and midazolam, respectively) was reported. Generic DDI prediction model with the induction mechanistic term (Table 2) was applied; however, intestinal interaction was not incorporated in the analysis. A subset of 35 rifampicin DDIs with victim drugs with known $F_G$ values was selected for the current analysis. The dataset included 18 victim drugs with a differential extent of intestinal first-pass ($F_G = 0.14–0.94$ in the case of tacrolimus and alprazolam, respectively). In vitro data from human hepatocytes (single donor) based on testosterone activity as an induction endpoint were used for the predictions. Intestinal interaction was incorporated as the predicted $F_G$ ratio obtained from the in vitro induction parameters and estimated $I_G$ concentration of rifampicin ($39 \, \text{mM}$ after 600 mg dose assuming average $Q_{\text{ent}}$ of 0.3 L/min). Analogous to the time-dependent DDIs, prediction accuracy was related to the $F_G$ of the victim drugs in the actual studies investigated (Fig. 2B, Table 5). Consistent with the inhibition database, significant outliers included interactions with atorvastatin, buspirone, simvastatin, saquinavir and tacrolimus, where in some instances the predicted/observed AUC ratio exceeded 2000% (saquinavir, tacrolimus).
In contrast to inhibition studies, significant inaccuracy was observed even when hepatic induction was considered in isolation, with 49% of the studies predicted outside of 2-fold of unity. Under-prediction trend was apparent, with a median predicted/observed AUC ratio of 68%, ranging from 6–272% (Fig. 3). Only 31% of the studies were predicted within 1.5-fold of in vivo, in contrast to 72% of time-dependent inhibition studies predicted within this range when no intestinal interaction was incorporated. Considering very poor prediction accuracy of hepatic induction, it is not surprising that the incorporation of the intestinal component resulted in 66% of the studies predicted outside 2-fold of the in vivo value and an increased numbers of over-predictions (Fig. 3). In the case of time-dependent DDIs, introduction of the intestinal interaction increased the bias by 33%; in contrast, a 99% increase was observed when intestine was incorporated in the induction DDI model.

However, the interpretation of this over-prediction trend has to be cautious and should not be associated entirely with the incorporation of the intestine. For a limited number of studies, induction data were available after both i.v. and oral administration of the victim drug in the presence of rifampicin, which allowed the delineation of the induction effect between liver and intestine. These studies also allowed us to assess the accuracy of the predicted extent of intestinal induction from in vitro data in the form of $F_G/F_I$ ratio. In the case of nifedipine, alfentanil and midazolam, DDI model predicted >70% reduction in $F_G$ due to induction (73, 81 and 84%, respectively), which was consistent with the observed in vivo changes (79, 75 and 72%, respectively). Despite the accuracy of $F_G/F_I$ ratio prediction, more than half of midazolam studies were over-predicted with the combined model (predicted/observed AUC ratio ranged up to 621%); similar trend was seen in the case of nifedipine (predicted/observed AUC ratio >700%) (Fig. 2B). In the case of cyclosporine and tacrolimus, model predicted 85–90% decrease in $F_G$, in contrast to clinical data where only 50–57% increase in the intestinal extraction was observed in the presence of rifampicin. Analysis of the in vivo data after i.v. and oral administration of tacrolimus (one of the most pronounced outliers, Fig. 2B) indicated that rifampicin administration had a minor effect on tacrolimus $F_I$ (decrease by 1%) consistent with low clearance of tacrolimus relative to hepatic blood flow. However, predicted change in the AUC assuming only hepatic interaction resulted in 5-fold overestimation of the actual magnitude of DDI reported after i.v. administration in the study by Hebert et al. (1.5-fold change in AUC). Combined over-estimation of both hepatic and intestinal interaction (as discussed above) leads to an overall 24-fold over-prediction of the observed change in AUC of orally administered tacrolimus. Unfortunately, i.v. data were not available for other induction pairs to increase our confidence in the predicted changes in $F_G$ in the presence of rifampicin using the current model.

