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Voriconazole efficacy against *Candida glabrata* and *Candida krusei*: preclinical data using a validated *in vitro* pharmacokinetic/pharmacodynamic model

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Background: Voriconazole exhibits *in vitro* activity against *Candida glabrata* and *Candida krusei* (EUCAST/CLSI epidemiological cut-off values 1/0.25 and 1/0.5 mg/L, respectively). Yet, EUCAST found insufficient evidence to set breakpoints for these species. We explored voriconazole pharmacodynamics (PD) in an *in vitro* dynamic model simulating human pharmacokinetics (PK).

Methods: Four *C. glabrata* and three *C. krusei* isolates (voriconazole EUCAST and CLSI MICs of 0.03–2 mg/L) were tested in the PK/PD model simulating voriconazole exposures ($t_{1/2}$ ~6 h q12h dosing for 3 days). PK/PD breakpoints were determined calculating the PTA for exposure indices *f*AUC_{0-24}/MIC associated with half-maximal activity (EI_{50}) using Monte Carlo simulation analysis.

Results: Fungal load increased from 3.60±0.35 to 8.41±0.24 log_{10} cfu/mL in the drug-free control, with a maximum effect of ~1 log_{10} kill of *C. glabrata* and *C. krusei* isolates with MICs of 0.06 and 0.25 mg/L, respectively, at high drug exposures. The 72 h log_{10} cfu/mL change versus *f*AUC_{0-24}/MIC relationship followed a sigmoid curve for *C. glabrata* (R^2=0.85–0.87) and *C. krusei* (R^2=0.56–0.76) with EI_{50} of 49 (32–76) and 52 (33–78) *f*AUC/MIC for EUCAST and 55 (31–96) and 80 (42–152) *f*AUC/MIC for CLSI, respectively. The PTAs for *C. glabrata* and *C. krusei* isolates with EUCAST/CLSI MICs ≤0.125/≤0.06 mg/L were >95%. Isolates with EUCAST/CLSI MICs of 0.25–1/0.125–0.5 would require trough levels 1–4 mg/L; isolates with higher MICs would not attain the corresponding PK/PD targets without reaching toxicity.

Conclusions: The *in vitro* PK/PD breakpoints for *C. glabrata* and *C. krusei* for EUCAST (0.125 mg/L) and CLSI (0.06 mg/L) bisected the WT populations. Trough levels of >4 mg/L, which are not clinically feasible, are necessary for efficacy against WT isolates.

Introduction

The incidence of candidaemia has increased in recent years due to numerous factors, most important being the exposure to broad-spectrum antimicrobial agents, the use of aggressive therapies such as cancer chemotherapy, use of indwelling vascular catheters, neutropenia, mucosal colonization of *Candida* spp. and prior surgery.[1,2] Although *Candida albicans* is the predominant cause of invasive candidiasis, *Candida glabrata* is the second most common species in northern Europe and America, and *Candida krusei* is an important pathogen in cancer patients and patients with prior fluconazole exposure.[1,3]

C. glabrata and *C. krusei* isolates demonstrate reduced susceptibility or resistance to fluconazole. Echinocandins and amphotericin B are thus first- and second-line agents, respectively, against these pathogens.[3,4] These two classes of antifungals are available as IV formulations only and not suitable for outpatient therapy. Furthermore, echinocandin resistance rates up to 15% among clinical isolates of *C. glabrata* have been reported in some settings, with a subset demonstrating an MDR phenotype, limiting the therapeutic options even further.[5]

Voriconazole exhibits potent *in vitro* antifungal activity against both *C. glabrata* and *C. krusei* isolates, with CLSI epidemiological cut-off values (0.25 and 0.5 mg/L, respectively) being significantly

lower than those of fluconazole (8 and 32 mg/L, respectively)[6] and close to the susceptibility breakpoint for voriconazole against *C. albicans* (0.125 mg/L).[7] However, EUCAST susceptibility breakpoints have not been established due to a paucity of efficacy data,[8] and CLSI breakpoints have been proposed only for *C. krusei* (0.5 mg/L) although with limited clinical data.[9] Due to the small number of patients with *C. glabrata* and *C. krusei* infections treated with voriconazole, clinical efficacy–pharmacokinetic (PK)–MIC data that might help describe exposure–effect relationships and determine clinical breakpoints are not foreseen to be collected in the future.

In vitro PK/pharmacodynamic (PD) models can offer an alternative reliable approach for the description of the exposure–effect relationships, the determination of PK/PD susceptibility breakpoints using Monte Carlo analysis and the estimation of target values for therapeutic drug monitoring (TDM). This is especially attractive in the case of voriconazole, which has complex PK properties and for which TDM is recognized as an important component to ensure effective, but not toxic, therapy.[10] In the present study, a previously described *in vitro* PK/PD model[11] was used in order to simulate human voriconazole PK against *C. glabrata* and *C. krusei* isolates with different voriconazole susceptibilities and to determine PK/PD susceptibility breakpoints for EUCAST and CLSI methodologies. Subsequently, voriconazole AUC and trough levels in serum necessary for optimal treatment and minimal toxicity were determined in relation to MICs.

Materials and methods

Candida isolates

Four clinical *C. glabrata* isolates and three clinical *C. krusei* isolates with increasing voriconazole EUCAST[12] and CLSI[13] MICs ranging from 0.03 to 2 mg/L were studied. The median EUCAST and CLSI MICs were determined after 24 h of incubation in triplicate experiments. The isolates were stored in normal sterile saline with 10% glycerol at −70°C and revived by subculturing on Sabouraud dextrose agar plates supplemented with gentamicin and chloramphenicol (SDA; bioMérieux) to ensure purity and viability. Inoculum suspensions were prepared in normal sterile saline from 24 h cultures and adjusted to a final inoculum of 10^4 cfu/mL using a counting chamber. The number of cfu was confirmed by quantitative cultures on SDA plates.

