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Predicting Fluconazole Drug-Drug Interactions Using a Physiologically-based Pharmacokinetic (PBPK) Model

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Abstract

Aim: Physiologically Based Pharmacokinetic (PBPK) model is developed to (1) simulate clinical trials involving the Drug-Drug Interactions (DDI) between fluconazole and drugs (substrates) metabolized primarily by CYP2C9 and CYP3A, and to (2) support dosing recommendations.

Methods: The plasma-concentration profiles were simulated in virtual individuals for each drug alone and in combination with fluconazole in B²O simulator. The effect of fluconazole on substrates was compared with published clinical data, and dose adjustment was carried out.

Results: The magnitude of inhibition tended to be more pronounced for substrates with predominant CYP3A4 metabolism, for example, lemborexant, than those with dual CYP3A4/2C9 metabolism. The dose of flurbiprofen was adjusted from 50 mg to 25 mg and 20 mg respectively, to counteract the DDI effects caused by fluconazole 200 mg/day and 400 mg/day.

Conclusion: The PBPK model established based on the mechanism of DDI and inhibitor's effect on enzyme activity can reasonably simulate the effect of fluconazole on drug substrates mainly metabolized by CYP2C9, CYP3A or both.

Keywords: CYP2C9; CYP3A; Inhibitor; Fluconazole; Pharmacokinetic/pharmacodynamic model; Drug-drug interaction; Candidemia

Introduction

As a triazole antifungal, fluconazole is one of the most prescribed antifungal drugs for candidemia. Candidemia is one of the most common bloodstream infections in the United States, with rate of new infections approximately 9 per 100,000 people [1]. It is estimated to have 25,000 cases of candidemia nationwide each year [2] including adults aged ≥ 65 years. People have candidemia may also have diseases such as cancer, asthma. Others may be suffering from insomnia or pain. Therefore, it is common to use a combination of anticancer drugs, asthma drugs, sleep-improving drugs, or pain relievers while treating candidiasis.

Fluconazole is also an inhibitor to Cytochrome P450 (CYP) 3A and CYP2C9 isoenzymes. The CYP superfamily is the primary oxidative drug metabolism mediator. CYP2C9 constitutes 10%-20% of the CYP protein content in the human liver, and it has been reported to catalyse approximately 20% of the CYP mediated drug oxidation reactions [3-5].

For drugs that are primarily metabolised by CYP3A and CYP2C9, we need to consider Drug-Drug Interactions (DDIs), because when fluconazole is co-administered with these drugs, the exposure of these drugs in humans may change and dose adjustments may be required. To optimise dose design and avoid unnecessary clinical trials, we used the PBPK simulation method to predict the effect of fluconazole on other drugs.

PBPK modelling has become an accepted tool in drug development and clinical DDI studies. In the FDA's 2020 *In vitro* DDI guidance [6], various modelling approaches are mentioned to help translate *in vitro* observations into *in vivo* predictions of potential clinical DDIs, including PBPK models. PBPK models can predict the DDI potential of investigational drugs or metabolites as enzyme substrate or enzyme perpetrators.

In the current study, following co-administration with fluconazole, drug exposures were simulated and compared using a PBPK model. Drugs (substrates) under investigation included vismodegib (anticancer agent), lemborexant (treat insomnia), ospemifene (treat dyspareunia), zafirlukast (treat chronic asthma), flurbiprofen (pain reliever), rivaroxaban (prevent blood clots) and avatrombopag (pain reliever). The PBPK model was developed using B²O simulator software (Shanghai Yinghan Pharmaceutical Technology Co., Ltd) and the same simulator has already been used in bioequivalence studies [7]. Elimination of these drugs involved multiple pathways in which important metabolites are formed by the CYP enzymes 2C9 and 3A4 [8-10]. Lemborexant is primarily metabolised through the cytochrome CYP3A pathway [11], while flurbiprofen is metabolised by CYP2C9 [12]. Rivaroxaban is metabolised by cytochrome CYP 3A4/3A5, CYP2J2 and other CYP-independent mechanisms [13]. As a member of CYP3A superfamily, CYP3A4 is the most abundantly expressed form in the liver [14]. CYP3A5 accounted for only 2% of the overall CYP3A protein among all studies samples, and CYP3A5 did not contribute significantly to hepatic drug metabolism in Caucasians [15]. CYP3A7 is considered a fetal-specific P450 enzyme [16], and CYP3A43 is extremely low in liver expression and contributes little to drug metabolism [17,18].

This PBPK model was established based on the mechanism of action of DDI and the effect of the inhibitor on enzyme activity. Changes in inhibitor/substrate concentration over time were considered. Plasma drug-concentration profiles were simulated in virtual individuals for each drug administered alone or in combination with fluconazole. The effect of fluconazole on substrates was compared with published clinical DDI data, and dose adjustment was carried out.

