In vitro pharmacokinetic/pharmacodynamic modeling of voriconazole activity against *Aspergillus* species in a new in vitro dynamic model

**Running title:** In vitro PKPD of voriconazole against *Aspergillus*

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ABSTRACT

The pharmacodynamics (PD) of voriconazole activity against Aspergillus spp. were studied in a new in vitro dynamic model simulating voriconazole human pharmacokinetics (PK) and the PK-PD data were bridged with human drug exposure to assess % target (near maximum activity) attainment of different voriconazole dosages. Three Aspergillus clinical isolates (1 A. fumigatus, 1 A. flavus, 1 A. terreus) with CLSI MICs 0.5 mg/l were tested in an in vitro model simulating voriconazole PK in human plasma with Cmax 7, 3.5 and 1.75 mg/l and t1/2 6h. The area under the galactomannan index-time curve (AUCGa) was used as the PD parameter. In vitro PK-PD data were bridged with population human PK of voriconazole exposure and % target attainment was calculated. The in vitro PK-PD relationship fAUC0-24-AUCGa followed a sigmoid pattern (global R²=0.97) with near maximum activities (10% fungal growth) observed at fAUC0-24(95%CI) of 18.9 (14.4-23.1) mg.h/l against A. fumigatus, 26.6 (21.1-32.9) mg.h/l against A. flavus and 36.2 (27.8-45.7) mg.h/l against A. terreus (F2,19=17.22, p<0.0001). Target attainment for 3, 4, and 5 mg/kg voriconazole dosages was 24(11-45)%, 80(32-97)% and 93(86-97)% for A. fumigatus, 12(5-26)%, 63(17-93)%, and 86(73-94)%, for A. flavus and 4(2-11)%, 36(6-83)%, and 68(47-83)% for A. terreus. Based on the in vitro exposure-effect relationships, a standard dosage of voriconazole may be adequate for most patients with A. fumigatus but not A. flavus and A. terreus infections, for which higher drug exposure may be required. This could be achieved using a higher voriconazole dosage, thus highlighting the usefulness of therapeutic drug monitoring in patients receiving standard dosage.
INTRODUCTION

Invasive aspergillosis is the most serious infection caused by _Aspergillus_ species particularly in patients with hematological malignancies or bone marrow transplantation (16). These infections are characterized by high morbidity and mortality despite available antifungal therapy (7), among which voriconazole is the drug of choice (24). Several factors may influence clinical outcome related to host (underlying condition, immunosuppression), drug (timing of administration, suboptimal exposure) and pathogen (resistance, virulence). Although voriconazole resistant isolates have been described, their low prevalence (<10%) cannot explain the high mortality of these infections (10, 21). Testing large sets of _Aspergillus_ isolates, showed that more than half of them exhibited similar in vitro susceptibility to voriconazole with a minimal inhibitory concentration (MIC) of 0.5 mg/l (5, 17). Furthermore, the most frequently isolated species, _A. fumigatus_, _A. flavus_ and _A. terreus_ demonstrated similar in vitro susceptibility although in vivo experimental and clinical data show that the efficacy of voriconazole differs for these species (22-24, 26). This lack of association between voriconazole MICs and clinical outcome implies that a different approach is required to study comparative in vitro-in vivo voriconazole responses against _Aspergillus_ infections in order to optimize therapeutic regiments (2).

The MIC as a marker of the in vitro activity of antifungal agents against _Aspergillus_ spp. is an important pharmacodynamic parameter, but it does not account for the pharmacodynamic properties of antifungal drugs, such as kinetics of growth inhibition, sub-MIC effects, rate and extent of killing and post-antifungal effect. In addition, during MIC testing drug concentrations remain stable over time, when, in vivo, the fungus is exposed to non-constant drug concentrations due to absorption, distribution, metabolism and excretion processes. The effect of decreasing drug concentrations to _Aspergillus_ spp. was unknown until recently, when we developed an in vitro model simulating human pharmacokinetics of antifungal drugs including voriconazole (11). This novel pharmacokinetic/pharmacodynamic (PKPD) model demonstrated a differential activity of
simulated standard dosages of antifungals against *Aspergillus* isolates with identical MICs. With this model, the time- and concentration-dependent pharmacodynamic properties of antifungal drugs can be studied and PKPD analysis simulating human pharmacokinetics can be performed. We therefore studied the effect of simulated human pharmacokinetics of increasing voriconazole dosages against the most frequently encountered clinical isolates *A. fumigatus*, *A. flavus* and *A. terreus* isolates with voriconazole MICs of 0.5 mg/l. The magnitude of PKPD parameters associated with maximal activity was determined for each species. Finally, in vitro PKPD data were bridged with previously obtained human PK data in order to assess the efficacy of clinically administered voriconazole dosages against these life-threatening infections.
MATERIALS AND METHODS

