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A Physiologically-Based Pharmacokinetic Model of Voriconazole Integrating Mechanism-based Inhibition of CYP3A4, Genetic Polymorphisms of CYP2C19 and Predictions of Drug-Drug Interactions

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ABSTRACT

Background: Voriconazole, a first-line anti-fungal drug, exhibits nonlinear pharmacokinetics together with large inter-individual variability but a narrow therapeutic range, and it markedly inhibits CYP3A4 *in vivo*. This causes difficulties in selecting appropriate dosing regimens of voriconazole and of co-administered CYP3A4 substrates.

Objective: This study aimed to investigate the metabolism of voriconazole in detail to better understand dose- and time-dependent alterations in the pharmacokinetics of the drug, to provide the model basis for safe and effective use according to CYP2C19 genotype, and to assess the potential of voriconazole to cause drug-drug interactions (DDIs) with CYP3A4 substrates in more detail.

Methods: *In vitro* assays were carried out to describe mechanism-based inactivation (MBI) of CYP3A4 by voriconazole. These results were combined with 93 published concentration-time curves of voriconazole from clinical trials in healthy volunteers to develop a whole-body physiologically-based pharmacokinetic (PBPK) model in PK-Sim®. The model was evaluated quantitatively with the predicted/observed ratio of AUC, C_{max}, and C_{trough}, the geometric mean fold error, as well as visually with the comparison of predicted with observed concentration-time profiles over the full range of recommended intravenous and oral dosage regimens.

Results: The result of the IC_{50} shift assay indicated that voriconazole causes MBI of CYP3A4. The PBPK model evaluation demonstrated a good performance of the model, with 71% of predicted/observed aggregate AUC ratios and all aggregate C_{max} ratios from 28 evaluation datasets being within a 0.5- to 2-fold range. For those studies reporting CYP2C19 genotype, 89% of aggregate AUC ratios and all aggregate C_{max} ratios were inside a 0.5- to 2-fold range of 44 test profiles. The model suggests that the standard maintenance dose of 200 mg bid is sufficient for CYP2C19 IMs (intermediate metabolizers: *1/*2, *1/*3, *2/*17, and *2/*2/*17) to reach the tentative therapeutic range of >1-2 mg/L to <5-6 mg/L for trough values, while 400 mg might be more suitable for RMs (rapid metabolizers: *1/*17, *17/*17) and NMs (normal metabolizers, *1/*1). When the model was integrated with independently developed CYP3A4 substrate models (midazolam and alfentanil), the observed AUC change of substrates by voriconazole was inside the 90% confidence interval of the predicted AUC change, indicating that CYP3A4 inhibition was appropriately incorporated into the voriconazole model.

Conclusions: Both the *in vitro* assay and model-based simulations confirmed MBI of CYP3A4 by voriconazole as a pivotal characteristic of this drug's pharmacokinetics. The PBPK model developed here could support individual dose adjustment of voriconazole according to genetic polymorphisms of CYP2C19, and DDI risk management.

Key Points:

1. A whole-body physiologically-based pharmacokinetic (PBPK) model of voriconazole incorporating mechanism-based inhibition of CYP3A4 was successfully developed to accurately capture the time- and dose-dependent alterations of voriconazole pharmacokinetics for different CYP2C19 genotypes.

2. Model-based simulations could i) elaborate potential exposure-equivalent dosing regimens for CYP2C19 genotype groups; ii) assess the dynamic inhibition of CYP3A4 by voriconazole in the liver and small intestine; iii) predict DDIs between voriconazole and other CYP3A4 substrates.

1 INTRODUCTION

Voriconazole is an essential drug in the treatment of severe fungal infections due to its activity against a wide range of clinically relevant fungal pathogens, including the most commonly occurring species of the genera *Aspergillus* and *Candida*, and some emerging fungi, such as *Scedosporium* and *Fusarium* species [1]. Moreover, voriconazole is well established as first-line therapy for patients with invasive aspergillosis [2–4]. However, the drug exhibits nonlinear pharmacokinetics with large inter-individual and intra-individual variability [5,6], which causes difficulties for clinicians to choose appropriate dosing regimens to target its narrow therapeutic range, especially in the case of high doses in severe infections, or for chronic treatments [7].

While underexposure of voriconazole may decrease the efficacy, overexposure increases the risk primarily for neural and hepatic toxicity [8,9]. Unitil now, no universally applicable therapeutic range has been established. Two Japanese societies in 2013 recommended voriconazole trough concentrations of >1-2 mg/L for clinical efficacy and of <4-5 mg/L for hepatic tolerability [10], while the British Society for Medical Mycology in 2014 recommended trough concentrations of >1mg/L for efficacy and of <4-6 mg/L for safety [11]. The Chinese Pharmacological Society recommended 0.5 mg/L as the lower limit and 5 mg/L as the upper limit of the voricoanzole trough concentration range [12]. In 2017, the Third Fungal Diagnosis and Management of *Aspergillus* diseases Clinical Guideline recommended a trough concentration range of 1-5.5 mg/L for most patients with voriconazole prophylaxis or treatment while the range for patients with severe infections be 2 to 6 mg/L [4]. In the present project, we selected lower and upper trough concentrations of >1-2 mg/L and <5-6 mg/L, respectively.

Voriconazole is extensively metabolized via the cytochrome P450 enzymes CYP2C19 and CYP3A4 [13], slightly by CYP2C9 and flavin-containing monooxygenase (FMO) [14], while less than 2% is excreted renally as the parent drug [15–17]. The main metabolite in plasma was reported as voriconazole N-oxide, accounting for 72% of circulating metabolites [1]. However, Geist et al. found that voriconazole N-oxide and its conjugates excreted in urine within 12 h during steady-state only accounted for 1% of the administered dose, while other metabolites, i.e., dihydroxy fluoropyrimidine-voriconazole and hydroxy fluoropyrimidine-voriconazole together with their conjugates accounted for 14% and 3%, respectively [17]. This was in agreement with another study where the major metabolite excreted in urine over 96 h was dihydroxy fluoropyrimidine-voriconazole, accounting for 13% of the administered dose of voriconazole [18]. Therefore, it seems reasonable to also consider dihydroxy-fluoropyrimidine voriconazole and hydroxy-fluoropyrimidine voriconazole as major metabolites of voriconazole, although both have low plasma concentrations due to their high renal clearances, which was reported to be approximately 150-fold and 55-fold higher, respectively, than that of voriconazole N-oxide [17]. However, two other groups found that the the main metabolite of voriconazole excreted in urine within 48 h after administration was voriconazole N-oxide with 10 to 21 % the administered dose [15,16]. The discrepancies between the studies may be explained by the length of urine collection periods together with a different elimination half-life of the metabolites and the mechanism-based inhibition (MBI) of CYP3A4. Thus, both fluoropyrimidine hydroxylation and N-oxidation pathways were considered as the main metabolic pathways, mainly mediated by CYP3A4 and CYP2C19, as shown in **Figure 1**.

Genetic polymorphisms of CYP2C19 are a major source for inter-individual variability, as reflected by 3-fold higher C_{max} values and 2- to 5-fold higher AUC values in CYP2C19 poor metabolizers (PMs) compared to those in normal metabolizers (NMs) or rapid metabolizers (RMs) [7,19,20].

Furthermore, voriconazole is also an inhibitor of CYP3A4 and 2C19 [21]. *In vitro*, voriconazole K_i for the competitive inhibition of CYP3A4-mediated metabolism of midazolam was reported to range from 0.15 to 0.66 μM [21,22]. *In vivo*, oral administration of therapeutic dosages of voriconazole increased the AUC of midazolam to 940% and 353% by oral and intravenous co-administration, respectively [23]. Also, voriconazole was reported to exhibit "autoinhibition" on CYP3A4 *in vivo* [15,24]. In addition, to properly describe the respective processes concerning enzyme inhibition by voriconazole *in vivo*, "time-dependent inhibition" and "autoinhibition" of voriconazole were integrated into the models reported by Friberg et al. and Kim et al., respectively [25,26].

