Physiologically-based pharmacokinetic modeling to evaluate in vitro-to-in vivo extrapolation for intestinal P-glycoprotein inhibition

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Abstract
As one of the key components in model-informed drug discovery and development, physiologically-based pharmacokinetic (PBPK) modeling linked with in vitro-to-in vivo extrapolation (IVIVE) is widely applied to quantitatively predict drug–drug interactions (DDIs) on drug-metabolizing enzymes and transporters. This study aimed to investigate an IVIVE for intestinal P-glycoprotein (Pgp, ABCB1)-mediated DDIs among three Pgp substrates, digoxin, dabigatran etexilate, and quinidine, and two Pgp inhibitors, itraconazole and verapamil, via PBPK modeling. For Pgp substrates, assuming unbound Michaelis-Menten constant ($K_m$) to be intrinsic, in vitro-to-in vivo scaling factors for maximal Pgp-mediated efflux rate ($J_{\text{max}}$) were optimized based on the clinically observed results without co-administration of Pgp inhibitors. For Pgp inhibitors, PBPK models utilized the reported in vitro values of Pgp inhibition constants ($K_i$), 1.0 μM for itraconazole and 2.0 μM for verapamil. Overall, the PBPK modeling sufficiently described Pgp-mediated DDIs between these substrates and inhibitors with the prediction errors of less than or equal to ±25% in most cases, suggesting a reasonable IVIVE for Pgp kinetics in the clinical DDI results. The modeling results also suggest that Pgp kinetic parameters of both the substrates ($K_m$ and $J_{\text{max}}$) and the inhibitors ($K_i$) are sensitive to Pgp-mediated DDIs, thus being key for successful DDI prediction. It would also be critical to incorporate appropriate unbound inhibitor concentrations at the site of action into PBPK models. The present results support a quantitative prediction of Pgp-mediated DDIs using in vitro parameters, which will significantly increase the value of in vitro studies to design and run clinical DDI studies safely and effectively.

Study Highlights
WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Physiologically-based pharmacokinetic (PBPK) modeling is increasingly being applied to predict transporter-mediated drug–drug interactions (DDIs); however,
there are currently knowledge gaps that limit the confidence of DDI predictions for modeling transporter kinetics of both substrates and inhibitors.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

The aim of this study was to quantitatively investigate an in vitro-to-in vivo extrapolation (IVIVE) for intestinal Pgp-DDIs between three Pgp substrates, digoxin, dabigatran etexilate, and quinidine, and two Pgp inhibitors, itraconazole and verapamil. The PBPK-IVIVE approach utilized Pgp kinetic parameters determined in vitro such as unbound Michaelis-Menten constants and maximal efflux rates for the substrates and inhibition constants for the inhibitors.

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

The present PBPK-IVIVE approach reasonably described clinically observed Pgp-DDI results, suggesting a consistent IVIVE for Pgp kinetics. The present modeling approach can be applicable to predict Pgp-DDIs with other Pgp substrates and inhibitors.

**HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?**

The present PBPK-IVIVE results support a quantitative Pgp-DDI prediction using in vitro Pgp kinetic parameters, thus presenting advancement toward quantitative Pgp-DDI prediction in clinical studies and/or case scenarios that have not been tested clinically yet.

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**INTRODUCTION**

Model-informed drug discovery and development (MID3) has become an important framework to quantitatively maximize the benefit-risk profiles of new molecular entities during their development. One of the critical components in the MID3 strategy is physiologically-based pharmacokinetic (PBPK) modeling, which is a mechanistic framework to quantitatively describe in vivo drug disposition profiles based on drug- and system-dependent parameters.\(^1\)\(^-\)\(^3\) By integrating in vitro-to-in vivo extrapolation (IVIVE) with PBPK modeling, PBPK-IVIVE is widely applied to predict in vivo disposition profiles of drugs in various clinical studies, such as drug-drug, drug-disease, and drug-gene interactions, that have not been tested yet. For the prediction of drug-drug interactions (DDIs), regulatory authorities in general have accepted the model outcomes on DDIs involving drug-metabolizing enzymes, particularly CYPs, whereas the predictive performance of transporter-mediated DDIs has not reached sufficient levels of confidence yet.\(^1\)\(^-\)\(^3\) One of the reasons for the latter is that the interpretation of clinical significance of transporter-mediated DDIs is typically more complicated than that of drug-metabolizing enzyme-mediated DDIs. In addition, there are knowledge gaps that limit the confidence of DDI predictions to model transporter kinetics with appropriate drug exposures at the site of action.\(^3\)\(^,\)\(^4\)

