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A pharmacokinetic/pharmacodynamic mathematical model accurately describes the activity of voriconazole against *Candida* spp. in vitro

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Abstract

We developed a pharmacokinetic/pharmacodynamic (PK/PD) mathematical model that fits voriconazole time–kill data against *Candida* isolates in vitro and used the model to simulate the expected kill curves for typical intravenous and oral dosing regimens. A series of $E_{\max}$ mathematical models were used to fit time–kill data for two isolates each of *Candida albicans*, *Candida glabrata* and *Candida parapsilosis*. PK parameters extracted from human data sets were used in the model to simulate kill curves for each isolate. Time–kill data were best fit by using an adapted sigmoidal $E_{\max}$ model that corrected for delays in candidal growth and the onset of voriconazole activity, saturation of the number of *Candida* and the steepness of the concentration–response curve. The rates of maximal killing by voriconazole ($k_{\max}$) were highly correlated with the growth rates (k_s) of the isolates (Pearson’s correlation coefficient = 0.9861). Simulations using PK parameters derived from the human data sets showed fungistatic effects against each of the isolates. In conclusion, we demonstrated that the activity of voriconazole against *Candida* isolates can be accurately described using a mathematical model. In the future, it might be possible to devise optimal dosing regimens of voriconazole using the model and PK data collected in vivo.

Keywords

Candida spp; Voriconazole; Time-kill assay; Mathematical modelling

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1. Introduction

Time–kill curves are attractive tools for studying the pharmacodynamics of antimicrobial agents as they provide detailed information about antimicrobial efficacy as a function of both time and concentration [1]. Although time–kill curves can be studied using animal models of infection, in vitro models offer significant advantages in cost, convenience and time, as well as permitting direct investigation of the antimicrobial–microbe interaction in a controlled and reproducible manner [2]. The results of specific time–kill experiments can be accurately described using pharmacokinetic/pharmacodynamic (PK/PD) mathematical models. By using PK data derived from clinical trials or animal experiments, mathematical models can be used to simulate the expected kill curves for different doses and dosing regimens of an antimicrobial agent [3]. As such, PK/PD modelling is a potentially powerful technique for defining optimal antimicrobial treatment strategies.

Voriconazole is a triazole antifungal agent effective in the treatment of patients with candidiasis [4,5]. We recently performed time–kill experiments against two strains each of *Candida albicans*, *Candida parapsilosis* and *Candida glabrata* using voriconazole concentrations of $0.25 \times$, $1 \times$, $4 \times$ and $16 \times$ the minimum inhibitory concentration (MIC) and measuring colony counts at 0, 2, 4, 8, 12, 24, 36, 48, 60 and 72 h [6]. We demonstrated that voriconazole exhibited dose–response effects against all isolates, as higher concentrations resulted in enhanced killing of *Candida* spp. The ranges of maximal growth inhibition of isolates at concentrations of $1 \times$ and $4 \times$ MIC were -0.61 to -2.78 log and -0.53 to -2.99 log, respectively, compared with controls. At $16 \times$ MIC, the range of maximal growth inhibition was -0.58 to -4.15 log. The objectives of the present study were to develop a PK/PD mathematical model to fit our time–kill data and to use the model to simulate the expected kill curves for typical intravenous and oral dosing regimens.

2. Materials and methods

2.1. Mathematical modelling of time–kill data

The methods and results of antifungal susceptibility testing and time–kill curve experiments for voriconazole against *Candida* isolates were described previously [6]. During time–kill experiments, aliquots were taken at each time point and examined under the microscope to exclude hyphal formation or clumping. The voriconazole MICs against the isolates ranged from $0.008 \mu\text{g/mL}$ to $0.19 \mu\text{g/mL}$. Fitting of time–kill data was performed with a series of models using the Scientist[®] 3.0 non-linear least squares regression software program (MicroMath, Salt Lake City, UT), as detailed in the Results and Discussion section. To determine the model that best fit the data for each isolate, graphs were visually inspected for goodness of fit and several criteria were considered, including model selection criterion (MSC), coefficient of determination (R^2) and the correlation between measured and calculated data points.

