Pharmacokinetics and Pharmacodynamics of Antifungals
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The role of pharmacokinetics and pharmacodynamics has gained increasing recognition as critical for selection and dosing of antimicrobial therapeutics, including antifungal agents. The study of pharmacokinetics involves understanding the interaction of a drug with the host, including measurements of absorption, distribution, metabolism and elimination. The study of antimicrobial pharmacodynamics provides insight into the link between drug pharmacokinetics, in vitro susceptibility, and treatment efficacy. Pharmacokinetic/pharmacodynamic (PK/PD) investigations have been valuable for defining optimal antifungal dosing regimens and developing in vitro susceptibility breakpoints. Numerous in vitro, animal, and clinical studies have been instrumental in characterizing the pharmacodynamic activity of the triazoles, polyenes, flucytosine, and echinocandins against \textit{Candida} species. Several studies have begun to apply these principles to optimize therapy against filamentous fungi. The principles that have been used to characterize pharmacodynamic characteristics of single antifungal drugs are also beginning to be used to examine the more complex relationship encountered with combinations therapy.

Understanding of PK/PD principles can provide useful information for the clinician, clinical trial development, and for development of microbiology laboratory guidelines [1–3]. Antifungal pharmacodynamics allows the clinician to choose the most potent drug and provides a guide to the most efficacious and safe dose and interval of administration for a particular pathogen and infection site. For the pharmaceutical industry, preclinical
PK/PD investigations help to predict the likelihood of success of a compound in development and can guide dosing regimen design for clinical trials. Understanding the relationship between antifungal drug exposure, in vitro potency (minimum inhibitory concentration [MIC]), and efficacy can be instructive for determining appropriate susceptibility breakpoints (i.e., should an organism with MIC X be classified as susceptible or resistant?) [4–6].

Pharmacokinetics

Pharmacokinetic studies describe how the body handles a drug, including absorption, distribution, binding to serum and tissue proteins, metabolism, and elimination. Comparison of antifungals is frequently based on their pharmacokinetic properties [7]. Antifungal drug concentrations have been well characterized in numerous body fluids and tissues, including serum, urine, cerebrospinal fluid, vitreous, epithelial lining fluid or bronchoalveolar lavage, brain, lung, and kidney. The pharmacokinetic goal of antifungal therapy is to achieve adequate drug concentrations at the site of infection. This begs the rather simplistic question, where is the fungus relative to the antifungal drug? The site of infection for fungal pathogens can range from the bloodstream, where one would expect serum measurements to be of importance, to various tissue sites for which tissue drug concentrations may be of greater interest. Most pathogenic fungi exist primarily in extracellular fluid, however, even at tissue sites of infection. Serum measurements thus serve as a reliable tissue concentration surrogate. The body sites for which tissue antifungal concentrations have been suggested to be most important include the brain parenchyma and the vitreous space in the eye [8–13]. Outcomes of infection at other tissue sites have correlated well with serum concentrations. The same is true for body fluid kinetics. For example, despite marked differences in antifungal measurements in urine and CSF, therapeutic outcome seems more dependent on extracellular parenchymal tissue concentrations (serum). For example, Groll and colleagues examined the relationship between CSF and brain kinetics of several amphotericin B (AmB) preparations and efficacy [8]. The CSF concentrations of four polyene compounds were remarkably similar. Brain tissue concentrations of liposomal AmB (AmBisome), however, were from 6- to 10-fold higher than the other polyene preparations. The burden of Candida in the brains of rabbits following therapy correlated well with brain tissue penetration of the various drugs. The relationship between urine antifungal pharmacokinetics and efficacy in fungal pyelonephritis has been similarly examined [13]. For example, marked differences in urine kinetics have been demonstrated among the triazole antifungals. Nearly all of the absorbed fluconazole is secreted as active drug into the urine. Conversely, almost none of the absorbed itraconazole or voriconazole is secreted into this body fluid [14,15]. Outcomes in the kidneys, however, have been linked more closely
to serum levels than urine. It has been theorized that tissue concentrations in extracellular space in the renal parenchyma are more relevant than urine concentrations for this infection.