Therefore, we investigated the inhibition of voriconazole and its metabolite voriconazole N-oxide on CYP3A4 and CYP2C19 *in vitro*. Based on the *in vitro* assay results, a whole-body physiologically-based pharmacokinetic (PBPK) model of voriconazole incorporating CYP3A4 MBI was then developed to describe dose- and time-dependent pharmacokinetics in the different CYP2C19 genotypes. Finally, model-based simulations were carried out to i) elaborate potentially exposure-equivalent dosing regimens for CYP2C19 genotype groups; ii) assess the dynamic inhibition of CYP3A4 by voriconazole in the liver and small intestine; iii) further evaluate drug-drug interactions (DDIs) between voriconazole and other CYP3A4 probe substrates. An early stage of this work has been presented in the Population Approach Group in Europe conference [27].

2 MATERIALS AND METHODS

2.1 *In vitro* assay for inhibition of CYP2C19 and CYP3A4

The *in vitro* assay for inhibition of human CYP2C19 and CYP3A4 by voriconazole and its metabolite voriconazole N-oxide, together with the respective measurements and data analysis were carried out according to the methods reported in the supplementary materials.

2.2 Model development

The PBPK model for voriconazole was developed by combining bottom-up and top-down approaches. An extensive literature search was performed to obtain (a) drug physio-chemical properties, (b) pharmacokinetic parameters describing absorption, distribution, metabolism and excretion processes and (c) clinical studies of intravenous and oral administration of voriconazole to healthy subjects with different dosing regimens. The clinical studies were screened and selected according to the following criteria: (i) intravenous or oral administration of voriconazole, (ii) healthy volunteers, (iii) plasma concentration-time profiles of voriconazole were available, and (iv) articles published in English. The training dataset for model development was selected based on (i) the information required for each step of model development, (ii) the optimized parameters, (iii) the number of studies available and (iv) the informative property of profiles for individual studies (genotype groups, dosing regimens, and routes of administration), as shown in **Figure 2**. Except datasets required and used for model development, all the remaining clinical trials datasets were utilized for model evaluation.

The modeling software PK-Sim® (version 7.3.0, part of the Open Systems Pharmacology suite) was used for model development, which consists of a system- and a drug-dependent component. System-dependent

physiological parameters (organ volumes, blood flow rates, hematocrit, etc.) were provided in PK-Sim® with the small molecule model [28–30]. Demographic characteristics of subjects were taken from each clinical study. Drug-specific physicochemical properties were obtained from the literature. Organ-plasma partition coefficients were determined by the Poulin and Theil method based on both the literature [31] and the best overlap between observed and predicted concentration-time profiles.

The workflow of model development is presented in **Figure 2**. For model development, the simplifying assumption was made that the metabolism of voriconazole is mediated exclusively by CYP3A4 and CYP2C19; the minor contributions of CYP2C9, FMOs and unchanged renal elimination of voriconazole were neglected [13,16]. Tissue expression distribution of enzymes was provided by the PK-Sim® expression database based on reverse transcription-polymerase chain reaction (RT-PCR) profiles [32] together with the reference value of 4.32 µmol CYP3A4 and 0.76 µmol CYP2C19 per liter liver tissue [33]. The relative CYP2C19 expression for different genotypes was obtained based on the CYP2C19 protein content ratio in genotype-defined pooled human liver microsomes [34]. The metabolism process of voriconazole was described by Michaelis-Menten kinetics [35]. As reported by Damle et al. [31], K_m for CYP3A4 and CYP2C19 were set to 15 and 3.5 µM, respectively, and V_{max} for CYP2C19 was fixed 1.19 pmol/min/pmol. V_{max} for CYP3A4 was optimized based on the concentration-time profile in CYP2C19 PMs [18] with the assumption that only CYP3A4 contributes to the metabolism of voriconazole in PMs. MBI was integrated into the model with **Eq. S4** in the supplementary materials based on the *in vitro* inactivity assay results of K_I. The other parameter k_{inact} was optimized based on concentration-time curves after multiple intravenous administrations [36], since the *in vitro* derived k_{inact} parameter value led to an overprediction of midazolam AUCs when evaluating the voriconazole-midazolam DDI studies.

The specific intestinal permeability was optimized based on the studies, including both intravenous and oral administration of voriconazole [6,37,38]. The dissolution of the formulation was assumed to follow a Weibull function and was estimated based on the concentration-time datasets after oral administration [18].

2.3 Model evaluation

Model-based simulations were created for visual comparison with the observed concentration-time profiles of voriconazole in different CYP2C19 genotype groups. For clinical trials not reporting CYP2C19 genotype information, the population was assumed to be NM as this genotype is the most common 2C19 polymorphism prevalent in more than 64% of "white", African American, Hispanic, and Ashkenazi populations [39]. The visual criteria for a good model performance were that 95% population prediction intervals should cover the observed individual plasma concentration-time profiles from original datasets, or that the observed aggregate plasma concentration-time profiles should be inside the 68% population prediction intervals. Predicted AUC, C_{max}, and C_{trough} values were compared to observed values via the goodness-of-fit plots.

The quantitative evaluation criterion for a good model performance was that the ratios of predicted to observed AUC, $C_{max,}$ and C_{trough} (trough concentration for multiple doses) values should be within 0.5- to 2.0-fold limits, as shown in **Tables 1, 2** and **S4**. As a quantitative summary of the predictive performance of the model, the geometric mean fold error (GMFE) was calculated with **Eq. 1** [40].

Eq. 1 $$\mathrm{GMFE} = 10^{(\sum|\log_{10}(\mathrm{pred\ P/obs\ P})|)/n}$$

GMFE: geometric mean fold error of all AUC, C_{max} or C_{trough} predictions from the respective model, pred P: predicted parameter (AUC, C_{max} or C_{trough}), obs P: observed parameter (AUC, C_{max} or C_{trough}), n: number of studies.

2.4 Drug-drug interactions with other CYP3A4 substrates

Published PBPK models of the CYP3A4 probe substrates midazolam or alfentanil were integrated with the model of voriconazole to assess the inhibitory effects of voriconazole on CYP3A4 *in vivo* and to verify the inhibition model of voriconazole meanwhile [40]. The DDI modeling performance was evaluated by both visual comparison of predicted versus observed probe substrates pharmacokinetic profiles, and by calculation of DDI AUC ratios and C_{max} ratios according to **Eq. 2-3**.

Eq. 2 $$\text{DDI AUC ratio} = \frac{\text{AUC}_{\text{treatment}}}{\text{AUC}_{\text{reference}}}$$

Eq. 3 $$\text{DDI } C_{\max} \text{ ratio} = \frac{C_{\max_{\text{treatment}}}}{C_{\max_{\text{reference}}}}$$

AUC (or C_{max}) treatment: AUC (or C_{max}) of victim drug with voriconazole co-treatment; AUC (or C_{max}) reference: AUC (or C_{max}) for victim drug administration alone.

2.5 Sensitivity Analysis

According to **Eq. 4**, the ratio of the relative change of AUC_{τ} (area under the plasma concentration-time curve during a dosage interval ($_{\tau}$)) versus the relative alteration of the evaluated parameter was calculated at steady-state after the standard therapeutic multiple dosages of voriconazole by oral administration. The sensitivity analysis was also conducted for the DDI between voriconazole and midazolam. Parameters selected for the sensitivity analysis fulfilled one of the following criteria [40]: i) optimized; ii) related to optimized parameters; iii) a strong influence on calculation methods used in the model; iv) significant impact in the model.

Eq. 4 $$S = \frac{\Delta \text{AUC}}{\text{AUC}} \div \frac{\Delta p}{p}$$

S: sensitivity of AUC to the evaluated parameter; ΔAUC: change of AUC; AUC: AUC with the initial value; Δp: change of the assessed parameter value; p: parameter with the initial value. A sensitivity value of +1.0 means that a 10% change of the examined parameter causes a 10% alteration of the predicted AUC_{τ}.

2.6 Virtual population characteristics

Based on the demographic characteristics from each clinical trial, virtual populations of 100 individuals were generated to assess the variability of the predicted concentration-time profiles quantitatively from the respective clinical trials. Information on age, body weight, body height and proportions of female participants was entered into the software for each clinical trial. The default population variabilities for enzyme expression in PK-Sim® were used. To compare the variability of observed and simulated pharmacokinetic profiles, 68% population prediction intervals (approx. mean±SD in case of assumed normal distribution) were plotted if the observed concentration-time profiles were reported as mean (±SD); while 95% population prediction intervals were described when all individual concentration-time profiles were available [41].

