Antifungal Pharmacokinetics and Pharmacodynamics

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Successful treatment of infectious diseases requires choice of the most suitable antimicrobial agent, comprising consideration of drug pharmacokinetics (PK), including penetration into infection site, pathogen susceptibility, optimal route of drug administration, drug dose, frequency of administration, duration of therapy, and drug toxicity. Antimicrobial pharmacokinetic/pharmacodynamic (PK/PD) studies consider these variables and have been useful in drug development, optimizing dosing regimens, determining susceptibility breakpoints, and limiting toxicity of antifungal therapy. Here the concepts of antifungal PK/PD studies are reviewed, with emphasis on methodology and application. The initial sections of this review focus on principles and methodology. Then the pharmacodynamics of each major antifungal drug class (polyenes, flucytosine, azoles, and echinocandins) is discussed. Finally, the review discusses novel areas of pharmacodynamic investigation in the study and application of combination therapy.

PHARMACOKINETICS

Pharmacokinetic (PK) studies describe the host exposure and elimination of a drug. Common PK measures include absorption, accumulation in various tissues or compartments, metabolism, and elimination. From a pharmacodynamic (PD) perspective, PK measures represent drug exposure with respect to the host, for example, translating a dose (i.e., mg/kg) to a drug exposure (e.g., area under the concentration curve, or AUC) (Drusano 2004). Traditional PK measures considered in PD analyses include the maximal concentration ($C_{\max}$), the total exposure or AUC, and assessment of concentration over time by use of the elimination

half-life. The most common site of PK assessment is the serum. However, other PK tissue sites of suggested clinical relevance include the cerebrospinal fluid, ocular compartments, the urine, and the epithelial lining fluid (ELF) compartment of the lung. Most fungal infections occur in the extracellular fluid compartment, and, therefore, serum or plasma drug concentrations correlate quite well with drug concentrations at the site of infection (Mukherjee et al. 2006). This has been shown to be true for candidiasis, including mucocutaneous and invasive/disseminated disease (Andes 2004; Mukherjee et al. 2006). However, a subset of fungal infections involves primarily pulmonary parenchymal disease, for example, aspergillosis and zygomycosis.

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cetes. The relevance of drug concentration within the ELF of the lung has been investigated, but the evidence that ELF concentrations are more predictive of serum or plasma concentrations is lacking. This is likely because of the fact that these disease syndromes are invasive in nature, with significant tissue involvement. However, ELF concentrations may be more relevant for prophylaxis as the organism must first colonize and infect the alveolar space, and prophylaxis studies in animal models using ELF concentrations are an emerging area of study (Rodvold et al. 2011). Infection of the central nervous system (CNS), eye, and lower urinary tract are examples in which drug penetration and PK measurement at the site of infection has been shown to be relevant (Perfect et al. 1986; Savani et al. 1987; Groll et al. 2000; Gauthier et al. 2005). For example, only flucytosine and fluconazole achieve reliably therapeutic urine concentrations for treatment of lower urinary tract infections (Fisher et al. 2011). For intraocular and CNS infections fluconazole, voriconazole, flucytosine, and liposomal amphotericin B would be optimal from the PK standpoint (Utz 1975; Brammer et al. 1990; Purkins et al. 2002; Sun et al. 2009; Riddell et al. 2011; Schwartz et al. 2011).

An additional PK measurement that warrants consideration is binding of drug to serum albumin (Kunin et al. 1973; Craig and Kunin 1976; Craig and Ebert 1989). A relatively large number of antimicrobial treatment studies have shown that only free, non-protein-bound drug is pharmacologically active (Craig and Welling 1977; Zeitlinger et al. 2011). This can be a result of limited penetration of protein-bound drug to the site of infection as well as limited ability of protein-bound drug to bind at the site of activity. In the context of antifungal drugs, the relevance of protein binding has been shown for both triazoles and echinocandins and will be elucidated in the following sections (Andes 2003a; Andes et al. 2010).

In Vitro Drug Potency

A unique factor in antimicrobial PD studies is ability to consider PK relative to a measure

of drug potency, specifically the in vitro susceptibility test most commonly expressed as the minimum inhibitory concentration (MIC). A variety of antifungal susceptibility testing protocols have been standardized using broth microdilution, disk-diffusion, and antifungal gradient strip (Etest) methodologies (Wanger et al. 1995; CLSI 2004, 2008a,b,c; EUCAST 2008, 2012). In each, the goal is to define the minimum concentration of drug that inhibits some degree of organism growth MIC. MIC end points and time at which they are read can differ from drug to drug and between standardized methodologies (Chrysanthou and Cuenca-Estrella 2006; Pfäler and Diekema 2012).

In Vitro PK/PD Models

In vitro PK/PD studies are often the first to define the PD characteristics. The majority of in vitro PK/PD studies use a nutrient broth system of variable complexity ranging from a single static environment to multiple chambers to allow the investigator to closely mimic human PK profiles. The advantages of in vitro systems include the ability to frequently assess microbiologic effect and closely parallel PK in patients. The disadvantages of in vitro approaches include the lack of the clinical infection site, which does not account for the host immune system and tissue PK, including protein binding. Additionally, in vitro models do not always account for variability in organism fitness that is often observed in vivo. However, the models provide an opportunity to accurately assess PD characteristics, such as the impact of concentration and can also be useful for estimating the PK/PD target (Lewis et al. 1998, 2000; Ernst et al. 1999; Te Dorsthorst et al. 2013). More recently, investigators have modified in vitro systems to more closely mimic the host and infection site. For example, a human cell culture with a bilayer of alveolar epithelial cells and pulmonary artery endothelial cells has been shown to be particularly useful to examine the pharmacology of invasive pulmonary aspergillosis treatment (Hope et al. 2007a; Gregson et al. 2012; Jeans et al. 2012a,b). This model has also been valuable in examining novel methods of drug delivery via

nebulized or airway route (Al-Nakeeb et al. 2012).