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Materials and Methods

Data collection

Drug interaction studies with inhibitor fluconazole and CYP substrates were searched by using PubMed, with the keywords' fluconazole AND substrate AND human AND clinical study.

Interaction studies that met the following requirements were selected for further study.

1. The plasma concentration-time curves for substrate alone and with fluconazole are available.

2. Pharmacokinetic parameters such as Area Under the Plasma Concentration Curve (AUC) and maximum Concentration in serum (C_{max}) are available.

3. Research published in the last 20 years.

4. The inhibitor fluconazole was pre-treated, and substrate was administered when fluconazole concentration reached a steady state.

PBPK model

The model included the substrate and inhibitor's gastrointestinal, liver absorption, and systemic elimination in the central and tissue compartments. When the drug elimination was single-phase elimination, a one-compartment model was used. A PBPK model describing the substrate and inhibitor concentration-time profile is shown in Figure 1.

The drug interaction model was adapted from the equations developed by Kato in 2005 and 2008 [19,20]. In this model, the DDI effect is specifically related to the local concentration of the perpetrators in the liver, which takes into account its nonspecific binding to liver tissue. When the substrate is co-administered with an inhibitor, the clearance rate of the substrate is:

$$CL_{\text{int},h,inhibition} = \frac{CL_{\text{int},h}}{1+\frac{I_h \bullet f_{u,i}}{K_{pliver,i} \bullet K_i}}$$

K_{pliver} is the ratio of liver to plasma concentration; K_i is the inhibition constant, which affects liver drug metabolism. $CL_{int,\ h}$ is the hepatic clearance rate and f_u is the unbound fraction of the drug in the liver.

Parameters of simulated drugs

The input parameters were acquired from *in vitro* and *in vivo* data for the following compound: fluconazole (DDI perpetrator) and seven substrates. Drug absorption, distribution, and elimination parameters, such as K_a, V_1, K_{12}, K_{21}, τ, were derived from their *in vivo* PK data. Referring to the physiological parameters and coefficient of variation of healthy people, the parameters were obtained from literature search [21,22]. The F_a and F_g referred to fractional absorption and intestinal availability, mainly derived from oral bioavailability. F_g was assumed to be 1 in the absence of data. In the B^2O simulator, the inter-subject variability was set to the default value of 30% and all the parameters were assumed to be normally distributed.

Design of simulated Drug-Drug Interaction (DDI) studies

Based on B^2O Caucasian population database, the virtual healthy Caucasian subjects were generated with age of 18-60 years, body weight of 50-100 kg, and a proportion of females of 0.5. The trial design used the same dose as the clinical trial study, and the dosing conditions were consistent. Fifty virtual individuals received different substrates and steady-state fluconazole 200 mg or 400 mg per day. Additional simulations were performed to determine dose adjustment of flurbiprofen to overcome the DDI of fluconazole. The criterion for dose adjustment is to obtain a drug exposure value as close as possible to that of the drug administered alone to provide an actual dose adjustment.

PBPK modelling software

The PBPK model was implemented using the software B^2O simulator, a web-based platform, to predict drug exposures. With lower and upper CI% (Confidence Interval) limits 2.5%-97.5%, the geometric mean of all C_{max} and AUC was calculated, and ratios between geometric means with and without inhibitor were calculated and compared to the reported geometric mean ratios (if any) in clinical studies. ≥ 1.5-fold change is considered significant. These data were further dissected to overall metabolic Clearance (CL_m) to investigate the effect of fluconazole on substrates.

Results

Parameters used in the PBPK model

The parameters were firstly used to simulate single drug plasma concentration to evaluate the performance of the model. Parameters were adjusted to best fit the single drug model (Table 1). When the adjustments finished, inhibitor and substrates parameters were input into B^2O simulator to simulate the DDI effect on the substrates. The contribution of each CYP isoform to the total hepatic metabolism of a substrate (f_m) was evaluated by *in vitro* inhibition studies using specific CYP inhibitors or antibodies. If there is no information on f_m, it is assumed to be 1. In Table 1, most f_m values can be obtained directly or inferred from literature, except ospemifene and rivaroxaban. The f_m of ospemifene was from the *in vitro* metabolic data and the relative abundance of each CYP enzyme in the liver [23,24]. Furthermore, that of rivaroxaban was calculated as the ratio of CYP3A4 contribution to total clearance and renal clearance [25]. K_{pliver} values were calculated by the method introduced by Poulin, et al. and Rodgers, et al. [26,27].