2.7 Model Applications

First, model-based simulations were performed according to the dosing regimens of the clinical trials in **Table 1** to compare the predicted versus observed data, capturing the nonlinear pharmacokinetics of voriconazole including dose- and time-dependence. Second, different CYP2C19 genotype groups, i.e., RMs, NMs, IMs (intermediate metabolizers) and PMs were simulated respectively to depict the effect of genetic polymorphisms of CYP2C19 on the metabolism of voriconazole in **Table 2**. Then, based on the PBPK model we explored the performance of various maintenance doses in different CYP2C19 genotype groups (RMs, NMs, and IMs). Virtual populations of 1000 individuals were generated based on the summary demographic characteristics from all clinical trials. The simulated dosing regimens were 400 mg b.i.d on the first day followed by 100-400 mg b.i.d on the following days for two weeks, which was considered to be sufficient to achieve steady-state. The probability of target attainment and potentially toxic trough concentrations was calculated based on two different definitions of therapeutic ranges to reflect the heterogeneity of guidelines. Thus, a therapeutic target of at least 1or 2 mg/L and at most 5 or 6 mg/L was defined. Third, the time course of active CYP3A4 content in both liver and small intestine during voriconazole treatment was simulated based on the most frequent oral therapeutic dosing regimen of voriconazole, i.e., 400 mg b.i.d on the first day and then 200 mg b.i.d on the following days. Fourth, by connecting the PBPK models of midazolam (or alfentanil) and voriconazole, DDIs models between voriconazole and the victim drugs was set up (see **Table 3**).

3 RESULTS

3.1 *In vitro* assays

The result of the IC_{50} shift assays indicated that voriconazole caused MBI on CYP3A4, with a 16-fold difference in the absence and presence of NADPH (see **Table 4**), supporting MBI to be introduced into the PBPK model. In contrast, inhibition of CYP2C19 was only within a 2-/3-fold range of IC_{50} shift and therefore was considered as negligible during model development. The inactivation kinetic assay gave a K_I of 9.33 (95% CIs: 2.56-34.0) μM and a k_{inact} of 0.0428 (95% CIs: 0.0171-0.107) min^{-1} for CYP3A4, which were used for the parametrization in the PBPK model (see **Table 5**).

3.2 Model development and evaluation

3.2.1 Clinical studies

Among all 93 concentration-time profiles of voriconazole from clinical trials, 21 were used for the model development and 72 for model evaluation (see **Tables 1** and **2**). The participants were all healthy volunteers, with an age range from 18 to 53 years and a body weight from 47 to 103 kg. CYP2C19 genotypes included 62 RMs ($^*1/^*17$, $^*17/^*17$), 101 NMs ($^*1/^*1$), 77 IMs ($^*1/^*2$, $^*1/^*3$, $^*2/^*17$, $^*2/^*2/^*17$), and 65 PMs ($^*2/^*2$, $^*2/^*3$, $^*3/^*3$) (see **Table 2**). Administration protocols included both oral and intravenous routes, both single and multiple doses, and individual doses ranging from 1.5 to 6 mg/kg and from 50 to 400 mg.

3.2.2 Model development

V_{max} for CYP3A4 was originally fixed to 0.31 according to the reported value by Damle et al. [31]. However, simulations resulted in a more than two-fold over-prediction for AUC for low doses of voriconazole. The reasons for over-prediction of AUC were explored. Simultaneous and separate optimization of V_{max} for CYP3A4 and CYP2C19 showed that the optimized value for CYP2C19 was approaching to the reported one, while for CYP3A4, the optimized value was far higher than the reported one. A possible reason was that the reported value for CYP3A4 was obtained without consideration of MBI on CYP3A4, which might lead to underestimation of V_{max}. Furthermore, the subjects in the clinical studies belonged to different CYP2C19 genotypes, which provided the possibility to optimize V_{max} of CYP3A4. Therefore, this parameter was optimized as 2.12 pmol/min/pmol based on the concentration-time datasets of CYP2C19 PMs with intravenous administration [18], assuming that only CYP3A4 mediated the metabolism of voriconazole in PMs due to the deficiency of CYP2C19. For other genotypes, both CYP2C19 and CYP3A4 contributed in the metabolism of voriconazole. The different CYP2C19 genotypes were integrated into the model for RMs, NMs, IM or PM with the reference CYP2C19 expression values of 0.79, 0.76, 0.40, and 0.01 μmol/L, respectively [34]. Therefore, in the absence of evidence for another root cause of AUC over-prediction,

MBI of CYP3A4 by voriconazole was introduced into the model with **Eq. S4** based on the *in vitro* inactivation kinetic parameter K_I of 9.33 μM. When the *in vitro* k_{inact} of 0.0428 min^{-1} served as model input, the predicted concentration-time profiles of midazolam in DDI with co-treatment of voriconazole were overestimated. Therefore, k_{inact} was finally optimized as 0.015 min^{-1} based on the concentration-time profiles with multiple intravenous dosing of voriconazole [36].

3.2.3 Model evaluation

The input parameters describing the PBPK model of voriconazole are listed in **Table 6**. The predicted pharmacokinetic results for the respective clinical trials in comparison with the observed aggregate values are presented in **Tables 1** and **2**, together with administration protocols and subjects' details. Prediction performance of the model was quantitatively evaluated by the ratios of predicted versus observed aggregate AUC and C_{max} with calculated GMFEs shown in **Tables 1** and **2**. Among the 28 test datasets for subjects with unspecified genotype, 71% of predicted/observed aggregate AUC ratios and all aggregate C_{max} ratios were within 0.5- to 2.0-fold limits (**Table 1**). Taking genotype of CYP2C19 into consideration, from 44 test profiles, 89% of aggregate AUC ratios and all aggregate C_{max} ratios were within 0.5- to 2.0-fold (**Table 2).** Also, 85% of predicted/observed aggregate C_{trough} ratios from clinical trials after multiple administration were within the 0.5- to 2.0-fold range (**Table S4**). The performance of the model was visualized by comparing predicted and observed concentration-time profiles as shown in **Figures 3-4** and **S1-2, S4-7**. The model-based simulations for multiple doses captured the dose- and time-dependent non-linear pharmacokinetics of voriconazole well (**Figure 3** and **S1, S4, S7**). Although the population predictions for low doses (i.e., 50 mg) reflected over-estimation compared to the observed individual data, for the therapeutic dose of 400 mg the 95% prediction interval covered the variability of the observed individual data sufficiently (**Figures 4** and **S5**), indicating that simulations grouped by different CYP2C19 genotype were suitable to describe the effect of genetic polymorphisms of CYP2C19 on the metabolism of voriconazole. This was further confirmed by the good population prediction for the observed aggregate concentration-time profiles for both a single and multiple doses in different CYP2C19 genotype groups, despite an over-prediction of exposure for multiple doses in PMs (**Figure S2** and **S7**). Also, plotting predicted versus observed AUC, C_{max} and C_{trough} from all the clinical studies confirmed a good fit of the final PBPK model of voriconazole for most clinical trials (**Figure 5**), despite an over-prediction of AUC for low doses.

3.3 Sensitivity analysis

A sensitivity analysis was performed based on the simulation of the therapeutic multiple oral dosing regimen (400 mg b.i.d on the first day and then 200 mg b.i.d on the following days until steady-state) to assess the impact of the parameters on the model. It was shown that the voriconazole model was most sensitive to CYP2C19 k_{cat}, K_m, and fraction unbound values (all taken from the literature) with sensitivity values ranging from -1.08 to 0.75 (**Figure S3A)**.

The sensitivity analysis of the parameters for DDI models between voriconazole and midazolam on the $AUC_{0\text{-}t}$ of [23] exhibited that sensitivity was most pronounced for midazolam lipophilicity, CYP3A4 k_{inact} and K_I with the sensitivity values beyond -1.0 or 1.0 (**Figure S3B**).

3.4 Model application

3.4.1 Suitable maintenance doses in CYP2C19 genotype groups

A separate simulation of specific CYP2C19 genotype groups could accurately describe both observed individual and aggregate concentration-time profiles for either a single dose or for multiple doses, as assessed by the respective criteria (Table 2, Figure 3 and S2, S5, S7). Therefore, model-based simulations were carried out to

explore the performance of voriconazole maintenance doses for different CYP2C19 genotypes (Figure 8). The standard dosage (oral 400 mg twice daily on the first day and 200 mg twice daily for the following days) was confirmed to be appropriate for IMs; while for RMs and NMs, the 200 mg maintenance dose seems to be insufficient. The model-based simulation suggests to double the maintenance dose for RMs and NMs to increase the probability of target attainment two-fold while maintaining a probability of toxic concentrations below 20%. The less reliable prediction for multiple doses in PMs precludes the suggestion of an appropropriate maintenance dose regimen in PMs, although it clearly shows that the 200 mg bid dose is too high.