Animal PK/PD Models

Most antifungal animal model studies are performed in mice; however, rats, rabbits, and other mammals have been used. The mice are often immunosuppressed as a result of cyclophosphamide treatment, which renders them neutropenic. Additional immune suppression with high-dose corticosteroids is used, for example, in pulmonary fungal infection models to reduce activity of alveolar macrophages. The purpose of the immune suppression is to ensure that reproducible infection is obtained in the minimal number of animals necessary for each treatment group. An additional factor of importance in animal model design is the infection itself, which should mimic as closely as possible what is observed clinically in patients. For example, invasive candidiasis animal models often involve infecting the animals by intravenous inoculation, which mimics disseminated disease in patients. Conversely, most invasive aspergillosis models use inhalational infection to mimic pulmonary disease.

Another critical component of an animal model PK/PD study includes the ability to reproducibly measure and quantify outcome. The most common measures of outcome include assessment of organism burden or animal survival. The former typically provides a larger range of effect to discern effective versus ineffective therapy and almost always requires use of a small number of animals. Assessment of mortality is complicated by nonpathogen causes of mortality in immunocompromised animal models and more recent changes in animal care regulations that introduce variability in determination of the animal distress level appropriate for euthanasia. Organism burden assessment is relatively straightforward for yeast pathogens because target organs can be homogenized and serial dilutions plated onto media to determine fungal burden by colony-forming unit (cfu) counts. This, however, is more problematic for filamentous fungi. These organisms, when grown from tissue homogenates,

do not reproducibly produce a 1:1 ratio of colony to fungal cell, as filamentous fungi grow as long, interconnected filaments of multiple cells. Therefore, a colony on a plate may represent a small fungal filament of only a few cells or may arise from a large mass of cells. Additionally, discrete colonies on a plate quickly coalesce into a large expanding fungal mass, making counting individual colonies difficult. There have been a number of nonculture approaches developed to assess organism burden in tissue for filamentous fungi. Examples include measurement of fungal cell wall component (galactomannan and β -glucan), pulmonary imaging, histopathology, and lung weight (Petratits et al. 1998, 2002, 2003, 2006, 2009; Petratiene et al. 2001, 2002; Francesconi et al. 2006; Meletiadis et al. 2006; Sheppard et al. 2006; Brock et al. 2008; Vallor et al. 2008; Wiederhold et al. 2008; Walsh et al. 2009; Howard et al. 2011; Lengerova et al. 2012; Galiger et al. 2013). Additionally, quantitative real-time polymerase chain reaction can be used to account for each organism. This nucleic acid assay provides a large dynamic range and has been shown to correlate well with mortality in animal models (Bowman et al. 2001; Francesconi et al. 2006; Sheppard et al. 2006; Vallor et al. 2008; Lengerova et al. 2012; Lepak et al. 2013a,b,c).

Experimental Determination of the PK/PD Measure and Magnitude Linked to Efficacy

PD studies integrate drug PKs, including drug concentration over time, in vitro potency (MIC), and treatment efficacy (Craig 1998; Mukherjee et al. 2006; Drusano 2007). There are two major PK/PD questions that are critical for optimizing therapy. First, which PK/PD measure (index) is most closely linked to efficacy? Traditionally, three PD indices have been used to describe the relationship among PK, MIC, and drug effect. These include peak drug concentrations in relation to the MIC ($C_{\max}/\text{MIC}$), the area under the drug concentration curve in relation to MIC (AUC/MIC), and the time (expressed as a percentage of the dosing interval) that drug concentrations are expected to exceed the MIC ($\%T > \text{MIC}$) (Fig. 1). The PD index

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that correlates best (i.e., predicts) with efficacy is largely dependent on the impact of drug concentration on organism survival over time. There are two patterns of activity, concentration dependent or concentration independent (also known as time dependent). Following exposure to concentration-dependent drugs, both the rate and extent of organism killing increase as the drug concentration is escalated. The PD indices associated with concentration-dependent action are $C_{\max}/\text{MIC}$ and AUC/MIC . The dosing strategy associated with maximal efficacy in this situation is administration of large doses infrequently to take advantage of the concentration-dependent action. Concentration-independent agents are characterized by a threshold of maximal activity that occurs at drug concentrations close to the MIC. The PD index most commonly associated with time-dependent action is $\%T > \text{MIC}$. The dosing strategy associated with maximal efficacy in this situation is administration of lower doses more frequently to increase the duration of exposure at levels just above the MIC.

A second exposure-response factor, the postantifungal effect (PAFE), can also influence PD relationships. The PAFE is the drug effect following drug exposures above the MIC (Vogelman et al. 1988). For some drug classes there is a period of prolonged growth suppression following drug exposure, whereas for others there is

rapid growth recovery. Extended PAFE periods allow dosing intervals to be lengthened. For time-dependent killing organisms the PAFE reduces reliance on concentrations above the MIC ($\%T > \text{MIC}$) and increases relevance of the total drug exposure or AUC/MIC index. This phenomenon was first observed and well described in bacteria (Eagle and Musselman 1949; Vogelman and Craig 1985; Craig and Vogelman 1987; Vogelman et al. 1988; Craig 1998) but has also been described in antifungal therapy (Fig. 2) (Turnidge et al. 1994; Andes 2003b, 2004; Mukherjee et al. 2006). For example, triazoles exhibit prolonged PAFEs, and it has been shown that AUC/MIC is the optimal predictive index (Turnidge et al. 1994; Andes and van Ogtrop 1999; Ernst et al. 2000; Andes et al. 2003a,b, 2004), whereas flucytosine exhibits little PAFE, and in this case $\%T > \text{MIC}$ is the most predictive PD index (Andes and van Ogtrop 2000). Finally, as elucidated previously, $C_{\max}/\text{MIC}$ is the predictive PD index for concentration-dependent drugs; however, whether there is evidence of prolonged PAFE, AUC/MIC and $C_{\max}/\text{MIC}$ can both be predictive of efficacy. This is particularly true for echinocandins, which exhibit concentration-dependent activity but also have a prolonged PAFE (Walsh et al. 1991; Ernst et al. 2000, 2002a; Andes et al. 2003c, 2008a). For this drug class, both $C_{\max}/\text{MIC}$

Figure 1. Pharmacokinetic/pharmacodynamic relationship of antimicrobial dosing over time relative to organism MIC. The three PD indices are also listed, including $C_{\max}/\text{MIC}$, AUC/MIC , and $\%T > \text{MIC}$.