One of the adenosine triphosphate-binding cassette-transporters, ABCB1 (P-glycoprotein [Pgp]), has been extensively studied.\(^5\)\(^-\)\(^7\) It has been recognized widely that Pgp plays a critical role for a variety of drugs in affecting the rate and extent of their absorption. Despite recently increased understanding of the role of Pgp in pharmacokinetics, it is still challenging to accurately predict the fraction of the dose absorbed (\(F_o\)) for Pgp substrates via PBPK modeling, largely due to several IVIVE factors, such as solubility/dissolution, permeability, and transporter kinetics. Consequently, it is difficult to quantitatively predict intestinal Pgp-mediated DDIs (Pgp-DDIs) from an IVIVE perspective for both substrates and inhibitors.\(^4\)\(^,\)\(^8\)\(^,\)\(^9\)

Knowledge gaps still remain in establishing an IVIVE for Pgp kinetics of both substrates (e.g., Michaelis-Menten constant \([K_m]\) and maximal efflux rate \([J_{max}]\)) and inhibitors (e.g., inhibition constant \([K_i]\)). In fact, the US Food and Drug Administration (FDA) indicates in their reviews that there is uncertainty in quantitatively translating Pgp \(K_i\) values from in vitro to in vivo in the PBPK approach.\(^10\)

In this study, we have investigated an IVIVE for Pgp-DDIs via PBPK modeling. We selected three Pgp substrates, digoxin, dabigatran etexilate, and quinidine, and two Pgp inhibitors, itraconazole and verapamil. Digoxin is largely excreted into urine as the unchanged drug whereas quinidine is mainly metabolized by CYP3A in liver with a moderate excretion into urine.\(^11\)\(^,\)\(^12\) Quinidine also inhibits CYP2D6, CYP3A, and Pgp.\(^13\)\(^,\)\(^14\) Dabigatran etexilate is a prodrug that is metabolized extensively by carboxylesterases to the pharmacologically active moiety, dabigatran, which is not a Pgp substrate.\(^15\)\(^,\)\(^16\) Pigoxin is categorized as class 3 in the Biopharmaceutics Classification System (BCS) whereas dabigatran etexilate and quinidine are BCS
METHODS

PBPK modeling outline

A commercially available dynamic PBPK model, Simcyp population-based simulator (version 19.1), was used to simulate pharmacokinetics of the Pgp substrates and inhibitors. The advanced dissolution, absorption, and metabolism (ADAM) model implemented in Simcyp was utilized to predict $F_a$ and a fraction of the dose escaping intestinal first-pass metabolism ($F_{a0}$). Simulation of all clinical trials was performed with a virtual default population of 100 healthy volunteers in 10 trials of 10 subjects, each aged 20 to 50 years with a female/male ratio of 0.5. Clinical trial designs in the simulations were primarily set as the study design reported in the literature described below.

PBPK model input parameters

Input parameters of Pgp inhibitors and substrates in the PBPK models are summarized in Appendix S1. For Pgp inhibitors, compound files of itraconazole and hydroxyitraconazole were obtained from the literature whereas those of verapamil and norverapamil were from the Simcyp library. These files were modified, such as Pgp $K_i$ inputs in the ADAM model, and then verified based on the clinical results, as described in Appendix S1. In vitro half maximal inhibitory concentration ($IC_{50}$) for Pgp inhibition by itraconazole, hydroxyitraconazole, verapamil, and norverapamil against digoxin in Caco-2 cell monolayers were obtained from the database in Drug Interaction Solutions (DIDB database; University of Washington, Seattle, WA). Assuming $K_i$ was half of the $IC_{50}$ (to be conservative), $K_i$ values used for PBPK modeling were 1.0 μM for itraconazole, 0.8 μM for hydroxyitraconazole, 2.0 μM for verapamil, 0.15 μM for norverapamil, and 0.8 μM for quinidine, as described in Appendix S1. In addition, itraconazole $K_i$ value of 0.22 μM toward dabigatran etexilate in Caco-2 cell monolayers was also explored in the DDI prediction between itraconazole and dabigatran etexilate. For Pgp substrates, the previously developed compound files of digoxin, dabigatran etexilate, dabigatran, and quinidine were primarily used in the present study. In vitro Pgp kinetic parameters, $K_m$ and $J_{max}$, were, respectively, 25 μM and 128 pmol/min/cm² for digoxin, 2.6 μM and 25 pmol/min/cm² for dabigatran etexilate and 1.0 μM and 21 pmol/min/cm² for quinidine. To adequately recover the plasma concentration-time profiles of the Pgp substrates in the control groups of each DDI study, the in vitro-to-in vivo Pgp scaling factors (Pgp-SFs) for $J_{max}$ were optimized by the sensitivity analysis for the ratios of transporter activity or abundance in intestine between in vivo and in vitro (relative activity/expression factors, RAF/REF, in Simcyp). In contrast, the unbound $K_m$ estimates in vitro were assumed to represent in vivo affinity (i.e., intrinsic values) as the general hypothesis. The refined compound files were then applied to the DDI prediction in the test groups with Pgp inhibitors.