3.4.2 Inhibition of CYP3A4 by voriconazole

The time courses of CYP3A4 activity in both liver and small intestine were assessed during chronic voriconazole treatment. The maximum inhibition was reached at 51.2 h in the liver and 52.5 h in the small intestine (**Figure 6**), resulting from the combination of the physiological CYP3A4 turnover and MBI of CYP3A4 (**Eq. S4**). The CYP3A activity was predicted to recover 90% of its baseline 5 days after the last voriconazole dose.

3.4.3 DDI modeling

The CYP3A4 inhibition model of voriconazole was further applied to the DDI between CYP3A4 probe substrates as victims (midazolam and alfentanil) and voriconazole as the perpetrator. **Figure 7** and **S8** demonstrate the good performance of DDI PBPK models for voriconazole and the two probe substrates. The observed AUC change of substrates during treatment with voriconazole versus control was inside the 90% confidence interval of the predicted AUC change, and the predicted/observed DDI AUC ratio of alfentanil was 0.86, indicating that this inhibition model was appropriate. The inhibition model was further confirmed to be suitable by the predicted/observed midazolam DDI AUC ratios of 1.09 and 0.76, respectively, for intravenous and oral administration.

4 DISCUSSION

A whole-body PBPK model of voriconazole integrating MBI of CYP3A4 has been successfully developed. Model-based simulations of voriconazole plasma concentrations were in good agreement with observations from clinical studies with both intravenous and oral administration of a wide range of single and multiple doses. The model was also appropriate to predict voriconazole plasma concentrations for individual CYP2C19 genotype groups and the extent of DDIs with the CPY3A4 probe substrates midazolam and alfentanil caused by voriconazole.

An MBI effect of voriconazole on its metabolism *in vivo* was reported previously [15,25]. Several lines of evidence supported that the incorporation of MBI should be considered to describe the pharmacokinetics of voriconazole accurately. First, Mikus et al. proposed that "autoinhibition" of CYP3A was the key to explain the observed dose nonlinearity of voriconazole elimination after administration of 50 and 400 mg in healthy volunteers [15,24]. Second, time-dependent disproportionately increasing exposure of voriconazole was found *in vivo* after multiple doses; e.g., AUC for multiple intravenous administration (3 mg kg^{-1} over 1 hour once on the first day and b.i.d. on the following days) on the 5^{th} day of treatment was more than 2-fold higher than the predicted value based on the results for the first dose under the assumption of dose-linearity and continued to increase until the 12^{th} day doses [36]. Third, both Friberg et al. and Kim et al. integrated "time-dependent inhibition" and "autoinhibtion" models of voriconazole to describe the respective processes concerning enzyme inhibition by voriconazole *in vivo*, respectively [25,26]. Fourth, our *in vitro* assays clearly showed a pronounced IC_{50} shift from 48.7 to 3 µM, verifying MBI of CYP3A4 by voriconazole. Indeed, incorporation of MBI into the PBPK model turned out to be essential to predict the dose- and time-dependent pharmacokinetic nonlinearity of voriconazole.

Beyond MBI, reversible inhibition of CYP3A4 and CYP2C19 by voriconazole was also explored. Our *in vitro* assay resulted in a competitive inhibition of CYP3A4 K_i of 0.47 (95% CIs: 0.344-0.636) µM, which is in agreement with results from other studies, e.g., competitive (K_i = 0.66 µM) and noncompetitive inhibition (K_i = 2.97 µM) in one study [21]; and solely competitive inhibition (K_i = 0.15 µM) in another study [22]. But *in vivo* evaluation of DDIs between voriconazole and midazolam indicated that assumption of a simple competitive inhibition only was explicitly not sufficient *in vivo* [42]. An MBI model of CYP3A was discussed in the previous research but not incorporated due to lack of *in vitro* data to support it. At that time, a hypothetical extra effect compartment was introduced to describe a time delay. Thus, we conducted an *in vitro* assay to explore MBI of voriconazole on CYP3A4 to fully understand the metabolism of voriconazole.

Also, our *in vitro* assay resulted in the competitive inhibition of voriconazole on CYP2C19 with K_i values of 1.08 (95% CIs: 0.815-1.43) µM and 1.26 (95% CIs: 0.839-1.82) µM with omeprazole and mephenytoin as substrates, respectively (in **Table 4**), which could provide some evidence for DDI between voriconazole and CYP2C19 probe substrates (e.g., omeprazole and mephenytoin). *In vivo*, voriconazole was reported to increase the C_{max} and AUC_T of omeprazole by 116% and 280% [43], respectively. However, detailed *in vivo* data were not available, which limited the evaluation of the PBPK DDI models between voriconazole and CYP2C19 substrates, which is one of the limitations of our PBPK model.

Beyond the effects of the parent drug, the inhibition of voriconazole N-oxide on CYP3A4 and CYP2C19 was also investigated. Although voriconazole N-oxide exhibited reversible inhibition on both enzymes, the effects were weaker with K_i 0.894 (95% CIs: 0.650-1.22) and 9.00 (95% CIs: 6.94-11.7) µM, respectively (see **Table 4**). Additionally, at therapeutic voriconazole doses, plasma concentrations of voriconazole N-oxide typically reach only about a third compared to that of its parent drug [17]. Thus, the inhibition by voriconazole N-oxide would be much less than that of the parent drug and was considered negligible during PBPK model development.

The advantages of the PBPK model approach presented here become evident compared to an empirical population pharmacokinetic model. PBPK models can depict a more precise mechanistic picture of inhibition processes. Based on the developed PBPK model, it was feasible to describe the time course of inhibition of CYP3A4 during and after voriconazole treatment by taking into account the dynamic nature of the inhibition process with a clear differentiation between liver and small intestinal enzyme activity (**Figure 6)**. Furthermore, this PBPK model could be applied to predict the effect of voriconazole dosing schemes on several other CYP3A4 substrate drugs and thus to manage respective clinical DDIs. It was verified by the observation that the predicted DDI was mostly suitable for oral and intravenous midazolam as well as for alfentanil (**Figure 7** and **S8**), both being established CYP3A4 probe substrates [44].

For a thorough understanding of voriconazole pharmacokinetics, CYP2C19 genotype groups were another important factor during model development, since the wide inter-individual variability mainly resulted from the genotypes of CYP2C19. Therefore, suitable maintenance doses for CYP2C19 genotype groups (RMs, NMs, and IMs) were suggested based on simulations. For PMs, the search for a dose to provide an appropriate exposure was less reliable due to the limited performance of the model for multiple doses in PMs. With MBI on CYP3A4 and deficiency of CYP2C19, voriconazole would accumulate in PMs and might reach extremely high concentrations after multiple administrations. Yet, the observations from one study showed that the increase of voriconazole concentrations in PMs after multiple doses was not as high as the prediction (**Figure S2 f)**[19], indicating that other elimination pathway may compensate to prevent drug accumulation in the body. However, for PMs, the experimental data to quantitatively describe voriconazole pharmacokinetics in individuals were sparse, limiting the integration of more complex pathways.