**Limitations and accuracy of different methods to estimate the extent of intestinal interaction:** As illustrated above, differential methods can be employed in order to incorporate the contribution of intestinal inhibition, induction or combined effect in the DDI prediction strategy. Recently, a comprehensive analysis of various approaches to assess the $F_G$ ratio has been performed using a dataset of 36 reversible and time-dependent inhibitors of CYP3A4 in the assessment. For a limited number of studies, data were available after both i.v. and oral administration of the victim drug in the presence of inhibitor, which allowed the assessment of the validity of the maximum inhibition assumption. The analysis indicated a very good agreement between maximum and observed $F_G$ ratios in vivo for interactions involving potent inhibitors (regardless of the mechanism) and substrates predominantly metabolised with no transporter issues (e.g., midazolam). Midazolam studies were the most abundant, with data available in the presence of 9 reversible and irreversible inhibitors (Table 1). For each of the inhibitors, $F_G$' approached 1 as expected (range from 0.83 to 1 in the interactions with erythromycin and itraconazole, respectively), confirming the application of the maximum $F_G$ approach. Analogous findings were observed for alfentanil, felodipine and nifedipine in the presence of grapefruit juice as a perpetrator drug.

However, the assumption of complete intestinal inhibition (1/$F_G$) over-predicted the $F_G$ ratio observed in vivo for dual P-gp-CYP3A4 substrates cyclosporine and tacrolimus up to 3.4-fold. This is not surprising considering that the estimated cyclosporine $F_G$ did not approach 1 in the presence of ketoconazole or grapefruit juice (0.69 and 0.66, respectively); a similar result was apparent in the case of tacrolimus, where the presence of ketoconazole increased its $F_G$ from 14 to only 30%. The lack of availability of i.v. data for other victim drugs in the presence of either ketoconazole or other inhibitors prevents a more extensive comparison. Therefore, the assumption of maximal intestinal inhibition may result in an over-estimation of the importance of intestinal inhibition in the case of moderate to weak inhibitors or in the interactions involving victim drugs with differential contribution of both metabolic enzymes and transporters to their disposition, as illustrated above.

Analysis of both inhibition and induction DDIs highlighted that prediction accuracy was lowest for the DDIs involving victim drugs with $F_G$<0.5 (Fig. 3), and in particular for the subset of highly extracted drugs with $F_G$<0.25 (Table 5). Use of the refined predicted $F_G$ approach and appropriate intestinal CYP3A4 $K_{deg,G}$ ($5 \times 10^{-4}$ min$^{-1}$) is recommended for DDIs with these victim drugs in order to avoid significant over-estimation of true
positives and increased number of false positive predictions. For drugs with intestinal extraction \( \leq 50\% \) (e.g., midazolam), the maximum fold change in \( F_G \) is 2, even in the cases of potent inhibition, i.e., when the perpetrator drug changes \( Cl_{in,G} \) of a victim drug by \( > 90\% \). Although this may suggest that the contribution of intestinal interaction is likely to be relatively minor, for some DDIs this 2-fold change may represent a significant improvement in DDI prediction and result in elimination of a false-negative result, or can result in over-prediction. Therefore, accurate estimate of the \( F_G \) control is essential for the assessment of the interaction magnitude, in addition to the inhibitor/inducer and enzyme properties, in particular for victim drugs where \( F_G < 0.25 \). The following section will address different methods to estimate the intestinal extraction from both in vivo and in vitro data and highlight their potential limitations.

**In vivo and in vitro methods for estimation of \( F_G \)**

Direct measurement of \( F_G \) is rarely performed in human for practical and ethical reasons, with the exception of studies in anhepatic patients during liver transplant operations or in patients whose portal blood circulation bypassed the liver.\(^{140,141}\) However, the severe state of illness in these patients makes the \( F_G \) estimate an unlikely representation of the \( F_G \) in healthy individuals. Different indirect methods have been proposed to estimate the \( F_G \), namely use of plasma concentration-time profiles after oral and i.v. administration and interaction data in the presence of grapefruit juice; these methods have recently been reviewed and the \( F_G \) estimates obtained by both methods have been revisited.\(^{86,122}\)

The estimation of \( F_G \) from i.v. and oral data is based on the assumption of negligible metabolism in enteroocytes after i.v. administration and that systemic clearance of a drug after i.v. dose (corrected for renal excretion) reflects only hepatic elimination.\(^{106,142}\) The extent of intestinal extraction of 25 drugs estimated by this method is shown in Table 3, ranging from 6 to 93\% in the case of alprazolam and lovastatin, respectively.\(^{122}\) In all the cases CYP3A4 contributes predominantly to elimination (\( fm_{CYP3A4} > 0.7 \)), with the exception of trazodone and zolpidem.\(^{63,95,103}\) In vivo \( F_G \) estimates obtained by this method are of limited availability and represent the measure of the net result of the transporter-enzyme interplay.