Validation of the PBPK Models

The PK parameters of each drug were simulated individually to evaluate the performance of the PBPK model. Parameters from observed clinical data and simulations, including area under the plasma concentration-time curve (AUC_t), maximum concentration (C_{max}) and the time to maximum concentration in serum (T_{max}) are collected in Table 2. The simulated plasma concentrations of the seven substrates were in good accordance with clinical data (Table 2 and Figure 2), such as avatrombopag. The observed value of AUC_t in clinical trials was 3.177h*μg/ml, and the predicted value (mean) was 3.417 h*μg/ml. The observed values of C_{max} in clinical trials was 0.0941 μg/ml, and the predicted value (mean) was 0.0946 μg/ml [28-39].

Simulations of DDIs between fluconazole and cytochrome P450 substrates

The simulated DDIs between fluconazole and cytochrome P450 substrates are presented as AUCR and $C_{max}R$ in Table 3. AUCR is the combination/single drug AUC_t ratio, and $C_{max}R$ is the combination/single C_{max} ratio. The dose used in each simulation were the same as in the relevant clinical studies. In clinical studies, substrates were administered when fluconazole concentrations reached a steady state. The model predicted the effects of fluconazole on substrates within a 1.5-fold difference of the observed corresponding pharmacokinetic parameters (Figure 3), demonstrating that the model reasonably predicted drug exposure.

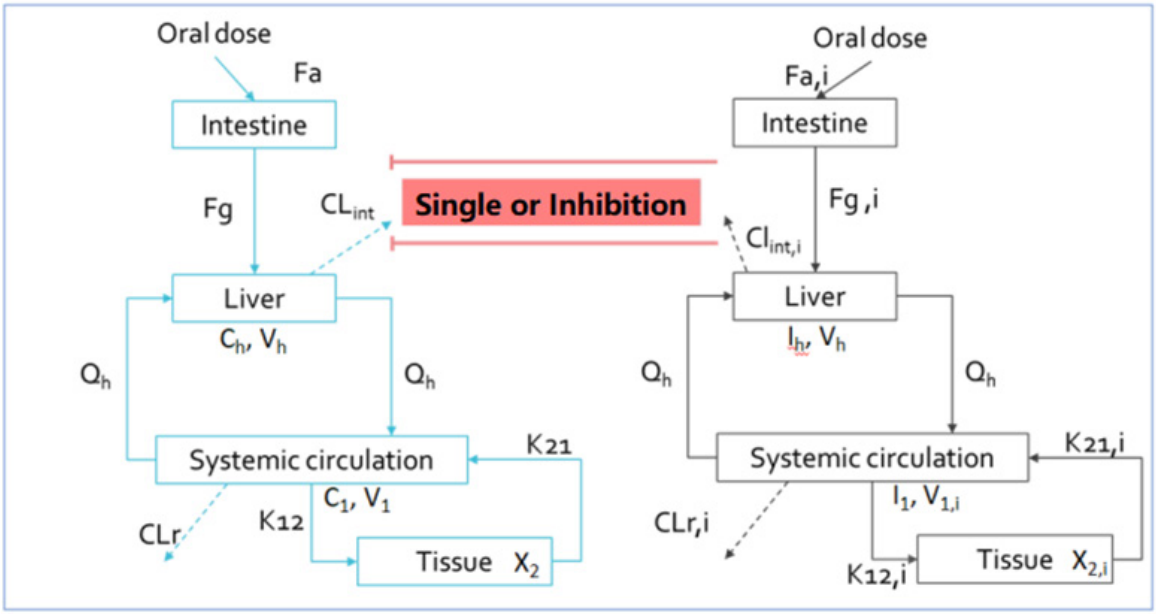

Figure 1: PBPK model describing the concentration-time curve of the substrate and inhibitor. C1 and I1 concentration in the systemic circulation, Ch and Ih concentration in the liver, CLr and CLr,i renal clearance. CLint and CLint,i the internal clearance rate of the liver, V1 and V1,i volume of the systemic circulation, Vh volume of the liver, K12 and K12,i transfer rate constant from the systemic circulation to the tissue compartment, K21 and K21,i transfer rate constant from the tissue compartment to the systemic circulation, Fa the fraction absorbed, F_g the intestinal availability, Qh the hepatic blood flow rate. X2, X2,i the content of the substrate in the tissue compartment. Subscript -i when a substrate is co-administered with an inhibitor.