Another pharmacokinetic factor shown to impact the availability of antimicrobial compounds in tissue is binding to serum proteins such as albumin [16]. In general it is accepted that only unbound (free) drug is pharmacologically active. This is related to the limited ability of protein-bound drug to diffuse across tissue and cellular membranes to reach the drug target. The relevance of protein binding has been most clearly demonstrated for drugs from the triazole class, in which there are marked differences in degree of binding among the drugs in this class. The studies demonstrating these findings are discussed later.

**Defining antifungal drug pharmacodynamic characteristics**

**Predictive parameter (how often do I give the drug?)**

Pharmacodynamics examines the relationship between pharmacokinetics and outcome. An added dimension of antimicrobial pharmacodynamics is consideration of the drug exposure relative to a measure of in vitro potency or the minimum inhibitory concentration (MIC) [1,3]. Three traditional pharmacodynamic parameters have been used to describe these relationships, including the peak concentration in relation to the MIC (C\text{max}/MIC), the area under the concentration curve in relation to the MIC (24 h area under the concentration curve [AUC]/MIC), and the time that drug concentrations exceed the MIC expressed as a percentage of the dosing interval (%T > MIC) (Fig. 1). Knowledge of which of the three pharmacodynamic parameters describes antifungal activity provides the basis for determining the frequency with which a drug is most efficaciously

![Diagram of Pharmacokinetics](image-url)

*Fig. 1. Pharmacokinetics of antimicrobial dosing relative to organism MIC. (From Andes D. Clinical pharmacodynamics of antifungals. Infect Dis Clin N Am 2003;17:635-49; with permission.)*
administered. For example, if the $C_{\text{max}}/\text{MIC}$ parameter relationship strongly correlates with activity of drug A, the optimal dosing schedule would provide large infrequent doses. Conversely, if the $\%T > \text{MIC}$ better describes drug activity, a dosing strategy may include smaller more frequent drug administration to prolong the period of time that drug levels exceed the MIC.

Two types of experimental studies have been used to examine these relationships. The first study design involves investigation of the antifungal drug activity over time. Two outcomes are commonly noted. First is the impact of increasing drug concentrations on the rate and extent of organism killing. When higher concentrations enhance killing, the drug is referred to as concentration dependent. The second study endpoint includes examination of antifungal activity after drug concentrations decrease to below the organism MIC. For some drugs there is a period of prolonged growth suppression following an initial supra-MIC exposure. This period of growth suppression is termed a post-antifungal effect (PAFE). Three combinations of these time kill endpoint characteristics have been observed and each combination is predictive of one of the pharmacodynamic parameters. The $C_{\text{max}}/\text{MIC}$ is associated with concentration-dependent killing and prolonged PAFEs. The $\%T > \text{MIC}$ is associated with concentration-independent killing and short PAFEs. The $\text{AUC}/\text{MIC}$ is associated with prolonged PAFEs and either concentration-dependent or -independent killing.

The second study type used to determine which pharmacodynamic parameter is predictive of efficacy is termed dose fractionation. Traditional dose escalation studies use a single dosing interval. With only a single dosing interval, escalating doses increase the values of all three parameters. Dose fractionation studies examine efficacy of various dose levels that are administered by using three or more dosing intervals. In examining treatment results, if the regimens with shorter dosing intervals are more efficacious, the time-dependent parameter ($T > \text{MIC}$) is the more important parameter. If the large, infrequently administered dosing regimens are more active, the peak level in relation to the MIC is most predictive. Finally, if the outcome is similar with each of the dosing intervals, the outcome depends on the total dose or the $\text{AUC}$ for the dosing regimen.

