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Susceptibility Breakpoints for Amphotericin B and *Aspergillus* Species in an *In Vitro* Pharmacokinetic-Pharmacodynamic Model Simulating Free-Drug Concentrations in Human Serum


Clinical Microbiology Laboratory, Attikon University Hospital, National and Kapodistrian University of Athens, Athens, Greece; Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands; Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands

Although conventional amphotericin B was for many years the drug of choice and remains an important agent against invasive aspergillosis, reliable susceptibility breakpoints are lacking. Three clinical *Aspergillus* isolates (*Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus terreus*) were tested in an *in vitro* pharmacokinetic-pharmacodynamic model simulating the biphasic 24-h time-concentration profile of free amphotericin B concentrations in human serum with free peak concentrations (*fC* max) of 0.1, 0.3, 0.6, 1.2, and 2.4 mg/liter administered once daily. Drug concentrations were measured with a bioassay, and fungal growth was monitored for 72 h with galactomannan production. The *fC* max/MICs correspond to half-maximal activity (*P* 50) was determined for each species, and the percentage of target attainment was calculated for different MICs for the standard (1 mg/kg of body weight) and a lower (0.6-mg/kg) dose of amphotericin B with Monte Carlo simulation analysis. The *fC* max/MICs (95% confidence intervals) corresponding to *P* 50 were 0.145 (0.133 to 0.158), 0.371 (0.283 to 0.486), and 0.41 (0.292 to 0.522) for *A. fumigatus*, *A. flavus*, and *A. terreus*, respectively. The median percentages of *P* 50 attainment were ≥88%, 47%, and 0% for *A. fumigatus* isolates with MICs of ≤0.5, 1, and ≥2 mg/liter, respectively, and ≥81%, 24%, and 0% and ≥75%, 15%, and 0% for *A. flavus* and *A. terreus* isolates with MICs of ≤0.25, 0.5, and ≥1 mg/liter, respectively. The lower dose of 0.6 mg/kg would retain efficacy for *A. fumigatus*, *A. flavus*, and *A. terreus* isolates with MICs of ≤0.25, ≤0.125, and ≤0.125 mg/liter, respectively. The susceptibility, intermediate susceptibility, and resistance breakpoints of ≤0.5, 1, and ≥2 mg/liter for *A. fumigatus* and ≤0.25, 0.5, and ≥1 mg/liter for *A. flavus* and *A. terreus* were determined for conventional amphotericin B with a pharmacokinetic-pharmacodynamic model simulating free-drug serum concentrations.

Invasive aspergillosis is a life-threatening disease among patients with leukemia and bone marrow and solid-organ transplantation (1). *Aspergillus fumigatus* is the most common pathogen causing invasive aspergillosis, accounting for >70% of the cases, followed by *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus niger* (2). Conventional amphotericin B was for many years the drug of choice and is still used against invasive aspergillosis. Despite its *in vitro* potent antifungal activity against *Aspergillus* spp., clinical trials have shown that conventional amphotericin B therapy is associated with <60% survival in patients with invasive aspergillosis (3–6). Although underlying disease, immunosuppression, toxicity, and timing of antifungal therapy affect the mortality of these infections, pathogen susceptibility and drug serum concentrations also represent important contributing factors. *In vivo* studies have shown that the best efficacy was achieved when the maximum concentration of amphotericin B in serum exceeded 2.4 times the MIC of the *A. fumigatus* isolate (7). However, *in vitro* antifungal susceptibility testing of amphotericin B is challenged by the lack of reliable susceptibility breakpoints for each *Aspergillus* species.