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Figure 2. The postantifungal effect. Shown in the figure is a representative example of a drug with a prolonged PAFE. Represented by solid circles is the growth of organism from the thighs of untreated infected mice (control). In comparison, shown by open triangles is the growth curve of organism from thighs of infected mice treated with a single 20-mg/kg dose of drug given at time point 0 h. The solid bar represents the time that drug concentrations are expected to exceed the MIC. The open circles represent the time at which drug concentration decreases below the MIC until the point at which 1- $\log_{10}$ growth was observed. This time period (38 h) minus the time period it takes the control group to grow 1 $\log_{10}$ (in this case 2.5 h) is the PAFE (i.e., ~35.5 h).

and AUC/MIC are robust PD indices (Walsh et al. 1991; Andes et al. 2003c, 2008a,b; Louie et al. 2005; Gumbo et al. 2006).

An additional exposure-response variable of significance for determination of the PD-linked measure is the impact of the dosing interval on treatment efficacy (Fig. 4).

The experiment by which the impact of the dosing interval is assessed allows one to reduce interdependence of the three PD indices. For example, if one increases the dose administered from 100 mg/kg once to 1000 mg/kg once, all three PD indices will increase proportionally. However, if the total dose over the dosing period is kept constant (as in a fractionation experiment), the interdependence is reduced. In this case, as the interval is increased (e.g., from once daily [q24h] to eight times daily [q3h]), each dose is appropriately decreased to keep the daily (or total dose) the same (Fig. 3; Table 1). Therefore, the AUC is approximately the same for each regimen as the same total daily dose is administered (i.e., the same drug exposure is occurring over the time period). However, as the interval is increased, and therefore the individual drug

dose is decreased, the $C_{\max}$ will proportionally decrease as the fractionation is increased. Conversely, time above MIC will increase as the fractionation is increased (Fig. 3). When efficacy is enhanced with shorter dosing intervals, the $\%T > \text{MIC}$ is most closely linked to efficacy. Conversely, when treatment effect is optimal when larger doses are administered infrequently, the $C_{\max}/\text{MIC}$ is the predictive index. When outcome is similar among various dosing intervals, typically the AUC/MIC measure is predictive of efficacy (Fig. 4). The strength of these relationships can be further examined with non-linear regression modeling to provide supportive PK/PD evidence (Fig. 5). Table 2 shows the major PD characteristics by fungal drug class.

The second common PK/PD question simply asks how much drug is needed for the intended effect, expressed as the numerical value of the PD index (AUC/MIC, $C_{\max}/\text{MIC}$, or $\%T > \text{MIC}$). Frequent outcome measures in experimental in vitro and in vivo systems include net stasis (lack of change in burden over the treatment period), 50% maximal effect, 75% maximal effect, 90% maximal effect, and a defined

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Figure 3. Concentration–time profile showing the effect of fractionating a total daily dose into once-, twice-, four-times-, and eight-times-daily fractions. AUC will remain the same because the total daily dose administered is the same in all four regimens; however, $C_{\max}$ will progressively decline and $\%T > \text{MIC}$ will progressively increase as the dose is fractionated into increasing fractions.

reduction in organism burden, such as a 1-log kill or 2-log kill. Which outcome is the most appropriate outcome depends on which correlates with outcome in humans. For invasive candidiasis, this has been well established for triazoles (50% maximal effect) and echinocandins (stasis). However, for other drug classes and especially for other disease states, such as invasive aspergillosis, the optimal outcome measure in the animal model is unknown. In this case, it is not uncommon for experimental model PD studies to report PD targets for multiple outcome measures, such as net stasis, 1-log kill, and 50% maximal effect.

Clinical Antifungal PK/PD Determination and Predictions

The process of translating preclinical PD studies to humans requires validation by analysis of clinical data sets. The optimal data needed to

perform these assessments are rare and include a combination of population PKs, an individual organism isolate, MIC, and outcome. There are several examples of data for both mucosal and invasive candidiasis and a single study for aspergillosis that provide this complement of information. These data are reviewed under each drug class below. In addition, there are additional clinical studies that provide solely the PK and outcome portion of these data. The latter can be useful for guiding therapeutic drug monitoring recommendations, which is also discussed below.

When a PD target is validated, the most robust method to bridge predictions to humans is the use of Monte Carlo simulation to optimize dosing regimen design and identify the MIC ceiling (highest MIC) for which the PD target can be achieved (Bradley et al. 2003, 2010; Hope and Drusano 2009). The optimal data sets for these analyses attempt to consider PK variability

Table 1. Total dose over dosing period

Total dose (mg/kg/24 h)	Interval	Dose (mg/kg) administered	AUC/MIC	$C_{\max}/\text{MIC}$	$\%T > \text{MIC}$
1600	q24h	1600×1	No change	↑↑↑	-
1600	q12h	800×2	No change	↑↑	↑
1600	q6h	400×4	No change	↑	↑↑
1600	q3h	200×8	No change	-	↑↑↑