Antifungal drugs and medium

Pure powder of voriconazole (Pfizer Inc., Athens, Greece) was dissolved in sterile DMSO (Carlo Erba Reactifs-SDS, Val de Reuil, France) and stock solutions of 10 mg/mL were stored at −70°C until use. The medium used in the *in vitro* PK/PD model was RPMI 1640 medium (with L-glutamine, without bicarbonate) buffered to pH 7.0 with 0.165 M MOPS and supplemented with 100 mg/L chloramphenicol (AppliChem GmbH, Darmstadt, Germany).

In vitro PK/PD model

A previously described two-compartment PK/PD diffusion/dialysis model simulating *in vivo* PK[11] was used. The model consists of an external compartment comprising a conical flask connected to a peristaltic pump (Minipuls Evolution®, Gilson Inc.) and an internal compartment comprising a 10 mL volume semi-permeable cellulose dialysis tube (mol. wt <20 kDa, Spectra/Por® Float-A-Lyzer® G2, Spectrum Laboratories Inc., Breda, The Netherlands) inoculated with a 10^4 cfu/mL conidial suspension. Repeated sampling of 100 μL was made from the internal compartment in order to ensure that drug concentrations in the internal compartment indeed mimic voriconazole drug concentration profiles in human plasma. Samples were stored at −70°C until tested. Replicate experiments were conducted in order to assess the reproducibility.

In vitro PK

In order to describe the exposure–effect relationship, different voriconazole concentration–time profiles were simulated in the *in vitro* PK/PD model, with fC_{max} of 7, 3.5, 1.75 and 0.8 mg/L and $t_{1/2}$ of ∼6 h.[14] Voriconazole levels were measured using a microbiological agar diffusion assay as previously described with a voriconazole-susceptible *Candida parapsilosis* isolate.[15] The lowest limit of detection was 0.25 mg/L and intraday/interday variation was <15%. A concentration–time curve was generated for each simulated dose and analysed by non-linear regression analysis using a one-compartment model described by the equation $C_t=C_0e^{-k/t}$, where C_t (dependent variable) is the concentration of drug at a given time (t) (independent variable), C_0 is the initial concentration of the drug at $t=0$ h, e is the physical constant 2.18 and k is the rate of drug removal. The $t_{1/2}$ was calculated using the equation $t_{1/2}=0.693/k$ and compared with the respective values observed in humans. Finally, the $f\text{AUC}_{0-24}$ was calculated for each simulated dosage by applying the trapezoidal rule.

In vitro PD

To estimate the fungal load inside the dialysis tubes (internal compartment) of each voriconazole dosing regimen, 100 μL samples were collected at regular intervals up to 72 h, 10-fold serially diluted in normal saline and subcultured on SDA plates. Plates were incubated at 30°C for 24 h and colonies were counted at each dilution. Dilutions that yielded 10–50 colonies were used in order to determine the $\log_{10}$ cfu/mL at each timepoint and to construct the time–kill curves. The lowest limit of detection was 10 cfu/mL.

PK/PD modelling

To determine the *in vitro* exposure–response relationship, the $\log_{10}$ cfu/mL at $t=0$ h was subtracted from the $\log_{10}$ cfu/mL at 72 h and plotted over the $f\text{AUC}_{0-24}$/MIC ratio for each simulated dose and isolate. The data were then analysed with non-linear regression analysis using the sigmoidal model with variable slope (E_{max} model) described by the equation $E=(E_{max}-E_{min})\times EI^n/(EI^n+EI_{50}{}^n)+E_{min}$, where E_{max} is the maximum increase in $\log_{10}$ cfu/mL of the drug-free control (kept constant at $\log_{10}$ cfu/mL in the drug-free control), E_{min} is the minimum $\log_{10}$ cfu/mL found at high drug exposures (kept constant at −1 $\log_{10}$ cfu/mL), EI is the exposure index $f\text{AUC}_{0-24}$/MIC, EI_{50} is the exposure index required to achieve 50% of $E_{max}-E_{min}$ and n is the slope of the dose–effect relationship (Hill coefficient). The goodness of fit of the E_{max} model was assessed by visual inspection of graphs, residuals analysis, post run's test and R^2. All data were analysed using the statistics software package GraphPad Prism, version 5.0, for Windows (GraphPad Software, San Diego, CA, USA).

Monte Carlo simulation

Monte Carlo simulation analysis was performed using the Normal random number generator function of EXCEL (MS Office 2007) for 5000 patients receiving the standard IV voriconazole dosage of 4 mg/kg IV or 300 mg orally twice daily, which corresponds to a total mean±SD steady-state $t\text{AUC}_{0-12}$ of 50.4±41.83 mg·h/L.[14] For the simulation analysis, the $f\text{AUC}_{0-24}$ used was calculated as $2\times f\text{AUC}_{0-12}$, where $f\text{AUC}_{0-12}$ was 21.4±17.57 mg·h/L based on the 42% unbound fraction of voriconazole in human serum.[16] The PTA for EI_{50} was estimated for isolates with MICs ranging from 0.008 to 4 mg/L and PK/PD susceptibility breakpoints were determined. Previously published MIC distribution data from CLSI[17] and EUCAST[8] were used. Since the uncertainty of EI_{50} is important for Monte Carlo simulations and PTA analysis,[18] the PTAs were determined for the mean and the upper and lower 95% CI limits of EI_{50} estimated with non-linear regression analysis.

Trough levels and MICs

The required trough levels in human serum necessary to attain the EI_{50}s were calculated for different MICs. For that reason, the previously described relationship between serum tAUC and trough concentrations (tC_{min}), namely $tAUC_{0-12}=7.011+12.687\times tC_{min}$,[19] was used taking into account the 58% protein binding of voriconazole in human serum.[16] The EUCAST and CLSI MICs for *C. glabrata* and *C. krusei* at which the corresponding PK/PD targets were attained were plotted against the $tAUC_{0-12}$ and tC_{min}.

