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PERSPECTIVE

Physiologically-based pharmacokinetic modeling for primary metabolites of CYP3A and P-glycoprotein inhibitors in drug–drug interactions: Should we assume the free drug hypothesis?

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INTRODUCTION

Metabolites of perpetrators may contribute to drug–drug interactions (DDIs) mediated by inhibition/induction of drug-metabolizing enzymes and transporters. Using physiologically-based pharmacokinetic (PBPK) modeling, we provide our perspective on the model-predicted site-of-action concentrations for metabolites of cytochrome P4503A (CYP3A) and P-glycoprotein (P-gp) dual inhibitors, itraconazole and verapamil, in DDI studies with midazolam (CYP3A substrate) and dabigatran etexilate (P-gp substrate), as representative examples. We focus on whether applying the free drug hypothesis improves DDI predictions in these cases.

PBPK models of itraconazole and verapamil

PBPK modeling is a mechanistic approach to quantitatively describe in vivo drug concentration-time profiles, and thus is widely applied to predict clinical outcomes including DDIs.[1,2] This modeling approach is one of the critical components in model-informed drug discovery and development (MID3). Regulatory authorities in general accept the modeling outcomes of drug-metabolizing enzyme-mediated DDIs, especially CYP enzymes, whereas sufficient confidence levels for predictive model-performance have not yet been reached for transporter-mediated DDIs due to, for instance, uncertainties around in vitro-to-in vivo scaling factors of transporter kinetics and drug concentrations at the site-of-action.[3–5] The uncertainties are typically addressed by sensitivity analyses to evaluate effects of the given parameters on overall outcomes.[5–7]

Itraconazole and verapamil are dual inhibitors of the major drug-metabolizing enzyme, CYP3A, and the efflux transporter, P-gp.[8,9] Their primary metabolites, hydroxy-itraconazole and norverapamil, also inhibit both CYP3A and P-gp. We recently reported PBPK modeling with in vitro-to-in vivo extrapolation (IVIVE) of P-gp kinetics in clinical DDI studies between itraconazole and verapamil as perpetrator drugs and digoxin, dabigatran etexilate, and quinidine as victim drugs.[7] We used the Simcyp

population-based simulator with the advanced dissolution, absorption, and metabolism (ADAM) model to predict DDIs, including the fraction of the dose absorbed (F_a) and the fraction of the dose escaping intestinal first-pass metabolism (F_g). The results revealed that the DDI results were reasonably described by the PBPK-IVIVE approach with P-gp kinetic parameters determined in vitro, such as inhibitor K_i. We incorporated the inhibition parameters of parent drugs in both liver and intestine whereas those of metabolites were only applied to the liver. The reason for this difference was because of the model-predicted different site-of-action concentrations between parent drugs and metabolites. That is, the predicted site-of-action concentrations for parent drugs were the ADAM-predicted unbound enterocyte concentrations ($C_{gut,u}$), whereas those for metabolites were the model-predicted unbound portal vein concentrations ($C_{portal,u}$). In most reported itraconazole and verapamil PBPK models, the unbound fractions in gut enterocytes ($f_{u,gut}$) of both the parent drugs and the metabolites are set at unity as often indicated as default values.[7–9] Several other perpetrator metabolite PBPK models accounting for intestinal DDIs on CYP3A also assume metabolite $f_{u,gut}$ of unity, as illustrated in Table S1. This implies that the reported PBPK models have utilized the model-predicted total portal vein concentrations (C_{portal}) of metabolites as the site-of-action concentration, which is not in line with the commonly applied free drug hypothesis.[10] Hence, the question arises whether the free drug hypothesis for metabolites should be applied to the PBPK modeling (i.e., $f_{u,gut} \approx f_{u,plasma}$ [unbound fraction in plasma]). To address this question, we focused on DDI prediction between itraconazole and verapamil as perpetrators and midazolam and dabigatran etexilate as victims, as representative DDI cases. First, the sensitivity analyses for the inhibition parameters for both the parent drugs and the metabolites were simultaneously performed in two scenarios with the metabolites' $f_{u,gut}$ of unity and $f_{u,plasma}$. Furthermore, the sensitivity analyses for the metabolite $f_{u,gut}$ ranging from 0.0001 to 1 were performed in these DDI studies. We believe that the present results could help understand the effects of the site-of-action concentrations of metabolites on DDI prediction. PBPK modeling outlines and results are summarized in the Supplementary Material.