Although the presented model performed well in several ways, it has several limitations. The first one is the assumption that only CYP3A4 and CYP2C19 mediate primary metabolism and elimination of voriconazole. This assumption may result in over-estimation of the role of CYP3A4 and CYP2C19 activity; the consequence of ignoring FMO and CYP2C9, however, should be acceptable in most CYP2C19 genotypes (RMs, NMs, and IMs). K_m values for FMO1 and FMO3 are in the millimolar range (about 3 mM) [14], which is far beyond the concentrations reached *in vivo*. A contribution of CYP2C9 was identified in only one paper [13] with a small V_{max} value, which was not confirmed in other *in vitro* assays [13,45]. Renal excretion of unchanged voriconazole is less than 2 %, and primary metabolism by glucuronidation is also negligible [17]. Thus, it is reasonable to simplify the primary metabolism of voriconazole as depending on CYP3A4 and 2C19 only. Also, the fact that our model was able to properly describe most published data supports a role of CYP3A4 and CYP2C19 also for unknown metabolic pathways. Another limitation was that the inhibitory effect of voriconazole N-oxide with less inhibitory effect and lower plasma concentrations was not taken into account, as well as the other metabolites. Also, we did not attempt to simultaneously describe the concentration-time profiles of voriconazole N-oxide and other metabolites (hydroxy-fluoropyrimidine voriconazole and dihydroxy-fluoropyrimidine

voriconazole) reported in a few published datasets to limit the complexity of the model and to limit the number of assumptions required. The third limitation was that during the model development, datasets with low doses, e.g., 50 mg, were not successfully integrated into the model. When extrapolating the model predictions to low dosages, the simulation showed some over-prediction of voriconazole concentrations. However, such low doses are not clinically relevant. Fourth, the uncertainty of K_I from *in vitro* assays could not be implemented into the PBPK model due to technical limitations of the software. Although the current model successfully described the complex metabolism of voriconazole, we suggest to further verify the model by additional clinical studies (e.g., studies quantifying the metabolites of voriconazole, i.e., voriconazole N-oxide, hydroxy-fluoropyrimidine voriconazole and dihydroxy-fluoropyrimidine voriconazole in plasma/urine/feces; and studies in PMs with low multiple doses; DDI studies between CYP3A4 substrates and voriconazole including quantification of its metabolites and different routes of administration of both substrates and voriconazole).

5 CONCLUSION

MBI of CYP3A4 by voriconazole is an important pharmacokinetic characteristic of the drug and needs to be taken into account along with CYP2C19 genotype to predict the exposure of voriconazole properly. By incorporating these elements, a PBPK model of voriconazole was developed which could accurately capture the time- and dose-dependent alterations of voriconazole pharmacokinetics as well as DDIs caused by voriconazole inhibitory effects on CYP3A4. This model could support individual dose optimization of voriconazole as well as DDI risk management. It will be provided as a public tool in the Open Systems Pharmacology (OSP) repository (http://www.open-systems-pharmacology.org/) to assess the DDI potential of investigational drugs, to support the design of clinical trials or to expand the model for predictions in special populations.

Compliance with Ethical Standards

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Conflict of interest

Sebastian Frechen is an employee and potential shareholder of Bayer AG, Leverkusen, Germany. Xia Li, Daniel Moj, Thorsten Lehr, Max Taubert, Chih-hsuan Hsin, Gerd Mikus, Pertti J. Neuvonen, Klaus T. Olkkola, Teijo I. Saari, Uwe Fuhr have no conflicts of interest to declare.

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Table 1 Clinical studies without information on CYP2C19 genotype used for voriconazole model development and evaluation

Dose [mg]	Route	n	Male [%]	Age [years]	Weight [kg]	Use of dataset	Pred AUC [mg*h/L]	Obs AUC [mg*h/L]	Pred/Obs AUC	Pred C_{max} [mg/L]	Obs C_{max} [mg/L]	Pred/Obs C_{max}	Ref
3/kg,QD D1	iv(1h)	9	100	24 (20-31)	72 (60-87)	d/a	7.90	5.22	1.51	2.45	2.14	1.14	[36]
3/kg,BID D3-11.5 (3/kg,QD D1)	iv(1h)	9	100	24 (20-31)	72 (60-87)	d/a	16.7	16.5	1.01	3.54	3.62	0.98	[36]
6/kg, BID D1	iv(1h)	9	100	28 (19-41)	73 (66-80)	d/a	16.2	13.2	1.23	5.12	4.70	1.09	[36]
3/kg,BID D2-9.5 (6/kg, BID D1)	iv(1h)	9	100	28 (19-41)	73 (66-80)	d/a	15.2	13.3	1.14	3.39	3.06	1.11	[36]
3/kg,BID D2-7 (6/kg BID D1)	iv(1h)	14	100	26.5±1.48*	78.7±1.93*	d/a	17.3	13.9	1.24	3.64	3.00	1.21	[6]
200,BID D8-13.5 (6/kg, BID D1, 3/kg,BID D2-7)	po(-)	14	100	26.5±1.48*	78.7±1.93*	d/a	13.7	9.77	1.40	2.17	1.89	1.15	[6]
4/kg,BID D2-7 (6/kg BID D1)	iv(1h)	7	100	24.7±2.37*	73.2±2.12*	d/a	34.4	29.5	1.17	5.82	5.40	1.08	[6]
300,BID D8-13.5 (6/kg BID D1, 4/kg,BID D2-7)	po(-)	7	100	24.7±2.37*	73.2±2.12*	d/a	20.6	30.9	0.67	2.95	4.84	0.61	[6]
5/kg,BID D2-7 (6/kg BID D1)	iv(1h)	14	100	26.5±1.48*	78.7±1.93*	d/a	44.5	43.4	1.03	7.46	7.18	1.04	[6]
400,BID D8-13.5 (6/kg BID D1, 5/kg,BID D2-7)	po(-)	14	100	26.5±1.48*	78.7±1.93*	d/a	31.8	37.6	0.85	4.48	5.27	0.85	[6]
100,SIG	iv(4h)	20	95	32 (23-52)	80.8±11.8*	e/a	3.25	2.63 [a]	1.24	0.51	0.48	1.06	[15]
400,SIG	iv(2h)	20	95	32 (23-52)	80.8±11.8*	e/a	16.5	21.1 [a]	0.78	3.14	3.73	0.84	[15]
400,SIG	iv(4h)	20	95	32 (23-52)	80.8±11.8*	e/a	16.1	18.8 [a]	0.86	2.23	2.67	0.84	[15]
400, SIG	iv(6h)	20	95	32 (23-52)	80.8±11.8*	e/a	15.9	17.6 [a]	0.90	1.81	1.83	0.99	[15]
200,SIG	iv(1.5)	52	100	26.9±4.9*	70.7±7.8*	e/a	7.53	8.13 [a,♦]	0.93	1.91	2.14 [♦]	0.89	[46]
1.5/kg,QD D1	po(-)	11	100	27 (20-45)	73 (60-90)	e/a	2.67	0.88	**3.03**	0.62	0.364	1.70	[47]
1.5/kg,TID D3-11.5 (1.5/kg,QD D1)	po(-)	11	100	27 (20-45)	73 (60-90)	e/a	6.48	3.79	1.71	1.34	1.11	1.21	[47]
2/kg,QD D1	po(-)	8	100	26 (20-36)	74 (66-89)	e/a	4.07	1.18	**3.45**	0.85	0.485	1.75	[47]
2/kg,BID D3-11.5 (2/kg,QD D1)	po(-)	8	100	26 (20-36)	74 (66-89)	e/a	9.52	4.30	**2.21**	1.61	1.01	1.59	[47]