Results

MICs

The MICs for the included strains determined by EUCAST and CLSI methodologies are shown in Table 1. Most MICs fell within one 2-fold dilution when comparing across the EUCAST and CLSI methods, with an absolute agreement of 64%.

PK

Figure 1 shows the steady-state plasma PK profiles based on human PK of twice-daily voriconazole dosages simulated in the *in vitro* PK/PD model. The mean±SD $t_{1/2}$ was 6.9±2.7 h, with fC_{max} of 6.49±0.98, 3.84±0.30, 1.76±0.60 and 0.86±0.10 mg/L, and AUC_{0-24} of 47.71±9.97, 28.12±5.82, 12.13±3.80 and 5.75±1.84 mg·h/L, respectively, for all species and strains.

Table 1. Median (range) of triplicate MICs (mg/L) for EUCAST and CLSI for *Candida* isolates used in the present study

Isolate no.	Reference code	EUCAST	CLSI
C. glabrata 3	SSI-W18236	0.06 (0.03–0.125)	0.03 (0.03)
C. glabrata 5	SSI-W17252	2 (1–4)	2 (2)
C. glabrata 9	SSI-W51696	0.5 (0.125–2)	0.5 (0.5)
C. glabrata 11	SSI-W42947	0.125 (0.125–0.25)	0.25 (0.125–0.25)
C. krusei 2	SSI-T5120	0.25 (0.125–0.25)	0.25 (0.25)
C. krusei 4	SSI-T3278	0.5 (0.5)	0.25 (0.25)
C. krusei 12	SSI-F49748	1 (0.125–2)	0.5 (0.125–0.5)

SSI, Statens Serum Institut.

PD

C. glabrata (Figure 2) and *C. krusei* (Figure 3) isolates in the drug-free control grew from 3.60±0.35 $\log_{10}$ cfu/mL at 0 h to 8.41±0.24 $\log_{10}$ cfu/mL at 72 h. Voriconazole decreased the fungal load in the tubes proportionally to the MIC for the isolates. The maximum effect of voriconazole corresponded to an ∼1 $\log_{10}$ cfu/mL reduction from the initial inoculum. This was found at high voriconazole exposures for the isolates with the lowest EUCAST MIC, which was 0.06 mg/L for *C. glabrata* and 0.25 mg/L for *C. krusei*.

The 72 h change in the $\log_{10}$ cfu/mL versus $fAUC_{0-24}$/MIC relationship for the *C. glabrata* and *C. krusei* isolates is displayed in Figure 4. The relationship followed a sigmoid curve for both species, but the relationship was much clearer for *C. glabrata* (R^2=0.85–0.87) than for *C. krusei* (R^2=0.56–0.76). For *C. glabrata*, curve fits and associated parameters of EUCAST- and CLSI-derived methods were comparable, with mean (95% CI) EI_{50}s of 49 (32–76) and 55 (31–96) $fAUC_{0-24}$/MIC, respectively. For *C. krusei* isolates, the difference in results according to susceptibility methods was more pronounced, with EI_{50} of 52 (33–78) for EUCAST and 80 (42–152) for CLSI. EI_{50}s determined from data at timepoints earlier than 72 h were different from those at 72 h, in particular for the 24 h timepoint (data not shown). This was mainly due to the drug-free control not reaching its maximum growth (Figures 2 and 3).

Monte carlo analysis

The simulated patients had a mean±SD $fAUC_{0-24}$ of 41.94±35.41 mg·h/L, very close to previously published voriconazole exposures.[14] The PTAs for 49 (32–76) and 52 (33–78) $fAUC_{0-24}$/

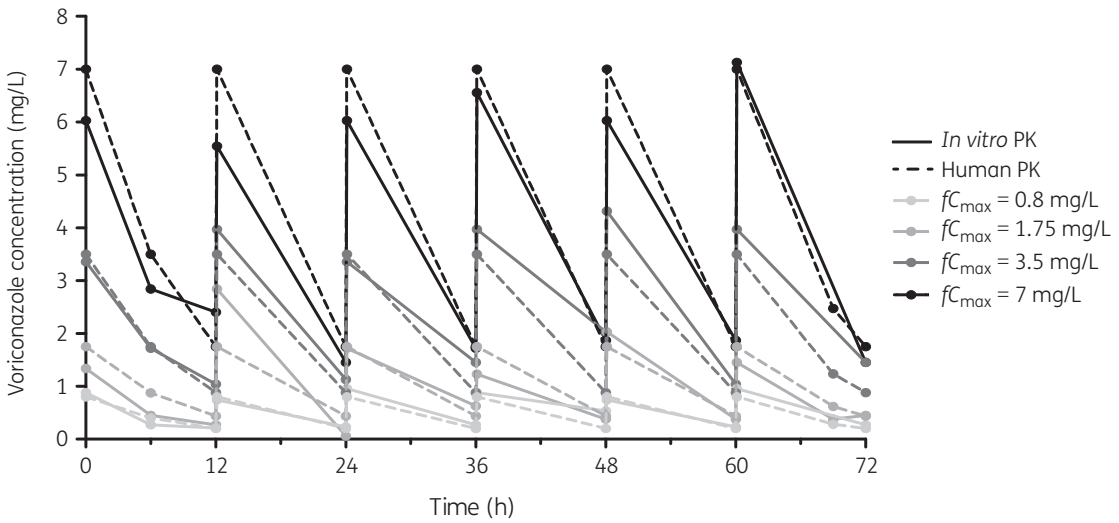

Figure 1. Representative concentration–time profiles of simulated q12h IV dosing regimens of voriconazole based on human PK in the *in vitro* PK/PD model with target fC_{max} of 0.8, 1.75, 3.5 and 7 mg/L and obtained C_{min} of 0.23, 0.67, 1.04 and 1.75 mg/L, respectively, and $t_{1/2}$ of ∼6 h. Data represent drug levels in the internal compartment of the *in vitro* model (solid lines) and the respective target values observed in human plasma (broken lines).