PBPK modeling for DDI prediction

The DDI results of itraconazole and verapamil with digoxin, dabigatran etexilate, and quinidine were obtained from the literature as described in Appendix S1. A brief outline of the DDI studies is as follows:
• Itraconazole 200 mg once-daily with digoxin 0.5 mg
  \( (n = 10) \).\textsuperscript{31}
• Itraconazole 200 mg once-daily with dabigatran etexilate 0.375 mg \( (n = 8) \).\textsuperscript{21}
• Itraconazole 100 or 200 mg once-daily with quinidine sulfate 100 or 200 mg \( (n = 6 \text{ or } 9) \).\textsuperscript{12,32}
• Verapamil 80 mg three-times-daily with digoxin 0.25 mg
  \( (n = 10) \).\textsuperscript{33}
• Verapamil 120 mg twice- or four-times-daily with dabigatran etexilate 150 mg \( (n = 20) \).\textsuperscript{34}
• Verapamil 80 or 120 mg three-times-daily with quinidine sulfate 400 mg \( (n = 6) \).\textsuperscript{35}

As indicated in Appendix S1, the PBPK modeling for the DDI studies with verapamil and digoxin was performed at digoxin doses of 0.25 and 1 mg because plasma concentrations of digoxin in the control group of this study were approximately fourfold higher than the mean values from the meta-analysis of six studies following the dose-normalization. In the verapamil DDI study with dabigatran etexilate, total dabigatran (unconjugated and conjugated) concentrations were measured, whereas conjugated dabigatran, mainly glucuronides, was approximately 20% of total dabigatran based on the assay results before and after alkaline cleavage. Assuming that the differences were within variability deriving from various factors, such as the differences in subjects, studies, and bioanalytical assays, the reported values were used in the present study. This assumption was also made in the previous report based on the meta-analysis.\textsuperscript{18}

Data analysis

Pharmacokinetic parameters of Pgp substrates, such as the maximal plasma concentrations \( (C_{\text{max}}) \) and the area under the plasma concentration-time curves (AUC), were obtained from the literature. When these parameters were not reported, the values were calculated from the reported clearance values with doses or the digitalized plasma concentration-time profiles by Digitizelt version 2.3.3 (Bormann, Germany). Pharmacokinetic parameters, such as \( C_{\text{max}} \), AUC, and the ratios of \( C_{\text{max}} (C_{\text{max}} \text{R}) \) and AUC (AUCR) in the test groups to the control groups are presented as either arithmetic mean, median, or geometric mean with standard deviations (SDs), 90% or 95% confidence intervals (CIs), or percent coefficients of variation (CV%) according to the literature. To estimate substrate Pgp-SFs, the local sensitivity analysis tool implemented in Simcyp were performed to assess the appropriate values. The simulations with the obtained Pgp-SF were thereafter performed with a virtual default population of 100 healthy volunteers in 10 trials of 10 subjects. The study conditions for the sensitivity analyses and the following simulations were based on the reported clinical study designs. To evaluate predictive model performance, the deviation of predicted from observed values was calculated as prediction error (PE):

\[
\text{PE} = \frac{\text{Predicted value} - \text{Observed value}}{\text{Observed value}} \times 100
\]

PE of less than or equal to \( \pm 25\% \) was provisionally used as the predefined criteria for the model verification.\textsuperscript{36,37}