Figure 4. Concentration–effect curves for a dose-fractionation experiment that included four fractionations. The total dose was kept the same for each regimen and is represented on the x-axis. Treatment outcome is represented on the y-axis as the change in fungal burden from the start of therapy. The dashed line represents net stasis over the duration (24 h). In this experiment, each of the fractionations essentially overlaps one another, indicating that AUC/MIC, as this is kept constant given that the same total dose is administered, is likely the PD index predictive of efficacy. If $C_{\max}$ was predictive, one would expect the q24h regimen to have enhanced effect (i.e., the concentration–effect curve would be significantly lower on the y-axis) compared with more fractionated regimens. However, if $\%T > \text{MIC}$ was the predictive index, one would expect the q3h regimen to have an enhanced effect (i.e., the concentration–effect curve would be significantly lower on the y-axis) compared with less fractionated regimens.

by use of population PKs (PK in patients with the disease state under treatment). This process accounts for common factors, such as age, weight, gender, CYP genotype, severity of illness, liver or renal function, and plasma protein levels. Monte Carlo simulation is a process whereby random repeat sampling is performed from a known distribution of PK measures and MIC based on the probability of each measure. In this respect, a small representative sample of patients in whom PK is directly measured can be used to estimate the PK measurements in thousands of patients. This is important because PK sampling and defining the variance in these measurements is impractical to perform on a large scale. The Monte Carlo simulation process occurs by the model program randomly selecting a drug exposure and an MIC from a large population and identifies the probability of PD target attainment for that drug dose population. The clinician then determines whether

this PD target attainment likelihood is acceptable. For example, if a PD target attainment is estimated at 80% for a *Candida* urinary tract infection, this may be a reasonable cure rate for an otherwise innocuous disease with little morbidity or mortality. However, an 80% PD target attainment may not be acceptable for invasive candidiasis, whereby 20% of the patient population is not receiving therapy that is expected to be adequate. For most serious infectious diseases, a PD target attainment of at least 90% is considered adequate. The primary goals of this clinical translational tool are to (1) identify drugs and dosing regimens expected to have success for the vast majority of patients, (2) identify drug exposures that may be inadequate, (3) identify alternative dosing strategies to enhance efficacy (such as dose escalation or increased frequency), (4) identify situations in which alternative drugs are necessary, and (5) define clinical susceptibility breakpoints.

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Figure 5. Dose–response curves for each of the three PD indices (displayed on the x-axis as AUC/MIC, $C_{\max}/\text{MIC}$, and time above MIC) in a dose-fractionation experiment. Shown on the y-axis is the change in organism burden from the start of the experiment, with the dashed line representing net stasis. Points above the dashed line represent an increase in organism burden (i.e., net growth), whereas those below the line represent a decrease in organism burden (i.e., net cidal activity). The curved line represents the best-fit line based on nonlinear regression modeling using the Hill equation (sigmoidal dose-response model) and in the legend is the coefficient of determination (R^2). In the above example, the PD index that best predicts efficacy is AUC/MIC.

Table 2. The major PD characteristics by fungal drug class

Drug class	Concentration dependent	Prolonged PAFE	PD index predictive of efficacy
Polyene	Yes	Yes	$C_{\max}/\text{MIC}$
Flucytosine	No	No	$T > \text{MIC}$
Azoles	No	Yes	AUC/MIC
Echinocandins	Yes	Yes	$C_{\max}/\text{MIC}$ or AUC/MIC

Monte Carlo simulation has been very helpful to define dosing regimens for difficult-to-study populations, such as children and neonates, and for certain infections in which the study of different dosing regimens is impractical based on the number of possible regimens or unethical because the study would include sub-optimal regimens that cannot be justified (Hope et al. 2007b, 2008, 2010; Ikawa et al. 2009; Warn et al. 2012). For example, studies using the bridging technique of Monte Carlo simulation were performed for neonates and children with hematogenous *Candida* meningoencephalitis, a rare but well-recognized and potentially lethal infection, with micafungin and anidulafungin (Hope et al. 2010; Warn et al. 2012). In both cases, the recommended dosing strategy would be expected to lead to suboptimal outcome, and therefore higher dosing regimens are recommended.

POLYENES

Concentration Effect, PD Index, and Target

The clinically available polyene class consists of conventional deoxycholate amphotericin B (AmB) and three lipid congeners: liposomal amphotericin B (LAmB), amphotericin B lipid complex (ABLC), and amphotericin B colloidal dispersion (ABCD). In vitro and animal model PD time-kill studies against *Candida* and filamentous fungi have shown concentration-dependent action as drug dose is escalated multiple times above the MIC (Turnidge et al. 1994; Kelpser et al. 1997; Denning and Warn 1999; Ernst et al. 2000, 2002b; Groll et al. 2000; Warn et al. 2000; Andes et al. 2001, 2006; Vitale et al. 2003; Gavalda et al. 2005; Lewis et al. 2005; Wiederhold et al. 2006; Seyedmousavi et al. 2013a). Long PAFE effects have also been noted, with one study showing a PAFE of almost 1 d

(>20 h) in a neutropenic mouse model (Turnidge et al. 1994; Ernst et al. 2000; Andes et al. 2001). Dose-fractionation studies have found $C_{\max}/\text{MIC}$ to be the PD index most predictive of efficacy (Andes et al. 2001, 2006). For example, when the dosing frequency was increased from q12h to q72h, the total dose (over 72 h) necessary to achieve a net static effect was 10-fold lower in the q72h treatment group when given as a single injection compared with the total dose necessary for net stasis when the total dose was fractionated into a q12h regimen (six divided doses). The AmB PD target against multiple *Candida* organisms in a murine model was a $C_{\max}/\text{MIC}$ of 2 for net stasis and 4 for 1-log kill end points. Maximal effects were noted as the $C_{\max}/\text{MIC}$ approached a value of 10.

AmB PD studies in *Aspergillus* models are less common; however, studies in vitro and in vivo were congruent with the *Candida* models (Lewis et al. 2005; Wiederhold et al. 2006). Concentration-dependent action was found, and dose-fractionation study revealed that a significantly lower total dose was required when administered in a single, large dose (q72h) compared with fractionating the total dose into q24h or q8h intervals. Both models found that dosing regimens that maximize peak drug concentration exposure were optimal. The $C_{\max}/\text{MIC}$ PD target in the *Aspergillus* model was also noted to be maximal at a range of 2–4. Thus, for amphotericin B the PD index and target appear to be relatively similar for both *Candida* and *Aspergillus* pathogens.