2.2. Simulations of expected time–kill curves using human PK data

PK parameters were extracted from published data sets of humans receiving voriconazole using XyExtract v2.5 (Wilton and Cleide Pereira da Silva, Campina Grande, Paraíba, Brazil) and the data were analysed by WinNonlin 5.2 (Pharsight Corporation, Mountain View, CA) to describe the best absorption model. The Scientist[®] 3.0 software program was then used to simulate plasma concentration–time profiles for multiple dosing of voriconazole ($\tau = 12$ h). Simulations of expected kill curves for each isolate were made using steady-state PK parameters in the mathematical model that was shown earlier to best fit the time–kill data.

3. Results and discussion

3.1. An adapted sigmoidal $E_{\max}$ model provides the best fit for voriconazole time–kill data against *Candida* isolates

Fitting of the time–kill data was started with a simple, commonly used $E_{\max}$ model [3]:

$$\frac{dN}{dt} = \left(k_s - \frac{k_{\max} \cdot C}{EC_{50} + C} \right) \cdot N \quad (\text{Model 1})$$

In this model, dN/dt is the change in number of *Candida* as a function of time, k_s (h^{-1}) is the candidal growth rate constant in the absence of voriconazole, $k_{\max}$ (h^{-1}) is the maximum killing rate constant (i.e. maximum effect), EC_{50} (mg/L) is the concentration of voriconazole necessary to produce 50% of maximum effect, C (mg/L) is the concentration of voriconazole at any time (t) and N (colony-forming units (CFU)/mL) is the number of viable *Candida*. Since voriconazole concentrations did not change during the time–kill experiments, C was constant for the entire fitted time period.

The $E_{\max}$ model does not account for the fact that isolates have not yet reached the logarithmic growth phase at time zero or for the delay in the onset of killing by voriconazole. To compensate for these delays, an exponential correction factor ($1 - \exp^{-\alpha t}$) was incorporated into the preceding model. Since the delays in the onset of candidal growth and voriconazole killing were not necessarily equivalent, they were assigned different adaptation rate constants (i.e. delay in growth = $(1 - \exp^{-\alpha t})$ and delay in killing = $(1 - \exp^{-\beta t})$):

$$\frac{dN}{dt} = \left[k_s \cdot (1 - e^{-\alpha t}) - \left(\frac{k_{\max} \cdot C}{EC_{50} + C} \right) \cdot (1 - e^{-\beta t}) \right] \cdot N \quad (\text{Model 2})$$

Model 2 was adapted by considering the maximum number of *Candida* ($N_{\max}$), which potentially corrected for the limitations of space and nutrients that are inherent in vitro systems:

$$\frac{dN}{dt} = \left[k_s \cdot \left(1 - \frac{N}{N_{\max}} \right) \cdot (1 - e^{-\alpha t}) - \left(\frac{k_{\max} \cdot C}{EC_{50} + C} \right) \cdot (1 - e^{-\beta t}) \right] \cdot N \quad (\text{Model 3})$$

In the final model, a Hill factor (h) was incorporated, which modified the steepness of the slopes and smoothed the curves:

$$\frac{dN}{dt} = \left[k_s \cdot \left(1 - \frac{N}{N_{\max}} \right) \cdot (1 - e^{-\alpha t}) - \left(\frac{k_{\max} \cdot C^h}{EC_{50}^h + C^h} \right) \cdot (1 - e^{-\beta t}) \right] \cdot N \quad (\text{Model 4})$$

The fitted time–kill curves of the six *Candida* isolates were generated using the different models in the absence of voriconazole (control) or in the presence of constant concentrations (mg/L) of voriconazole. In Model 4, Hill factor values ranging from 0 to 4 were tested. In general, the curve fittings for all PD data were acceptable. The best fits for each isolate were obtained using Model 4; the PD parameters and goodness of fit criteria were excellent for all voriconazole–*Candida* isolate pairings, with $MSC \geq 2.46$ and $R^2 \geq 0.95$. The fitted curves for Model 4 are shown in Fig. 1a. The PD parameters of voriconazole against the six *Candida* isolates and the goodness of fit criteria are presented in Table 1.