2/kg,QD D1	po(-)	8	100	31 (21-44)	74 (64-87)	e/a	3.46	1.44	**2.40**	0.82	0.646	1.27	[47]
2/kg,TID D3-11.5 (2/kg,QD D1)	po(-)	8	100	31 (21-44)	74 (64-87)	e/a	9.23	9.04	1.02	1.88	2.18	0.86	[47]
3/kg,QD D1	po(-)	8	100	25 (18-30)	73 (61-87)	e/a	5.65	3.15	1.79	1.22	1.19	1.03	[47]
3/kg,BID D3-11.5 (3/kg,QD D1)	po(-)	8	100	25 (18-30)	73 (61-87)	e/a	15.4	11.2	1.38	2.50	2.36	1.06	[47]
4/kg,QD D1	po(-)	8	100	25 (20-37)	74 (66-94)	e/a	7.67	5.90	1.30	1.35	1.57	0.86	[47]
4/kg,QD D3-11.5 (4/kg,QD D1)	po(-)	8	100	25 (20-37)	74 (66-94)	e/a	14.3	13.2	1.08	1.98	2.07	0.96	[47]
200,BID D1-6.5	po(-)	9	100	22 (19-25)	74 (67-91)	d/a	14.4	12.9	1.12	2.40	2.24	1.07	[37]
200,BID D1	po(cap)	6	100	29 (23-36)	74 (67-82)	d/a	4.58	3.14	1.46	1.23	0.96	1.28	[38]
200,BID D2-6.5 (200,BID D1)	po(cap)	6	100	29 (23-36)	74 (67-82)	d/a	12.0	12.5 [a]	0.96	2.20	2.04	1.08	[38]
400,QD D1	po(-)	18	100	26 (20-40)	75 (66-92)	e/a	9.22	9.31	0.99	1.92	2.31	0.83	[48]
200,BID D2-9.5 (400,QD D1)	po(-)	18	100	26 (20-40)	75 (66-92)	e/a	12.5	11.2	1.12	2.23	2.08	1.07	[48]
200,BID D2-4 (400 BID D1)	po(-)	12	-	18-50	>40	e/a	12.4	15.2 [a,♦]	0.82	2.23	2.60 [♦]	0.86	[49]
200,BID D22-24 (400 BID D21)	po(-)	12	-	18-50	>40	e/a	12.0	13.6 [a,♦]	0.88	2.21	2.50 [♦]	0.88	[49]
200,BID D2-2.5 (400 BID D1)	po(tab)	13	100	31 (19-52)	78 (62-88)	e/a	13.0	26.5 [a,♦]	**0.49**	2.24	3.60 [♦]	0.62	[50]
200,BID D2-2.5 (400 BID D1)	po(tab)	16	100	40 (26-54)	80 (65-95)	e/a	13.1	26.8 [a,♦]	**0.49**	2.24	3.36 [♦]	0.67	[50]
200,BID D1-6.5	po(tab)	10	100	25 (20-30)	73 (62-85)	d/a	13.1	10.5	1.25	2.32	1.87	1.24	[51]
200,BID D1-6.5	po(-)	12	100	29 (21-39)	75 (67-82)	d/a	12.1	13.6	0.89	2.19	2.25	0.97	[52]
200,BID D1-6.5	po(-)	11	100	29 (20-42)	77 (61-91)	d/a	12.0	9.42	1.27	2.16	2.00	1.08	[53]
200,BID D2-3.5 (400 BID D1)	po(-)	14	0	35 (19-51)	74 (52-87)	e/a	13.5	17.6 [a]	0.77	2.32	2.80	0.83	[54]
200,BID D2-2.5 (400 BID D1)	po(tab)	16	100	34 (20-48)	79 (59-92)	e/a	13.0	26.3 [a,♦]	**0.49**	2.22	3.06 [♦]	0.73	[55]
200,BID D2-3.5 (400 BID D1)	po(-)	16	0	26 (19-36)	-	e/a	18.5	14.9 [♦]	1.24	2.91	2.64 [♦]	1.10	[56]
200,BID D2-3.5 (400 BID D1)	po(-)	16	100	30 (20-42)	-	e/a	12.6	24.0 [♦]	0.53	2.10	2.74 [♦]	0.77	[57]

200,BID D2-6.5 (400 BID D1)	po(tab)	20	50	28 (20-43)	-	e/a	12.9	11.2	1.15	2.33	2.37	0.98	[58]
200,BID D2-7.5 (400 BID D1)	po(-)	14	100	29 (18-45)	-	e/a	14.6	14.7 [a,♦]	0.99	2.47	2.87 [♦]	0.86	[59]
200,BID D2-3.5 (400 BID D1)	po(-)	18	100	28 (20-40)	-	e/a	13.2	29.9 [b,♦]	**0.44**	2.25	3.96 [♦]	0.57	[60]
								GMFE(range)	1.39(0.44-3.45)			1.20(0.57-1.75)	
								Pred/Obs within 2-fold	36/44			44/44	

AUC values are AUC_{τ} if not specified otherwise, [a]: AUC_{obs}, [b]: AUC at steady-state; Observed aggregate values are reported as geometric mean if not specified otherwise, ♦: arithmetic mean; *: standard error; /kg: per kg of body weight; D: day of treatment according to the numbering in the reference; SIG: single dose, QD: once daily, BID: twice daily, TID: three times daily; iv: intravenously, po: orally; e: datasets for model evaluation, d: dataset for model development; i: individual datasets; a: aggregate datasets; tab: tablet, cap: capsule; Obs: observed aggregate value from literature, Pred: predicted value based on the model; GMFE: geometric mean fold error; -: not available. The ratios of predicted versus observed AUC and C_{max} outside 0.5- to 2.0-fold limits were printed in bold.

Table 2 Clinical studies with information on CYP2C19 genotype used for voriconazole model development and evaluation

CYP2C19 genotype	Dose [mg]	Route	n	Male [%]	Age [years]	Weight [kg]	Use of dataset	Pred AUC [mg*h/L]	Obs AUC [mg*h/L]	Pred/Obs AUC	Pred C_{max} [mg/L]	Obs C_{max} [mg/L]	Pred/Obs C_{max}	Ref.
RM(*1/*17, *17/*17)	50,SIG	iv(2h)	8	63	30 (24-53)	71 (55-96)	e/i	1.66	1.02	1.63	0.39	0.320	1.22	[24]
	50,SIG	po(tab)	8	63	30 (24-53)	71 (55-96)	e/i	1.08	0.40	**2.70**	0.27	0.167	1.62	[24]
	400,SIG	iv(2h)	7	71	30 (24-53)	73 (58-96)	e/i	17.5	16.5	1.06	3.49	3.29	1.06	[24]
	400,SIG	po(tab)	7	71	30 (24-53)	73 (58-96)	e/i	9.37	15.3	0.61	1.6	3.21	0.50	[24]
	400,SIG	iv(2h)	6	67	25 (23-28)	75 (61-93)	e/i	17.4	18.8	0.93	3.56	4.05	0.88	[18]
	400,SIG	po(tab)	6	67	25 (23-28)	75 (61-93)	d/i	10.3	13.6	0.76	1.66	2.90	0.57	[18]
	200,SIG	po(tab)	4	100	21±2*	-	e/a	6.07	3.39	1.79	1.22	1.15	1.06	[61]
	400,SIG	po(cap)	3	0	29 (24-37)	69 (64-74)	e/i	13.9	15.9	0.87	1.83	2.97	0.62	[62]
	400,SIG	po(tab)	5	100	26 (24-31)	80 (71-87)	e/i	11.2	11.6	0.97	1.79	2.22	0.81	[63]
	400,SIG	po(cap)	8	100	27 (24-37)	-	e/a	12.0 [a]	13.3 [a]	0.90	1.69	2.16	0.78	[20]
									GMFE(range)	1.36(0.61-2.70)			1.37(0.50-1.62)	
NM(*1/*1)	50,SIG	iv(2h)	4	100	35 (24-46)	77 (65-86)	e/i	1.69	1.24	1.36	0.38	0.345	1.10	[24]
	50,SIG	po(tab)	3	100	35 (24-46)	77 (65-86)	e/i	1.12	0.53	**2.11**	0.27	0.167	1.62	[24]
	400,SIG	iv(2h)	4	100	35 (24-46)	77 (65-86)	e/i	18.1	21.4	0.85	3.33	3.61	0.92	[24]
	400,SIG	po(tab)	3	100	35 (24-46)	77 (65-86)	e/i	11.2	13.6	0.82	1.79	2.21	0.81	[24]
	200,SIG	iv(1h)	6	100	26.7±2.9*	71.2±4.3*	e/a	9.03 [a]	6.51 [a]	1.39	2.48	2.74	0.91	[19]
	200,QD D1	po(-)	6	100	26.7±2.9*	71.2±4.3*	e/a	6.16 [b]	4.64 [b]	1.33	1.24	2.32	0.53	[19]
	200,BID D2-7 (200,QD D1)	po(-)	6	100	26.7±2.9*	71.2±4.3*	e/a	16.4 [b]	19.3 [b]	0.85	2.41	3.21	0.75	[19]
	400,SIG	iv(2h)	2	50	31 (24-38)	76 (69-83)	e/i	19.9	18.8	1.06	3.28	4.05	0.81	[18]
	400,SIG	po(tab)	2	50	31 (24-38)	76 (69-83)	d/i	13.4	13.6	0.99	1.87	2.90	0.64	[18]
	200,SIG	po(tab)	7	100	22±1.5*	59.4±6.2*	e/a	6.04	5.16 ♥	1.17	1.41	1.45 ♥	0.97	[64]
	200,SIG	po(tab)	8	100	21±2*	-	e/a	6.97	6.18	1.13	1.46	1.65	0.88	[61]
	200,BID D2-2.5 (400,BID D1)	po(-)	24	83	27 (18-45)	69 (49-103)	e/a	13.9 [b]	12.9 [b,♦]	1.08	2.32	3.01 ♦	0.77	[65]