Amphotericin B Lipid Formulations

Lipid formulations of amphotericin B (LAmB, ABLC, and ABCD) are in general less potent in vivo on a milligrams-per-kilogram basis when compared with AmB. For example, PD studies

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in *Candida* models with the lipid formulations have shown a four- to sixfold-higher PD target compared with conventional AmB (Andes et al. 2006). Although these formulations are similar in the presence of a lipid component, they are distinct in structure. These differences result in several unique PK characteristics. One potentially relevant PK/PD difference is penetration into certain tissues, such as the CNS. For example, LAmB is composed of small unilamellar particles (liposomes) that exhibit high serum and CNS concentrations in comparison with other lipid preparations (Groll et al. 2000). The relevance of this PK difference was found to be important in a CNS-invasive candidiasis model, whereby the difference in drug concentration in brain parenchyma was closely related to treatment efficacy in favor of LAmB over the other formulations (Groll et al. 2000). Conversely, both ABLC and ABCD achieve much higher concentrations in the intracellular space and in organs of the reticuloendothelial system (Janknegt et al. 1992). In a disseminated candidiasis model, differences in PD potency in the liver, kidney, and lung closely followed the differences in tissue kinetics for each drug in each target organ system (Andes et al. 2006). Several animal model and human investigations have shown that ABLC attains higher concentrations in the lung relative to other formulations (Groll et al. 2006; Lewis et al. 2007). This PK difference was associated with some degree of enhanced efficacy in these models. However, the clinical relevance of these differences remains less clear.

Clinical Implications

Clinical PD studies of amphotericin B for which PK, MIC, and outcome data are available are limited to a single pediatric cohort (Hong et al. 2006). In this study, a $C_{\max}/\text{MIC}$ ratio of >40 was associated with maximal efficacy for a lipid-associated AmB formulation. After accounting for potency differences, this value is relatively similar to the preclinical models that have identified a $C_{\max}/\text{MIC}$ of ratio of 10 as maximally efficacious. Most other studies that have attempted to correlate dose, MIC, and outcome with AmB have been hampered by the very

narrow MIC and dose range, making it very difficult to correlate the PD indices with outcome (Park et al. 2006).

Escalating the dose of amphotericin B, and thus increasing the $C_{\max}/\text{MIC}$, is a strategy that one would presume would increase efficacy given the PD characteristics of this class. This has been investigated to some degree in a prospective clinical study comparing conventional dosing of LAmB (3–5 mg/kg/d) to higher initial doses (10 mg/kg/d) for the first 14 d of a proven or probable mold infection (Cornely et al. 2007). However, there was no difference in the outcome with either regimen, although there was decreased toxicity (renal) and decreased rates of drug discontinuation in the low-dose arm. Thus, in this clinical study, increasing drug concentration exposure did not increase efficacy and unfortunately increased adverse effects. The PD explanations for these results include the possibility that (1) the concentration–effect relationship may be maximal at 5 mg/kg/d, (2) a two- to threefold increase in drug concentration was not enough to discern a meaningful difference in efficacy, or (3) the toxicity of high concentrations outweighed any efficacy benefit. The latter toxicodynamic concept has also been further examined clinically with a suggestion of reduced renal toxicity when amphotericin B has been administered as a continuous infusion compared with once-daily bolus dosing (Eriksson et al. 2001; Imhof et al. 2003; Peleg and Woods 2004; Hall et al. 2005). Unfortunately, these studies were primarily PK based and did not include analysis of this dosing strategy and treatment outcome in patients with proven fungal infection. The PD studies from preclinical models would suggest that this potential toxicodynamic benefit may negatively impact therapeutic efficacy based on lower $C_{\max}/\text{MIC}$ attainment with a continuous-infusion dosing strategy (Seyd-mousavi et al. 2013b).

FLUCYTOSINE

Concentration Effect, PD Index, and Target

Several studies have examined the effect of escalating concentrations of flucytosine on efficacy

against *Candida* (Turnidge et al. 1994; Andes and van Ogtrop 2000; Lewis et al. 2000; Ernst et al. 2002b; Hope et al. 2006). The results of these studies have shown concentration-independent action with maximal activity at concentrations near the MIC. Additionally, there is rapid regrowth of organism once concentrations decrease below the MIC, and therefore the PAFE is very short. Dose-fractionation studies confirmed the concentration-independent nature of flucytosine, as 10-fold-less total drug was needed for efficacy using the most fractionated dosing strategy (Andes and van Ogtrop 2000). Time course, PAFE, and dose-fractionation studies consistently support $\%T > \text{MIC}$ as the most predictive index. Therefore, small but frequent administration of the drug to prolong the $\%T > \text{MIC}$ is the optimal dosing strategy for flucytosine. The PD target associated with net stasis from independent animal model studies is a $\%T > \text{MIC}$ of $\sim 40\%$ for invasive candidiasis (Andes and van Ogtrop 2000; Hope et al. 2006). Similar PK/PD studies for *Cryptococcus* species are limited but do suggest that the current human regimen is sufficient for stasis (O'Connor et al. 2013). This analysis does not provide an estimate of $\%T > \text{MIC}$.

Clinical Implications

There are no clinical data sets that allow for robust PD efficacy analysis for flucytosine. A single study has bridged animal model PK/PD study of flucytosine to humans (Hope et al. 2006). The investigators integrated flucytosine dose ranges, PKs, and MIC distribution for *C. albicans*. Interestingly, doses as low as 25 mg/kg/d (two- to fourfold lower than is currently recommended) would be predicted to achieve the PD target against *C. albicans* given the current MIC distribution.