In time–kill experiments, PD effects can be described by at least three parameters: k_s , the growth rate constant of the organism in the absence of drug; $k_{\max}$, the maximum killing rate constant of the drug against the organism; and EC_{50} (the concentration of drug necessary for 50% of its maximum effect [1,3]. The EC_{50} values calculated in the present study demonstrate that voriconazole was highly effective at easily achievable serum levels against each of the six *Candida* isolates (EC_{50} range 0.002–0.05 mg/L) (Table 1). Moreover, the rates of maximal killing by voriconazole were highly correlated with the growth rates of the isolates (Pearson's

correlation coefficient = 0.9861). In other words, the data suggest that voriconazole achieved its highest kill rates against the most rapidly proliferating organisms.

3.2. Human PK data for voriconazole can be used to simulate expected time–kill curves

Mathematical models can be used to simulate the expected kill curves for different doses and dosing regimens of antimicrobials by substituting the concentration term with PK parameters collected in vivo and accounting for protein binding [3]. To simulate time–kill curves that might be expected for voriconazole under typical dosing regimens, we first extracted PK parameters from published data sets of humans receiving the drug intravenously (3 mg/kg twice a day (bid)) and orally (200 mg bid) in steady state [7]. Plasma concentration profiles for intravenous and oral dosing were found to be best characterised by two-compartment models of zero- and first-order absorption, respectively. Some of the PK parameters are listed in Table 2.

Applying the PK parameters for the two dosing regimens, we simulated plasma concentration–time profiles for multiple dosing of voriconazole ($\tau = 12$ h), assuming plasma protein binding of 58% (Fig. 1b) [5]. We then used the steady-state PK parameters to generate simulations for voriconazole activity against each of the *Candida* isolates in the present study (Fig. 1b). As shown, voriconazole showed fungistatic activity against all six strains. For five of the six strains, simulated kills were virtually identical for the intravenous and oral regimens. The exception was *C. parapsilosis* 2, for which the effects of intravenous dosing were greater.

By collecting PK data for a range of voriconazole doses and dosing regimens during clinical trials or animal models, we should be able to use the mathematical model to predict treatment strategies that might be most effective against a given *Candida* isolate. Although PD studies comparing the efficacy of various voriconazole regimens against *Candida* isolates are feasible in animal models [8], they are laborious, expensive and complicated. In vitro models permit direct a study of the interaction between fungi and fungal agents in a controlled and reproducible manner and allow direct comparisons of different agents and dosing strategies in a more convenient and faster way [2].

In conclusion, we have shown that it is feasible to describe precisely the time–kill activity of voriconazole against *Candida* isolates by using an adapted sigmoidal $E_{\max}$ model that corrects for delays in candidal growth and the onset of voriconazole activity, saturation of the number of *Candida* that can grow in the in vitro system and the steepness of the concentration–response curve. We established the PK/PD models and simulated the expected voriconazole activity for typical intravenous and oral dosing regimens by using PK data collected in vivo and accounting for protein binding. A limitation of the present study design was that voriconazole concentrations were constant. In the future, we will characterise voriconazole–*Candida* interactions using a dynamic in vitro system in which drug concentrations change over time in a manner consistent with the PK profile in humans. Based on the success of the present study, it should also be feasible to model accurately the time–kill data for changing voriconazole concentrations. We anticipate that this approach will be an efficient method for devising optimal voriconazole treatment strategies against candidiasis.

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Fig. 1.