RESULTS

Itraconazole DDIs with Pgp substrates

Clinically observed and PBPK model-predicted plasma concentration-time profiles of digoxin, dabigatran, and quinidine in the itraconazole DDI studies are presented in Figure 1. In the DDI study with digoxin, Pgp-SF for digoxin was estimated at 0.75 in the control group with PE of \( \pm 4\% \) for \( C_{\text{max}} \) and AUC (Table 1). PBPK modeling sufficiently predicted the observed \( C_{\text{max}} \text{R} \) with PE of \( -11\% \), whereas AUCR was slightly underpredicted with PE of \( -27\% \). The predicted \( F_a \) in the control and test groups were \( -0.8 \) and \( -0.9 \), respectively. It is noteworthy that the predicted \( C_{\text{max}} \text{R} (1.20 \pm 0.10) \) and AUCR \( (1.11 \pm 0.07) \) were within the regulatory agency’s criteria of negligible DDIs \( (\pm 25\%) \) whereas the observed ratios \( (1.34 \text{ and } 1.52, \text{ respectively}) \) were above the criteria. This could be the potential limitation on the use of PE\% as the predictive model performance; therefore, we should carefully account for the variability in the predicted results (e.g., SDs and CIs) for making decisions.

In the DDI study with dabigatran etexilate, Pgp-SF for dabigatran etexilate was estimated at 90 in the control group with PE of \( -15\% \) for \( C_{\text{max}} \) and 30\% for AUC (Table 1). Using itraconazole in vitro \( K_i \) of 1 \( \mu \text{M} \) (against digoxin), PBPK modeling slightly underpredicted the observed \( C_{\text{max}} \text{R} \) and AUCR in the test group with PE of \( -30\% \) to \( -50\% \) (Table 1 and Figure 1). The predicted \( F_a \) increased from \( -0.1 \) to \( -0.3 \). In contrast, using in vitro \( K_i \) of 0.22 \( \mu \text{M} \) (against dabigatran etexilate), the modeling results showed PE of 32\% for \( C_{\text{max}} \text{R} \) and \( -4\% \) for AUCR. The predicted \( F_a \) in the control and test groups were \( -0.1 \) and \( -0.5 \), respectively.

In the DDI study of quinidine sulfate (100 mg) with itraconazole, Pgp-SF for quinidine was estimated at five in the control group with PE of \( \pm 8\% \) for \( C_{\text{max}} \) and AUC (Table 1). PBPK modeling adequately predicted the observed \( C_{\text{max}} \text{R} \) and AUC with PE of \( \pm 18\% \) (Table 1 and
PBPK-IVIVE FOR Pgp-MEDIATED DRUG-DRUG INTERACTIONS

Figure 1. The predicted $F_a$ in the control and test groups were ~0.4 and ~0.5, respectively, while the predicted $F_g$ was near-unity in both the groups. In another DDI study of quinidine sulfate (200 mg) with itraconazole, quinidine Pgp-SF was estimated at nine in the control group with PE of ±6% for $C_{max}$ and AUC (Table 1). PEs for $C_{max}$R and AUCR in the test group were within ±25%. The predicted $F_a$ was ~0.5 in the control group and ~0.6 in the test group, whereas the predicted $F_g$ was near-unity in both the groups.

Overall, these results indicated that the PBPK-IVIVE for itraconazole reasonably described the clinical DDI results with digoxin, dabigatran etexilate, and quinidine.

**Verapamil DDIs with Pgp substrates**

Clinically observed and PBPK model-predicted plasma concentration-time profiles of digoxin, dabigatran, and quinidine in the verapamil DDI studies are presented in Figure 2. In the DDI study with digoxin, Pgp-SF for digoxin was estimated at 2.5 in the control group with PE of −16% for both $C_{max}$ and AUC. In this study, PBPK modeling was performed at digoxin doses of 0.25 and 1 mg, as indicated above. The predicted $C_{max}$R and AUCR were comparable between the two doses (i.e., 1.59 vs. 1.61 and 1.41 vs. 1.44, respectively). The predicted $C_{max}$R and AUCR were also consistent with the observed results (1.44 and 1.50, respectively) with PE of ±12% (Table 2). The predicted $F_a$ in the control and test groups were −0.6 and −0.7, respectively.

In the DDI study with dabigatran etexilate, Pgp-SF for dabigatran etexilate was estimated at 70 in the control group with PE of −17% for $C_{max}$ and 13% for AUC (Table 2). PBPK modeling sufficiently predicted the observed $C_{max}$R and AUCR in three test groups following the different dosing regimens with PE of ±22%. The predicted $F_a$ in the control group was 0.10, which increased to 0.12 to 0.15 in the test groups.