The epidemiological cutoff values 2, 2, and 4 mg/liter for the CLSI method and 1, 4, and 4 mg/liter for the EUCAST method were determined for *A. fumigatus*, *A. flavus*, and *A. terreus*, respectively, in order to detect isolates with extreme MICs (8, 9). The current susceptibility breakpoints for *Aspergillus* spp. with the CLSI and EUCAST methodologies are ≤1 mg/liter (9, 10). However, the supporting clinical and experimental *in vivo* data are poor, and no pharmacokinetic-pharmacodynamic (PK-PD) studies have validated this breakpoint (9). In a retrospective case-control study of 29 patients, 22/23 patients with isolate MICs of ≥2 mg/liter died, whereas 6/6 patients with isolate MICs of ≤1 mg/liter survived (11). Of note, 5/6 survivors with isolate MICs of ≤1 mg/liter underwent surgery or resolved their neutropenia. In two other large retrospective studies of 160 and 40 patients, no correlation was found between an *Aspergillus* MIC of ≥2 mg/liter and clinical outcome in patients with invasive aspergillosis treated with conventional amphotericin B (12, 13). Notably, more than 95% of *A. fumigatus* isolates have MICs of ≤1 mg/liter (8). Therefore, the majority of the isolates would be considered wild-type susceptible based on the 1-mg/liter cutoff, while conventional amphotericin B therapy has been associated with <60% of survival in all clinical trials (3, 4, 14). Furthermore, treatment failure was observed in patients infected with *A. fumigatus* and *A. flavus* isolates with CLSI MICs of 0.25 to 0.5 mg/liter and 1 mg/liter, respectively, when treated with the standard dose of 1 mg/kg of conventional amphotericin B (15, 16). Thus, a clinically relevant and
useful susceptibility breakpoint for amphotericin B and Aspergil-
lus spp. is warranted.

An in vitro pharmacokinetic-pharmacodynamic model was re-
cently developed for Aspergillus spp., simulating pharmacokinetics
of antifungal drugs (17). This model revealed considerable phar-
macodynamic differences among Aspergillus isolates reflecting
differences in time- and concentration-dependent inhibitory and
fungicidal activity that were not captured by the MIC (18). Using
the same model and simulating multidose human pharmacoki-
netics of voriconazole, in vitro data were predictive of experimen-
tal animal data and clinical outcome for A. fumigatus, enabling the
determination of susceptibility breakpoints and target values for
therapeutic drug monitoring (19).

Therefore, in the present study, we simulated multidose phar-
macokinetics of amphotericin B and studied its activity against A.
fumigatus, A. flavus, and A. terreus. The in vitro PK-PD relation-
ship was described and bridged with human PKs in order to de-
termine susceptibility breakpoints for each species.

MATERIALS AND METHODS
Isolates. Three clinical strains of A. fumigatus (AFM 4215; ATCC MYA-
1163), A. flavus (AFL 113), and A. terreus (AT 137) isolated from patients
with invasive aspergillosis were studied. The MICs of amphotericin B as
determined thrice according to the CLSI broth microdilution method
were 1 mg/liter for all Aspergillus species. The strains were maintained
at −70°C in 10% glycerol and cultured twice in Sabouraud dextrose agar
at 30°C for 5 to 7 days. A conidial suspension was prepared in normal
saline at 1% Tween 20. Conidia were counted with a Neubauer cham-
ber in order to obtain a final suspension of 1 × 10⁶ CFU/ml, and their
concentration was confirmed by quantitative cultures on Sabouraud dext-
rose agar.

Antifungal drug and medium. Pure powder of amphotericin B (Sig-
ma-Aldrich, Bioline, Athens, Greece) was reconstituted at 5,000 mg/liter
in dimethyl-sulfoxide and was stored at −70°C. The medium contained
10.4 g/liter RPMI 1640 with glutamine without sodium bicarbonate (Sig-
ma-Aldrich, St. Louis, MO) and 0.165 M buffer morpholinepropanesul-
fonic acid (MOPS) (In vitrogen, Carlsbad, CA), pH 7.0, with 100 mg/liter
chloramphenicol (Sigma-Aldrich, St. Louis, MO).

In vitro pharmacokinetic-pharmacodynamic model. The in vitro
pharmacokinetic simulation model consisted of (i) a glass beaker contain-
ing 700 ml medium (external compartment [EC]) in which was placed (ii)
a dialysis tube of a 10-ml volume (internal compartment [IC]) made of a
permeable cellulose membrane, which allowed free diffusion of molecu-
es with a molecular mass of <20 kDa, and (iii) a peristaltic pump (Miniplus
Evolution, Gilson, France), which removed the content of EC and added
fresh medium within it at a rate equivalent to drug removal from human
growth at each dose was calculated based on the AUCGI of each dose
and drug-free control. The percentage of fungal

determination at 0 h, 4 h, 6 h, 8 h, 20 h, 24 h, 44 h, 48 h, and 72 h after the
introduction of the drug in the IC using the bioassay. The data were
analyzed with nonlinear regression based on a two-compartment model
described by the equation C = Ceαt + Ceβt, where α and β are the
rate constants, Ce and Cg are the y intercepts for alpha and beta, respec-
tively, and C is the concentration at a given time t. The half-lives of alpha
and beta phases were calculated using the equations t1/2,α = ln(2)/kα and t1/2,β = ln(2)/kβ.