The i.v./oral approach is based on several assumptions that may lead to inaccuracies in \( F_G \) estimates. The assumption that the extent of intestinal metabolism after i.v. administration is negligible may not necessarily be valid as an average 8\% extraction ratio after an i.v. dosed midazolam has been reported in anhepatic patients (up to 26\% in one patient).\(^{140}\) Variation in drug-dependent parameters such as \( Cl_{in,F} \), \( F_a \) and blood:plasma ratio contributes to differential \( F_G \) estimates and these parameters are not generally reported in the clinical studies used to estimate the \( F_G \). For example, in the case of tacrolimus, the blood:plasma ratio has been reported to range from 12 to 67 (average value used in this analysis was 35).\(^{143,144}\) Although these parameters are generally available in the literature, they are associated with considerable inter-individual variability. In the absence of accurate estimates of \( F_a \), the indirect method assumes complete absorption (i.e., \( F_a = 1 \)) when estimating the hepatic extraction ratio (\( E_H \)). This can result in an under-estimation of \( F_G \) and subsequently over-estimation of the \( F_G \) ratio in the DDI prediction, in particular if the latter is obtained assuming maximum intestinal inhibition. Additional errors may occur due to the use of average \( Q_{H,H} \), despite its range from 17.1 to 25 mL/min/kg,\(^{145}\) which may result in biased estimates of \( E_H \) and consequently \( F_G \) for drugs with blood clearance (\( Cl_b \)) approaching hepatic blood flow. A number of drugs in the current analysis have \( Cl_b \) values \( > 70\% \) of the average \( Q_{H,H} \), namely atorvastatin, buspirone, felodipine, repaglinide, saquinavir and verapamil. DDIs associated with some of these drugs represent significant outliers in the case of both induction and inhibition interactions (Figs. 2A and 2B). This can be rationalised by the sensitivity of i.v./oral \( F_G \) esti-

![Fig. 4. Impact of \( Q_{in} \) on the estimation of \( F_G \) (grey) and \( F_a \) (white) for three Ca\textsuperscript{2+}-channel antagonists reported to increase \( Q_{in} \); A: \( F_G \) and \( F_a \) estimates based on the mean population \( Q_{in} (20.7 \text{ mL/min/kg}) \); error bars indicate the physiological range of \( Q_{in} (17.1-25.5 \text{ mL/min/kg}) \); B: \( F_G \) and \( F_a \) estimates based on the reported increase in \( Q_{in} \) by 71, 47 and 36\% for felodipine, verapamil and nifedipine, respectively.\(^{85,146,147}\)](image-url)
mates on $Q_{H}$ and consequently sensitivity of the DDI models on the $F_G$ parameter when estimated to be in the range $<0.25$, as is the case for buspirone and atorvastatin. In addition, certain drugs, for example felodipine, verapamil and nifedipine, were previously reported to temporarily increase $Q_{H}$ by 36 to 71%\cite{85,146,147} and this is generally not taken into account when estimating $F_G$ from i.v./oral data. Figure 4 highlights the potential bias in $F_G$ and $F_H$ estimates for these drugs associated with alterations in $Q_{H}$. The $F_H$ and subsequent $F_G$ estimates of felodipine and verapamil are highly dependent on $Q_{H}$, whereas the effect of $Q_{H}$ is minor for the estimation of nifedipine $F_H$ and $F_G$. This can be explained by the generally higher $CL_b$ values of felodipine and verapamil (approximately 75% of average $Q_{H}$ in comparison to 50% in the case of nifedipine). Assuming that the bioavailability remains unaffected, the increasing $Q_{H}$ in the presence of the Ca$^{2+}$-channel antagonists decreases the $F_G$ estimate of nifedipine, nifedipine and verapamil by 67%, 16% and 86%, respectively, as shown in Figure 4. Therefore, it must be emphasized, that especially for drugs with high hepatic extraction, an accurate assessment of $Q_{H}$ is of pivotal importance for the estimate of $F_G$, and as long as $Q_{H}$ is not reported in studies aiming to determine intestinal availability, $F_G$ estimates for these drugs should be regarded with caution.