**Parameter magnitude (how much drug do I give?)**

Knowledge of the pharmacodynamic characteristics of a compound allows one to better design a dosing interval strategy. This knowledge can also be useful to design studies to determine the amount of drug or parameter magnitude that is required for treatment efficacy [1,2,17]. These studies can be used to help answer numerous questions related to the exposure response relationship. For example, what pharmacodynamic magnitude of a drug is needed to treat a *Candida* infection? Is this pharmacodynamic magnitude the same as that needed to treat a drug-resistant *Candida* infection? Is
the magnitude similar for other fungal species, for different infection sites, in different animal species? The answers to these questions have been explored and most successfully addressed using various in vivo infection models. The results of these studies have demonstrated that the magnitude of a pharmacodynamic parameter associated with efficacy is similar for drugs within the same class, provided that free drug levels are considered. Furthermore, these data show that the parameter magnitude associated with efficacy is independent of the animal species, dosing interval, site of infection, and most often, the infecting pathogen. Most important, correlation of human pharmacokinetics and clinical trial outcome with several antifungal agents has suggested that the pharmacodynamic parameter magnitude that produces efficacy in animal models also predicts efficacy in humans. The pharmacodynamic evaluation of each antifungal drug class and the clinical implications of these studies are detailed here.

**Triazole pharmacodynamics**

*Predictive pharmacodynamic parameter*

In vitro and in vivo time kill studies have been undertaken with all of the clinically available triazole compounds [18–26]. Studies have shown that over a wide triazole concentration range (starting below the MIC [sub-MIC] to those more than 200-fold in excess of the MIC), growth of *Candida* organisms are similarly inhibited [22]. In other words, increasing drug concentrations do not enhance antifungal effect. Furthermore, in vitro studies demonstrated organism regrowth soon after drug removal [20,21]. In vivo studies, however, demonstrated prolonged growth suppression after levels in serum decreased to below the MIC [22–25]. These prolonged in vivo PAFEs have been theorized to be caused by the profound sub-MIC activity of these drugs (ie, effect of the triazoles after concentrations fall below the MIC in vivo). The time kill combination of concentration-independent killing and prolonged PAFEs suggest that the 24 AUC/MIC parameter is most closely tied to treatment effect. Dose fractionation studies in several in vivo models with each of the triazole compounds have corroborated these results [22–25,27]. The earliest fluconazole dose fractionation studies with fluconazole examined the impact of dividing four total dose levels into one, two, or four doses over a 24-hour period [27]. The results clearly demonstrated that outcome depended on the total amount of drug or AUC rather than the dosing interval. Subsequent studies with fluconazole, posaconazole, ravuconazole, and voriconazole similarly demonstrated that outcome was independent of fractionation of the total drug exposure supporting the 24-hour AUC/MIC as the pharmacodynamic parameter driving treatment efficacy [22–25]. These later observations demonstrate that the pharmacodynamic parameter associated with efficacy was similar within the triazole drug class.
Predictive pharmacodynamic magnitude

The usefulness of knowing which parameter predicts efficacy is being able to then determine the magnitude of that parameter needed for successful outcome. The most efficient experimental way to define the magnitude of the predictive parameter is to examine treatment efficacy against organisms with widely varying MICs. For example, the efficacy of posaconazole over a more than 1000-fold AUC range was studied in therapy against 12 *C. albicans* with MICs varying nearly 100-fold [24]. Results from these studies showed that the AUC/MIC exposure associated with treatment efficacy was similar across the group of strains. Similar studies have now been undertaken with four triazole compounds that include more than nearly 40 drug/organism combinations for which MICs and dose levels varied more than 1000-fold each (Fig. 2) [22–25]. The consistency of data with these triazoles demonstrates that when protein binding is considered (ie, free drug concentrations), the antifungal pharmacodynamic target is similar among drugs within a mechanistic class (triazoles).

Several host, pathogen, and infection site factors have also been investigated to determine if and how they might impact the pharmacodynamic magnitude necessary for efficacy. For example, intuitively one may expect that more drug would be required to achieve an efficacy endpoint in absence of host neutrophils. Data from two murine candidiasis models differing only in the presence (or absence) of neutrophils, however, found a similar AUC/MIC associated with fluconazole efficacy [22,27]. Study with several triazoles has also investigated the impact of resistance mechanism on antifungal pharmacodynamics [22–25,28]. The triazole AUC/MIC associated with