Determination of fungal growth. Fungal growth in the IC was as-
essed in samples of 100 µl at regular time intervals by determining galac-
tomannan production using an enzyme-linked immunosorbent assay
(ELISA) (Plateelia; Bio-Rad, Athens, Greece). Samples were diluted with
200 µl saline in order to reach the final volume of 300 µl before process-
ing. Results were expressed as a galactomannan index (GI) according to
the manufacturer’s instructions. Galactomannan levels were also deter-
mined in the EC in order to ensure that no galactomannan escaped from the
IC.

Pharmacodynamic analysis. In vitro pharmacodynamics of each
amphotericin B dose and Aspergillus spp. species were determined based on
the GI-time relationship analyzed with the Emax model: E = E_min + E_max ×
T/(T² + T₀⁻⁻), where E is the GI (dependent variable), E_min and E_max are
the maximum and minimum GI, respectively. T is the incubation time
(independent variable), T₀ is the time corresponding to 50% of the E_max,
and y is the slope of the curve. As shown previously, the parameters E_max,
y, and T₀ describe the extent, rate, and time of galactomannan produc-
tion, respectively, whereas the area under the galactomannan index-time
curve (AUCGI) is a surrogate marker of fungal growth capturing any dif-
fferences in extent, rate, and time of antifungal activity. The higher the
AUCGI, the greater is the fungal growth over time. The area under the
galactomannan index-time curve (AUCGI) was calculated for each
amphotericin B dose and drug-free control. The percentage of fungal
growth at each dose was calculated based on the AUCGI of each dose
divided by the AUCGI of the drug-free growth control. The percent
growth-FCmax/MIC relationship was analyzed with the Emax model: E =
E_min + E_max × P/(P² + P₀⁻⁻), where E is the percent growth (dependent variable), E_max and E_min are the maximum and minimum percent growth,
respectively, P is the PK-PD index FCmax/MIC (independent variable), P₀ is
the FCmax/MIC corresponding to 50% of E_max, and y is the slope of the
curve. In addition, the FCmax/MIC corresponding to near-maximum an-

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Amphoterocin B Breakpoints for Aspergillus

22319 (18, 21). Specifically, P. variotii conidia at final concentration of 5 ×
In vitro pharmacokinetics of AMB

![Graph showing in vitro pharmacokinetics of amphotericin B](image)

**FIG 1** Multidose pharmacokinetics of amphotericin B in the *in vitro* model with different $fC_{\text{max}}$ and $t_{1/2}$ of 0.2 to 2 h and 10 to 17 h for alpha and beta phases, respectively. Coefficient of variations was ±15% between two replicates.

Amphotericin B, 0.6 mg/kg, used in clinical practice. This dose resulted in a serum $t_{C_{\text{max}}}$ of 1.43 ± 0.2 mg/liter, corresponding to an $fC_{\text{max}}$ of 0.0715 ± 0.01 mg/liter. The median, upper, and lower 95% CI limits of percentage of $P_{50}$ target attainment were calculated for each species and for different MICs. Finally, because trough levels ($C_{\text{min}}$) are clinically easier obtained than $C_{\text{max}}$ levels, the trough levels were assessed using linear regression analysis with $C_{\text{max}}$ concentrations in the *in vitro* PK-PD model.

**Statistical analysis.** All analysis was performed with the Prism 5.01 software (GraphPad Inc., La Jolla, CA). All $E_{\text{max}}$ models were globally fitted to the data, with $E_{\text{max}}$ and $E_{\text{min}}$ shared among data sets. Comparisons between $E_{\text{max}}$ model parameters among *Aspergillus* species were assessed using the extra sum-of-squares F test. Monte Carlo analysis was performed with the random function of an Excel spreadsheet (MS Office 97). A P value of <0.05 was considered statistically significant.

**RESULTS**

**Pharmacokinetic analysis.** The time-concentration profiles of amphotericin B doses are shown in Fig. 1. The calculated $fC_{\text{max}}$ in the IC were within ±20% of the target $fC_{\text{max}}$ of 0.1, 0.3, 0.6, 0.8, 1, 1.2, and 2.4 mg/liter. The half-lives of alpha and beta phases were 0.2 to 2 h and 10 to 17 h, respectively.