Strains. Three clinical strains of *A. fumigatus*, *A. flavus* and *A. terreus* isolated from patients with invasive pulmonary aspergillosis were used. The CLSI MICs were 0.5 mg/L for voriconazole and confirmed by testing in triplicate in our laboratory (4). The strains were maintained at -70°C in 10% glycerol and were subcultured twice on Sabouraud Dextrose agar at 30°C for 5-7 days before testing. Conidia suspension was prepared in normal saline with 1% Tween 20 and the inoculum size was adjusted to 1x10^5 CFU/ml using a Newbauer chamber slide; concentration was quantified by spread plate cultures on Sabouraud Dextrose Agar.

Antifungal drug and nutrient medium. A clinical formulation of voriconazole (Vfend, Pfizer) was reconstituted according to manufacturer’s instructions at 10,000 mg/L and stored at -70°C. The nutrient medium used throughout contained 10.4 g/l RPMI1640 with glutamine without sodium bicarbonate (Sigma-Aldrich, St. Luis, MO) and 0.165M buffer MOPS (Invitrogen, Carlsbad, CA), pH 7.0, with 100 mg/L chloramphenicol (Sigma-Aldrich, St. Luis, MO). CLSI MICs were determined in medium without chloramphenicol.

In vitro pharmacokinetic-pharmacodynamic model. The in vitro PKPD simulation model is shown in Figure 1. It consists of a) a 10 ml-volume dialysis tube (internal compartment, IC) made of semi-permeable cellulose membrane (Float-A-Lyzer, Spectrum Europe B.V, Breda, The Netherlands) allowing free diffusion of small molecules (molecular weight <20 kDa). This is placed into b) a glass beaker containing 700 ml medium (external compartment, EC), the content of which is continuously diluted by c) a peristaltic pump (Minipuls Evolution®, Gilson Inc., Villiers le Bel, France) removing drug-containing medium from the EC and adding drug-free medium into the EC at a rate equivalent to drug clearance from human plasma. Dialysis tubes, glass beakers and tubings were sterilized by gamma irradiation 20G, autoclavage 121°C for 20 min, and 70% ethanol, respectively while the system was kept free of bacterial contamination using chloramphenicol.
The IC was inoculated with 10 ml medium containing \(1 \times 10^5\) CFU/ml of *Aspergillus* conidia. The cellulose membrane of the IC allowed free diffusion of nutrients and voriconazole until an equilibrium was reached with the EC, while at the same time it retained the conidia, hyphae and macromolecular products such as galactomannan (molecular weight of 25-75kDa). Thus, galactomannan was concentrated within the IC and was used as a biomarker of fungal growth. In order to assure that galactomannan did not diffuse into the EC, galactomannan levels were determined in the EC.

At time zero, voriconazole was injected into the EC and the IC simultaneously in order to achieve rapid equilibration of drug concentration between the two compartments. The drug-containing medium in the EC was then continuously diluted with drug-free medium by the peristaltic pump adjusted to a specific flow rate in order to reproduce average drug half-lives observed in human plasma after intravenous administration of voriconazole (6h) in accordance with previous clinical studies (18, 19). The EC was then placed on a heated magnetic stirrer adjusted at 37°C and 2 rpm. The temperature and the flow rate were checked regularly throughout the experiment using a thermometer and by measuring the volume of medium pumped out of the EC within 1 min, respectively.

**Bioassay of voriconazole.** The drug levels in EC and IC were determined by a microbiological method using the voriconazole susceptible strain *Candida kefyr* NCPF 3234 (15). Briefly, \(3 \times 10^5\) CFU/ml of *C. kefyr* were inoculated in pre-warmed medium (54°C) containing 15 g/L agar (Bacto Agar Difco, BD Hellas SA, Athens, Greece). The medium was poured into square (10x10cm) plastic petri dishes and after solidification, 2 mm diameter wells were made by a 2 mm diameter cork borer. Hundred microliters of serial twofold drug dilutions (range 0.25-16 mg/L) and 100μl of samples obtained from the EC or IC medium were added into the holes. The plates were incubated at 37°C for 24h and the diameter of the inhibition zones around the holes was measured with a ruler. A standard curve between drug concentration and diameter of inhibition zones was constructed and analyzed with linear regression. Based on this standard curve, the drug
concentrations of the EC and the IC were determined at any time point. In order to assure that drug concentration within the IC was uniform; samples from the center and periphery of the top, middle and bottom parts of the IC were tested.

