Pharmacodynamics of Voriconazole in a Dynamic In Vitro Model of Invasive Pulmonary Aspergillosis: Implications for In Vitro Susceptibility Breakpoints

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Background. Voriconazole is a first-line agent for the treatment of invasive pulmonary aspergillosis (IPA). There are increasing reports of *Aspergillus fumigatus* isolates with reduced susceptibility to voriconazole.

Methods. An in vitro dynamic model of IPA was developed that enabled simulation of human-like voriconazole pharmacokinetics. Galactomannan was used as a biomarker. The pharmacodynamics of voriconazole against wild-type and 3 resistant strains of *A. fumigatus* were defined. The results were bridged to humans to provide decision support for setting breakpoints for voriconazole using Clinical Laboratory Standards Institute (CLSI) and European Committee of Antimicrobial Susceptibility Testing (EUCAST) methodologies.

Results. Isolates with higher minimum inhibitory concentrations (MICs) required higher area under the concentration time curves (AUCs) to achieve suppression of galactomannan. Using CLSI and EUCAST methodologies, the AUC:MIC values that achieved suppression of galactomannan were 55 and 32.1, respectively. Using CLSI and EUCAST methodologies, the trough concentration:MIC values that achieved suppression of galactomannan were 1.68 and 1, respectively. Potential CLSI breakpoints for voriconazole are ≤0.5 mg/L for susceptible and >1 mg/L for resistant. Potential EUCAST breakpoints for voriconazole are ≤1 mg/L for susceptible and >2 mg/L for resistant.

Conclusions. This dynamic model of IPA is a useful tool to address many remaining questions related to antifungal treatment of *Aspergillus* spp.

Invasive pulmonary aspergillosis is a common and frequently lethal infectious syndrome. Despite advances in diagnostics and therapeutics, the mortality is approximately 50% [1]. There are increasing reports of triazole resistance in *Aspergillus fumigatus* and this appears to be associated with increased mortality [2, 3]. Relatively little is known about the pharmacodynamics (PD) of antifungal agents against *Aspergillus* spp. A better understanding represents an important step toward improved clinical outcomes for patients.

Voriconazole is a first-line agent for the treatment of invasive aspergillosis [4, 5]. The efficacy of voriconazole was established in an era when triazole resistance was exceedingly uncommon. Similarly, currently accepted drug-exposure targets for therapeutic drug monitoring are largely predicated on the assumption of infection with fully susceptible fungi. The PD of voriconazole against less susceptible *Aspergillus* spp. remains poorly defined.

Voriconazole is a notoriously difficult compound to study in murine and rat models of invasive
aspergillosis. The guinea pig is potentially a more reliable laboratory animal to study the efficacy of voriconazole, but this model is not widely available. Here we describe a novel dynamic in vitro model of the human alveolus in which we were able to replicate humanlike voriconazole pharmacokinetics (PK). We used this model to study the PD of both wild-type and mutant strains of *A. fumigatus* with varying susceptibilities to voriconazole. We then bridged the results to humans to gain a further insight into targets for therapeutic drug monitoring and potential in vitro susceptibility breakpoints.

**METHODS**

**Aspergillus Isolates and In Vitro Susceptibility Testing**

The strains used in this study are summarized in Table 1. A previously described *A. fumigatus* transformant expressing green fluorescent protein was used as the wild-type [6, 7]. Isolates with reduced susceptibility to voriconazole and carrying previously defined substitutions [2] within the triazole target Cyp51A were also studied: L98H, G138C, and G434C. The isolates were stored in glycerol broth at −80°C. Prior to each experiment, isolates were subcultured to potato dextrose agar (Oxoid), and incubated at 37°C for 3 days. Suspensions of conidia were prepared by flooding with phosphate buffered saline (PBS) (Invitrogen) and gently abrading the surface with a sterile swab. Conidial suspensions were filtered through sterile gauze. The resulting suspension was washed twice by centrifugation at 1000 g and resuspending the pellet in PBS. The final inoculum was prepared in endothelial basal medium 2 (EBM-2) (Lonza Biologics) using a hemocytometer. A final density of 2.5 x 10⁵ conidia/mL was verified using quantitative cultures.