In the DDI study of quinidine sulfate with verapamil (80 and 120 mg three-times-daily), Pgp-SF for quinidine was estimated at two in the control group with PE of ±5% for $C_{max}$ and AUC (Table 2). PBPK modeling sufficiently predicted the observed $C_{max}$R and AUCR in two test groups following the different dosing regimens with PE of ±18%. The predicted $F_a$ and $F_g$ were, respectively, 0.89 and 0.98 in the control group and 0.91 and 1.0 in the test group.
| Substrate                        | Group             | Analysis | $C_{\text{max}}$ (ng/ml) | AUC (ng∙h/ml) | $C_{\text{max}}$R | AUCR |
|---------------------------------|-------------------|----------|--------------------------|---------------|-------------------|------|
| Digoxin 0.5 mg p.o.             | Control           | Obs      | 2.7 ± 1.6                | 23 ± 6        | −                 | −    |
|                                 |                   | Pred     | 2.7 ± 0.9                | 24 ± 9        | −                 | −    |
|                                 |                   | $PE\%$  | 0                        | 4             | −                 | −    |
| Itraconazole 200 mg p.o. q.d.   | Obs               | 3.7 ± 1.3| 34 ± 6                   | 1.34          | 1.52              |      |
|                                 | Pred              | 3.2± 0.9 | 26 ± 9                   | 1.20 ± 0.10   | 1.11 ± 0.07       |      |
|                                 | $PE\%$  | −12      | −25                      | −11           | −27               |      |
| Dabigatran Etexilate 0.375 mg p.o. | Control           | Obs      | 0.17 (0.13 – 0.23)       | 1.4 (1.0 – 2.0)| −                 | −    |
|                                 | Pred              | 0.15 (0.13 – 0.16) | 1.9 (1.4 – 2.3) | − | − |
|                                 | $PE\%$  | −15      | 30                       | −             | −                 | −    |
| Itraconazole 200 mg p.o. q.d.   | Obs               | 1.1 (0.85 – 1.5) | 10 (7.5 – 13) | 6.4 | 6.9 |
|                                 | Pred              | 0.62 (0.57 – 0.68) | 6.3 (5.2 – 7.4) | 4.3 (4.1 – 4.4) | 3.4 (3.2 – 3.6) |
|                                 | $PE\%$  | −43      | −37                      | −34           | −51               |      |
|                                 | Pred              | 1.2 (1.2 – 1.3) | 12 (11 – 14) | 8.5 (8.0 – 9.0) | 6.6 (6.0 – 7.3) |
|                                 | $PE\%$  | 13       | 24                       | 32           | −4                |      |
| Quinidine 100 mg p.o.           | Control           | Obs      | 174 ± 59                 | 1980 ± 760    | −                 | −    |
|                                 | Pred              | 160 ± 80 | 1921 ± 1010              | −             | −                 | −    |
|                                 | $PE\%$  | −8       | −3                       | −             | −                 | −    |
| Itraconazole 200 mg p.o. q.d.   | Obs               | 276 ± 112| 3890 ± 1460              | 1.59          | 1.96              |      |
|                                 | Pred              | 269 ± 95 | 3745 ± 1467              | 1.87 ± 0.49   | 2.14 ± 0.51       |      |
|                                 | $PE\%$  | −2       | −4                       | 18           | 9                 |      |
| Quinidine 200 mg p.o.           | Control           | Obs      | 616 (487 – 1298)         | 5348 (4363 – 9211) | − | − |
|                                 | Pred              | 635 (513 – 595) | 5041 (4372 – 5255) | − | − |
|                                 | $PE\%$  | 3        | −6                       | −             | −                 | −    |
| Itraconazole 100 mg p.o. q.d.   | Obs               | 811 (519 – 1038) | 13817 (8290 – 19057) | 1.32 | 2.58 |
|                                 | Pred              | 817 (725 – 811) | 9622 (8562 – 10058) | 1.39 (1.36 – 1.42) | 1.94 (1.89 – 1.98) |
|                                 | $PE\%$  | 1        | −30                      | 5            | −25               |      |

Note: Values are expressed as mean ± SD, median (95% confidence interval) or geometric mean (90% confidence interval).
Abbreviations: −, not reported or available; AUC, area-under-the-plasma-concentration-time curve; AUCR, area-under-the-plasma-concentration-time curve ratio; $C_{\text{max}}$, maximal plasma concentration; $C_{\text{max}}$R, maximal plasma concentration ratio; DDI, drug-drug interaction; $K_i$, inhibition constant; Obs, observed; PBPK, physiologically-based pharmacokinetic; PE, prediction error (%); Pred, predicted.

aItraconazole Pgp $K_i$ (1.0 μM) for digoxin as substrate.
bItraconazole Pgp $K_i$ (0.22 μM) for dabigatran etexilate as substrate.
cQuinidine sulfate doses (100 – 200 mg = 83 – 166 mg equivalents).
Overall, these results indicated that the PBPK-IVIVE for verapamil reasonably described the clinical DDI results with digoxin, dabigatran etexilate, and quinidine.