**Pharmacokinetic analysis.** The in vitro system simulated steady state voriconazole pharmacokinetics observed in patients with maximum total plasma concentrations 1.75, 3.5 and 7 mg/L respectively, and an average half life of 6h (18, 19). Voriconazole concentration at 0h, 4h, 6h, 8h, 20h and 24h after the introduction of the drug in the system was determined both in the IC and EC with the bioassay. The data were analyzed by nonlinear regression (Prism 5.0, GraphPad Inc., La Jolla, CA) based on the compartment pharmacokinetic model described by the equation $C_t = C_0 e^{-kt}$, where $C_t$ (dependent variable) the concentration of drug at a given time t (independent variable), $C_0$ the initial concentration of the drug at time $t = 0h$, e the physical constant 2.18 and k the rate of drug removal. The half-life was calculated using the equation $t_{1/2} = k/0.693$ for EC and IC separately and compared with the respective values obtained with human plasma. Finally, the calculated area below the curve of drug concentration and time (Area Under the Curve, AUC) within 24 hours ($fAUC_{0-24}$) was determined.

**Galactomannan levels for determination of fungal growth.** Fungal growth in the IC was monitored by galactomannan production. Galactomannan levels were measured by ELISA (Platellia, Biorad Laboratories, Athens, Greece) and results were expressed as a galactomannan index (GI) according to the manufacturer's instructions. In order to correlate GI levels with fungal growth, IC tubes were inoculated with $10^3$, $10^4$, $10^5$ or $10^6$ CFU/ml of *A. fumigatus*, incubated without drug for 24 hours at 37°C and GI levels were determined at regular time intervals. The kinetics of galactomannan production were analyzed with nonlinear regression analysis (Prism 5.0, GraphPad Inc., La Jolla, CA) based on the $E_{max}$ model described by the equation $E = E_{max} * T^\gamma / (T^\gamma + T_{50})$, where E the GI (dependent variable), $E_{max}$ the maximum GI, T (independent variable) the time, $T_{50}$ the time corresponding to 50% of $E_{max}$ and $\gamma$ the slope of the curve. In order to capture differences in the extent, rate and time of galactomannan production reflected by $E_{max}$, $\gamma$ or $T_{50}$...
parameters, respectively, the area under the galactomannan index-time curve (AUC GI) was calculated for each inoculum. The AUC GI was then correlated with the initial inoculum using linear regression analysis.

**Pharmacodynamic analysis.** The IC was inoculated with $10^5$ CFU/ml *A. fumigatus*, *A. flavus* or *A. terreus* and incubated for 72h. Galactomannan levels in the IC were measured at regular time intervals with ELISA (Platellia, Biorad Laboratories, Athens, Greece) and results were expressed as a GI according to the manufacturer's instructions. The kinetics of galactomannan production were studied by nonlinear regression (Prism 5.0, GraphPad Inc., La Jolla, CA) based on the Emax model described above. The $E_{\text{max}}$, $\gamma$ or $T_{50}$ parameters at each voriconazole dose ($E_{\text{max},D}$, $\gamma_D$ or $T_{50,D}$) were compared with the corresponding values of drug-free growth control ($E_{\text{max},GC}$, $\gamma_{GC}$ or $T_{50,GC}$). The AUC GI for the growth control and each dosing regimen at 24h, 48h, and 72h was estimated and used as a surrogate marker of fungal growth. All the experiments were repeated at least twice.