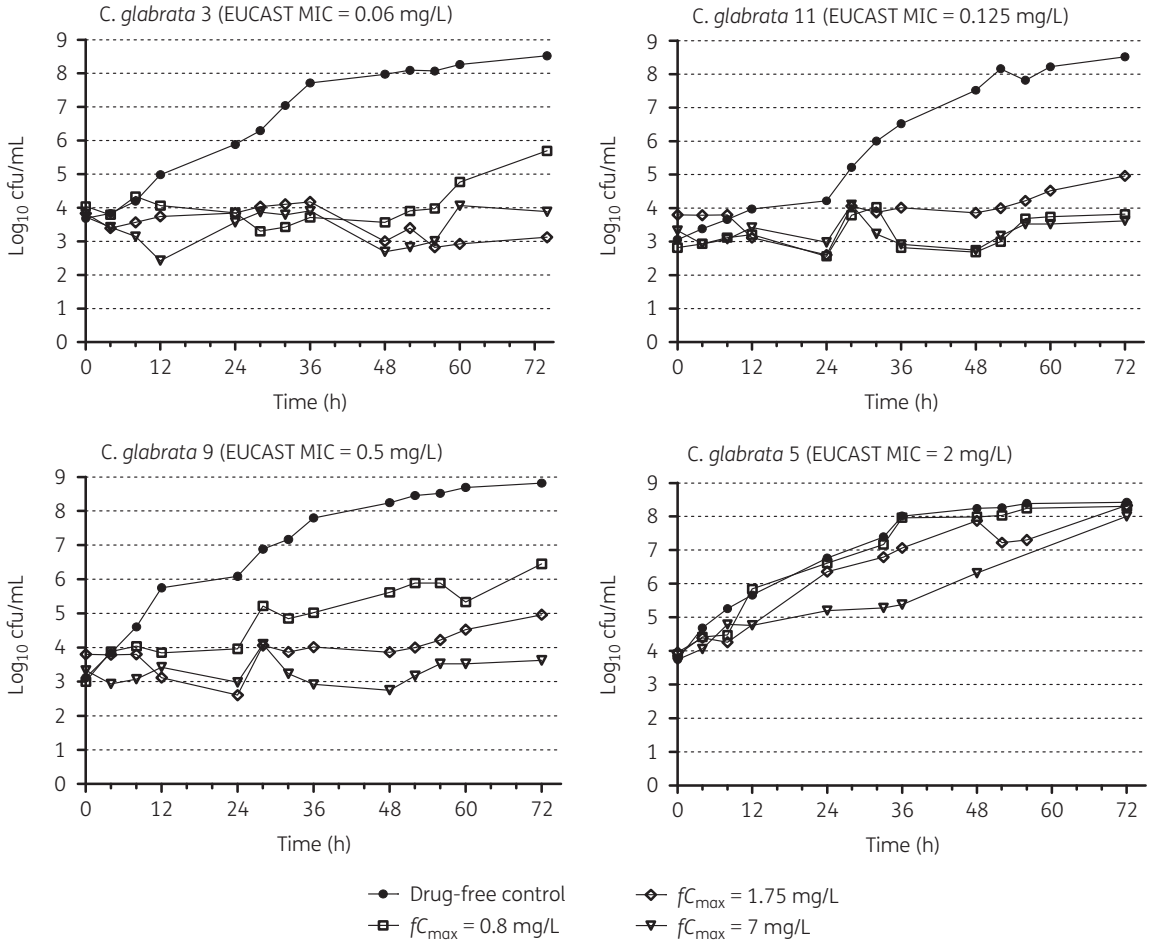

Figure 2. Time–kill curves in the *in vitro* PK/PD model simulating q12h IV dosing regimens of voriconazole against *C. glabrata* isolates with fC_{max} of 0.8, 1.75 and 7 mg/L, and $t_{1/2}$ of ∼6 h.

MIC for EUCAST and 55 (31–96) and 80 (42–152) $fAUC_{0-24}$/MIC for CLSI for *C. glabrata* and *C. krusei* isolates with increasing MICs are shown in Figure 5. The PTAs for the mean EI_{50} (solid black lines) were >95%, 10%–95% and <10% for *C. glabrata* and *C. krusei* isolates with EUCAST and CLSI MICs of ≤0.125, 0.25–1 and ≥2 mg/L, whereas the PTAs for the upper 95% CI limit of EI_{50} (lower broken black lines) were >95%, 10%–95% and <10% for *C. glabrata* and *C. krusei* isolates with EUCAST MICs of ≤0.125, 0.25–1 and ≥2 mg/L and CLSI MICs of ≤0.06, 0.125–0.5 and ≥1 mg/L, respectively (Figure 5).

Trough levels and MICs

The voriconazole trough levels in human serum required to attain the corresponding PK/PD targets for *C. glabrata* and *C. krusei* isolates with increasing EUCAST and CLSI MICs are shown in Figure 6. The corresponding PK/PD targets could be attained for *C. glabrata* and *C. krusei* isolates with EUCAST/CLSI MICs of 0.25–1/0.125–0.5 mg/L, with trough levels of 1–4 mg/L. In contrast, for isolates with higher MICs, trough levels of >5.5 mg/L, which are associated with increased risk of toxicity,[10] will be required.