![Fig. 2. Relationship between the 24-hour AUC/MIC parameter and efficicay of four triazoles against Candida in mice. (From Andes D. Clinical pharmacodynamics of antifungals. Infect Dis Clin N Am 2003;17:635–49; with permission.)]
efficacy in these studies was similar for susceptible *C. albicans* and those with reduced susceptibility caused by target site mutations and over expression of several drug efflux pumps. Finally, pharmacodynamic analysis of triazole studies can be used to examine the impact of treatment in different animal species. Study results in mice, rats, and rabbits are remarkably similar, suggesting that the pharmacodynamic magnitude target associated with treatment outcome is similar in different mammals [2]. One may expect differences in pharmacokinetics in different animal species to impact the pharmacodynamic target. Consideration of drug exposures in pharmacodynamic terms (relative to MIC of the organism), however, corrects for interspecies kinetic differences. Simply put, the drug target is in the organism and not in the host and thus host pharmacokinetic differences should not change the antimicrobial exposure the organism needs to see for effect. This knowledge allows one to use results from preclinical animal pharmacodynamic target studies to estimate antifungal dosing efficacy in humans.

The important next question is what endpoint in these preclinical animal models is relevant to treatment outcome in patients. Numerous microbiologic and survival endpoints are routinely examined using these in vivo infection models. The most reproducible endpoint that has correlated well with outcome following triazole therapy in patients is the drug exposure associated with 50% of the maximal effect (ED50) [2]. For each of the triazoles examined in pharmacodynamic animal model studies, the 24-hour AUC/MIC necessary to produce the ED50 corresponds to a value near 25. For the non-pharmacokinetically oriented, this is essentially the same as averaging a drug concentration near the organism MIC for a 24-hour period (1 × MIC × 24 h = AUC/MIC of 24).

**Clinical impact**

The logical next step is to determine if and how the experimental pharmacodynamic studies relate to outcome in patients. Data from antibacterial pharmacodynamics provide a compelling precedence for the predictive value of animal model pharmacodynamics and clinical therapeutic efficacy [1]. The complexities surrounding patients who have fungal disease are well known and undoubtedly contribute to outcome independent of antifungal pharmacodynamics. The most important confounding host variable is the underlying host immune deficiency, which has been shown to be perhaps the most important factor influencing patient survival [29,30].

Despite this limitation, there are several data sets that allow one to consider the relationship between antifungal dose, organism MIC, and clinical outcome. The largest of these is summarized in the Clinical Laboratory Standards Institute (CLSI) antifungal susceptibility breakpoint guideline publication [6]. Data from six fluconazole trials include nearly 500 episodes of oropharyngeal candidiasis in which the organism MIC, drug dose, and clinical outcomes were available. One can use the organism MIC and dose
in these patients to estimate a 24-hour AUC-MIC value and then examine the relationship between this value and treatment success. When the 24-hour fluconazole AUC/MIC exceeded a value of 25, clinical treatment success was observed in 91% to 100% of patients. When this pharmacodynamic value decreased to less than 25, however, treatment failures were reported in 27% to 35% of cases. The association between the 24-hour AUC/MIC and outcome is similar to that observed in animal model pharmacodynamic studies. The fluconazole AUC/MIC magnitude of near 25 is supportive of the susceptibility breakpoint guidelines suggested in the CLSI publication. Of additional interest in this publication was the proposal of a new susceptibility category, termed “susceptible-dose dependent,” in which the organism is considered susceptible if a higher drug dose is used. In this particular case a fluconazole dose escalation to 400 or 800 mg/d achieves a 24-hour AUC-MIC value of approximately 25 for organisms with MICs up to 16 and 32 mg/L, respectively. There are numerous additional publications of smaller series of patients (in total more than 1000 patients) with oropharyngeal candidiasis in which treatment failures were associated with an elevated MIC and the fluconazole drug dose was provided [31–50]. For nearly all treatment failures reported, the estimated fluconazole AUC/MIC value would have decreased to less than a value of 25, again in line with predictions from animal models.