**Pharmacokinetic-pharmacodynamic analysis.** The pharmacodynamic parameter AUC GI was associated with the pharmacokinetic parameter $f_{\text{AUC}0-24}$ for each species with nonlinear regression analysis using the Emax model described above. The near-maximum activity $f_{\text{AUC}0-24}$ was calculated for each species as the $f_{\text{AUC}0-24}$ corresponding to 10% of AUC GI. Differences among the species were assessed with analysis of variance (ANOVA). In order to validate the in vitro PKPD model, the AUC/MIC associated with near-maximum activity was compared with the corresponding value found in an experimental animal model of *A. fumigatus* infection (9). Finally, in vitro PKPD data were bridged to human PK data combining in vitro AUC GI/$f_{\text{AUC}0-12}$ and clinical $f_{\text{AUC}0-12}$ taking into account the inter-patient variation. The % target attainment of the upper and lower 95% confidence interval limits of voriconazole $f_{\text{AUC}0-12}$ observed previously in patients (18, 19) were calculated based on the in vitro AUC GI/$f_{\text{AUC}0-12}$ relationship for each species.
183 **RESULTS**

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**Bioassay of voriconazole.** The standard curves of the diameter of inhibition zone - drug concentration are shown in Figure 2. The lowest concentration of drug detected by these techniques was 0.5 mg/L and the concentration range 0.5 mg/L to 16 mg/L. The diameter of inhibition zone correlated linearly with drug concentration of \( r^2 > 0.86 \). The coefficient of variation ranged from 5% to 20% (median 8%).

**Surrogate marker of fungal growth.** The production of galactomannan for each clinical Aspergillus isolate followed a sigmoid curve described very well with the E\text{max} model \( r^2 > 0.98 \) (Figure 3 depicts results obtained by testing *A. fumigatus*). The area under the GI-time curve \( \text{AUC}_{GI} \) was linearly associated with the initial inoculum \( r^2 = 0.96 \), slope \( 46 \pm 6 \), Figure 3). Therefore, the \( \text{AUC}_{GI} \) was used as a surrogate marker of fungal growth.

**Pharmacokinetic analysis.** The one compartment pharmacokinetic model described well the drug levels in the IC \( R^2 > 0.97 \). Intra-and inter-experimental variation of the in vitro PK data was <10 % (Figure 4). In vitro voriconazole pharmacokinetics were close to the target values observed in human plasma after administration of 3, 4 and 5 mg/kg voriconazole (18, 19). The half-life of voriconazole in the in vitro system was 5.7-6.5h, which was similar to the half-life of 4.7-7.3h observed in patients’ plasma. The voriconazole f\( \text{AUC}_{0-12} \) was 11.6, 23.5 and 43.8 mg.h/l for the three doses; likewise, in human plasma, the respective values for voriconazole dosages 3, 4 and 5 mg/kg corresponded to 5.8, 12.4 and 18.2 mg.h/l (calculated based on 58% protein binding and 13.9, 29.5 and 43.4 mg.h/l total \( \text{AUC}_{0-12} \)) (18, 19).

**Pharmacodynamic analysis.** The pharmacodynamic data for each simulated voriconazole dose against the three strains are shown in Figure 5. The \( E_{\text{max}} \) model described the data well \( \text{R}^2 > 0.86 \). The three simulated voriconazole doses resulted in different GI-time curves in terms of the extent and rate of galactomannan production as reflected by the different \( E_{\text{max}} \), \( T_{50} \) and \( \gamma \) parameters of the \( E_{\text{max}} \) model. In particular, all three voriconazole doses delayed galactomannan production by
A. fumigatus (T_{50,GC}=15h vs. T_{50,D}=19-29h) whereas only the two highest doses decreased the maximum galactomannan production compared to growth control (E_{max,GC}=3.8 vs. E_{max,1.75}=3.8, E_{max,3}=2, E_{max,7}=1.8). In the case of A. flavus, there were no significant differences in T_{50} parameters (T_{50,GC}=5.8h vs T_{50,GC}=4.2-5.3), whereas maximum galactomannan production was reduced as voriconazole dose increased (E_{max,GC}=3.9 vs. E_{max,1.75}=3.6, E_{max,3.5}=3.2, E_{max,7}=2.6).

Regarding A. terreus, voriconazole delayed galactomannan production (T_{50,GC}=10.5h vs T_{50,1.75}=14h, T_{50,3}=18h, T_{50,7}=21h) whereas maximum galactomannan production was reduced as voriconazole dose increased (E_{max,GC}=4 vs. E_{max,1.75}=3.7, E_{max,3.5}=3.2, E_{max,7}=3).