Discussion

The present study showed that voriconazole PK/PD targets cannot be attained for the vast majority of clinical *C. glabrata* and *C. krusei* isolates with the standard doses. Based on the *in vitro* PK/PD targets determined in the present study for *C. glabrata* and *C. krusei*, high PTAs were found only for isolates with EUCAST and CLSI MICs of ≤0.125 and ≤0.06 mg/L, respectively. These PK/PD breakpoints are two to three 2-fold dilutions lower than the corresponding epidemiological cut-off values for EUCAST (1 mg/L for both species) and CLSI (0.25 mg/L for *C. glabrata* and 0.5 mg/L for *C. krusei*). This questions the efficacy of voriconazole for those infections.

Voriconazole produced a small killing effect against *C. glabrata* and *C. krusei* isolates (∼1 $\log_{10}$ reduction) at high drug exposures against the most susceptible isolates also in line with previous *in vitro* PK/PD studies for low-MIC isolates.[20–22] For *C. glabrata* there are no clinical breakpoints. The epidemiological cut-off values are 1 mg/L for EUCAST[8] and 0.25 mg/L for CLSI.[6] Based on the PK/PD target determined in the present study (∼50 $fAUC_{24}$/MIC), the corresponding PK/PD susceptibility breakpoint of ≤0.125 mg/L for EUCAST and ≤0.06 mg/L for CLSI methodologies would bisect the WT MIC distributions. The PD target could be attained for isolates

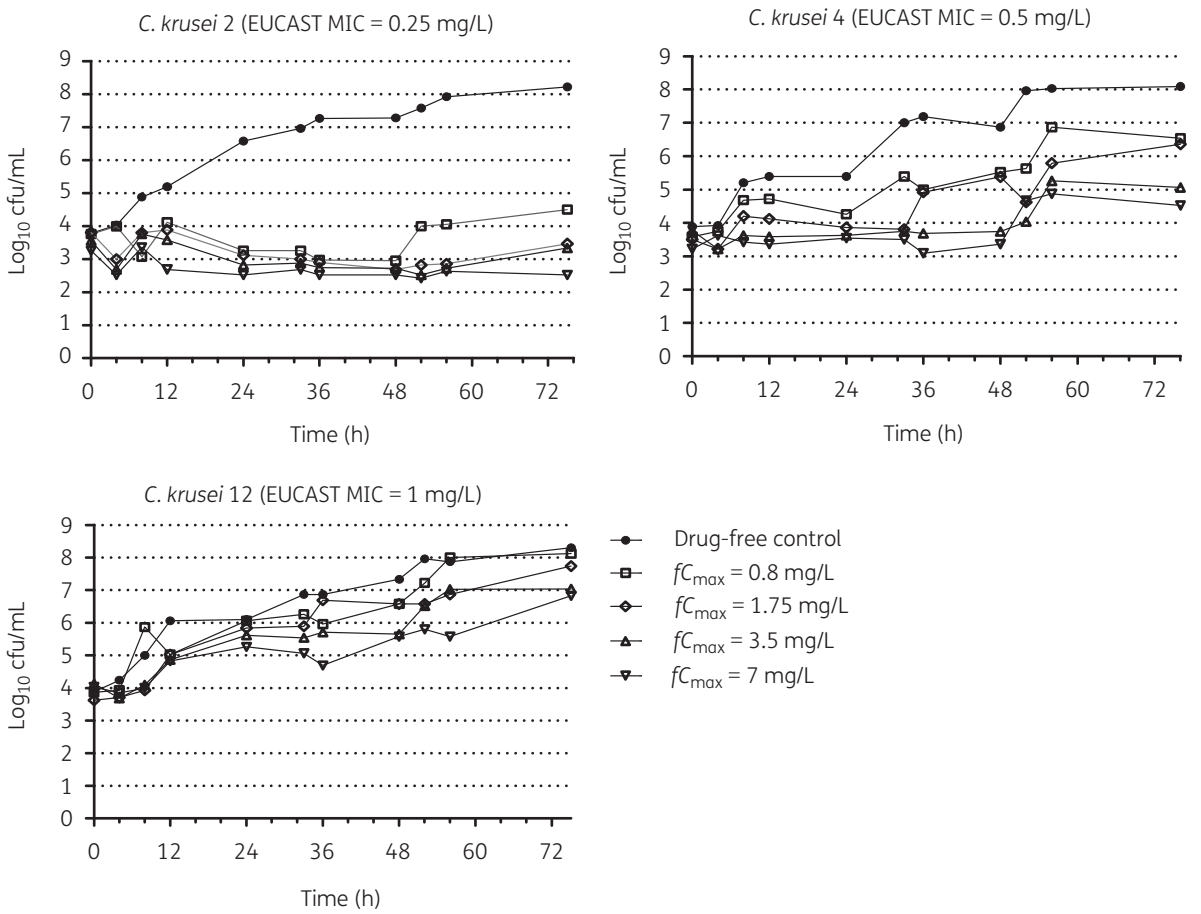

Figure 3. Time–kill curves in the *in vitro* PK/PD model simulating q12h IV dosing regimens of voriconazole against *C. krusei* isolates with fC_{max} of 0.8, 1.75, 3.5 and 7 mg/L, and $t_{1/2}$ of ∼6 h.