**Pharmacodynamic analysis.** Fungal growth was progressively inhibited at higher concentrations of amphotericin B. The GI-time curves followed a sigmoid pattern described well by the $E_{\text{max}}$ model ($R^2 > 0.82$), and they were characterized by the same $E_{\text{max}}$ but different slopes and $T_{50}$s for the different doses of amphotericin B and *Aspergillus* species tested (Fig. 2). Complete inhibition of fungal growth was found for *A. fumigatus* with an amphotericin B $fC_{\text{max}}$ of ≥0.3 mg/liter, while for *A. flavus* and *A. terreus*, complete inhibition was obtained with an amphotericin B $fC_{\text{max}}$ of ≥0.8 mg/liter. In particular, the percentages of fungal growth at $fC_{\text{max}}$ of 0.1, 0.3, and 0.6 mg/liter were 97%, 78%, and 74% for *A. flavus* and 96%, 82%, and 67% for *A. terreus*, respectively.

The $fC_{\text{max}}$/MIC relationship followed a sigmoid pattern (global $R^2 = 0.96$) for all three species (Fig. 3). The $fC_{\text{max}}$/MIC (95% CI)

![Graphs showing pharmacodynamic analysis](image)

**FIG 2** Multidose pharmacodynamic analysis of simulated amphotericin B doses with $fC_{\text{max}}$ of 0.1, 0.3, 0.6, 0.8, 1, 1.2, and 2.4 mg/liter against *A. fumigatus*, *A. flavus*, and *A. terreus* isolates with a modal CLSI MIC of 1 mg/liter as determined by the galactomannan index in the *in vitro* PK-PD model.
associated with 50% growth for *A. fumigatus* was 0.145 (0.133 to 0.158), which was statistically significantly lower than the corresponding \(f_{C_{\text{max}}}/\text{MIC}\) of *A. flavus* (0.371; 0.283 to 0.486) and *A. terreus* (0.41; 0.292 to 0.522) \((P < 0.001)\). The \(f_{C_{\text{max}}}/\text{MICs}\) corresponding to near maximal activity (20% growth) were 0.188 (0.166 to 0.213), 0.474 (0.362 to 0.655), and 0.545 (0.376 to 0.714) for *A. fumigatus*, *A. flavus*, and *A. terreus*, respectively.

**Monte Carlo simulation analysis.** Monte Carlo simulation analysis of 1,000 patients receiving the standard amphotericin B dose of 1 mg/kg resulted in mean \(C_{\text{max}}\) ± standard deviation (SD) values of 2.8 ± 1.2 mg/liter \((f_{C_{\text{max}}} \pm SD\) of 0.14 ± 0.06 mg/liter). These values are close to the target values previously found in clinical studies (23). The percentage of patients attaining the PK-PD target \(\left(f_{C_{\text{max}}}/\text{MIC}\right)\) associated with half-maximal \(P_{50}\) activity is shown in Fig. 4. Higher-than-75% and lower-than-15% \(P_{50}\) target attainment was found for *A. fumigatus* isolates with MICs of \(\geq 0.5\) and \(\geq 2\) mg/liter and for *A. flavus* and *A. terreus* isolates with MICs of \(\leq 0.25\) and \(\leq 1\) mg/liter. In particular, the median (range) percentages of target attainment for *A. fumigatus*, *A. flavus*, and *A. terreus* isolates with a MIC of 1 mg/liter were 47% (39 to 57%), 0% (0 to 0%), and 0% (0 to 0%) and with a MIC of 0.5 mg/liter were 88% (87 to 90%), 24% (5 to 50%), and 15% (2 to 46%), respectively. The percentages of \(P_{50}\) target attainment for *A. fumigatus*, *A. flavus*, and *A. terreus* isolates with a MIC of 1 mg/liter were 22%, 0%, and 0%, respectively (data not shown).

For the lower amphotericin B dose of 0.6 mg/kg, a >98% \(P_{50}\) target attainment was found for MICs of \(\leq 0.25\), \(= 0.125\), and \(\leq 0.125\) mg/liter for *A. fumigatus*, *A. flavus*, and *A. terreus*, respectively (Fig. 4). A linear correlation \((r^2 = 0.98)\) was found between the \(C_{\text{max}}\) and the \(C_{\text{max}}\) levels in the *in vitro* model with a slope of 0.20 ± 0.03, indicating that \(C_{\text{max}}\) levels correspond to 1/5 of the \(C_{\text{max}}\) concentrations, as previously found in humans (22, 23).