Predicted metabolite contribution to overall DDIs

In the midazolam DDI studies, the sensitivity analyses for CYP3A4 inhibition parameters of the parent drugs and the metabolites showed modest differences in the predicted midazolam F_g between the metabolite $f_{u,gut}$ of unity and $f_{u,plasma}$ (Figures 1 and 2). The differences were pronounced in the areas around the weaker inhibition potency of the parent drugs than the metabolites. Noteworthily, the midazolam F_g at the original inputs of parent drugs (itraconazole K_i of 0.001 μM and verapamil k_{inact} of 1.2 h^{-1}) was near-unity among the ranges of the metabolite inhibition potency tested due to the parent drug-mediated near-complete CYP3A4 inhibition. This led to the negligible differences in the predicted F_g at the metabolite $f_{u,gut}$ of 0.0001 to 1 between the two sets of analyses with and without the metabolite inhibition parameters (Figure S2). Thus, the differences in the predicted ratios of the maximal plasma concentrations and the area under the plasma concentration–time curves ($C_{max}R$ and AUCR, respectively) suggested that the contribution of metabolites to the overall DDIs would be mainly due to hepatic CYP3A4 inhibition. The ADAM-predicted maximal $C_{gut,u}$ for both itraconazole and verapamil reached around 1–10 μM (Figure S1). Accordingly, in these cases, additional effects of the metabolites on intestinal DDIs were negligible due to near-complete intestinal CYP3A4 inhibition by the parent drug alone.

In the dabigatran etexilate DDI studies, the sensitivity analyses for the parent drug and metabolite P-gp K_i showed considerable differences in the predicted substrate F_a between the metabolite $f_{u,gut}$ of unity and $f_{u,plasma}$ (Figures 1 and 2). Notably, the predicted F_a was nearly independent of the metabolite P-gp K_i when the metabolite $f_{u,gut}$ was set at $f_{u,plasma}$, suggesting the negligible contributions of metabolites to the intestinal DDIs. This is mostly explained by the model-predicted steady-state site-of-action concentrations of metabolites (i.e., $C_{portal,u}$) not reaching the P-gp K_i values (Figure S1). When the metabolite $f_{u,gut}$ was set at unity, the predicted site-of-action concentrations (i.e., C_{portal}) were two to three-fold higher than the P-gp K_i (Figure S1). This resulted in pronounced differences in the predicted substrate F_a in the sensitivity analyses where the metabolites were more potent than the parent drugs. In the case of itraconazole DDIs, there were negligible differences in the DDI prediction ($C_{max}R \approx 8$ and AUCR ≈ 7) among the range of hydroxyitraconazole $f_{u,gut}$ largely due to that hydroxyitraconazole was approximately four-fold less potent than itraconazole (P-gp K_i of 0.8 vs. 0.22 μM; Figure S2). In contrast, the contribution of metabolite to the overall results depended on the metabolite $f_{u,gut}$ in the case of verapamil DDIs because norverapamil was approximately 10-fold more potent than verapamil (P-gp K_i of 0.15 vs. 2.0 μM; Figure S2). The predicted F_a increased from ~0.1 at a norverapamil $f_{u,gut}$ of less than 0.1 to ~0.2 at $f_{u,gut}$ of unity, causing an increase in $C_{max}R$ and AUCR from 1.5 to 2.5. Norverapamil $f_{u,gut}$ corresponding to $f_{u,plasma}$ of 0.083 was around the point where the predicted F_a increased. To exemplify DDI prediction