Voriconazole minimum inhibitory concentrations (MICs) were determined for each isolate using both Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodologies with the single modification of using a maximum voriconazole concentration of 32 mg/L and 64 mg/L for CLSI and EUCAST, respectively (microscopy was performed to confirm that the voriconazole did not crystallize or precipitate during incubation). The MICs were determined in 13 independent experiments performed in 2 separate laboratories (Manchester and Copenhagen), and the geometric mean was calculated.

**Dynamic In Vitro Model of the Human Alveolus**

A previously described cell culture model of the human alveolus [6] was modified for use with 12-well cell culture plate inserts. A cellular bilayer was constructed using human pulmonary artery endothelial cells (HPAECs) (Lonza Biologics) and human alveolar epithelial cells (A549) (LGC Standards), which were used in passages 4 and 79–86, respectively. The HPAECs were grown in EBM-2 supplemented with 2% fetal bovine serum (FBS), ascorbic acid, heparin, hydrocortisone, human endothelial growth factor, vascular endothelial growth factor, human fibroblast growth factor B, and R-3-insulinlike growth factor 1 according to the manufacturer’s instructions to produce endothelial growth medium 2 (EGM-2). Amphotericin B and gentamicin, which are ordinarily components of EGM-2, were not included. The A549 cells were grown in EBM-2 supplemented with 10% FBS (Lonza Biologics) only. Cells were grown to near-confluence in humidified 5% carbon dioxide (CO₂) at 37°C. The HPAECs and A549s were harvested with warmed 0.25% trypsin–ethylenediaminetetraacetic acid (Sigma-Aldrich), centrifuged, and resuspended in warm fresh media. Final densities of HPAEC and A549 cells of 1 x 10⁶ cells/mL and 5.5 x 10⁵ cells/mL, respectively, were achieved by dilution in their respective media.

ThinCert cell culture well inserts for 12-well plates (transparent polyethylene terephthalate membranes with 3 µm pores; Greiner Bio-One) were inverted, and 400 µL of HPAEC suspension was placed on the underside of the membrane. The well inserts were then incubated for 2 hours to allow cell attachment before being righted and placed in 12-well cell culture plate wells containing 1.5 mL of EGM-2. Four hundred microliters of EBM-2 supplemented with 10% FBS were then added to the upper (alveolar) compartment of each well insert before incubation at 37°C in humidified 5% CO₂ for 24 hours. Well inserts were then transferred to a cell culture plate containing fresh EGM-2. Spent medium from each alveolar compartment was removed, and 400 µL of A549 suspension was then added. After 2 hours of incubation, the medium in each alveolar compartment was removed to create an air-liquid interface. The EGM-2 in the lower (endothelial) compartment was changed daily, and any medium that accumulated in the alveolar compartment was also removed. Experiments were performed 5 days after seeding of the well inserts with A549 cells. The integrity of the cellular bilayer at this time was assessed by the extent of translocation of 1% dextran blue (Sigma-Aldrich). Four hundred microliters were placed in the alveolar compartment, and 1.5 mL of PBS warmed to 37°C were placed in the endothelial compartment.

**Table 1. Strains Used in This Study**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Cyp51A Amino Acid Substitution</th>
<th>CLSI MIC Range (mean)</th>
<th>EUCAST MIC Range (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFP</td>
<td>Wild-type</td>
<td>0.5–1.0 (0.56)</td>
<td>0.5–2.0 (0.95)</td>
</tr>
<tr>
<td>F16216</td>
<td>L98H</td>
<td>4–8 (4.45)</td>
<td>2–16 (7.19)</td>
</tr>
<tr>
<td>F11628</td>
<td>G138C</td>
<td>16 (16.00)</td>
<td>8–32 (25.85)</td>
</tr>
<tr>
<td>F13747</td>
<td>G434C</td>
<td>16 (16.00)</td>
<td>8–32 (25.85)</td>
</tr>
</tbody>
</table>

The minimum inhibitory concentrations (MICs) were determined in 13 independent experiments. The mean is the geometric mean.

Abbreviations: CLSI, Clinical Sciences Laboratory Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; GFP, green fluorescent protein.
The concentration of dextran blue in the endothelial compartment was determined spectrophotometrically after 1 hour of incubation using a wavelength of 620 nm.