**DISCUSSION**

The multidose pharmacodynamic study of amphotericin B using an *in vitro* pharmacokinetic-pharmacodynamic model that simulated free serum amphotericin B concentrations revealed differences among the three *Aspergillus* species. Complete growth inhibition was observed at an \(f_{C_{\text{max}}}/\text{MIC}\) of \(= 0.3\) mg/liter for *A. fumigatus* and \(\geq 0.8\) mg/liter for *A. flavus* and *A. terreus* despite their identical MIC values. The \(f_{C_{\text{max}}}/\text{MICs}\) (95% CI) corresponding to near-maximal activity were 0.188 (0.166 to 0.213), 0.474 (0.362 to 0.655), and 0.545 (0.376 to 0.714) for *A. fumigatus*, *A. flavus*, and *A. terreus*, respectively. These differences are in agreement with our previous study where single-dose pharmacokinetics and a higher initial inoculum were used, providing better discrimination of the *in vitro* activity of amphotericin B against the three species (18). The observed differences among the three species were attributed to the fast inhibitory action and increased killing rate against *A. fumigatus*, a slower inhibitory action and reduced killing efficiency against *A. flavus*, and the slowest inhibitory action and no killing against *A. terreus* (18).

The PK-PD target of near maximal antifungal activity found in the present study against *A. fumigatus* is in agreement with that previously obtained in a murine neutropenic model of experimental pulmonary aspergillosis demonstrating that the \(C_{\text{max}}/\text{MIC}\) of 2.4 was associated with near-maximal survival (7). Considering that the free-drug percentage of conventional amphotericin B in mouse serum corresponded to 7.4% (25), the *in vivo* \(f_{C_{\text{max}}}/\text{MIC}\) corresponds to 0.188, which is very close to the \(f_{C_{\text{max}}}/\text{MIC}\) of 0.178 found in the present study. In addition, the percentage of patients attaining the PK-PD target associated with half-maximal activity \(P_{50}\) for *A. fumigatus*, *A. flavus*, and *A. terreus* isolates with different MICs based on the Monte Carlo analysis simulating 1,000 patients treated with 1 or 0.6 mg/kg of conventional amphotericin B. Error bars represent the range of percentage of target attainment for the upper and lower 95% CI limit of the \(P_{50}\) target.

**FIG 3** Multidose exposure-efficacy relationship of amphotericin B against each *Aspergillus* species with modal CLSI MICs of 1 mg/liter for *A. fumigatus*, *A. flavus*, and *A. terreus* in the *in vitro* PK-PD model simulating amphotericin B human serum levels based on the increasing amphotericin B \(f_{C_{\text{max}}}/\text{MIC}\) (maximum concentration) and the galactomannan index as a marker of fungal growth.

**FIG 4** The percentage of patients attaining the PK-PD target associated with half-maximal activity \(P_{50}\) for *A. fumigatus*, *A. flavus*, and *A. terreus* isolates with different MICs based on the Monte Carlo analysis simulating 1,000 patients treated with 1 or 0.6 mg/kg of conventional amphotericin B. Error bars represent the range of percentage of target attainment for the upper and lower 95% CI limit of the \(P_{50}\) target.
of 1 to 2 mg/liter (26 to 29). The dose of 5 mg/kg, which corresponds to a $C_{\text{max}}$ around 2.5 mg/liter (i.e., $f_{\text{C}}_{\text{max}}$ of 0.185 mg/liter) in mouse serum (7, 30), would attain the PK-PD targets determined in the present study for A. fumigatus but not for A. terreus and A. flavus isolates. However, there are many confounding factors, like immunosuppression, type and severity of infection, dosing regimens, time of initiation of antifungal therapy, pharmaco-tors, like immunosuppression, type and severity of infection, dos- 

The percentage of 1-week survival, whereas the percentage of 6-week survival found previously in a retrospective study being nonsusceptible (3). Further- 

the choice of the in vitro PK-PD target ($P_{50}$ or $P_{90}$) for corre- 

The present in vitro model indicated that the standard dose of 1 mg/kg of amphotericin B is associated with 47% target attainment using a half-maximum activity PK-PD target ($P_{50}$) and 22% using a near-maximum activity PK-PD target ($P_{90}$) for Aspergillus fumigatus isolates with an amphotericin B MIC of 1 mg/liter. This is in agreement with a previous retrospective study where the MICs of 274 clinical Aspergillus isolates from transplant recipients with proven or probable invasive aspergillosis were analyzed togeth- 