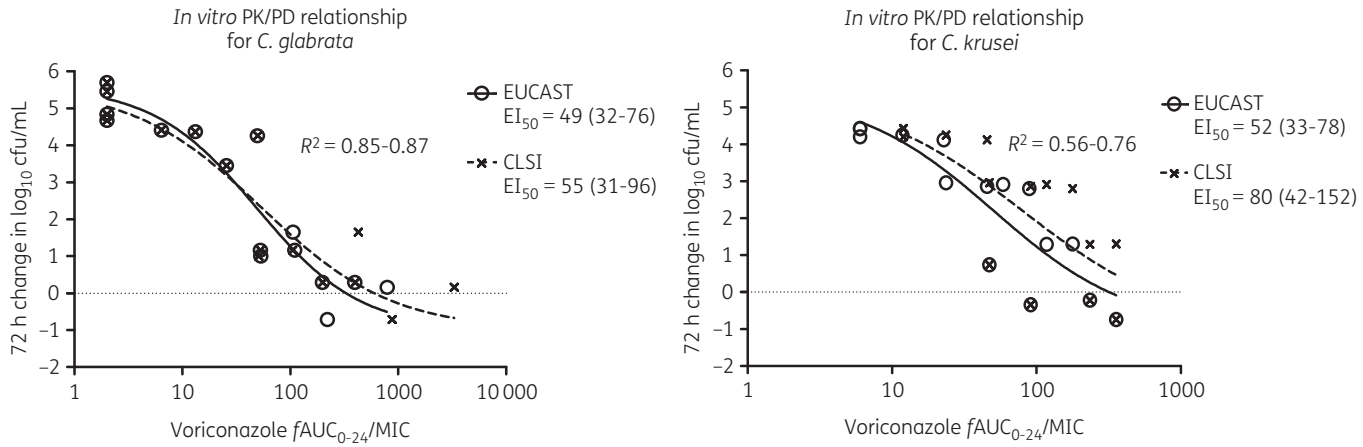

Figure 4. *In vitro* PK/PD relationship of voriconazole against *C. glabrata* and *C. krusei* isolates tested in the *in vitro* PK/PD model using EUCAST and CLSI MICs. The mean (95% CI) $fAUC_{0-24}$/MICs are shown for each species for EUCAST and CLSI.

with EUCAST/CLSI MICs of 0.25–1/0.125–0.5 mg/L, provided serum trough levels of at least 1–4 mg/L were ensured, thus necessitating TDM and dose adjustment in most cases. Previous animal studies showed that voriconazole at 40 mg/kg once daily was effective at reducing statistically significantly kidney fungal burden >1 $\log_{10}$ cfu/g (average ∼2 $\log_{10}$ cfu/g) compared with untreated controls after 7 days of treatment against *C. glabrata* isolates with CLSI MICs of ≤0.125 mg/L in grapefruit-fed neutropenic mice.[23]

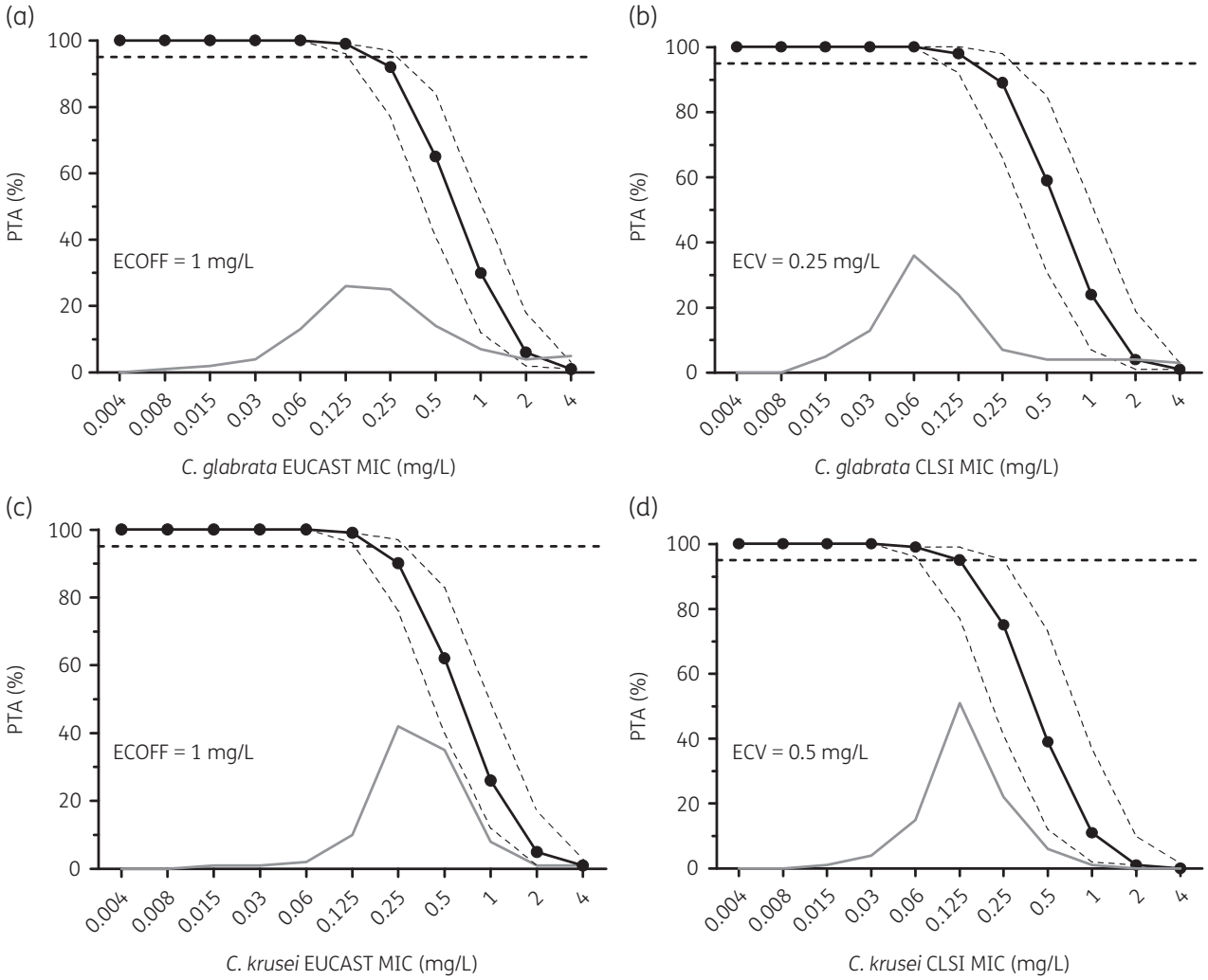

Figure 5. PTA for 5000 patients receiving standard voriconazole dosages of 4 mg/kg IV twice daily for which the AUCs were simulated with Monte Carlo analysis for different EUCAST and CLSI MICs. Broken lines around the PTA curve represent the 95% CI calculated using the 95% CI limit of EI_{50} obtained from non-linear regression analysis of exposure–effect relationships for each *Candida* species. The horizontal broken line represents 95% PTA. The epidemiological cut-off values for EUCAST (ECOFF) and CLSI (ECV) are shown for each species.