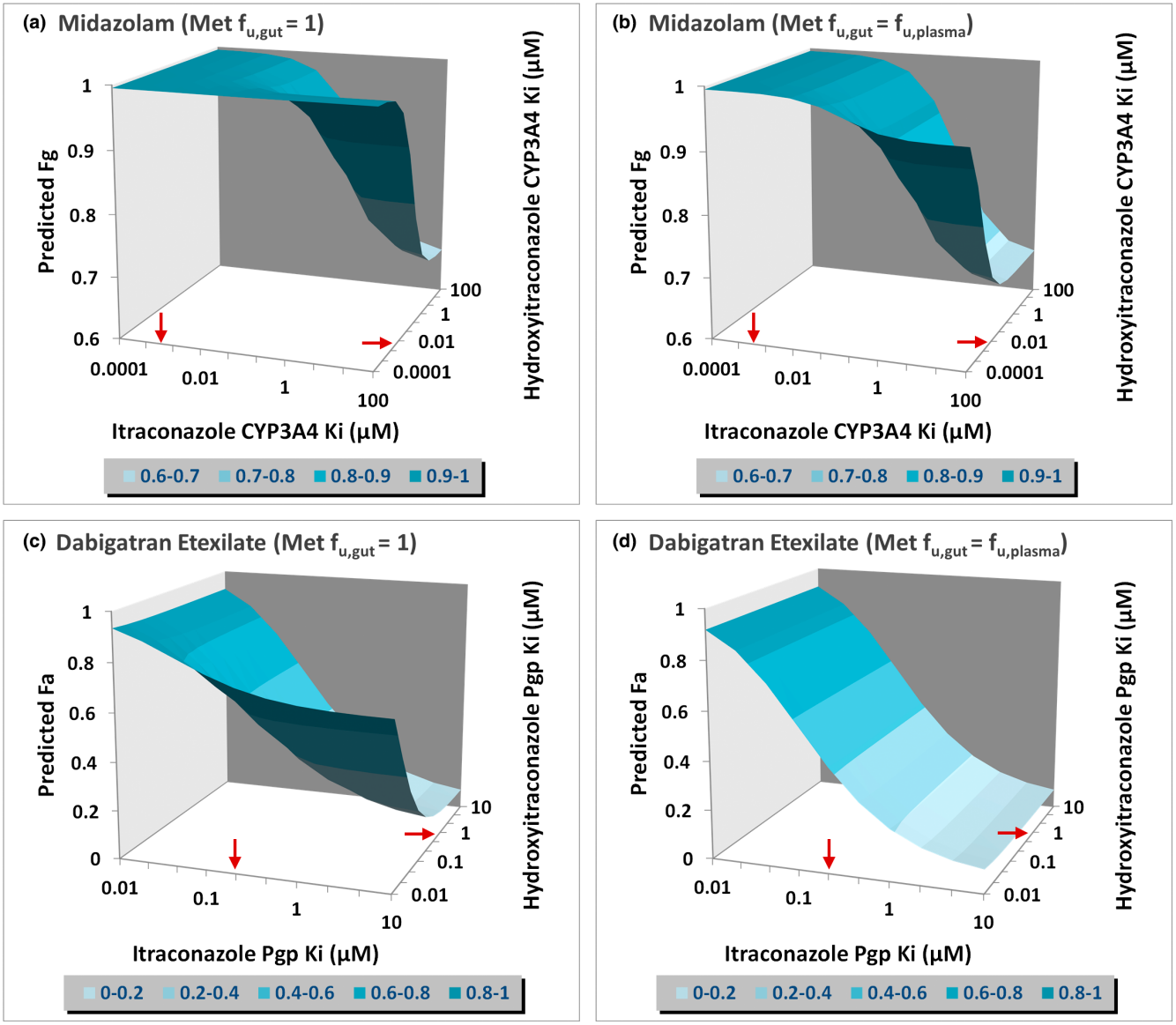

FIGURE 1 Sensitivity analyses for the CYP3A4 and P-gp inhibition parameters of itraconazole and hydroxyitraconazole in the DDI studies with midazolam at 7.5 mg (a,b) and dabigatran etexilate at 0.375 mg (c,d) in healthy subjects following a single oral administration of the substrate with multiple-dose oral administration of itraconazole at 200 mg once-daily. The simulations were performed for two scenarios assuming the metabolite (Met) $f_{u,gut}$ of unity (a,c) and $f_{u,plasma}$ (b,d). The red arrows represent the CYP3A and P-gp K_i input values of itraconazole (0.001 and 0.22 μM, respectively) and hydroxyitraconazole (0.008 and 0.8 μM, respectively). DDI, drug–drug interaction.

depending on the inhibition potency of parent drugs versus metabolites, additional sensitivity analyses were performed by changing the parent drug and metabolite P-gp K_i. When itraconazole and hydroxyitraconazole P-gp K_i values were changed to 10-fold higher and lower values, respectively, the effects of hydroxyitraconazole $f_{u,gut}$ were significantly more pronounced on the DDI prediction with increasing the predicted F_a up to ~0.3 (Figure S3). This example appeared to be similar to the DDI prediction between verapamil and dabigatran etexilate because of more potent inhibition by metabolites than parent drugs. Reversely, when verapamil and norverapamil P-gp K_i values were changed to 10-fold lower and higher values, respectively, the effects of norverapamil $f_{u,gut}$ on the DDI prediction disappeared from the original analyses (Figures S2 and S3). The predicted F_a of ~0.4 in the test group was higher than that in the original analyses (0.1–0.2) because of the strong P-gp inhibition by the parent drug.