Drug	Fluconazole	emborexant	Avatrombopag	Vismodegib	Rivaroxaban	Ospemifene	Zafirlukast	Flurbiprofen
Category	Inhibitor	Substrate	Substrate	Substrate	Substrate	Substrate	Substrate	Substrate
K_{pliver}	0.889	5.422	5.259	3.656	1.041	5.122	1.171	1.172
F_a	0.95	0.87	0.675	0.318	0.9	0.3	0.2	1
F_g	0.948	1	1	1	1	1	1	1
K_a(1/h)	2.727	1.369	0.366	2.529	0.449	0.52	0.481	0.925
f_u	0.89	0.113 [28]	0.037 [29]	0.01 [30]	0.065 [31]	0.01 [30]	0.01 [30]	0.01 [30]
BP	1	0.636 [28]	0.66 [29]	0.745 [32]	0.71 [31]	0.564 [33]	0.55	0.55 [34]
V_1 (L)	21.8	75.3	122.4	13.9	37.6	7.78	2.242	3.25
CL_r (L/h)	0.475	0.145	0.181	0.039	2.91	0.002	0.0935	0.0306
$CL_{int,h}$ (L/h)	0.134	181.6	89.6	0.501	86.1	325.1	184.5	112.8
K_{12} (1/h)	0	0.313	0	0	0.0884	0.808	0.669	0
K_{21} (1/h)	0	0.0568	0	0	0.253	0.0292	0.0113	0
Main metabolic CYP enzyme inhibited by fluconazole	/	CYP3A	CYP2C9 CYP3A	CYP2C9 CYP3A	CYP3A4	CYP2C9 CYP3A	CYP2C9 CYP3A	CYP2C9
f_m	/	1 [30]	1 [30]	1 [30]	0.28 [25]	0.83 [23]	1[35]	1
τ	0	0	1	0.45	0.75	0.5	0.45	0.25 [30]
Ki(mg/L)	2.91	2.91	2.14 [36]	2.14 [36]	2.91	2.14 [36]	2.14 [36]	2.14 [36]

Parameter description: K_{pliver}: liver tissue partition coefficient to plasma; F_a: absorption fraction; F_g: intestinal availability;K_a : the absorption rate; F_u: plasma unbound drug fraction; BP: whole Blood Plasma fraction;V_1: central compartment volume; CL_r: renal Clearance; CL_{int}: inherent Liver Clearance; K_{12}/K_{21} : absorption and elimination rate of the tissue compartment in the two-compartment model; f_m: the contribution of each CYP isoform to the metabolism of a substrate; τ: delay time; K_i: Inhibition constant of the corresponding enzyme that fluconazole inhibits (if there are more than one enzyme, the smallest value is chosen).

Table 1: Prediction of PK parameters for use in the PBPK model.

Drug (Dose mg/day)	AUC_t (h*μg/ml)	C_{max} (μg/ml)	T_{max} (h)	References
Fluconazole (150 mg/day) [a]				
Simulated	149.2	4.21	1.9	[37]
Observed (n=25)	151.7	4.38	1.5	
Lemborexan (10 mg/day)				
Simulated	0.324	0.04598	1.3	[11]
Observed (n=14)	0.2816	0.04663	1	
Avatrombopag (20 mg/day)				
Simulated	3.417	0.0946	8.7	[10]
Observed (n=16)	3.177	0.0941	7.0[b]	
Vismodegib (150 mg/day)				
Simulated	173	8.419	2.3	[38]
Observed (n=22)	176.9	8.175	2.0[b]	
Rivaroxaban (20 mg/day)				
Simulated	1.615	0.1746	3.7	[13]
Observed (n=13)	1.541[c]	0.1866[c]	4.0[b]	
Ospemifene (60 mg/day)				
Simulated	4.482	0.466	2.3	[9]
Observed (n=12)	3.871	0.4923	3	
Zafirlukast (20 mg/day)				
Simulated	1.78	0.3058	3.3	[39]
Observed (n=12)	1.535	0.3492	3.0[b]	
Flurbiprofen (50 mg/day)				
Simulated	30.46	5.387	1.9	[12]
Observed d (n=11)	30.50[b]	6.015[b]	1.5[b]	

Note: [a] Fluconazole formulation: Diflucan®, Pfizer, S.A. de C. V., Mexico City, Mexico
[b] Only median (min, max were available from the tables in the papers)
[c] Subjects having genotype CYP2C9*1/*1
All observed data were obtained from the figures as an arithmetic mean
Abbreviation: AUC_t: Area Under the plasma Concentration-time Curve over a dosing interval; C_{max}: Maximum Concentration in serum; T_{max}: Time of Maximum Concentration in serum

Table 2: Validation of PBPK models: simulated versus observed clinical data of each drug.