(a) Fitted time-kill curves derived using the mathematical model for constant concentrations of voriconazole (mean $\pm$ standard deviation; $n = 3$ for *Candida albicans* ATCC 90029 and $n = 2$ for the other strains, without significant differences in results by analysis of variance (ANOVA)). Voriconazole concentrations were $0 \times$, $0.25 \times$, $1 \times$, $4 \times$ and $16 \times$ the minimum inhibitory concentrations (listed as N1–N5, respectively, in the individual figure legends). (b) Simulations of candidal time-kills (upper curves) and plasma voriconazole concentration-time profiles (lower curves). The simulations of time-kills are shown for short-term intravenous (i.v.) infusion (····) and oral dosing of voriconazole (---). Control growth is in the absence of drug (—). The simulated plasma concentration-time profiles for multiple dosings of

voriconazole ($\tau = 12$ h) are shown for short-term i.v. infusion ($\tau = 1$ h, infusion rate = 3 mg/kg/h, twice a day (bid)) and oral administration (200 mg, bid, $F = 0.9$). Free voriconazole concentrations were calculated assuming plasma protein binding level of 58%.

Table 1
Pharmacodynamic parameters and goodness of fit criteria against *Candida* isolates

Parameter	<i>C. albicans</i> ATCC 90029	<i>C. albicans</i> SC5314	<i>C. glabrata</i> 1	<i>C. glabrata</i> 2	<i>C. parapsilosis</i> 1	<i>C. parapsilosis</i> 2
C_{max} (mg/L) ^a	0.002/0.008/0.032/0.128	0.003/0.012/0.048/0.192	0.0475/0.19/0.76/3.04	0.008/0.032/0.128/0.512	0.002/0.008/0.032/0.128	0.004/0.016/0.064/
k_s (h ⁻¹)	0.28	0.87	0.24	0.26	0.22	0.66
k_{max} (h ⁻¹)	0.34	0.72	0.29	0.35	0.24	0.63
EC ₅₀ (mg/L)	0.0015	0.0030	0.05	0.0042	0.0066	0.012
α	1.24	0.19	0.14	0.58	0.26	0.072
β	0.069	0.11	0.032	0.059	0.23	0.19
N_{max} (CFU/mL)	1.09E+07	1.21E+06	3.85E+06	6.75E+06	3.48E+07	6.95E+06
h	2.13	2.77	1.16	2.32	1.49	3.84
MSC/ R^2	4.68/0.99	3.58/0.99	4.76/0.995	4.33/0.99	5.63/0.997	4.91/0.95

C_{max} , maximum voriconazole concentration; k_s , fungal growth rate constant in the absence of voriconazole; k_{max} , maximum killing rate constant (maximum effect); EC₅₀, concentration of voriconazole necessary to produce 50% of maximum effect; α , constant used to fit the initial lag phase for the growth; β , constant used to fit the initial lag phase for the inhibition or killing; N_{max} , maximum number of *Candida*; CFU, colony-forming units; h , Hill factor; MSC, model selection criteria.

^aValues using voriconazole concentrations of 0.25 ×, 1 ×, 4 × and 16 × the minimum inhibitory concentration, respectively.

Table 2
Steady-state pharmacokinetic parameters in plasma calculated by two-compartment model analysis [7]

Parameter	Regimen	
	200 mg oral	3 mg/kg/h i.v.
$C_{\max}$ (mg/L)	1.59	2.66
$T_{\max}$ (h)	1.72	1.00
α (h ⁻¹)	0.443	1.260
β (h ⁻¹)	0.139	0.120
F	0.9	1.0
CL/F (L/h)	17.9	14.2
Vd_{ss}/F (L)	98.9	109.7
Vd_{area}/F (L)	128.5	117.9

i.v., intravenous; $C_{\max}$, maximum voriconazole concentration; $T_{\max}$, time to $C_{\max}$; α , macro rate constant associated with the distribution phase; β , macro rate constant associated with the elimination phase; F , bioavailability; CL/F , systemic clearance; Vd_{ss}/F , apparent volume of distribution at steady-state; Vd_{area}/F , apparent volume of distribution.