Voriconazole has been successfully used for *C. glabrata* infections,[24,25] whereas breakthrough *C. glabrata* isolates have been reported in patients with voriconazole trough levels ≤0.63 mg/L.[26]

For *C. krusei*, the PD targets of 52 $fAUC_{0-24}$/MIC for EUCAST and 80 $fAUC_{0-24}$/MIC for CLSI were determined, suggesting a PK/PD breakpoint of 0.125 and 0.06 mg/L, respectively, which are three 2-fold dilutions lower that the corresponding epidemiological cut-off values of 1 mg/L (EUCAST) and 0.5 mg/L (CLSI).[8,27] Currently, only CLSI has defined the susceptibility breakpoints of susceptible/intermediate/resistant (S/I/R) 0.5/1/2 mg/L although there are only a few cases (n=9) of invasive candidiasis caused by *C. krusei* available for analysis.[9] Of note, the response rate in the latter study was 78% for isolates with CLSI MICs of 0.125 mg/L (5/7) and 0.25 mg/L (2/2). However, the 0.125 mg/L breakpoint again bisects the WT distributions of EUCAST and CLSI methods. Voriconazole at 5 and 10 mg/kg twice daily was effective in reducing tissue fungal load by 1–2 $\log_{10}$ cfu/g in neutropenic guinea pigs infected with *C. krusei* isolates with an MIC of 0.5 mg/L.[28] Considering that 10 mg/kg results in a tAUCτ of 31 mg·h/L and a protein binding of 45%,[29] the PK/PD target in the former study was around 68 $fAUC_{24}$/MIC, which is close to the PK/PD target found in the present study. Voriconazole at 40 mg/kg once daily was effective in reducing tissue fungal load by 2 and 1 $\log_{10}$ cfu/g compared with untreated controls of *C. krusei* isolates with CLSI MICs of 0.125 and 0.25 mg/L, respectively, in grapefruit-fed neutropenic mice.[30] Voriconazole has been successfully used for *C. krusei* infections,[25,31] whereas breakthrough infection of *C. krusei* isolates has been reported in patients with trough levels of 0.53 mg/L,[26] which hardly covers most isolates with MICs >0.06 mg/L as shown in the present study.

Since *in vitro* data in artificial growth media simulating serum PK cannot fully compensate for the lack of clinical data, the clinical significance of the chosen EI_{50} endpoint, which corresponds to an ∼2 $\log_{10}$ cfu/mL increase from the initial inoculum for azoles and *Candida* species, is unknown. Usually, stasis or 1 log kill is used, although again with no solid support for the clinical significance