**Bioreactors and Construction of the In Vitro Dynamic Model**

During each experiment, well inserts were housed in specially designed and constructed stainless steel bioreactors (see Figure 1). These were designed to house the insert with the cellular bilayer and allow the flow of media through the endothelial compartment using a peristaltic pump. Each bioreactor was connected to a circuit formed from 1.6-mm bore platinum-cured silicone tubing (Dow Corning) connected by polypropylene barbed luer adapters (Cole-Parmer). A central reservoir containing 200 mL of warmed media was housed within a 250-mL Duran bottle. These were connected to the circuit using 1.5-mm bore semirigid polytetrafluoroethylene (PTFE) tubing (Diba Industries Ltd) and Omnimif Q-series bottle caps with one-quarter-inch 28 Unified National Fine thread ports (Diba Industries Ltd). The central reservoirs contained magnetic stir bars and were placed on a stirrer plate to ensure adequate mixing of circulating media. Duran bottles containing fresh media and empty bottles to collect waste were also connected to each circuit in the same way. Two 3-way taps (Infusion Concepts Ltd) within the circuit enabled sample collection and the administration of drug to the central reservoir through separate access points. A 205-U multichannel cassette pump (Watson-Marlow) fitted with 1.52-mm bore Marprene manifold tubing (Watson-Marlow) was used and enabled multiple bioreactors to be run simultaneously.

All components of the circuit were sterilized prior to use. Each circuit was assembled in a class 2 safety cabinet before being transferred to a 37°C environment. Warmed Dulbecco’s modified Eagle medium (DMEM) containing 4500 mg/L D-Glucose, L-Glutamine, and HEPES buffer (Invitrogen) was used within the circuit. This was supplemented with 2% FBS (Lonza Biologics). A penicillin-streptomycin solution was also added (Sigma-Aldrich) to give a final concentration of 100 U/mL of penicillin and 0.1 mg/mL of streptomycin. The circuits were primed with media before connecting the bioreactors. During each experiment, the pump was run at 1.9 rpm to produce a flow rate of approximately 10 mL/hour within the circuit.

**Inoculation of the Dynamic Model**

Inserts were inoculated while in the cell culture plate. Four hundred microliters of the conidial suspension was placed in each alveolar compartment (ie, the absolute number of inoculated conidia was 100 000). After 6 hours of incubation at 37°C, the residual fluid from the conidial suspension was removed from each alveolar compartment, and the well inserts were transferred into the bioreactors.

**Treatment With Voriconazole**

The clinical formulation of voriconazole (Pfizer) was reconstituted in DMEM supplemented with 2% FBS. Serial dilutions in DMEM and 2% FBS were performed to create a range of concentrations required for each experiment. Voriconazole was administered at 6, 18, 30 and 42 hours postinoculation. For the wild-type (green fluorescent protein), 0 mg, 0.01 mg, 0.02 mg, and 0.2 mg were administered every 12 hours. For the mutants L98H, G138C, and G434C 0 mg, 0.2 mg, 1 mg, and 3 mg were administered every 12 hours. Samples for voriconazole concentrations and galactomannan levels were obtained from the circuit 6–54 hours postinoculation. At each sampling point, the first 0.5 mL of media drawn were discarded, and a second 0.5 mL sample was taken for analysis.

**PK and PD of Voriconazole**

The PK and PD of voriconazole against each isolate were estimated using 4 bioreactors/circuits administered with various voriconazole dosages, including 1 untreated circuit that served as a control. Voriconazole was administered at 6, 18, 30 and 42, hours postinoculation. For the wild-type (green fluorescent protein), 0 mg, 0.01 mg, 0.02 mg, and 0.2 mg were administered every 12 hours. For the mutants L98H, G138C, and G434C 0 mg, 0.2 mg, 1 mg, and 3 mg were administered every 12 hours. Samples for voriconazole concentrations and galactomannan levels were obtained from the circuit 6–54 hours postinoculation. At each sampling point, the first 0.5 mL of media drawn were discarded, and a second 0.5 mL sample was taken for analysis.
High Performance Liquid Chromatography (HPLC)