	200,BID D2-3.5 (400,BID D1)	po(-)	8	100	29 (22-43)	70 (56-77)	e/a	17.9 [c]	31.0 [c,♦]	0.58	2.75	4.02 [♦]	0.68	[31]
	400,SIG	po(tab)	4	100	25 (22-31)	78 (70-88)	e/i	11.5	16.9	0.68	1.69	3.11	0.54	[63]
	400,SIG	po(cap)	5	100	28 (25-31)	78 (71-85)	e/i	12.0	15.9	0.75	1.69	2.97	0.57	[62]
	400,SIG	po(cap)	9	100	27 (22-31)	-	e/a	9.82 [a]	16.4 [a]	0.60	1.59	3.10	0.51	[20]
									GMFE(range)	1.31 (0.58-2.11)			1.38(0.51-1.62)	
IM (*1/*2,*1/*3,*2/*17, *2/*2/*17)	50,SIG	iv(2h)	4	75	30 (25-34)	71 (56-78)	e/i	1.86	1.13	1.65	0.42	0.32	1.31	[24]
	50,SIG	po(tab)	4	75	30 (25-34)	71 (56-78)	e/i	1.29	0.58	**2.22**	0.31	0.22	1.41	[24]
	400,SIG	iv(2h)	4	75	30 (25-34)	71 (56-78)	e/i	22.8	25.0	0.91	3.70	3.82	0.97	[24]
	400,SIG	po(tab)	4	75	30 (25-34)	71 (56-78)	e/i	14.2	23.2	0.61	2.14	3.32	0.64	[24]
	200,SIG	iv(1h)	6	100	24.7±2.7*	74.2±7.3*	e/a	9.96 [a]	10.1 [a]	0.99	2.45	3.36	0.73	[19]
	200,QD D1	po(-)	6	100	24.7±2.7*	74.2±7.3*	e/a	7.07 [b]	7.02 [b]	1.01	1.22	1.81	0.67	[19]
	200,BID D2-7 (200,QD D1)	po(-)	6	100	24.7±2.7*	74.2±7.3*	e/a	29.7	42.4 [b]	0.70	3.50	5.78	0.61	[19]
	400,SIG	iv(2h)	8	63	26 (24-32)	76 (65-103)	e/i	22.9	37.4	0.61	3.53	4.33	0.82	[18]
	400,SIG	po(tab)	8	63	26 (24-32)	76 (65-103)	d/i	14.9	30.9	**0.48**	1.89	3.28	0.58	[18]
	400,SIG	po(tab)	5	100	27 (26-31)	80 (68-93)	e/i	12.8	22.2	0.58	1.79	3.15	0.57	[63]
	400,SIG	po(cap)	8	78	26 (22-33)	76 (62-84)	e/i	15.6	20.7	0.75	1.83	2.85	0.64	[62]
	400,SIG	po(cap)	14	100	26 (22-33)	-	e/a	13.2 [a]	25.7 [a]	0.51	1.77	2.84	0.62	[20]
									GMFE(range)	1.51(0.48-2.22)			1.46(0.57-1.41)	
PM(*2/*2, *2/*3,*3/*3)	50,BID D2-2.5 (100,BID D1)	po	8	100	29 (24-45)	76 (68-102)	e/a	5.07 [b]	6.00 [b,♦]	0.85	0.72	0.760 [♦]	0.95	[65]
	200,SIG	iv(1h)	6	100	27.3±3.6*	68.9±3.5*	e/a	14.3 [a]	20.5 [a]	0.70	2.71	2.92	0.93	[19]
	200,QD D1	po(-)	6	100	27.3±3.6*	68.9±3.5*	e/a	9.23 [b]	9.25 [b]	1.00	1.35	2.41	0.56	[19]
	200,BID D2-7 (200,QD D1)	po	6	100	27.3±3.6*	68.9±3.5*	e/a	122 [b]	58.7 [b]	**2.08**	12.1	7.21	1.68	[19]
	400,SIG	iv(2h)	4	50	30 (20-37)	69 (58-79)	d/i	38.8	44.4	0.87	3.94	4.30	0.92	[18]
	400,SIG	po(tab)	4	50	30 (20-37)	69 (58-79)	d/i	25.2	41.6	0.61	2.08	3.91	0.53	[18]
	400,SIG	po(tab)	4	33	29 (19-37)	67 (47-85)	e/i	30.2	42.4	0.71	2.19	3.24	0.68	[62]

200,SIG	po(tab)	7	100	21.6±2.2*	58.4±8.1*	e/a	11.7	17.2♥	0.68	1.7	1.36♥	1.25	[64]
200,SIG	po(tab)	8	100	21±2*	-	e/a	11.3	16.3	0.69	1.63	1.89	0.86	[61]
200,BID D2-3.5 (400,BID D1)	po(-)	8	100	29 (22-43)	70 (56-77)	e/a	79.9 [c]	77.1 [c,♦]	1.04	8.76	10.9 ♦	0.80	[31]
400,SIG	po(cap)	4	100	31 (19-37)	-	e	25.0 [a]	45.7 [a]	0.55	2.26	3.13	0.72	[20]
								GMFE(range)	1.39(0.55-2.08)			1.34(0.53-1.68)	
								GMFE(range)	1.39(0.48-2.70)			1.39(0.50-1.68)	
								Pred/Obs within 2-fold	44/49			49/49	

AUC values are AUC_{obs} if not specified otherwise, [a]: $AUC_{0-\infty}$, [b]: AUC_{τ}, [c]: AUC_{12}. Observed aggregate values are reported as arithmetic mean if not specified otherwise, ♦: geometric mean, ♥: median; *: standard deviation; D: day of treatment according to the numbering in the reference; SIG: single dose, QD: once a day, BID: twice daily; iv: intravenously, po: orally; e: datasets for model evaluation, d: dataset for model development; i: individual datasets; a: aggregate datasets; Obs: observed aggregate value from literature, Pred: predicted value based on the model; tab: tablet, cap: capsule, GMFE: geometric mean fold error; RM: rapid metabolizers, NM: normal metabolizers, IM: intermediate metabolizers, PM: poor metabolizers; -: not available. The ratios of predicted versus observed AUC and C_{max} outside 0.5- to 2.0-fold limits were printed in bold.

Table 3 DDI study dosing regimens, populations, predicted and observed AUC and C_{max} ratios

Perpetrator [mg]	Victim	n	Male [%]	Age [years]	Weight [kg]	Use of dataset	Pred AUC ratio with/without VCZ (90% CI)	Obs AUC ratio with/without VCZ (90% CI)	Pred AUC ratio / Obs AUC ratio	Pred C_{max} ratio with/without VCZ (90% CI)	Obs C_{max} ratio with/without VCZ (90% CI)	Pred C_{max} ratio /Obs C_{max} ratio	Ref.
voriconazole	alfentanil												
400 BID D1,200 BID D2,po	0.02mg/kg,iv	12	58	19-31	65-105	e/a	3.41(1.69-5.28)	3.97 (3.39-4.66) [a]	0.86	-	-	-	[61]
voriconazole	midazolam												
400 BID D1,200 BID D2,po	0.05mg/kg,iv	10	100	19-26	65-100	e/i	3.95 (1.96-6.41)	3.61 (3.20-4.08) [b]	1.09	-	-	-	[17]
400 BID D1,200 BID D2,po	7.5mg,po	10	100	19-26	65-100	e/i	7.51 (2.83-12.0)	9.85 (8.23-11.8) [b]	0.76	2.44(1.90-3.44)	3.56 (2.85-4.44) [b]	0.69	[17]

[a]: AUC_{0-10}, [b]: $AUC_{0-\infty}$; Observed aggregated values are reported as geometric mean if not specified otherwise; D: day of treatment according to the numbering in the reference; BID: twice daily; e: datasets for model evaluation, d: dataset for model development; i: individual datasets; a: aggregate datasets; iv: intravenously, po: orally; Obs: observed aggregated value from literature; Pred: predicted value based on the model; CI: confidence interval; -: not available.