Alternatively, $F_G$ can be estimated from the large number of interaction studies reported with grapefruit juice.\cite{87,123,148-150} This approach is based on the assumption of complete and irreversible inhibition of CYP3A-mediated metabolism in the intestine but no effect on hepatic enzymes or the fraction absorbed of the investigated drug. The application of this method was addressed in detail recently,\cite{186} highlighting the limitation of this method for the estimation of $F_G$ for drugs whose disposition is co-dependent on efflux/uptake transporters and metabolic enzymes. The authors have reported the highest discrepancy between the i.v./oral and GFJ method in the case of atorvastatin, cyclosporine and saquinavir, all substrates for P-gp and OATP transporters.\cite{58,151,152} For example, analysis of i.v./oral data for atorvastatin estimated an $F_G$ value of 0.24,\cite{153} whereas grapefruit juice interaction data suggested a far less extensive intestinal contribution to atorvastatin first-pass metabolism ($F_G,GFJ = 0.56$).\cite{86} In contrast, good agreement between the GFJ and i.v./oral $F_G$ estimates was observed for purely metabolised drugs, supporting the interchangeable use of these methods for drugs where transporter mediated uptake/eflux is insignificant.

One key assumption in the estimation of $F_G$ from GFJ data is the complete and selective inhibition of CYP3A4 metabolism in the intestine. Assessment of this assumption was undertaken for drugs where i.v./oral data in the absence and presence of GFJ were reported in the same individuals.\cite{82-85,88,159} In the case of complete intestinal in-
availability of GFJ data allowed the assessment of both inter-individual and inter-study variation in $F_G$. The inter-individual variation in cyclosporine $F_G$ estimates (33% and 27% for healthy and kidney transplant patients, respectively) was more pronounced in comparison to the inter-study variability (9.2% and 5.7% for healthy and kidney transplant patients, respectively). A similar trend was seen for felodipine, with inter-individual and inter-study variability of 24–54% and 13%, respectively.

Reduction in intestinal extraction was observed in studies investigating cirrhotic patients, as reported for verapamil, nifedipine and midazolam. Increase in splanchnic blood flow and consequently decreased contact with intestinal CYP3A in the case of cirrhotic patients with portosystemic shunts has been suggested as a rationale for increased $F_G$ observed for midazolam in this group of patients. In contrast, Chalasani and coworkers proposed that a decrease in intestinal CYP3A4 activity in cirrhotic patients causes the increase in $F_G$, as changes in splanchnic blood flow alone would be insufficient to explain the findings. Recently, McConn et al. confirmed that duodenal CYP3A expression and total midazolam hydroxylation were lower by 47 and 34%, respectively in patients with cirrhosis; the severity of the condition correlated with the decrease in intestinal expression levels and activity.

**Prediction of $F_G$ from in vitro data:** In silico approaches to estimate $F_G$ using physiologically-based pharmacokinetic models with differential ability to incorporate in vitro metabolic and transporter data are becoming increasingly important in the current integrative and mechanistic systems biology approaches. Modelling of intestinal first-pass requires incorporation of drug absorption, zonal and cellular heterogeneous distribution of metabolic enzyme and efflux/uptake transporters along the length of the intestine and enterocytic rather than total organ blood flow. A number of physiologically-based models have been reported with different levels of complexity and integration of passive permeability with the activity of metabolic enzymes and transporters.

In contrast to complex physiologically-based models, ‘minimal’ $Q_{Gal}$ model is also proposed to predict $F_G$ from in vitro clearance and permeability data by overcoming the inadequacy of the perfusion limited approaches adapted from the corresponding liver model. The $Q_{Gal}$ model and models adapted from the original Compartmental Absorption Transit (CAT) and Advanced CAT model have now been incorporated in the commercially available softwares (GastroPlus and Simcyp). These integrated dynamic models for the prediction of oral drug absorption and metabolism have recently been discussed. The major disadvantage of some of the models proposed is their complexity and the limited availability of all the parameters required for the prediction of $F_G$. Therefore, addressing all the complexities of the intestinal first-pass metabolism adequately and yet retaining a relatively practical model remains a challenge. In addition, comprehensive analysis of the utility of the $F_G$ estimates obtained from in vitro clearance and permeability data for the prediction of inhibition and induction DDIs is lacking.