Voriconazole concentrations in media were measured using HPLC with a Shimadzu Prominence (Shimadzu). Thirty microliters of extracted sample were injected onto a Kinetex 2.6-µ C18 100A 75 × 4.6-mm column 250 × 4.6 mm (Phenomenex). A standard curve encompassing 0.0625–8 mg/L was constructed in media from stock solutions of voriconazole pure powder (Pfizer) at 1000 mg/L in methanol (Fisher Scientific). The internal standard was diazepam 1 µg/L (Sigma-Aldrich). A gradient method was used with starting concentrations of 80% 0.1% trifluoroacetic acid in water and 20% 0.1% trifluoroacetic acid in acetonitrile (Fisher Scientific) changing to 35% and 65%, respectively, over 10 minutes. An overall run time of 13 minutes and flow rate of 1 mL/minute was used. Voriconazole and the internal standard were detected using UV at 254 nm; they eluted after 5.9 and 4.7 minutes, respectively. The coefficient of variation percentage was <9% over the concentration range of 0.0625–8 mg/L. The limit of detection was 0.0625 mg/L. The intra- and interday variation was <9%.

Galactomannan

The antifungal effect of voriconazole was assessed by measuring levels of galactomannan within the central reservoir of the experimental circuit. A commercially available double-sandwich enzyme linked immunosorbent assay was used (Platelia Aspergillus kit; Bio-Rad) according to the manufacturer’s instructions with a single modification. Because of the low volumes of experimental samples, 50 µL of serum treatment solution were used to pretreat 150 µL of sample (rather than the recommended 100 µL for 300 µL of sample). As previously described, this modification did not have an impact upon the levels of galactomannan measured [8].

Mathematical Modeling

The PK and PD of voriconazole against each isolates were described using the following mathematical model, which consisted of 2 inhomogeneous ordinary differential equations.

\[
\frac{dX_1}{dt} = B(1) - \left( \frac{SCL}{V_c} \right) \times X_1 \quad (1)
\]

\[
\frac{dN}{dt} = K_{gmax} \times \left( 1 - \left( \frac{N}{P_{OPMAX}} \right) \right) \times N \quad (2a)
\]

\[
\times \left( 1 - \frac{(X_1/V_c)_{Hg}}{(X_1/V_c)^{Hg} + C_{50g}^{Hg}} \right) \quad (2b)
\]

In this model, \( X_1 \) is the amount of voriconazole (mg); \( B(1) \) is the bolus input of voriconazole; \( SCL \) is the clearance of voriconazole from the circuit (L/h); \( V_c \) is the volume of the circuit (L); \( N \) is the galactomannan concentration; \( K_{gmax} \) is the maximal rate of growth (GMI/h); \( P_{OPMAX} \) is the theoretical maximal density within the circuit (GMI); \( Hg \) is the slope function for the suppression of growth; and \( C_{50g} \) (mg/L) is the concentration of voriconazole in the circuit where there is half-maximal suppression of growth.

Equation 1 describes the rate of change of voriconazole concentrations in the circuit. Equation 2 describes the rate of change of galactomannan in the circuit and contains terms that describe fungal growth in the absence of voriconazole (Equation 2a) and the voriconazole-induced suppression of growth (Equation 2b).

The mathematical model was fitted to the data using a population methodology with the Big version of the program Nonparametric Adaptive Grid [9]. The goodness-of-fit of the model to the data was assessed using the log-likelihood value, the coefficient of determination of the regression of observed-vs-predicted values after the Bayesian step, and a visual inspection of this relationship.

Bridging to Humans

The mathematical model was used to further define the relationship between drug exposure and the antifungal effect for each isolate. Drug exposure was quantified in 2 ways: (1) area under the concentration time curve (AUC) and (2) the trough concentration. Both measures were then divided by MIC to yield the AUC:MIC and trough concentration:MIC values for each isolate. The relationship between the 2 measures of drug exposure and the probability of suppressing galactomannan concentrations ≤1 was explored using logistic regression.

The experimental results were bridged to humans using a population PK model for voriconazole fitted to data from healthy volunteers and patients [10]. Using the simulation module of the PK program ADAPT 5 [11], a standard intravenous regimen of 6 mg/kg for 2 dosages followed by 4 mg/kg every 12 hours was administered to 5000 simulated patients, and the resultant AUCs were obtained at the end of the first week of therapy. The proportion of patients that exceeded AUC:MIC values of 55 and 32.1 for CLSI and EUCAST methodologies, respectively, was determined. The MIC distributions determined using CLSI methodology were obtained from the publication of Espinel-Ingroff et al [12]. For EUCAST methodology, data were obtained and pooled from Denmark (M. C. A.), Spain (M. C. E.), and Austria (C. L. F.) (1426 clinical A. fumigatus isolates).