Table 4 IC_{50}, IC_{50} shift, K_i assay results (point estimates with 95% confidence intervals)

Enzyme	Inhibitor	IC_{50}	K_i	IC_{50}		IC_{50} Shift
				Without NADPH	With NADPH	
		μM	*μM*	*μM*		-fold difference
CYP3A4 (midazolam)	VRZ	6.04(3.41-10.7)	0.470(0.344-0.636)	48.7(18.5-128)	3.00(0.465-19.3)	16
	VRZ N-oxide	3.52(2.08-5.95)	0.894(0.650-1.22)	32.3(21.1-49.4)	5.24(0.814-33.7)	6
CYP2C19 (mephenytoin)	VRZ	17.1(11.7-25.0)	1.08(0.815-1.43)	47.6(8.47-267)	24.1(17.6-33.0)	2
	VRZ N-oxide	119(49.0-289)	9.00(6.94-11.7)	145(71.6-295)	44.0(26.8-72.4)	3
CYP2C19 (omeprazole)	VRZ	5.29(3.98-7.02)	1.26(0.839-1.82)	17.9(11.9-27.1)	5.46(1.10-27.0)	3
	VRZ N-oxide	40.4(5.78-282)	7.43(5.58-9.80)	121(72.0-202)	21.0(12.6-34.8)	6

The inactivity pre-incubations time was 30 min and the secondary activity incubation time was 10 min. VRZ: voriconazole.
K_i: inhibitor constant, IC_{50}: half maximal inhibitory concentration of inhibitor.

Table 5 Mechanism-based inactivation K_I/k_{inact} assay conditions and results (point estimates with 95% confidence intervals)

Enzyme	Substrate	voriconazole concentrations	Duration of pre-incubation	Incubation time	K_I	k_{inact}	k_{inact}/K_I
		μM	*min*	*min*	*μM*	min^{-1}	*ml/min/μmol*
CYP3A4	midazolam	0,4,12,40,120,400	0,1,3,6,12,18,24,30	10	9.33 (2.56-34.0)	0.0428 (0.0171-0.107)	0.00459

K_I: the inhibitor concentration when reaching half of k_{inact}, k_{inact}: maximum time-dependent inactivation rate constant.

Table 6 Physicochemical and pharmacokinetic parameters of the voriconazole PBPK model

Parameter	Units	Value used in voriconazole model	Source of values	Description
MW	g/mol	349.3	349.3	Molecular weight
fu	%	42 [1,24,62,63]	0.42[1,24,62,63]	Fraction unbound
logP		1.8 [24,63]	1.75[64],1.65*,1.8[24,63] 2.56[62]	Lipophilicity
pKa		1.60(base) [65]	1.60[65], 1.76[24,62,63],12.71(acidic)*, 2.27(basic)*	Acid dissociation constant
Solubility (pH)	mg/mL	3.2(1.0)[65], 2.7(1.2)[66], 0.1(7.0)*	0.2[63],0.0978*,3.2(1.0)[65],2.7(1.2)[66]	Solubility
Specific intestinal permeability	cm/s	$2.71*10^{-4}$	Optimized, $2.81*10^{-5}$ [24]	Normalized to surface area
Partition coefficients		Poulin and Theil [24,62]	Poulin and Theil [24,62]	Organ-plasma partition coefficients
Cellular permeabilities		PK-Sim standard	-	Permeation across cell membranes
CYP3A4 K_m	µmol/L	15 [24]	15[24],11[24], 16±10[67], 11±3[67], 235[8], 834.7±182.2 [63]	Michaelis-Menten constant of CYP3A4 #
CYP3A4 k_{cat}	min^{-1}	2.12	Optimized, 0.31[24], 0.1[24], 32.2±28.4[63], 0.05±0.01[67], 0.10±0.01[67], 0.14[8]	CYP3A4 catalytic rate constant#
CYP2C19 K_m	µmol/L	3.5 [24]	3.5[24], 9.3±3.6[63], 14±6[67], 3.5[8]	Michaelis-Menten constant of CYP2C19#
CYP2C19 k_{cat}	min^{-1}	1.19 [24]	1.19[24], 40±13.9[63], 0.22±0.02[67], 0.39[8]	CYP2C19 catalytic rate constant#
GFR fraction		1	-	Fraction of filtered drug reaching the urine
CYP3A4 K_I	µmol/L	9.33	*in vitro* result from this study	Voriconazole inhibition constant on CYP3A4
CYP3A4 k_{inact}	min^{-1}	0.015	Optimized from *in vitro* results from this study (0.04)	Voriconazole inactivation rate constant on CYP3A4
$D_{T,50}$ for tablet	min	30	Optimized	Dissolution time (50% dissolved) for Weibull function
Shape factor for tablet		1.29	Optimized	Dissolution shape parameter for Weibull function

* drug bank; all three reported solubility values were used for interpolation; # values apply for global voriconazole metabolism via this enzyme irrespective of the metabolic pathway; Specific intestinal permeability $2.71*10^{-4}$ cm/s were optimized; CYP: cytochrome P450; CYP3A4 k_{cat} 2.12 min^{-1} were optimized; GFR: glomerular filtration rate; -: not available.

Figure legends

Figure 1 Metabolic pathway for voriconazole

*Indirect evidence from different CYP2C19 genotype groups [18].

Figure 2 Workflow of voriconazole PBPK model development and evaluation

The PK profiles used to select the distribution model were also utilized to optimize V_{max} and k_{inact} for CYP3A4. There were 21 PK datasets for model development and 72 for model evaluation in total. ADME: absorption, distribution, metabolism, elimination; PK: pharmacokinetics; MBI: mechanism-based inactivation; PMs: poor metabolizers; DDIs: drug-drug interactions.

Figure 3 Prediction performance of voriconazole PBPK model on aggregate plasma concentrations for multiple doses

Observed aggregate data reported in the literature are shown as dot, triangle, square, cross, or crossed square [6,36–38,47–60]. Population simulation medians are shown as lines; the shaded areas illustrate the 68% population prediction intervals. Details of dosing regimens, study populations, predicted versus observed pharmacokinetic parameters are summarized in **Table 1**. iv: intravenously, po: oral; D: day; QD: once daily, BID: twice daily, TID: three times daily; Plasma conc: voriconazole plasma concentration.

Figure 4 Prediction performance of voriconazole PBPK model on individual plasma concentration in different CYP2C19 genotype groups for a single dose

Observed individual data reported in the literature are shown as dots [18,24,62,63]. Population simulation medians are shown as lines; the shaded areas illustrate the 95% population prediction intervals. Details of dosing regimens, study populations, predicted versus observed PK parameters are summarized in **Table 2**. iv, intravenously, po: oral; Plasma conc: voriconazole plasma concentration; RM: rapid metabolizers, NM: normal metabolizers, IM: intermediate metabolizers, PM: poor metabolizers; Rengel: Rengelshausen.

Figure 5 Goodness of fit plot of the PBPK model of voriconazole

Predicted versus observed aggregate AUC (a), C_{max} (b) and C_{trough} (c) of the voriconazole from all clinical studies. The identity line and 0.5- to 2.0-fold acceptance limits are shown as solid and dashed lines, respectively. Different colors represent different clinical trials.

Figure 6 Effect of therapeutic multiple oral dosages of voriconazole on hepatic and small intestinal CYP3A activity

Predicted change of relative hepatic (green line) and small intestinal (red line) CYP3A activity over time after therapeutic multiple oral dosages of voriconazole. The blue line represents voriconazole plasma concentration. Arrows indicate dosing events of a standard therapeutic dosing schedule for oral voriconazole.

Figure 7 Prediction performance of voriconazole PBPK model in DDI with CYP3A4 probe substrates

The voriconazole model integrated with the models of CYP3A4 probe substrates predicted inhibitory effects of voriconazole on CYP3A4 *in vivo*. Population predictions of a) alfentanil or b, c) midazolam plasma concentration-time profiles, with and without voriconazole treatment were compared to observed data shown as

green triangles (control) or red dots (VCZ co-administration) or symbols ± SD [23,66]. Population simulation median are shown as green lines (control) or red lines (VCZ co-administration); the shaded areas illustrate the respective a) 68% and b, c) 95% population prediction intervals. iv: intravenously; po: oral. Details of dosing regimens, study populations, predicted and observed DDI AUC ratios and C_{max} ratios are summarized in **Table 3.**

Figure 8 Probability of target attainment for therapeutic and toxic trough concentrations in different CYP2C19 genotype groups for chronic dosing

Red and green lines represent the probability of therapeutic target attainment based on trough plasma concentration above 1 mg/L and above 2 mg/L, respectively. Blue and purple lines show probability of toxicity target attainment based on trough plasma concentration above 5 mg/L and above 6 mg/L, respectively. IM, intermediate metabolizers; NM, normal metabolizers; RM, rapid metabolizers