Pharmacodynamic analysis of studies in patients who have candidemia and deep Candida infection is more difficult. Most of the larger trials in treatment of candidemia provide a paucity of data with organisms for which the MIC is elevated. In this case it is difficult to show a relationship between MIC and outcome, because the AUC/MIC values are above a value at which one expects failures related to drug therapy. For example, in the large candidemia trial examining the efficacy of fluconazole, among the C albicans isolates from patients treated with fluconazole the MIC for more than 90% of organisms was less than 1 mg/L, where the 24-hour AUC/MIC value is many fold higher than that expected to be associated with treatment failure [51]. In addition, outcome in candidemia can be impacted not only by antifungal therapy and underlying host immune state, but also by management of intravascular catheters, adding yet another confounding variable. Four studies, however (in total more than 600 patients), of invasive candidiasis allow consideration of fluconazole dose, MIC, and outcome [6,52–54]. Examination of data from these studies also demonstrates a strong relationship between MIC, fluconazole AUC, and outcome. Taken together these studies showed that clinical success was observed in 70% of patients when the fluconazole AUC/MIC ratio was 25 or greater and was 47% when the value decreases to less than 25. When the pharmacokinetics of fluconazole in humans are considered, these AUC/MIC ratios would support in vitro susceptibility breakpoints of 8 mg/L for doses of 200 mg/d and susceptibility breakpoints of 16 to 32 mg/L for doses of 400 to 800 mg/d for candidemia and mucosal disease.
Most recently attempts have been made to similarly correlate the pharmacokinetics of the recently approved triazole, voriconazole, with MIC, and outcome [5]. If one considers the kinetics of voriconazole in humans, an intravenous dose of 4 mg/kg every 12 hours would produce free drug AUCs of approximately 20 \( \mu \text{g} \cdot \text{h/ml} \). Given a pharmacodynamic target of a free drug AUC/MIC ratio of 20–25, one could predict that these voriconazole dosing regimens could successfully be used for treatment of infections caused by *Candida* spp. for which MICs are as high as 1 mg/L. Indeed, maximal efficacy was observed with *C. albicans* isolates for which MICs were less than 1. The highest failure rates (45%) were observed with *C. glabrata* isolates for which many MICs were greater than 1 mg/L. These data were used in the development of susceptibility breakpoints for voriconazole [5].

Unfortunately there are no complete clinical databases (kinetics, MIC, and outcome) to examine these relationships for voriconazole or other antifungals in treatment of filamentous fungal infections. There is, however, an accumulating body of evidence from which one can attempt to draw pharmacodynamic information. There have been more than 40 reported patients who have developed breakthrough infections while receiving voriconazole [55–59]. A common feature of nearly all of these cases was infection with an organism for which the voriconazole MIC was greater than 1 mg/mL. Unfortunately voriconazole serum concentrations were not available for these patients. A recent case series did, however, identify a relationship between voriconazole serum concentration and patient outcome [59]. Patients who have concentrations less than 2 mg/L were more likely to die from invasive fungal infection (mostly aspergillosis) than those who had serum concentrations exceeding this value. Considering free drug concentrations and the MICs of organisms involved in these case series, one can estimate that treatment failure was associated with 24-hour free drug AUC/MIC values less than 20 to 50. Again, these values are similar to those with fluconazole for treatment of *Candida* infections in clinical trials.

**Polyene pharmacodynamics**

*Predictive pharmacodynamic parameter*

In vitro polyene time kill studies have been undertaken with numerous yeast and filamentous fungal pathogens [18,20,21,60]. Each of these studies has demonstrated marked concentration-dependent killing and maximal antifungal activity at concentrations exceeding the MIC from 2- to 10-fold. Several of these in vitro models have demonstrated prolonged persistent growth suppression following drug exposure and removal (PAFE) [18,20,21]. In vivo time kill studies with AmB and each of the lipid preparations against several *Candida* species have also demonstrated an enhanced rate and extent of killing with increasing AmB concentrations [61,62]. Maximal killing was similarly observed with doses that produce serum
concentrations exceeding the MIC from 4- to 10-fold. The AmB products also produced prolonged in vivo PAFEs. The duration of these persistent effects was also linearly related to the concentration of the AmB exposure. For example, the longest periods of in vivo growth suppression were nearly an entire day (> 20 h) following a single high dose of AmB in neutropenic mice. For drugs displaying this pattern of activity the C max/MIC ratio has most often been the PK/PD parameter predictive of efficacy [1].