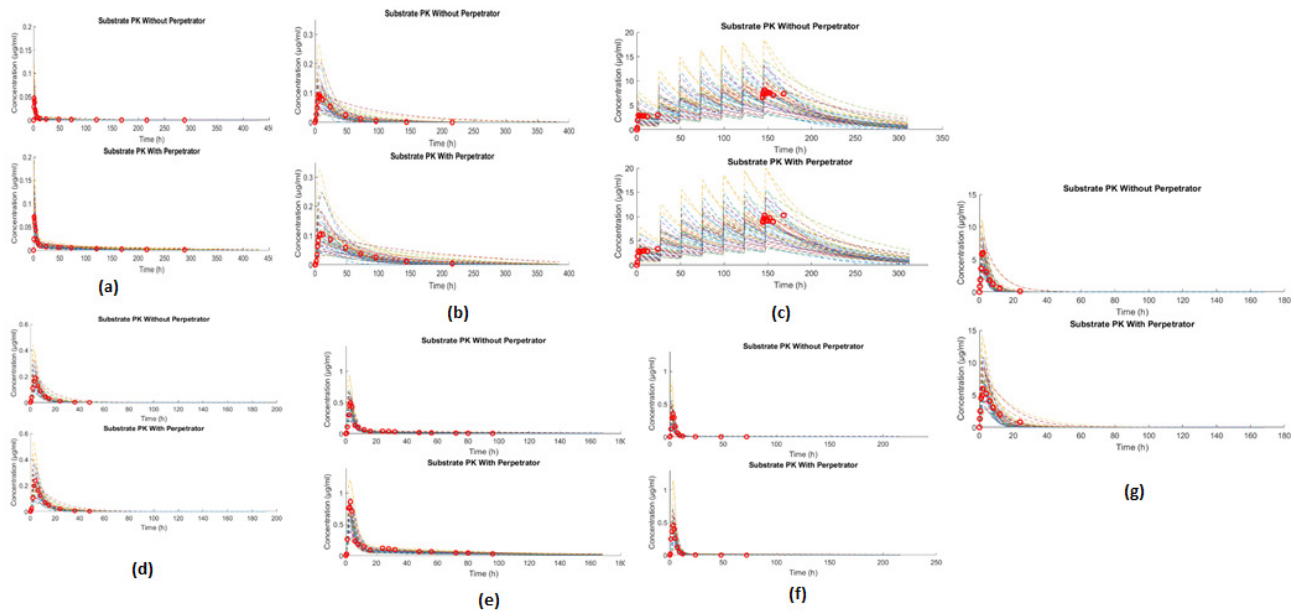

Figure 2: Simulated plasma concentration of (a) Lemborexant, (b) avatrombopag, (c) vismodegib, (d) rivaroxaban, (e) ospemifene, (f) zafirlukast, (g) flurbiprofen (dashed lines, n=50) with observed data (red dots) in the clinical study; perpetrator: fluconazole.

Parameter	AUC_R (CI%)	$C_{max}R$ (CI%)	References
Lemborexan (10 mg/day)+fluconazole (200 mg/day)			
Simulated	4.027(2.307-7.164)	1.486 (1.141-2.096)	Landry 2021[11]
Observed	4.439	1.538	
Avatrombopag (20 mg/day)+fluconazole (400 mg/day)			
Simulated	2.271 (1.814-2.675)	1.176 (1.054-1.384)	Nomoto 2018[10]
Observed	2.143	1.102	
Vismodegib (150 mg/day)+fluconazole (400 mg/day)			
Simulated	1.096 (1.023-1.195)[a]	1.080 (1.023-1.147)[a]	Malhi 2016 [38]
Observed	1.289[a]	1.257[a]	
rivaroxaban (20 mg/day)+fluconazole (400 mg/day)			
Simulated	1.208 (1.124-1.262)	1.089 (1.031-1.159)	Mueck 2013 [13]
Observed	1.405	1.267	
Ospemifene (60 mg/day)+fluconazole (200 mg/day)			
Simulated	2.231 (1.744-3.046)	1.308 (1.171-1.521)	Lehtinen 2013 [9]
Observed	2.504	1.756	
Zafirlukast (20 mg/day)+fluconazole (200 mg/day)			
Simulated	1.105 (1.379-2.269)	1.321 (1.105-1.678)	Karonen 2012 [39]
Observed	1.53	1.287	
Flurbiprofen (50 mg/day)+fluconazole (200 mg/day)			
Simulated	2.087 (1.656-2.592)	1.270 (1.105-1.537)	Kumar 2008 [12]
Observed	1.992	0.9887	
Flurbiprofen (50 mg/day)+fluconazole (400 mg/day)			
Simulated	2.424 (1.974-2.857)	1.319 (1.123-1.602)	Kumar 2008 [12]
Observed	2.658	1.021	

Note: AUC_R is the ratio of the AUC of fluconazole with substrate to the AUC of the substrate alone; $C_{max}R$ is the ratio of the exposure C_{max} of fluconazole with substrate to the C_{max} of the substrate alone.
[a] PK on day seven after multi-dose administration.
[b] Subjects having genotype CYP2C9*1/*1

Table 3: Simulated versus observed values of drug-drug interactions between fluconazole and substrates.