**Sensitivity analyses for substrate Pgp-SF and inhibitor $K_i$**

The sensitivity analyses for the substrate Pgp-SF and the inhibitor Pgp $K_i$ were performed to investigate the impacts of these parameters on the substrate $F_a$ in the DDI studies. As presented in Figure 3, the predicted $F_a$ for the substrates would significantly decline with the increases in the substrate Pgp-SFs (i.e., the increases in Pgp $J_{max}$) when the inhibitor $K_i$ were relatively less potent (e.g., $\geq 0.1 \mu M$). Similarly, the predicted substrate $F_a$ would decline with the increases in the inhibitor $K_i$ when the substrate Pgp-SFs were relatively higher (e.g., $\geq 5$). In contrast, when either substrate Pgp-SF or inhibitor $K_i$ was lower (e.g., $\leq 4$ and $\leq 0.06 \mu M$, respectively), the predicted $F_a$ was less sensitive to these parameters. The lower Pgp-SF would readily cause saturation of Pgp activity whereas the lower (or more potent) inhibitor $K_i$ could potentially lead to near-complete Pgp inhibition; thus, both the cases would result in higher $F_a$ (e.g., $\geq 0.7$). Overall, the modeling results suggested that both the substrate Pgp-SF and the inhibitor $K_i$ would be key for prediction and/or understanding of Pgp-DDIs.

**DISCUSSION**

In the PBPK-IVIVE for clinical Pgp-DDIs, we first focused on the model verification of three Pgp substrates, digoxin, dabigatran etexilate, and quinidine, with Pgp-SF for $J_{max}$. This is based on the general hypothesis that unbound $K_m$ for enzymes and transporters is intrinsic. The modeling results suggest that the optimization of substrate Pgp-SF is critical to adequately recover the clinical results. We then applied the PBPK models of two Pgp inhibitors, itraconazole and verapamil, with the in vitro Pgp $K_i$ values to recover the clinical Pgp-DDIs with the Pgp substrates. The results suggest that the PBPK-IVIVE could adequately recover the Pgp-DDI results between these substrates and inhibitors. Thus, the present PBPK-IVIVE approach appears to be successful in describing clinical Pgp-DDIs. However, the results clearly underscore the current challenges...
on PBPK-IVIVE on Pgp-DDIs. Some potential issues are identified and warrant further investigation and discussion.

One of the main challenges on the Pgp-DDI prediction associated with PBPK-IVIVE is to accurately determine substrate Pgp kinetics in vitro ($K_m$ and $J_{max}$).
PBPK-IVIVE FOR PGP-MEDIATED DRUG-DRUG INTERACTIONS

because of a large inter-laboratory variability associated with various factors such as different cell lines and transporter kinetic equations/models.4,38 The current strategy to overcome these limitations is to adequately model in vitro data, where transporter kinetics with unbound substrate concentrations at the binding site can be taken into account along with flux through two diffusional barriers.4,38 We used the previously reported Pgp kinetic parameters, which were determined by fitting in vitro Caco-2 data (from a laboratory) to the compartmental model.18 In the compartment model used, $K_m$ is defined as an intracellular unbound concentration and governed by the substrate-Pgp interaction, yielding independent $K_m$ estimates from Pgp expression levels.39 In vitro unbound $K_m$ estimates are thus assumed to represent in vivo affinity (i.e., intrinsic values). In contrast, Pgp-SFs for $J_{\text{max}}$ could account for the differences in Pgp expression or functional activity between in vitro and in vivo, although this might be drug-dependent due to some other factors, such as the regional difference of Pgp abundance along the various regions of intestine and drug absorption sites.40,41 Accordingly, we have estimated substrate Pgp-SFs to adequately recover the observed results of control groups in each study, given an expected variability derived from various factors such as intra- and intersubjects and studies.