**Summary and Conclusions**

The contribution of intestinal interaction is incorporated into the prediction equation based on hepatic enzyme interaction data in the form of the ratio of $F_G$ in the presence and absence of the perpetrator; this approach is applicable for both inhibition and induction interactions. The variable success of current prediction strategies might pose the question of the benefit of incorporation of the intestinal component in the prediction models. Specificities of intestinal physiology and prevalence of clinical evidence support the importance of intestinal CYP3A; therefore, its contribution to the overall magnitude of inhibition or induction DDIs should not be ignored. Differences observed in the prediction success cannot be associated exclusively with the incorporation of the intestine in the prediction strategy, as values of certain key parameters used in conjunction with the in vitro data are often inconsistent between the datasets. In some instances, use of unrealistically high hepatic input concentrations may compensate for ignoring the contribution of the gut and result in comparable prediction outcome to the scenarios where the inhibitor concentration corrected for the plasma binding is used in conjunction with intestinal inhibition. This review emphasizes the importance of a valid mechanistic model and appropriate interpretation of clinical observations to increase our confidence in the quantitative prediction of the intestinal contribution to the overall pharmacokinetics in human.

As the current analysis has shown, incorporation of the intestinal component reduces the number of false negatives; yet, in some instances, depending on the initial model assumptions, it can lead to increased numbers of false-positives, as illustrated in particular in the case of induction DDIs. However, over-prediction of hepatic induction interaction is evident for certain drugs (e.g., tacrolimus) warranting further refinement of the hepatic component in the induction model per se to increase our confidence in overall prediction of these DDIs. In the case of inhibition, prediction models provide more options for the incorporation of the intestinal contribution, using either the pragmatic approach assuming complete inhibition ($1/F_G$) or the more refined predicted $F_G$ ratio. A very good agreement between maximum and observed $F_G$ ratios in vivo for interactions involving potent inhibitors (regardless of the mechanism) and substrates predominantly metabolised with no transporter issues (e.g., midazolam, nifedipine) supports the utility of this
approach. However, limitations exist for the assessment of DDIs with moderate to weak inhibitors or victim drugs with contribution from both metabolic enzymes and transporters in their disposition (e.g., cyclosporine, tacrolimus). Use of the predicted $F_G$ ratio with appropriate intestinal CYP3A4 $k_{deg}$ is recommended for time-dependent DDIs, in particular for the interactions involving victim drugs with high intestinal first-pass ($F_G < 0.25$).

In addition, current estimates of the predicted $F_G$ ratio for induction DDIs are based on the use of induction in vitro data obtained in hepatocytes due to limited availability of induction data in intestinal in vitro systems.\(^{172,173}\) Consideration of the physiological specificities associated with the intestine and the use of corresponding in vitro data may improve our prediction success for induction DDIs.

Due to the sensitivity of the DDI prediction models to the accuracy of the $F_G$ estimates, the advantages and limitations of different in vivo and in vitro methods to assess $F_G$ and inter-individual variability associated with this parameter were discussed. The $F_G$ estimates obtained via different approaches in vivo (anhepatic patients, i.v./oral or from grapefruit juice interactions) have been shown to be comparable in the case of midazolam. However, it is evident that this is not always the case, as a large discrepancy between grapefruit juice and i.v./oral $F_G$ estimates was seen for drugs with $F_G < 0.25$ (e.g., saquinavir). Considering the range of assumptions in both indirect methods and the generally limited number of studies reported for highly extracted drugs (e.g., buspirone), it is advisable to consider the available in vivo data very critically.

A number of recent reports have assessed differential prediction strategies (e.g., inclusion of the time course in the model) using midazolam as the only CYP3A4 victim drug in the dataset. Alternatively, midazolam DDIs constituted a large proportion in the database driving the overall prediction success and general recommendations. As illustrated here, midazolam DDIs show good prediction success regardless of the method applied to implement the contribution of intestine (maximum inhibition or predicted $F_G$ ratio).

Although this is reassuring, the prospective specification of CYP3A4 DDI for new molecular entities should not entirely rely on the midazolam scenario. A number of drug-dependent properties discussed here and variability associated with these parameters need to be considered in order to avoid under-estimation of the $F_G$ and subsequent over-estimation of the $F_G$ ratio in the DDI prediction. In addition, variability observed in $F_G$ estimates due to either health status or gender raises concern in the extrapolation across different populations. The incorporation of the inter-individual variability in $F_G$ as a result of differential enzyme/transporter abundance, contribution of polymorphic variants and disease- and age-specific differences in demography is essential in order to progress further in DDI prediction. In addition, broader application of current physiologically-based pharmacokinetic models as tools to rationalise the alterations in the intestinal clearance in the presence of inhibitor/inducers of either transporters or enzymes is crucial, as well as consideration of any spatial disparities in the location of absorption of victim and perpetrator drugs.

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