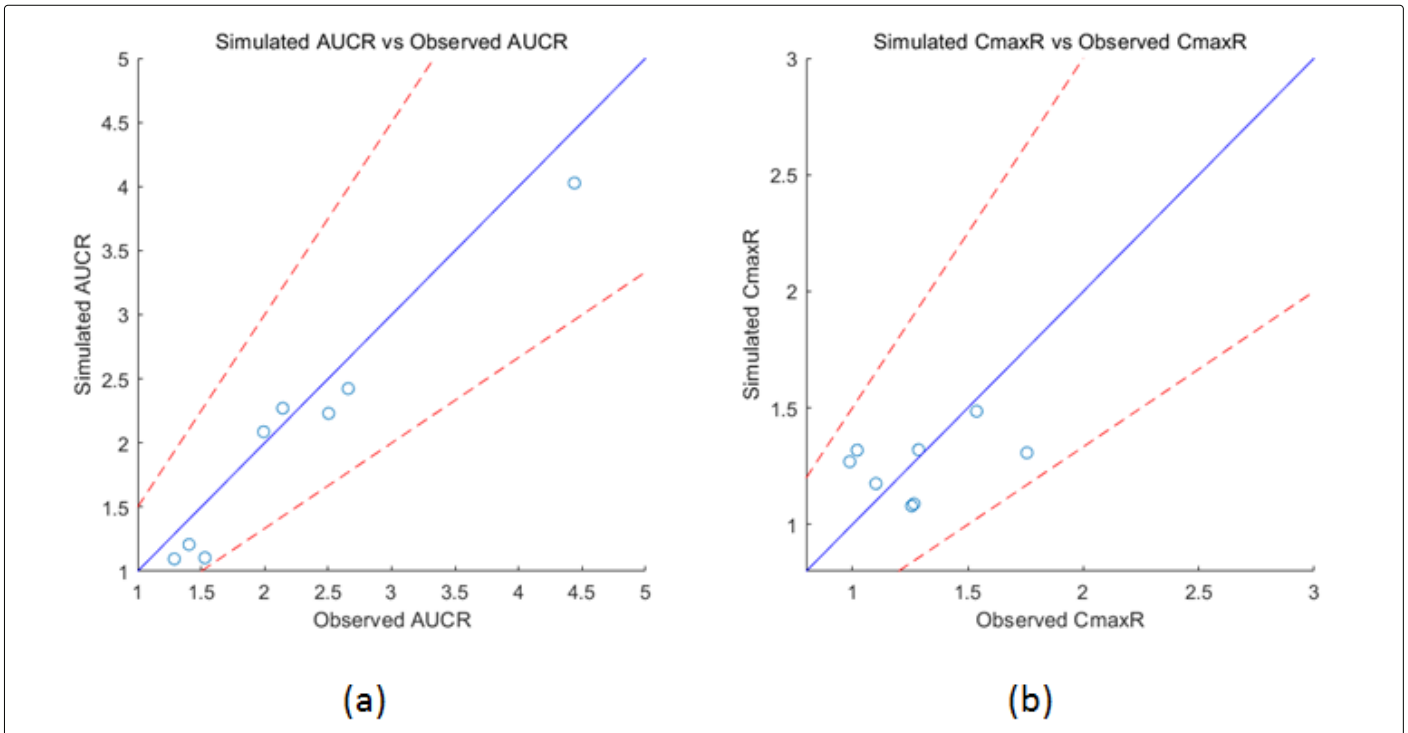

Figure 3: Simulated (N=50) versus observed (blue dots) of AUC_R (a) and $C_{max}R$ (b) for all substrates studied in the study. The red dashed lines represent the 1.5-fold difference from the observed pharmacokinetic parameters. The slope of the blue line is 1.

Lemborexant, mainly metabolised by CYP3A and CYP2B6 : Co-administration of lemborexant with fluconazole resulted in an approximately 1.5-fold increase in lemborexant C_{max} and an approximately 4-fold increase in lemborexant AUC_t (Table 3). Fourteen healthy subjects were randomly dosed and completed the study (six males and eight females, mean ± SD age were 37.4 ± 10.4 years) [11]. Since fluconazole inhibited CYP3A4 approximately 15-fold more than CYP2B6, CYP3A played a more critical role in the metabolic clearance of lemborexant [40].

Avatrombopag, mainly metabolised by CYP2C9 and CYP3A : Co-administration of avatrombopag and fluconazole increased the C_{max} and AUC_t of avatrombopag by 1.2-fold and 2.3-fold, respectively (Table 3). In the clinical study, sixteen subjects were randomly assigned and completed study. There were fourteen male subjects and two females, with an average age of 38.4 years. There were eight (50.0%) Black or African American subjects, six (37.5%) White, one (6.3%) Asian and one (6.3%) multiracial subject [10]. Both CYP2C9 and CYP3A contributed to the metabolic clearance of avatrombopag.

Vismodegib, mainly metabolised by CYP2C9 and CYP3A : Co-administration of fluconazole with vismodegib increased the C_{max} and AUC_t of vismodegib by 1.08-fold and 1.10-fold, respectively (Table 3). Eligible subjects for clinical studies were non-fertile females aged 18- 70 years and in good health [38]. Both CYP2C9 and CYP3A contributed to the metabolic clearance of vismodegib.