As expected, one of the key parameters for DDI prediction is the ratios of unbound inhibitor concentrations over inhibition potency. The contribution of metabolites to overall DDIs could depend on the differences in these ratios between parent drugs and metabolites. The metabolite contribution could also underlie the baselines of substrate F_a and F_g, such as near-complete intestinal

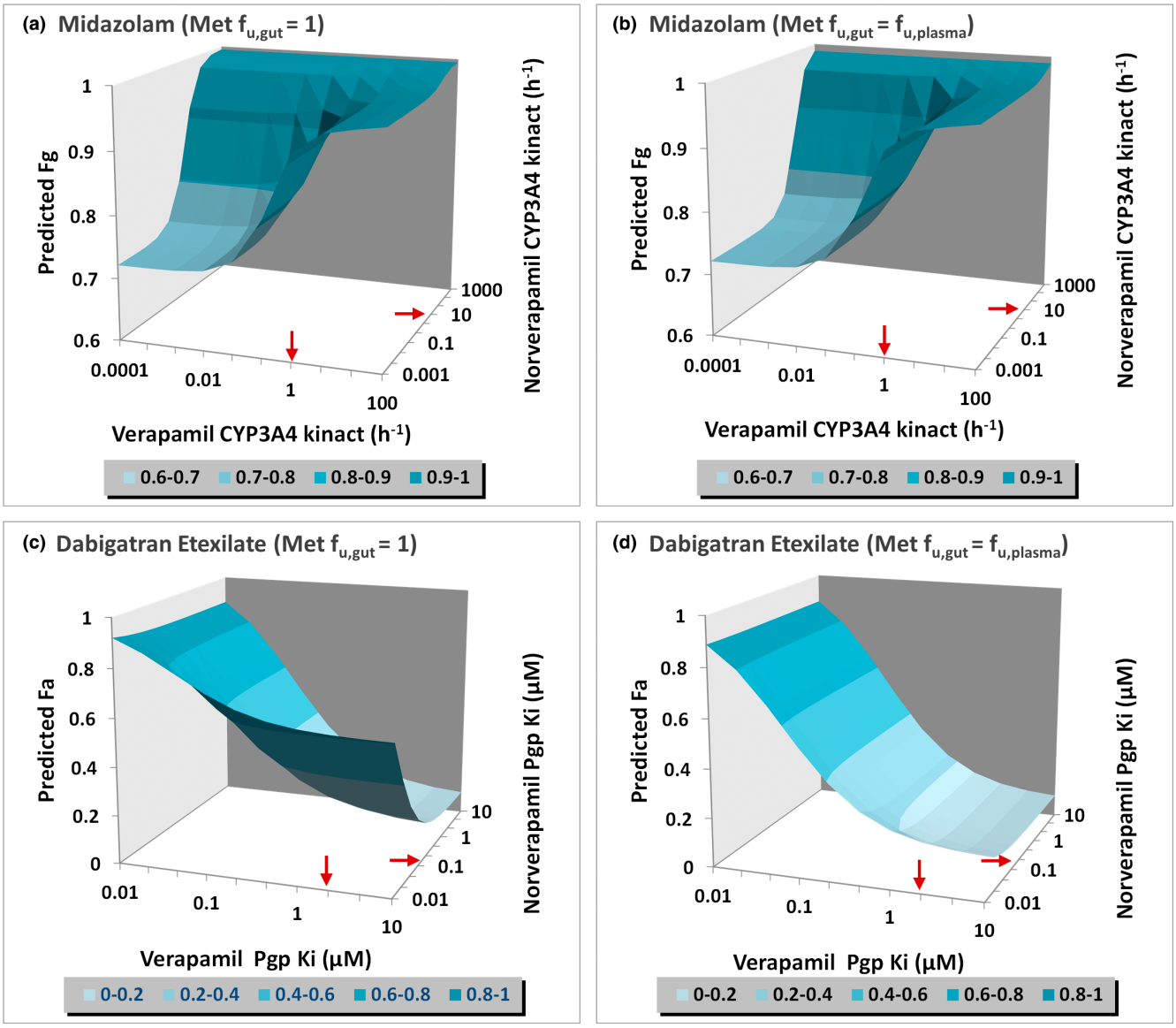

FIGURE 2 Sensitivity analyses for the CYP3A4 and P-gp inhibition parameters of verapamil and norverapamil in the DDI studies with midazolam at 15 mg (a,b) and dabigatran etexilate at 150 mg (c,d) in healthy subjects following a single oral administration of the substrate with multiple-dose oral administration of verapamil 80 mg three-times-daily or 120 mg twice-daily. The simulations were performed for two scenarios assuming the metabolite (Met) $f_{u,gut}$ of unity (a,c) and $f_{u,plasma}$ (b,d). The red arrows represent the CYP3A k_{inact} and P-gp K_i input values of verapamil (1.2 h^{-1} and 2.0 μM, respectively) and norverapamil (10.8 h^{-1} and 0.15 μM, respectively). DDI, drug–drug interaction.

absorption or availability in control groups without inhibitors or test groups without metabolites.

CONCLUSION

As the main MID3 component, PBPK modeling is a powerful tool to address key questions in clinical studies, including untested scenarios. Currently, it is challenging to predict unbound metabolite concentrations at the site-of-action. To overcome uncertainty in the prediction outcomes, sensitivity analyses for key parameters, such as metabolite $f_{u,gut}$, should be performed to evaluate their effects on overall results. When the degree of predicted overall DDIs depend on metabolite $f_{u,gut}$, the input values of $f_{u,gut}$ should be considered carefully. In some cases, such as where potential safety concerns should primarily be considered, the $f_{u,gut}$ of unity could be assumed as the most conservative scenario to predict *maximal* intestinal DDIs by metabolites. In this case, particular attention should also be paid to the differences in outcomes over the range of $f_{u,gut}$, (e.g., $f_{u,gut} \approx 1$ vs. $f_{u,plasma}$). Depending on metabolite $f_{u,plasma}$, the predicted site-of-action concentrations could be significantly different in some cases, for example, ~100-fold for hydroxyitraconazole ($f_{u,plasma} \approx 0.012$) and ~10-fold for norverapamil ($f_{u,plasma} \approx 0.083$). Although the