Pharmacokinetic-pharmacodynamic analysis. In order to capture the above described changes in E_{max} model parameters of GI-time curves, the AUC_GI, which was used as surrogate marker of fungal growth, was correlated to the area under the concentration-time curve for each voriconazole dose (fAUC_{0-24}). The AUC_GI at 24h decreased from 34 GIxh in the growth control to 10.5 GIxh at the highest voriconazole dose for A. fumigatus following a sigmoid pattern (R^2>0.97) (Figure 6, left graph). The same pattern of AUC_GI reduction was observed for A. flavus (60.3 GIxh in growth control to 42.3 GIxh at the highest voriconazole dose) and for A. terreus (50.1 GIxh to 20.5 GIxh, respectively). Because of the different dynamic AUC_GI ranges among the three Aspergillus spp., the AUC_GI was normalized from 0% to 100% based on the minimum and maximum AUC_GI, respectively (Figure 6, right graph). Based on the normalized PKPD relationship, the fAUC_{0-24} associated with near-maximum activity (10% AUC_GI) was 18.9 (14.4-23.1) mg.h/l against A. fumigatus, 26.6 (21.1-32.9) mg.h/l against A. flavus and 36.2 (27.8-45.7) mg.h/l against A. terreus (F_{2,19}=17.22, p<0.0001). These fAUC_{0-24}s were similar when the AUC_GI at 48h and 72h were used as markers of fungal growth. Of note, the fAUC_{0-24} of 18.9 mg.h/l for the tested A. fumigatus isolate (MIC=0.5 mg/l) corresponds to an fAUC/MIC of 37.8 which is close to the fAUC/MIC of 36.4 associated with 90% survival in an animal model of experimental aspergillosis(9); thus providing a validation of the in vitro PKPD model.
Bridging the in vitro PKPD data with human PK data, low median % target attainment (<45%) was found for 3 mg/kg voriconazole dosing for all species (Table 1). The standard dosage of 4 mg/kg was associated with high median % target attainment (80%) for *A. fumigatus* but not for *A. flavus* and *A. terreus* for which as low as 17% and 6% target attainment, respectively, was found for fAUC$_{0-12}$ at the lower 95% confidence interval limit observed in patients. Even for *A. fumigatus* a wide range of % target attainment (32-97%) was detected reflecting the wide variation of voriconazole fAUC$_{0-12}$ among patients receiving 4 mg/kg. In agreement with these findings is the range of 50 to 80% survival rate reported in clinical trials among patients with aspergillosis treated with voriconazole (2, 6). Importantly, for patients infected by *A. fumigatus* isolates with voriconazole MICs of 0.5 mg/l the 6-week survival rate was ~75% (2), which is very close to the 80% median % target attainment recorded in the present study (2) providing, thus, clinical validation of the in vitro model. With a dosage of 5 mg/kg more patients will attain the target for *A. fumigatus* infections (>86%) whereas >73% and >47% will achieve the target for *A. flavus* and *A. terreus* infections. Of note, the % of target attainment for the upper 95% confidence interval of 4 mg/kg voriconazole dosage was >83% for all *Aspergillus* species (Table 1).
The in vitro PKPD modeling of voriconazole activity against *A. fumigatus*, *A. flavus* and *A. terreus* isolates with identical MICs showed important pharmacodynamic differences in a new dynamic model simulating human plasma voriconazole pharmacokinetics. The voriconazole fAUC$_{0-24}$ associated with near maximum activity differed among the three *Aspergillus* spp. with fAUC$_{0-24}$ of 18.9 (14.4-23.1) mg.h/l for *A. fumigatus*, 26.6 (21.1-32.9) mg.h/l for *A. flavus* and 36.2 (27.8-45.7) mg.h/l for *A. terreus*. Bridging these data with human PK showed that the standard dosage of 4 mg/kg was associated with a high median % target attainment for *A. fumigatus* (80%) but not for *A. flavus* (63%) and *A. terreus* (36%), although a wide range of % target attainment was observed reflecting the large inter-patient variation of voriconazole fAUC$_{0-12}$.