Toxicodynamic relationships, however, have been well established from clinical data (Kauffman and Frame 1977; Stamm et al. 1987; Francis and Walsh 1992; Pasqualotto et al. 2007; Smith and Andes 2008). Toxicity of flucytosine therapy has been associated with peak drug concentrations. This makes dosing strategies that maximize $\%T > \text{MIC}$ even more attractive as this

dosing scheme will also lead to decreased peak ($C_{\text{max}}/\text{MIC}$) concentrations. Thus, efficacy can be enhanced and toxicity limited by administering low doses very frequently (at least every 6 h) to keep drug concentrations above the MIC for 40%.

TRIAZOLES

Concentration Effect and PD Index and Target

The azole drug class has undergone the most extensive PD study. Concentration-escalation studies have consistently shown concentration-independent action against *Candida* species, with maximal growth inhibition at concentrations near the MIC (Ernst et al. 1998, 2002b; Louie et al. 1998; Andes and van Ogtrop 1999; Andes et al. 2003a,b, 2004; Warn et al. 2009). For example, in a dose-escalation in vitro experiment in which the azole drug concentration was varied from sub-MIC levels to more than 200-fold in excess of the MIC, the growth of *Candida* was similarly inhibited once levels exceeded the MIC. In vivo animal models have also shown maximal growth inhibition associated with concentrations near the MIC. However, these in vivo studies also reveal prolonged PAFEs (Andes and van Ogtrop 1999; Andes et al. 2003a,b, 2004). The integration of concentration-independent action with prolonged PAFE would suggest that AUC/MIC is the PD index most closely associated with efficacy, which has been validated in dose-fractionation studies (Louie et al. 1998; Andes and van Ogtrop 1999; Andes et al. 2003a,b, 2004).

PD target studies have been performed in a number of models that have included *Candida*, *Aspergillus*, and *Cryptococcus* as the infecting pathogens. PK/PD studies have been undertaken with five triazoles in therapy for invasive candidiasis. The PD target for a treatment outcome (defined as a 50% maximal effect) against *Candida* species in these studies has consistently been found to be a free-drug AUC/MIC of 25–50 (Andes and van Ogtrop 1999; Andes et al. 2003a,b, 2004; Lepak et al. 2013d). This would mean that drug concentrations that re-

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main near the MIC over a 24-h period would be expected to achieve this target ($1 \times \text{MIC} \times 24 \text{ h} = \text{AUC/MIC}$ of 24). There are a number of interesting observations from these studies. First, the PD index predictive of efficacy is similar for drugs within a class as long as free-drug (non-protein-bound) concentrations are considered. Second, the PD target is remarkably similar against different *Candida albicans*, *Candida tropicalis*, and *Candida krusei* species. Additionally, the PD target was congruent across levels of resistance and resistance mechanisms.

PD target measure in *Aspergillus* animal models has also been recently reported. Interestingly, although azoles are static against *Candida*, they show marked killing activity against *Aspergillus* species. At optimal dosing levels, one can see up to 4-log reduction in organism burden in murine pneumonia models (Lepak et al. 2013c). Similar to *Candida*, there is a very strong relationship between treatment efficacy and the PD index AUC/MIC (Mavridou et al. 2010a,b; Howard et al. 2011; Lepak et al. 2013a,c). This finding affirms that the PK/PD measure linked to efficacy is similar across fungal genera. However, the PD targets associated with efficacy were significantly lower for *Aspergillus*-active azoles in models that have included both disseminated infection by tail-vein injection of the organism and invasive pulmonary models. The PD target free-drug AUC/MIC for *Aspergillus* associated with either ED₅₀ or net stasis targets was only 1.7–11 (Mavridou et al. 2010a,b; Howard et al. 2011; Lepak et al. 2013a,c). Perhaps this lower PD target is a result of the more differential fungicidal activity against *Aspergillus* species. Similar to the *Candida* studies, the PD target for *Aspergillus* studies was similar for drug-susceptible and drug-resistant strains.

An additional study has examined the fluconazole PD target in a murine cryptococcal meningitis model (Sudan et al. 2013). Therapy against a group of three *Cryptococcus neoformans* isolates produced a stasis end point with a fluconazole AUC/MIC value near 400, which, interestingly, is higher than is observed for other pathogens.

Clinical Implications

Although clinical PK/PD data sets are in general uncommon, there is a relatively large experience with fluconazole and voriconazole (Rex et al. 1997; Lee et al. 2000; Takakura et al. 2004; Clancy et al. 2005; Pfaller et al. 2006; Pai et al. 2007; Rodríguez-Tudela et al. 2007; Baddley et al. 2008). In a study of >1000 patients receiving fluconazole for oropharyngeal candidiasis, clinical success was noted in 91%–100% of patients in whom the free-drug AUC/MIC was >25, whereas it was only 27%–35% in patients with a free-drug AUC/MIC of <25 (Rex et al. 1997). Another study in oropharyngeal disease provides similar results, with 92% success noted in patients with a free-drug AUC/MIC of >25 and only 9% in those with a free-drug AUC/MIC of <25 (Rodríguez-Tudela et al. 2007). Comparable analysis has been undertaken for invasive candidiasis. In aggregate, the individual studies include fluconazole drug exposure (PK/PD) for >600 patients with invasive candidiasis (Rex et al. 1997; Lee et al. 2000; Takakura et al. 2004; Clancy et al. 2005; Baddley et al. 2008). There is a remarkably strong relationship between AUC/MIC and treatment outcome, with a free-drug AUC/MIC target of 25–50 associated with maximal survival. A more contemporary analysis with voriconazole shows congruent results (Pfaller et al. 2006). These studies suggest that efficacy in humans is predicted by the animal model ED₅₀ end point.