Similarly, in vitro Pgp $K_i$ values have been reported to show a large variability among laboratories due to multiple factors, including different substrates and cell lines with various Pgp expression levels.9,42 For instance, the in vitro $K_i$ values for digoxin varied from 20 to 800-fold for 15 Pgp inhibitors among 22 laboratories.43,44 In the present study, we primarily used the median $K_i$ values of Pgp inhibitors against digoxin in Caco-2 cell monolayers obtained from the DIDB database. The Pgp IC$_{50}$ values varied considerably from 0.46 to 6.0 μM (median 2.0 μM) for itraconazole and 0.06 to 17 μM (4.0 μM) for verapamil. Median IC$_{50}$ value for itraconazole against dabigatran etxilate in Caco-2 cell monolayers (0.44 μM) was ~5-fold lower than that against digoxin (2 μM). In the Pgp-DDI prediction between itraconazole and dabigatran etxilate, the PE for $C_{\text{max}}R$ and AUCR were −34% and

FIGURE 3 The relationships among the PBPK model-predicted Pgp substrate $F_a$, intestinal Pgp $J_{\text{max}}$ scaling factor (Pgp-SF), and Pgp inhibitor $K_i$ in healthy subjects following a single oral administration of Pgp substrates with multiple-dose oral coadministration of itraconazole or verapamil. Oral doses were digoxin 0.5 mg (a), dabigatran etxilate 0.375 mg (b), quinidine sulfate 100 mg (c), digoxin 1 mg (d), dabigatran etxilate 150 mg (e), and quinidine sulfate 400 mg (f) with itraconazole 200 mg once-daily (a, b, c) or verapamil 80 mg three-times-daily (d), 120 mg twice-daily (e), and 120 mg three-times-daily (f). The ranges of Pgp-SF were 1 to 100 against the Pgp inhibitor $K_i$ of 0.001 to 10 μM. $F_a$, fraction of the dose absorbed; $J_{\text{max}}$, maximal efflux rate; $K_i$, inhibition constant; PBPK, physiologically-based pharmacokinetic.
-51%, respectively, when using the $K_i$ value for digoxin whereas those were 32% and -4%, respectively, when using the $K_i$ value for dabigatran etexilate (Table 1). The sensitivity analysis revealed that itraconazole Pgp $K_i$ of -0.3 μM yielded a reasonable prediction with PE of ±16% for $C_{\text{max}}R$ and AUCR, suggesting that itraconazole $K_i$ in the best fit was relatively closer to that for dabigatran etexilate relative to digoxin. This suggests that it is important to determine in vitro $K_i$ toward appropriate Pgp substrates, which can be supported with the reasonable prediction of verapamil DDIs with digoxin and quinidine because of comparable verapamil IC$_{50}$ between digoxin (4.0 μM) and quinidine (3.9 μM). We could not further investigate this point because in vitro $K_i$ values for itraconazole and verapamil against other Pgp substrate were limited in the DIDB database. Despite the reasonable Pgp-DDI prediction, one of the limitations of the present modeling is that the median IC$_{50}$ values obtained from the DIDB database were used whereas the compartment modeling analyses for Pgp kinetic estimation have been recommended.\textsuperscript{38,42} Therefore, this point should be addressed in the future, although, at this moment, the traditional extracELLular concentration-based analyses for estimating Pgp IC$_{50}$ is still widely used and in general accepted by regulatory agencies.\textsuperscript{45-47} Another limitation is that inhibitors’ $K_i$ is incorporated into only intestine and/or liver to focus on Pgp-DDIs on absorption, including re-absorption via biliary excretion. As digoxin and quinidine are excreted into urine, there is a possibility for Pgp-mediated DDIs in the kidneys.\textsuperscript{14,15} However, the modeling results exhibited that Pgp $K_i$ values used in the present studies were at least several fold higher than steady-state unbound $C_{\text{max}}$ of Pgp inhibitors; for instance, ~10-fold for itraconazole and 20-fold for hydroxyitraconazole following itraconazole 200 mg once-daily, and 40-fold for verapamil and 5-fold for norverapamil following verapamil 120 mg twice-daily. Therefore, systemic effects of Pgp inhibitors on Pgp-DDIs can be expected to be minimal unless a significant accumulation of unbound drugs takes place in certain tissues.