In vitro PKPD analysis may reveal differences in antifungal activity that cannot be predicted by an MIC value. Studying the effect of decreasing concentrations of voriconazole provides information about drug pharmacodynamic properties related with sub-MIC effect, post-antifungal effect, time- and concentration-dependent activities. These effects can be quantified by a surrogate marker of fungal growth based on galactomannan production kinetics which captures any difference on the above antifungal effects. Differential antifungal activity against three *Aspergillus* species with identical MICs of voriconazole, amphotericin B and caspofungin was recently found emphasizing the importance of studying non-constant drug concentrations (11). The growth rate may be an important determinant of antifungal activity. Voriconazole activity was found to be correlated with growth rates of *Candida* isolates as determined in a pharmacokinetic model (8) and of *Aspergillus* species using a microdilution assay measuring metabolic activity (1). The three *Aspergillus* species are characterized by different growth rates with *A. terreus* growing slower and *A. flavus* faster than *A. fumigatus* (12, 25). Indeed, in the present model a larger voriconazole fAUC$_{0-24}$ was required to inhibit the slow growing *A. terreus* than the other two species. However, the fAUC$_{0-24}$ against the slower growing *A. fumigatus* was smaller than the fAUC$_{0-24}$ against the faster growing *A. flavus*.
indicating that other factors influence voriconazole activity in the present model such as time-
dependent effects.

Time-dependent activity of voriconazole inhibition may differ for each *Aspergillus* species.

Exposure of *Aspergillus* conidia to concentrations near voriconazole MIC for 6 h resulted in
significant amount of fungal growth for *A. terreus* (64%), *A. flavus* (24%) and *A. fumigatus* (15%)
isolates. (1) After 8 h of exposure, voriconazole MICs against *A. fumigatus* isolates were lower than
the respective MICs after 48 h of incubation whereas the MICs against *A. flavus* isolates remained
the same, indicating that voriconazole needs more time to act against *A. flavus*. This may explain
the smaller fAUC$_{0-24}$ observed in the present model for *A. fumigatus* in comparison to *A. flavus*.

In agreement with a differential voriconazole time-dependent inhibition among species is
the fact that complete inhibition of galactomannan production was not observed for any of the
strains tested in the present dynamic model. This effect may be related to drug mechanism of action
since azole-induced complete inhibition of ergosterol synthesis requires at least 1 h and complete
exchange of ergosterol by its methylated precursors occurs after about 6 h of azole exposure (20).
The kinetics of voriconazole inhibition may differ among *Aspergillus* species as reflected in the
present dynamic model by the different maxima of galactomannan production for each species.

These differences were taken into account during the analysis by normalization of data.

In order to validate the present in vitro PKPD model, the obtained results were compared
with those found in a murine model of experimental aspergillosis (9). In the latter, voriconazole
efficacy was tested against four *A. fumigatus* isolates demonstrating increasing MIC values. The
AUC/MIC associated with 90% survival in mice was 36.4, which was very close to the AUC/MIC
of 37.8 associated with near maximum activity (i.e. 10% fungal growth) in the present study.

Voriconazole dosage of 10 and 40 mg/kg, which corresponded to an fAUC$_{0-24}$ of 1 and 20 mg.h/ml,
against a wild-type *A. fumigatus* isolate resulted in 90% and 0% mortality, respectively. (9)
Similarly in our in vitro dynamic model, fAUC$_{0-24}$ of 1 and 20 mg.h/ml (0 and 1.3 log$_{10}$fAUC$_{0-24}$,
respectively) corresponded to 100% and 10% of fungal growth (right graph of Fig. 6). Finally,
voriconazole at a dosage of 10 mg/kg was active against *A. fumigatus* (13, 23) but not *A. flavus* (27) infections in murine models of invasive aspergillosis. Likewise, the present study demonstrated that voriconazole was more active against *A. fumigatus* than *A. flavus*. Thus, the results of the in vitro dynamic model can predict the outcome of voriconazole treatment in vivo.

The results of the PKPD model are in agreement with clinical data since the % target attainment (32-97%) for the 4 mg/kg dosage of voriconazole was similar to the survival rates among patients with *A. fumigatus* infections who were treated with standard dosing (50-80%) (2, 6). In addition, the median % target attainment for this species (80%) was very close to the median 6-week survival (~75%) previously observed in hematological patients infected by *A. fumigatus* isolates with MICs corresponding to 0.5 mg/l (2). The AUC/MIC index of 37.8 which found to be associated with near maximum activity for *A. fumigatus* in the present study can be reached with standard dosing of 4 mg/kg for isolates with MIC 0.5 mg/l supporting thus the voriconazole ECVs of 1 mg/l (5). Regarding the other species, the survival rate of patients with invasive aspergillosis due to *A. terreus* was generally lower than due to *A. fumigatus* after treatment with voriconazole (6, 22). This was also reflected in the present study by a higher % target attainment for *A. fumigatus* compared to *A. terreus*. These correlations provide further clinical validation of the in vitro model.