Clinical PD analyses for the mold-active triazoles in therapy for *Aspergillus* are less common. The sole study that includes analysis of PK, MIC, and outcome is with voriconazole (Troke et al. 2011). The analysis found that maximal outcome was associated with a trough-to-MIC ratio near 2. There are numerous additional analyses that include PK and outcome (Janknegt et al. 1992; Groll et al. 2006; Lewis et al. 2007; Bradley et al. 2010). The results for voriconazole are congruent and suggest that trough concentrations ranging from 1–2 µg/ml are associated with optimal outcome for invasive aspergillosis. Given the MIC distribution for *Aspergillus* spp. (MIC₉₀ of 1 µg/ml), the results are relatively similar to the full PK/PD

analysis. Similar PD analysis based on therapeutic drug monitoring (TDM) is available for itraconazole and posaconazole. The TDM results for these compounds are relatively similar. For treatment of a variety of invasive disease caused by a variety of fungal pathogens, optimal outcome is described with trough concentrations near 1 $\mu\text{g}/\text{ml}$ (Andes et al. 2009). These antifungals have also been studied in the setting of prophylaxis. Interestingly, TDM evaluation of both antifungals suggests that the PD target is roughly half of that needed for treatment of documented infection (Boogaerts et al. 1989; Jang et al. 2010; Campoli et al. 2013).

A single clinical PK/PD study has examined the relationship between fluconazole serum and cerebrospinal fluid PK and outcome in cryptococcal meningitis. The relationship between PK and outcome was significant and favored the 800-mg/d-dosing regimen (Manosuthi et al. 2010).

ECHINOCANDINS

Concentration Effect, PD Index, and Target

The most recently developed antifungal class is the echinocandins (caspofungin, micafungin, and anidulafungin). As a group they exhibit activity against most common *Candida* species, with less potency in vitro for *C. parapsilosis*. Both in vitro and animal model studies of invasive candidiasis have revealed pronounced concentration-dependent killing effects with prolonged PAFEs (Groll et al. 2001; Petratis et al. 2001, 2002; Ernst et al. 2002a; Andes et al. 2003c, 2008a,b; Louie et al. 2005; Gumbo et al. 2006, 2007; Hope et al. 2008). Investigations examining the impact of dose and dosing interval found that five- to nearly eightfold less total drug was necessary to achieve treatment end points in the animal model when large, single doses were used in comparison with fractionating the dose into two to six doses over the study (Andes et al. 2003c; Gumbo et al. 2007). Thus, a dosing strategy that includes infrequent administration of large doses is optimal for the echinocandin class. PD index analysis suggests that

both $C_{\text{max}}/\text{MIC}$ and AUC/MIC are linked to treatment efficacy, although there may be a slight advantage to optimizing peak concentration ($C_{\text{max}}/\text{MIC}$) (Andes et al. 2003c, 2008a,b; Louie et al. 2005). An interesting observation with the echinocandin class in experimental studies against *Candida* and *Aspergillus* is a concentration–effect ceiling, above which reduced activity and paradoxical growth are observed (Stevens et al. 2004, 2005, 2006; Clemons et al. 2006; Fortwendel et al. 2010). This observation, though, has not been universally noted for all echinocandins, species, or strains, and mechanistically appears complex (Vanstraelen et al. 2013). It is postulated that cell wall compensatory mechanisms and stress response pathways are the major factors contributing to this phenomenon. For example, it has been noted in isolates that exhibit paradoxical growth to echinocandins that there is a compensatory up-regulation of the synthesis of the cell wall component chitin (Stevens et al. 2005; Bizerra et al. 2011). A confirmatory study design showed abolition of the paradoxical effect following the addition of a chitin synthase inhibitor to echinocandin therapy (Shields et al. 2011; Szilagyi et al. 2012). The stress response pathways that have been implicated in this phenomenon include protein kinase C (Wiederhold et al. 2005; Shields et al. 2011), the calcineurin pathway (Wiederhold et al. 2005; Shields et al. 2011), the high-osmolarity glycerol response pathway (Popolo et al. 2001), and heat shock protein 90 (Cowen and Lindquist 2005; Kaneko et al. 2009). Interestingly, all of these pathways seem to have a similar end product as they lead to elevated cell wall chitin content (Munro et al. 2007).

The echinocandins also exhibit activity against *Aspergillus* species, and animal model studies show similar PK/PD characteristics including concentration-dependent effects and efficacy linked to AUC/MIC and $C_{\text{max}}/\text{MIC}$ (Wiederhold et al. 2004; Clemons and Stevens 2006; Lewis et al. 2008, 2011; Lepak et al. 2013b). However, unlike the cidal activity observed against *Candida* species, drug exposure against *Aspergillus* results in growth inhibition without significant organism killing. It is theo-

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rized that this lack of killing activity is related to the apparent limitation of drug activity concentrated at the hyphal tips based on morphology analysis following drug exposure (Kurtz et al. 1994).

Studies examining the PD target for the echinocandins have identified species-specific results. Investigations with *C. albicans* in animal models found that a free-drug $C_{\max}/MIC$ of >1 or AUC/MIC of 10–20 was the PD target associated with a stasis end point (Groll et al. 2001; Andes et al. 2003c, 2008a; Louie et al. 2005; Gumbo et al. 2006). Interestingly, similar study with *C. parapsilosis* and *Candida glabrata* in the same models found that the echinocandin exposure needed for stasis was two- to threefold lower than for *C. albicans* (Andes et al. 2008b, 2010). The mechanistic basis for these differences remains unclear. However, it has been hypothesized that the disparity may be associated with the reduced fitness of these non-*albicans* species in vivo. These observations have led to species-specific susceptibility breakpoints for *Candida* species (Pfaller et al. 2011a). Although the *Candida* species has been found to impact treatment response, drug resistance within species does not appear to influence the PD target. Animal model PK/PD studies with wild-type susceptible and drug-resistant *C. glabrata* clinical isolates resulting from a variety of *Fks* mutations found that a similar AUC/MIC is needed for efficacy (Lepak et al. 2012). This observation is consistent with PK/PD observations with azoles in therapy against drug-resistant strains. Given the relatively wide therapeutic window with the echinocandins in patients, the potential to escalate drug doses could have important clinical implications given the recent emergence of drug-resistant *C. glabrata* infections (Pfaller et al. 2011b, 2012; Alexander et al. 2013).