The choice of the in vitro PK-PD target ($P_{50}$ or $P_{90}$) for corre- 

The percentage of $P_{90}$ target attainment was comparable to 12-week survival, whereas the percentage of $P_{50}$ target attainment was comparable to 6-week survival. High serum drug exposure ($P_{90}$) may result in high concentrations at the site of infection and/or fungicidal actions that will likely produce a long-lasting effect (12-week survival) compared to the lower drug exposure ($P_{50}$) that will produce a short-lasting effect (6-week survival). However, since most deaths during the second 6-week period of 12-week therapy were attributed to causes other than invasive aspergillosis, albeit with some uncertainty, the 6-week survival endpoint was suggested to be the preferable endpoint to judge the effectiveness of antifungal therapy (35). Because the percentage of $P_{90}$ target attainment found by Monte Carlo analysis of the present study for A. fumigatus isolates with an amphotericin B MIC of 1 mg/liter was correlated with the 6-week survival found previously in a ret- 

An important assumption when bridging in vitro concentrations to serum PK is that the serum concentrations correspond to the tissue concentrations at the site of infection, which is the lung in the case of invasive pulmonary aspergillosis. In vivo pharmacokinetic data of conventional amphotericin B in mice showed that although the $C_{\text{max}}$ in the lung was lower than the $C_{\text{max}}$ in serum (3.33 and 4.58 mg/liter, respectively), the $C_{\text{max}}$ values were similar (0.33 and 0.34 mg/liter, respectively) based on the different degree of protein binding in the lung (10%) compared to that in serum (7.4%) (30). In two clinical studies, a wide range of amphotericin B lung concentrations (0.1 to 23.3 µg/g) were detected with high-performance liquid chromatography (HPLC) in patients with mean concentrations of 11.29 and 5.29 µg/g (36, 37). However, only a small (1.5% to 30%) proportion of HPLC-detected ampho- 

The percentage of 1-week survival) compared to the lower drug exposure ($P_{90}$) respectively. 

The present study, all patients with A. flavus isolate MICs of $\geq$1 mg/liter died. These clinical data provide further support of the breakpoints determined in the present PK-PD model. 

In order to maximize the efficacy and minimize the toxicity of conventional amphotericin B treatment, one could increase or decrease the dose based on the MIC of isolated pathogens or epidemiological data of each center. Thus, for a small number of A. fumigatus, A. flavus, and A. terreus strains with MICs of $\leq$0.25, $\leq$0.125, and $\leq$0.125 mg/liter, respectively, the lower dose of 0.6 mg/kg would retain efficacy of the 1-mg/kg dosage but reduce its toxicity. The 0.6-mg/kg dose was associated with 30% renal toxicity compared to $>50%$ renal toxicity associated with the 1-mg/kg dose of amphotericin B (5, 40). For isolates with inter- 

A. fumigatus, A. flavus, and A. terreus isolates estimated in the present study being nonsusceptible (16). Interest- 

Since in vivo amphotericin B is bound by tissue and slowly released, accounting for the prolonged (serum) terminal elimination half-life of the drug, the correlation between $f_{\text{C}}_{\text{max}}$ and $C_{\text{max}}$ may be clinically relevant only for the first few days of therapy. However, the unavoidable toxicity of conventional amphotericin B, which often results in drug discontinuation, will be the limiting factor even for susceptible isolates, limiting the use of conventional amphotericin B, in agreement with the ECIL guidelines for the management of invasive aspergillosis (42). Therefore, lipid formulations of amphotericin B could be used efficiently even for isolates with reduced susceptibility to conventional amphotericin
B, as previously shown in an animal model infected by an A. fumigatus isolate with a MIC of 1 mg/liter (43). Lipid formulations can safely deliver high concentrations of amphotericin B in the fungal target and at the site of infection (44).

In conclusion, a PK-PD model was used to simulate free amphotericin B serum concentrations and to study the in vitro activity against three Aspergillus species. The PK-PD targets were 0.145, 0.371, and 0.41 fCmax/MIC for A. fumigatus, A. flavus, and A. terreus, respectively, and the susceptibility breakpoints derived were \( \leq 0.5, 1, \) and \( \geq 2 \) mg/liter for A. fumigatus and \( \leq 0.25, 0.5, \) and \( \geq 1 \) mg/liter for A. flavus and A. terreus. The suggested breakpoints are substantiated with results obtained from previously published clinical studies of invasive aspergillosis.

REFERENCES