Rivaroxaban, mainly metabolised by CYP3A4 and CYP2J2 : Co-administration with fluconazole significantly increased the primary pharmacokinetic parameters of rivaroxaban. Mean AUC_t and C_{max} increased by approximately 44% and 18%, respectively, compared with rivaroxaban alone (Table 3). In the clinical study, thirteen healthy male subjects between 18-55 years were randomised. CYP3A4/3A5 accounted for approximately 18% of total rivaroxaban elimination and CYP2J2 approximately 14% [13].

Ospemifene, mainly metabolised by CYP2C9 and CYP3A : The concentrations of ospemifene increased after fluconazole pre-treatment. The ratio of the geometric means for AUC_t for ospemifene after fluconazole compared with ospemifene alone was 2.23. After fluconazole administration, C_{max} was 31% higher than ospemifene alone (Table 3). In the clinical study, all subjects were white, with a mean age of 62.5 years [9]. Both CYP2C9 and CYP3A contributed to the metabolic clearance of ospemifene.

Zafirlukast, mainly metabolised by CYP2C9 and CYP3A4 : Co-administration of fluconazole increased zafirlukast plasma concentrations. During the fluconazole phase, the geometric mean ratio of zafirlukast AUT_t was 1.11, and its C_{max} was 1.32. Twelve healthy volunteers with genotypes CYP2C9 and CYP2C19 (eight men, four women; age range 20-27 years) were randomised [39]. Both CYP2C9 and CYP3A contributed to the metabolic clearance of zafirlukast.

Flurbiprofen, mainly metabolised by CYP2C9 : The ratio of the AUC_t of co-administration to the AUC_t of the flurbiprofen alone (200 mg and 400 mg) in subjects CYP2C9*1/*1 is described in Table 3. Co-administration of flurbiprofen and fluconazole (200 mg) increased AUC_t of flurbiprofen by 2.09-fold, and co-administration with fluconazole (400 mg) increased AUC_t by 2.42-fold. CYP2C9 played a significant role in the metabolic clearance of flurbiprofen.

Dosage adjustments to overcome the interaction with fluconazole

Dose adjustment simulations showed that a flurbiprofen dose of 25 mg per day resulted in a mean AUC_t of 31.25 (h*μg/ml; observed flurbiprofen AUC_t when administered alone at 50 mg daily: 30.11 h*μg/ml), which was sufficient to overcome the effect of fluconazole (200 mg) on flurbiprofen exposure (Table 4). Also, reducing the dose to 20 mg per day resulted in a mean AUC_t of 29.07 h*μg/ml (observed AUC_t for flurbiprofen at 50 mg per day: 30.06 h*μg/ml), which was sufficient to overcome the effect of fluconazole (400 mg) on flurbiprofen exposure (Table 4).

Administration (dose after adjustment)	AUC_t (h*μg/ml)	AUC_R (% CI)
Flurbiprofen (25 mg)+fluconazole 200 mg	31.25	1.038 (0.9022-1.137)
Flurbiprofen (20 mg)+fluconazole 400 mg	29.07	0.967 (0.8468-1.063)
Note: AUC_R is the ratio of the AUC_t after dose adjustment of co-administration of flurbiprofen and fluconazole to the AUC_t of flurbiprofen alone.		

Table 4: Comparison of AUC and AUC_R of the substrate alone before dosage adjustment and co-administration of fluconazole after dosage adjustment.

Discussion

Fluconazole is a prototypical CYP2C9 inhibitor with almost complete bioavailability, and less than 10% of the dose being metabolised [12]. Gastrointestinal symptoms associated with fluconazole use are rare, and when they do, they are usually considered mild [41]. Fluconazole is also water-soluble and exists as polymorphic forms [42]. Because CYP3A4 is expressed in the intestine as well as in the liver and some CYP3A4 substrates are metabolized by intestinal first-pass metabolism, it is better to select drug candidates that exhibit no intestinal first-pass metabolism during the drug discovery and development processes [20]. In the B^2O simulator, drug interaction was assumed to take place both in the hepatic and intestinal metabolism, and the unknown parameters were K_i and F_aF_g [20]. When the intrinsic hepatic clearance of substrates exceeded 100 ml/min*kg, the estimated value of F_aF_g increased significantly by more than 1.3 times and further adjustment of F_aF_g will be required. In this study, the CL_{int} for fluconazole was 0.118 L/h and the F_aF_g was 0.901, no more adjustment for F_aF_g was made [20]. When the logP of the inhibitor was more than 1, there was a relatively significant difference between the *in vivo* and *in vitro* K_i values [20]. Since the logP of water-soluble fluconazole is less than 1, the K_i value was no longer adjusted.