RESULTS

Minimum Inhibitory Concentrations

The MICs for voriconazole against A. fumigatus determined using both CLSI and EUCAST methodologies are summarized in Table 1. In general, EUCAST methodology resulted in MICs that were 1 dilution higher compared with CLSI. The geometric means that were used in the PD analyses from 13 separate experiments are also summarized in Table 1.
The dynamic model of the human alveolus enabled humanlike voriconazole concentration time profiles to be replicated. The integrity of the cellular bilayer was maintained after 48 hours in the circuit, demonstrated by a lack of translocation of 1% dextran blue.

**PK and PD of Voriconazole in the Dynamic Model**

The kinetics of galactomannan release into the central circuit was observed in untreated organisms and was similar for all 4 strains (Panel B in Figures 2–5). Galactomannan concentrations began to increase approximately 16–24 hours postinoculation, after which a maximum was reached after approximately 36 hours. The rate of increase and the maximum galactomannan concentrations were comparable between each strain, with similar estimates for Kgmmax (data not shown).

Human like concentration–time profiles for voriconazole were achieved (see Figures 2–5). The exposure–response relationship for voriconazole against each of the 4 strains was distinct. The trough concentration that was required to achieve near-maximal suppression of circulating galactomannan concentrations in the wild-type was approximately 1 mg/L (see Figure 2). In contrast, a trough concentration of approximately 4 mg/L was required to suppress galactomannan production from isolate F16216 carrying L98H (Figure 3). Moreover, trough concentrations of approximately 15 mg/L were required to suppress isolates F11628 and F13747 carrying G138C and G434C, respectively (Figures 4 and 5).

**Mathematical Modeling**

The fit of the mathematical model to the PK and PD data was highly acceptable for each strain. A linear regression of observed–predicted values after the Bayesian step from each model for each strain had an intercept and slope that approximated 0 and 1, respectively (data not shown). The fit of each model to the data is shown in Figures 2–5.

The mathematical model was used to construct the relationship between the AUC24–48 and the predicted galactomannan concentrations at the end of the experimental period (ie, 48 hours post–treatment initiation, 54 hours postinooculation). The AUC values that were associated with near-maximal antifungal effect varied by approximately 10-fold between isolates. Importantly, however, the AUC:MIC values (regardless of methodology) that were associated with near-maximal antifungal effect only varied by approximately 2-fold. The AUC:MIC values determined according to CLSI and EUCAST methodologies that resulted in a 90% probability of galactomannan concentrations ≤1 for all strains were 55 and 32.1, respectively. Similarly, the trough concentration:MIC values determined according to CLSI and EUCAST methodologies that resulted in a 90% probability of galactomannan concentrations ≤1 for all strains were 1.68 and 1, respectively.

**DISCUSSION**

Invasive aspergillosis remains a lethal infectious syndrome that leads to suboptimal clinical outcomes in patients [1]. Although the anti-Aspergillus triazoles are safe and effective agents for the treatment of this syndrome, relatively little is known about the PD of these agents against either wild-type or resistant isolates. This is primarily related to limitations of currently available experimental models. Here, we describe the development of a novel dynamic in vitro model of the human alveolus that enables an estimate of the magnitude of drug exposure associated with near-maximal antifungal activity against *A. fumigatus*.

This dynamic in vitro model of the human alveolus enables some of the inherent limitations in laboratory animal models of invasive fungal infections to be circumvented. The model mimics the architecture of the human alveolus, as well as a number of important factors that can influence drug exposure–response relationships (eg, 2% protein that enables drug–protein interactions). The most important advantage of this model is the ability to reproduce humanlike PK. The PK of voriconazole in mice are characterized by nonlinear PK, rapid elimination, and auto-induction of clearance [13]. These differences may limit the broad applicability of findings from laboratory animals for humans. Despite these advantages, there are a number of potential limitations of this model. Most important, the dynamic model is a mimic of early invasive disease and there are no immune effector cells, which have an impact upon antifungal exposure–response relationships.