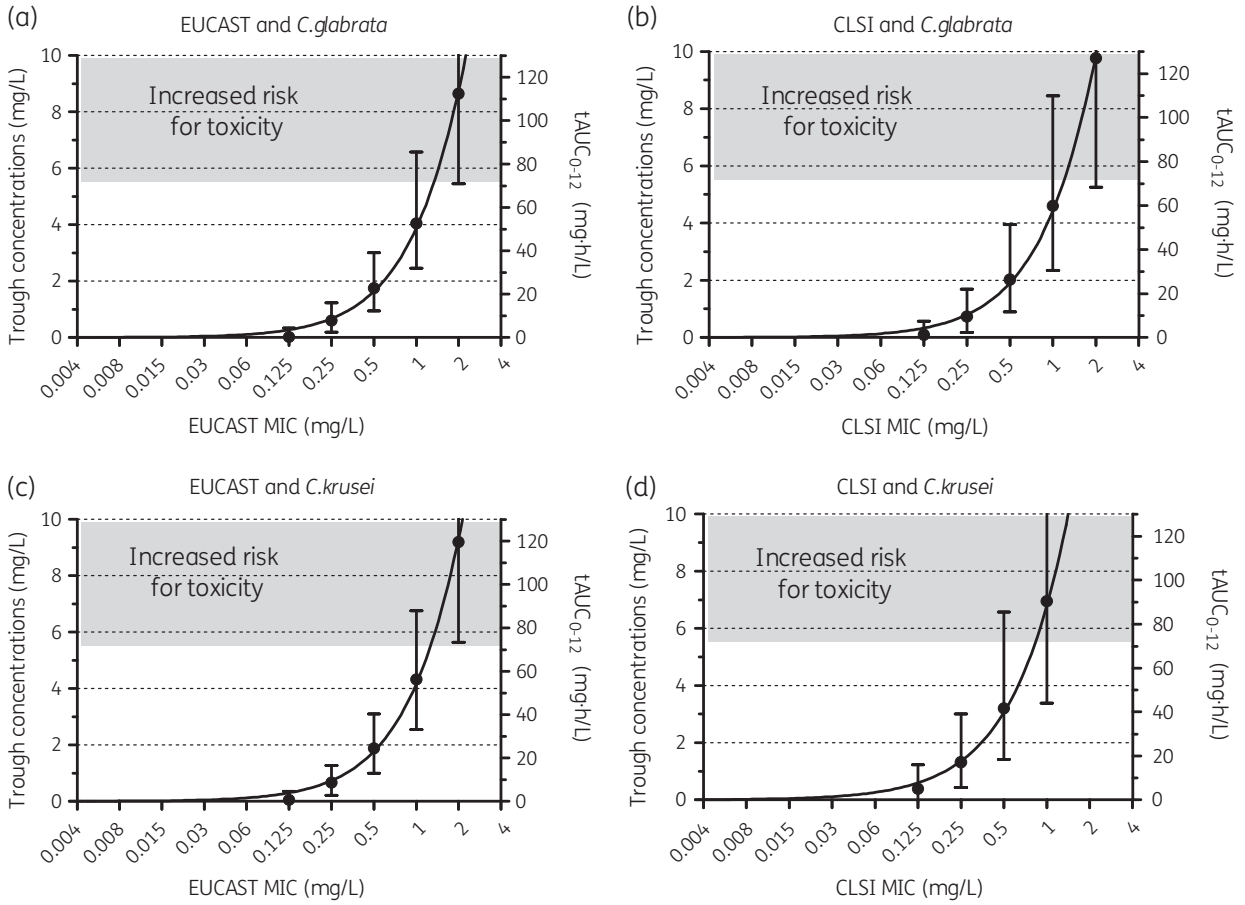

Figure 6. Correlation between voriconazole trough concentrations in human serum and EUCAST/CLSI MICs of *C. glabrata* and *C. krusei* in order to attain the corresponding PK/PD targets of 49 and 52 for EUCAST and 55 and 80 for CLSI, respectively. Error bars represent 95% CI.

of those effects. The 2 $\log_{10}$ cfu/mL increase from the initial inoculum is further supported by the clinical AUC/MIC PD target of 100 for fluconazole,[32,33] which corresponds to a 1–2 $\log_{10}$ cfu/kidney increase from the initial inoculum in neutropenic animals.[34] One explanation might be the absence of neutrophils both in *in vitro* and in neutropenic animal studies that usually exist in patients with invasive candidiasis, particularly in ICU patients. Indeed *in vivo* studies in neutropenic and non-neutropenic mice showed that median survival was prolonged and fungal load in kidney decreased by 1 $\log_{10}$ cfu, whereas fluconazole reduced the fungal load by a further 1 $\log_{10}$ cfu in non-neutropenic mice compared with neutropenic mice.[35] In addition, voriconazole increased phagocytosis of *Candida* conidia by monocytes/polymorphonuclear leucocytes.[36] Thus, the 2 $\log_{10}$ cfu/mL increase from the initial inoculum in preclinical neutropenic models for azoles may result in clinical stasis.

The PK/PD breakpoints determined for *C. glabrata* and *C. krusei* for EUCAST (0.125 mg/L) and CLSI (0.06 mg/L) are symmetrical to the WT MIC distributions of both methodologies since they are two to three 2-fold dilutions lower than EUCAST epidemiological cut-off values (1 mg/L for both species) and CLSI epidemiological cut-off values (0.25 mg/L for *C. glabrata* and 0.5 mg/L for *C. krusei*). Of note, weighted analysis of *C. glabrata* MIC WT distribution indicated a CLSI epidemiological cut-off value of 0.5 mg/L,[27] which increases symmetry of current PK/PD breakpoints for both species and methodologies. The PK/PD breakpoints cut exactly in the middle the WT *C. glabrata* MIC distribution and at the left side of the WT *C. krusei* MIC distribution for both EUCAST and CLSI methodologies. This implies that 47% of *C. glabrata* and 85% of *C. krusei* WT isolates with EUCAST MICs of 0.25–1 mg/L in previously published MIC distributions[8] will be above the PK/PD breakpoint and, similarly, 31% (35% based on 0.5 mg/L CLSI epidemiological cut-off value) of *C. glabrata* and 79% of *C. krusei* WT isolates with CLSI MICs of 0.125–0.25 and 0.125–0.5 mg/L, respectively, in previously published CLSI MIC distributions.[17] In addition, the modal MICs for EUCAST and CLSI MIC distributions differ by one 2-fold dilution (Figure 5), with the CLSI modal MIC being lower than the EUCAST modal MIC,[37,38] further supporting the one dilution lower PK/PD susceptibility breakpoint of CLSI compared with EUCAST. However, one should also take into account the lower virulence of *C. krusei* compared with *C. glabrata*[39] that may affect *in vivo* PD.

In conclusion, the present study suggests that voriconazole cannot be recommended for *C. glabrata* and *C. krusei* infections since the probability of attaining the PK/PD targets determined in the study is too low. The corresponding PK/PD targets for *C. glabrata* and *C. krusei* are 50–80 $fAUC_{24}$/MIC, resulting in a PK/PD susceptibility breakpoint of $\leq$0.125 mg/L for EUCAST and $\leq$0.06 mg/L for CLSI methodologies bisecting WT MIC distributions.

Isolates with EUCAST and CLSI MICs >1 and >0.5 mg/L, respectively, should be considered resistant as toxic levels would be required to attain the PK/PD targets. For *C. glabrata* and *C. krusei* WT isolates with intermediate EUCAST/CLSI MICs of 0.25–1/0.125–0.5 mg/L, TDM will be required to optimize drug exposure targeting trough levels of 1–4 mg/L. However, due to the inherent variation in the susceptibility testing methods, in practice all WT isolates would require TDM to ensure that PK/PD targets are attained targeting trough concentrations of >4 mg/L that will cover up to EUCAST and CLSI epidemiological cut-off values. This may be difficult in clinical practice because of a narrow therapeutic window (4–5.5 mg/L) and the large interindividual and interoccasion variability requiring real-time TDM and dose adjustment[40] when no other alternatives exist (e.g. echinocandin-resistant infection in patients with kidney injury) or when a patient should be discharged with an oral therapy. However, those levels would be even more difficult to attain with the oral formulation (300 mg q12h) in haematological patients because of the 65% oral bioavailability.[19] Since MIC clinical outcome data for *C. glabrata* and *C. krusei* species are difficult to collect, the findings of the present study provide a unique opportunity to propose PK/PD breakpoints and help define the role of voriconazole in the management of those infections.

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Transparency declarations

None to declare.

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