difference in the modeling results between $f_{u,gut}$ of unity and $f_{u,plasma}$ were minimal in three out of the four cases presented, the modeling approach utilizing metabolite C_{portal} with $f_{u,gut}$ of unity should not be simply extrapolated to other DDI scenarios, especially when metabolites are more potent inhibitors than parent drugs. As presented in this study, verapamil DDIs with dabigatran etexilate were overpredicted when norverapamil $f_{u,gut}$ was set at unity, whereas those were reasonably predicted assuming $f_{u,gut}$ equal to $f_{u,plasma}$. This case underscores that the metabolite $f_{u,gut}$ is a critical parameter for predicting the relevant site-of-action concentrations. Furthermore, PBPK modeling with the metabolite $f_{u,gut}$ equal to $f_{u,plasma}$ sufficiently predicted the DDI results in all the cases tested. It is our belief that, unless there is any direct evidence to the contrary, such as low parent drug F_g, metabolite $C_{portal,u}$ (i.e., $f_{u,gut} \approx f_{u,plasma}$) should be the more appropriate site-of-action concentration than C_{portal} (i.e., $f_{u,gut} \approx$ unity). This is in line with the free drug hypothesis generally assumed for modeling.[10] To quantitatively predict metabolite $C_{gut,u}$, the model should account for the metabolite disposition profiles, such as the metabolite formation in the gastrointestinal (GI) tract and the distribution between the GI tract and portal vein (including recirculation if occurring). Hence, more mechanistic intestinal PBPK models will be required to further support decision making on MID3.

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CONFLICT OF INTEREST

All authors are employees of Janssen Research & Development, LLC, and are shareholders in the parent company (Johnson & Johnson).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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