An AUC:MIC value of 55 and a trough concentration:MIC value of 1.68 are associated with a 90% probability of suppression of galactomannan ≤1 for all isolates (CLSI methodology). Similarly, if MICs are measured using EUCAST methodology, an AUC:MIC value of 32.1 and a trough concentration:MIC value of 1 are associated with successful therapy. Ultimately, we could have chosen any number of PD cut-off values. A value of 0.5 is used for a diagnosis of invasive aspergillosis, but the appropriate cutoff value for assessing response to antifungal therapy is not known. If this lower cutoff of 0.5 is used, the
Figure 2. The pharmacokinetics (A, C, E, and G) and pharmacodynamics (B, D, F, and H) of various dosages of voriconazole for the wild-type strain. The open squares are the individual pharmacokinetic and pharmacodynamic data points from each circuit, and the solid line is the fit of the mathematical model (Bayesian posterior estimates).
Figure 3. The pharmacokinetics (A, C, E, and G) and pharmacodynamics (B, D, F, and H) of various dosages of voriconazole for Aspergillus fumigatus F16Z16 carrying L98H. The open squares are the individual pharmacokinetic and pharmacodynamic data points from each circuit, and the solid line is the fit of the mathematical model (Bayesian posterior estimates).
Figure 4. The pharmacokinetics (A, C, E, and G) and pharmacodynamics (B, D, F, and H) of various dosages of voriconazole for Aspergillus fumigatus F11628 carrying G138C. The open squares are the individual pharmacokinetic and pharmacodynamic data points from each circuit, and the solid line is the fit of the mathematical model (Bayesian posterior estimates).
Figure 5. The pharmacokinetics (A, C, E, and G) and pharmacodynamics (B, D, F, and H) of various dosages of voriconazole for Aspergillus fumigatus F13747 carrying G434C. The open squares are the individual pharmacokinetic and pharmacodynamic data points from each circuit, and the solid line is the fit of the mathematical model (Bayesian posterior estimates).
AUC:MIC and trough concentration:MIC values associated with a 90% chance of success using CLSI methodology are 75.56 and 2.32, respectively. For EUCAST methodology, an AUC:MIC value of 45.59 and a trough concentration:MIC value of 1.39 are associated with a 90% chance of success. Use of these higher PD targets slightly alters the fractional attainment rates shown in Figure 6 but does not alter the proposed breakpoints. Interestingly, the targets using CLSI methodology are similar to those derived for voriconazole against Candida albicans, for which a total drug AUC:MIC value of approximately 60 is associated with half-maximal antifungal effect in mice [14]. Furthermore, a recent analysis of patients infected with a variety of fungal pathogens treated with voriconazole suggests a trough concentration:MIC value of 2–5 is associated with a suitably high probability of clinical response [15]. The PD targets reported in this study may not apply to non-*fumigatus* species, and further specific studies are required to derive these values.

The human population PK model and Monte Carlo simulations enable the drug exposure targets to be placed in a clinical context and provide decision support for setting in vitro susceptibility breakpoints. The simulations suggest that for both CLSI and EUCAST methodologies, isolates with MICs < 1 mg/L are susceptible; whereas, isolates with MICs > 2 mg/L could be classified as resistant. Isolates with an MIC of 1 mg/L determined using CLSI methodology are only predicted to be successfully treated in 75.3% of cases (Figure 6). Therefore, a reasonable classification for isolates with an MIC of 1 and 2 mg/L using CLSI could be “intermediate” and “resistant,” respectively. Because MICs obtained using EUCAST methodology tend to be approximately 1 dilution higher than those obtained using CLSI methodology, the same isolates with an MIC using this method could be classified as “sensitive” and “intermediate,” respectively. Our experimental data suggest that underlying resistance mechanisms can be overcome with higher drug exposures. Thus, an isolate with an MIC of 2 mg/L determined using EUCAST methodology may be treatable if higher than average drug exposures can be obtained. Dosage escalation requires careful clinical monitoring given the nonlinear PK and well-described toxicodynamics of voriconazole [10, 16, 17].

In conclusion, this dynamic in vitro model of the human alveolus is a potentially useful model to investigate the PD of antifungal agents against *Aspergillus* spp. This model circumvents many of the limitations of laboratory animal models of voriconazole therapy and provides valuable insights for the treatment of patients with invasive pulmonary aspergillosis.

**Notes**

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