The model-predicted substrate $F_s$ decreases with increases in Pgp-SFs for $J_{\text{max}}$ when Pgp activity is neither saturated nor inhibited at given doses (Figure 3). Pgp-SFs used in this study ranged from 0.75 to 2.5 for digoxin at 0.25 to 1 mg, 70 to 90 for dabigatran etexilate at 0.375 to 150 mg, and 2 to 9 for quinidine sulfate at 100 to 400 mg, corresponding to the predicted $F_s$ of 0.54 to 0.85, 0.06 to 0.14, and 0.41 to 0.93, respectively. Hence, the predicted $F_s$ showed roughly two-fold difference among the studies, which could be conceivable given the different doses and study conditions along with expected variability. In addition, quinidine is known to exhibit supra-proportional increases in oral exposures at the doses used in this study.\textsuperscript{12,13,32,35} These differences potentially lead to different degrees of Pgp-DDIs depending on substrate $F_s$, as simulated in Figure 3. Therefore, it would be critical to optimize substrate Pgp-SF to sufficiently predict Pgp-DDIs, as the FDA guidance indicates that the sponsor should establish and verify PBPK models for transporter substrates before applying for DDI predictions.\textsuperscript{45} The range of predicted $F_s$ for digoxin (0.54 to 0.85) corresponds to the extent of increases in oral exposure by 1.2 to 2-fold when digoxin $F_s$ increases up to unity due to a complete Pgp inhibition. The European Medicines Agency (EMA) guidance recommends that dabigatran etexilate is a better probe Pgp substrate for clinical DDI studies because of its lower $F_s$ (0.1).\textsuperscript{46} In the clinical DDI studies used for the present study, the observed $C_{\text{max}}R$ and AUCR for digoxin and quinidine were within two-fold with the exception of AUCR (~2.6-fold) in one of four DDI results with quinidine. In contrast, those for dabigatran were six to seven-fold at the microdose of dabigatran etexilate (0.375 mg) with itraconazole and ~1.5-fold at the clinically recommended dose of dabigatran etexilate (150 mg) with verapamil. This may suggest that the microdose of dabigatran etexilate is more sensitive for clinical Pgp-DDI studies, which can also be reasonably predicted by the present PBPK-IVIVE.

In the itraconazole and verapamil PBPK models, Pgp $K_i$ values for parent drugs were incorporated into intestine and liver whereas those for metabolites were only in liver. This is because the ADAM model is not available for inhibitor metabolites in Simcyp, resulting in metabolite-mediated intestinal Pgp inhibition being dynamically predicted by plasma concentrations in portal vein corrected for unbound fraction in enterocytes ($f_{\text{u,gut}}$), e.g., total plasma concentrations when $f_{\text{u,gut}}$ is unity. In both the compound files of hydroxyitraconazole from the literature and norverapamil from the Simcyp library, the input values of $f_{\text{u,gut}}$ are set at unity.\textsuperscript{25} In contrast, unbound enterocyte concentrations predicted by the ADAM model are used for the prediction of parent drug-mediated intestinal Pgp inhibition. It is noteworthy that the predicted $F_s$ for itraconazole and verapamil were ~1 and ~0.8, respectively, suggesting minimal metabolite formations in enterocytes. The present PBPK modeling therefore assumed that metabolite-mediated Pgp inhibition was negligible in the intestines, which will (and should) be investigated further. This also includes the distribution (rate and extent) of metabolites (and parent drug administered intravenously) from the portal vein to the lipid bilayer in the apical membrane of enterocytes where Pgp interacts with substrates and inhibitors via conformational changes coupling ATP hydrolysis.\textsuperscript{48-50}

In summary, the present study has demonstrated that clinical Pgp-DDIs among three substrates and two inhibitors could be reasonably described by PBPK-IVIVE with
Pgp kinetic parameters determined in vitro. The present modeling approach can be applicable to predict Pgp-DDIs with other Pgp substrates and inhibitors. In addition, the modeling results also suggest that Pgp kinetic parameters of both the substrates (\(K_m\) and \(J_{\text{max}}\)) and inhibitors (\(K_i\)) are key for successful DDI prediction because these parameters are sensitive to substrate \(F_a\) in Pgp-DDIs. It would also be critical to incorporate appropriate unbound inhibitor concentrations at the site of action into PBPK modeling. These points are graphically summarized in Figure 4 as the PBPK-IVIVE scheme for Pgp-DDI prediction, which is in line with the FDA guidance and the industry review.4,45 The present results support a quantitative prediction of Pgp-DDIs using in vitro parameters, which will significantly increase the value of in vitro studies to design and run clinical DDI studies safely and effectively.

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CONFLICT OF INTEREST

The authors are employees of Janssen Research & Development.

AUTHOR CONTRIBUTIONS

S.Y., R.E., and L.DZ. wrote the manuscript. S.Y. designed and performed the research and analyzed the data.

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