The PBPK model was used to quantitatively predict the DDI effects between fluconazole, a moderate CYP3A and CYP2C9 inhibitor, and seven substrates metabolised primarily by CYP3A or CYP2C9 or of both. The simulated results approved that the model reasonably predicted the effects of fluconazole on drug substrates. The same PBPK model was also validated with other inhibitors such as gemfibrozil (inhibiting CYP2C8), clopidogrel (inhibiting CYP2C8) and enoxacin (CYP1A2) (Data not published).

Predicting DDI interactions can be challenging due to the complex exposure-response relationship of each drug and the lack of a complete understanding of the underlying mechanisms. Avatrombopag is a substrate of CYP2C9 and CYP3A. Co-administration results of fluconazole or itraconazole suggested that CYP2C9 played a more important role than CYP3A in the metabolic clearance of avatrombopag [10]. Zafirlukast is also a substrate of CYP2C9 and CYP3A4 *in vitro*. Fluconazole, but not itraconazole, increased zafirlukast plasma concentrations, strongly suggesting that CYP2C9 but not CYP3A4 is involved in zafirlukast metabolism *in vivo* [39]. In the ospemifene study, 4-hydroxylation, a hydroxylated metabolite involved in the

metabolism of ospemifene, appeared to be catalysed by many CYPs, whereas 4'-hydroxylation appeared to be predominantly catalysed by CYP3A4. In conclusion, CYP3A4 was the most critical CYP isoform for ospemifene metabolism, and CYP2C9, CYP2C19 and other isoforms also contribute to ospemifene metabolism [9]. One of the substrates metabolised by CYP3A but not CYP2C9 is rivaroxaban. CYP3A4/3A5 accounted for approximately 18% of total rivaroxaban elimination and CYP2J2 approximately 14% [13]. As a result, co-administration resulted in a 1.6-fold increase in rivaroxaban exposure. Co-administration of lemborexant (substrate mainly metabolised by CPY3A) with fluconazole resulted in a 4-fold increase in lemborexant, and the total CL_m was decreased from 15.37 L/h (without fluconazole) to 0.4980 L/h (with fluconazole). The magnitude of inhibition appears to be more significant for substrates with major CYP3A4 metabolism such as lemborexant. Patients should avoid taking fluconazole with lemborexant.

CYP2C9 polymorphisms are responsible for 10% of human drug metabolism [43]. Although more than 100 single nucleotide polymorphisms (SNPs) have been identified, only homozygous CYP2C9*3/*3 is considered to have clinically significant low CYP2C9 activity [43]. In the clinical study reported by Kumar, et al. the apparent oral clearance, AUC_t, and half-life of flurbiprofen at baseline in subjects with CYP2C9*1/*1 or CYP2C9*1/*3 genotypes were significantly different with post-coadministration parameters. However, there were no statistically significant changes in the above parameters following fluconazole co-administration in CYP2C9*3/*3 subjects [12]. No statistically significant differences were observed in C_{max} and T_{max} for any of the three genotype groups after the same treatment [12]. Since homozygotes for CYP2C9*3/*3 constitute only 0.5% of the population, in clinical study, the sample size of subjects with the CYP2C9*3/*3 genotype was small (N=2), it is difficult to conclude that parameters such as AUC_R or $C_{max}R$ are significantly affected depending on genotypes. In cases where dose adjustment is required following co-administration with CYP2C9 inhibitor, subjects with genotype CYP2C9*3/*3 may require different dose suggestion due to low CYP2C9 activity. Furthermore, subjects of different ages, such as the elderly and children, may require different simulations due to the physiological changes associated with ageing. Because most of the substrates are metabolised mainly by CYP3A4 and CYP2C9, the simulator didn't consider transporter-medicated drug interactions. The nonspecific or tissue binding for $CL_{int,h}$, K_{pliver} and K_i were not considered either.

Conclusion

The PBPK model established based on the compartment model can reasonably simulate the effect of fluconazole on drugs mainly metabolised by CYP2C9 or CYP3A or both. This model has the potential to be used to predict the effects of inhibitors on drugs with CYP mechanisms. Based on the simulated AUCR, the dose of flurbiprofen was adjusted from 50 mg to 25 mg and 20 mg respectively, to counteract the DDI effect caused by fluconazole 200 mg/day and 400 mg/day.

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Author Contributions

Conceptualization, Bo Liu; Data curation, Jingxi Li, Xue Li; Formal analysis, Keheng Wu; Investigation, Xue Li; Methodology, Keheng Wu; Project administration, Bo Liu; Software, Keheng Wu and Youni Zhao; Validation, Keheng Wu; Writing – original draft, Xue Li; Writing–review and editing, Xue Li, Zhou Zhou and Ranran Jia.

Declaration of Conflicting Interests

X.L., K.W., Z.Z., R.J., Y. Z. were employees of Yinghan Pharmaceutical Technology (Shanghai) at the time of study conduct.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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