In summary, higher voriconazole exposure was required to inhibit *A. terreus* than *A. flavus* and *A. fumigatus*. Based on the in vitro exposure-effect relationships found in the novel PKPD model, a standard dosage of voriconazole may be adequate for most patients with *A. fumigatus* infections; higher drug exposure appears to be required for *A. flavus* and *A. terreus* infections. This could be achieved using a higher voriconazole dosage or by therapeutic drug monitoring in patients receiving the standard dosage given the wide distribution of voriconazole fAUC$_{0-12}$ among them. Optimizing voriconazole exposure in order to obtain fAUC$_{0-12}$ at the upper 95% confidence interval limit, would result in % target attainment >83% for all three *Aspergillus* species. Therapeutic drug monitoring for voriconazole is becoming an important tool to improve efficacy and safety of this agent (3). This is corroborated by clinical studies where adequate voriconazole levels have been
associated with efficacy, while drug underexposure was associated with worse outcome (14, 15).

There is a multitude of factors that can influence patient outcomes. In that respect, in vitro PKPD modeling assists in providing information on critical parameters such as drug kinetics and dynamics, thus offering a powerful tool for estimating the impact of drug underexposure and improving efficacy through drug exposure optimization.

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REFERENCES


Figure 1. In vitro pharmacokinetic/pharmacodynamic model that simulates the pharmacokinetics of voriconazole in humans and determines a drug’s effect on *Aspergillus* growth. The model consists of an external compartment (EC), a glass beaker containing 700 ml broth medium, and an internal compartment (IC), a dialysis tube containing 10 ml broth medium; the tube is made of a semi-permeable cellulose membrane that allows free passage of small molecules (<20kD) like antifungals, but not galactomannan. The EC is placed on a heated magnetic stirrer (37°C and 2 rpm). A peristaltic pump introduces drug-free medium in the EC and removes its content concurrently, in order to maintain a constant volume. The flow rate is adjusted to achieve drug concentrations corresponding to their clearance from human plasma. At time zero, 10^5 CFU/ml of *Aspergillus* conidia are inoculated in the IC; while the drug is introduced into both EC and IC for rapid concentration equilibration. Subsequently, drug concentration declines over time. Galactomannan levels of the IC medium are measured at regular time points.
Figure 2. Microbiological method for determining voriconazole levels. Linear regression analysis between the diameter of inhibition zone and drug concentration. The intra-and inter-experimental variation was <20%. Error bars represent standard deviations.
Figure 3. Use of the area under the galactomann index–time curve (AUC_{GI}) as surrogate marker of fungal growth. Kinetics of galactomannan production by increasing inocula of *A. fumigatus* (left graph) and correlation of the AUC_{GI} with the initial inoculum (right graph).
Figure 4. Pharmacokinetic analysis of simulated doses with Cmax 7, 3.5 and 1.75 mg/l of voriconazole in the in vitro pharmacokinetic/pharmacodynamic system. Dotted lines represent the lower limit of detection. Error bars represent standard deviations.
Figure 5. Galactomannan index-time curves in the internal compartment of the in vitro PKPD model for each voriconazole simulated dose against the three Aspergillus species.
Figure 6. In vitro PKPD relationship of voriconazole. The relationship between the area under the galactomannan index curve (AUC GI) (left graph) or normalized AUC GI (right graph) and the area under the concentration time curve (fAUC0-24) for each Aspergillus species. The AUC GI for the first 24h was used as the surrogate marker of fungal growth.
Table 1. Percent target attainment of the in vitro PKPD parameter associated with near maximum activity for each voriconazole dosage.

<table>
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<tr>
<th>Voriconazole Dose (mg/kg)</th>
<th>Mean (95%CI) fAUC&lt;sub&gt;0-12&lt;/sub&gt; (mg·h/l)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean (95%CI) % target attainment</th>
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<td>3</td>
<td>5.85(4.4-7.75)</td>
<td>24(11-45)</td>
</tr>
<tr>
<td>4</td>
<td>12.4(6.55-23.35)</td>
<td>80(32-97)</td>
</tr>
<tr>
<td>5</td>
<td>18.2(14.15-23.5)</td>
<td>93(86-97)</td>
</tr>
</tbody>
</table>

<sup>a</sup>From ref. 19.