PD target study in the setting of invasive aspergillosis is limited. A single in vivo observation found that a quite high $C_{\max}/MIC$ (10–20) was needed to achieve a stasis end point in monotherapy (Wiederhold et al. 2004). This may not be surprising, though, based on the relatively modest effect echinocandins have as monotherapy against *Aspergillus*.

Clinical Implications

Clinical PD studies with the echinocandins have in general revealed results congruent with studies in preclinical animal models. Study from two phase 3 clinical trials of micafungin use in patients with candidemia or invasive candidiasis was recently used for PK/PD analysis (Andes et al. 2012). Among the nearly 500 patients, a total drug AUC/MIC of >3000 was found to be associated with optimal treatment outcome (98% success, compared with 84% in those with an AUC/MIC of <3000). If one considers the protein binding of micafungin (99.75%), the free-drug concentration would be ~ 8 , which is very similar to the PD target identified in preclinical animal models. Interestingly, subgroup analysis based on *Candida* species identified a 10-fold lower PK/PD target for patients infected with *C. parapsilosis*, also consistent with the animal model data. In addition to clinical investigation of the amount of echinocandin needed for efficacy, one study sought to explore the impact of dosing interval on treatment outcome. The impact of high-dose, extended-interval micafungin was compared to the approved daily regimen in patients with esophageal candidiasis. Outcomes were statistically similar with both treatment strategies, consistent with animal model studies linking efficacy to the AUC/MIC index (Andes et al. 2013). Clinical studies in which echinocandin dose levels have been escalated have not identified a paradoxical effect (Pappas et al. 2007; Betts et al. 2009; Shields et al. 2011; Szilagyi et al. 2012; Andes et al. 2013; Elefanti et al. 2013).

PK/PD ANALYSIS OF COMBINATION THERAPY

An emerging area of study is PK/PD analysis of combination therapy. There are a number of fungal infections for which monotherapy yields suboptimal outcomes. In such instances, the study and application of combination therapy is an alternative strategy that has been attempted to enhance outcome. The best example of the utility of combination antifungal therapy is in the treatment of cryptococcal meningitis

(Perfect et al. 2010), and, until recently, although clinical efficacy was clear with this strategy, formal PK/PD studies were lacking for this infection and drug combination. The PK/PD of LAmB and flucytosine alone and in combination in a murine model of cryptococcal meningoencephalitis was recently reported (O'Connor et al. 2013). Monotherapy LAmB at a dose of 3 mg/kg/d and even at the higher dose level at 5 mg/kg/d was less effective than the combination with flucytosine at 50 or 100 mg/kg/d.

The fungal infection that has received the most attention in the area of combination therapy is invasive aspergillosis. A number of in vitro studies have examined combinations of polyene/azole, polyene/echinocandin, and azole/echinocandin (Vazquez 2008; Seyedmousavi et al. 2013c,d). The analysis of these data is conflicting, with some combination experiments revealing enhanced effects, whereas others fail to confirm this. Azoles in combination with echinocandins in theory may provide the most promising combination strategy given that they act at completely distinct sites (the cell membrane and cell wall, respectively). An in vitro PD study of combination therapy with these two classes supports this tenet (Jeans et al. 2012b). In vivo animal model experiments are also common, but few have been designed to analyze the combination effects from a pharmacodynamic perspective. For example, a number of fixed-dose studies have suggested potential benefits of combination therapy (Kirkpatrick et al. 2002; Petraitis et al. 2003, 2009; Chandrasekar et al. 2004; MacCallum et al. 2005; van de Sande et al. 2009). However, synergistic interaction may not occur at all doses, and therefore combination PK/PD analysis requires dose-ranging experiments. An additional in vivo study has incorporated a wide dose-ranging combination therapy design for invasive aspergillosis (Lepak et al. 2013b). Both wild-type and drug-resistant (*Cyp51* mutant) *Aspergillus* isolates were included in the study of echinocandin/azole combination therapy in the neutropenic murine invasive pulmonary aspergillosis model. Combination therapy did not appear to markedly impact outcome against wild-type isolates when the azole drug concentration was

optimized. However, striking synergistic results were noted for combinations of posaconazole and caspofungin against azole-resistant isolates. In fact, at maximal synergistic efficacy a $>2\text{-log}_{10}$ increase in microbiological effect was noted in certain combinations in comparison with what would have been predicted. Therefore, combination therapy may be particularly useful in treatment of the azole-resistant (*Cyp51* mutant) *Aspergillus* infections that appear to be emergent in many parts of the world (Snelkers et al. 2008).

Combination therapy may be also a useful strategy for mucormycosis, and animal model data suggest utility of polyene/echinocandin therapy against *Rhizopus oryzae* (Spellberg et al. 2005; Ibrahim et al. 2008). A similar study with polyene/posaconazole combination did not show enhanced efficacy (Ibrahim et al. 2009). This may have been because of the fact that posaconazole shows modest activity against *R. oryzae*, the most common species of mucormycosis (Dannaoui et al. 2003; Barchiesi et al. 2007; Rodriguez et al. 2008; Ibrahim et al. 2009). However, escalation of posaconazole exposure has shown efficacy, and combination with tacrolimus also showed enhanced effect (Rodriguez et al. 2010; Lewis et al. 2013). Continued PD study and analyses are important for future investigation to close knowledge gaps related to therapy for mucormycosis and other emerging fungal pathogens.

CONCLUSIONS

Application of PK/PD principles to antifungal drug therapy has provided an understanding of the relationship between drug exposure and outcome. The results from experimental PK/PD investigations have been critical for designing optimal dosing strategies to improve clinical efficacy while decreasing toxicities. There remain numerous knowledge gaps in the area of combination therapy and PK/PD against emerging fungal pathogens.

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