Dose fractionation studies with AmB in vivo against Candida and Aspergillus demonstrated superior efficacy when administered as large doses as infrequently as every 3 days [61,63]. In the study against Candida, the total drug required to produce microbiologic efficacy was nearly eightfold less when administered every 3 days compared with daily dosing. The results of these experiments corroborate the importance of the C max/MIC pharmacodynamic parameter.

Predictive pharmacodynamic magnitude

In vivo study with AmB against multiple Candida species in a neutropenic disseminated candidiasis model observed a net static effect (growth inhibition) when the C max/MIC ratio approached values of 2 to 4 [61]. Maximal microbiologic efficacy was observed with ratios near 10. Similar investigation of efficacy in a murine pulmonary aspergillosis model found near maximal efficacy with C max/MIC exposures in the range of 2 to 4 [63]. These most recent studies with aspergillus address a critical gap in knowledge and suggest that at least for AmB, pharmacodynamic relationships are similar among fungal species.

It is generally accepted that the lipid formulations of AmB are not as potent as conventional AMB on a mg/kg basis [62]. Each of the lipid formulations is complexed to a different lipid and exhibits unique pharmacokinetic characteristics. For example, the liposomal formulation of AmB achieves high serum concentrations relative to those achieved by the other formulations. Conversely, following administration of the lipid complex formulation of AmB, serum levels are low, yet the distribution to certain organs, such as the lungs, is reported to exceed those of the other formulations. Recently murine candidiasis models (lung, kidney, and liver) were used to discern if pharmacokinetic differences in serum or tissue could explain these in vivo potency differences [62]. Similar to prior in vivo experiments, the lipid formulations were 4.3- to 5.9-fold less potent than conventional AmB. The pharmacokinetic differences in serum accounted for much of the difference in potency between conventional AmB and the lipid complex formulation. The differences in the kinetics in the various end organs between AmB and the liposomal product were better at explaining the disparate potencies at these infection sites. Groll and colleagues performed a similar investigation with Candida in a rabbit CNS infection model. There was a poor relationship between CSF concentrations and microbiologic
efficacy [8]. The brain tissue C\textsubscript{max}/MIC ratio, however, was a reliable predictor of outcome. The liposomal formulation of AmB seemed to provide a pharmacokinetic/outcome advantage over the other formulations in this CNS infection model.

**Clinical impact**

The pharmacokinetics of AmB and the various lipid formulations have been carefully characterized in serum and tissues for several patient populations. Several investigations have attempted to demonstrate a correlation between AmB MIC and outcome. Most of these studies have found it difficult to discern MIC impact, likely related to the narrow MIC range observed with current testing methods [64]. The author is aware of only a single investigation that has attempted to correlate individual patient level pharmacokinetics, MIC, and outcome with polyenes [65]. This recently published study examined liposomal AmB kinetics and outcome of invasive fungal infections in pediatric patients. In this small study, data from a subset of patients provided detailed kinetics, MIC, and outcome. The results demonstrate a statistically significant relationship between C\textsubscript{max}/MIC ratio and outcome. Maximal efficacy was observed with liposomal AmB serum C\textsubscript{max}/MIC ratios greater than 40. This value is similar to that observed in the animal model studies described earlier when using serum liposomal AmB measurements. This small study demonstrates that pharmacodynamic investigation with a drug from the polyene class can produce meaningful results that are congruent with those from preclinical infection models.

**Flucytosine pharmacodynamics**

*Predictive pharmacodynamic parameter*

In vitro and in vivo studies have examined the pharmacodynamics of flucytosine [21,66–69]. Results from these models have been consistent. Increasing drug concentrations in vitro and larger doses in vivo produced minimal concentration-dependent killing of *Candida* species and soon after exposure organism growth resumes. Dose fractionation studies in vivo against *Candida* spp demonstrated that efficacy was optimal when drug was administrated in smaller dose levels more frequently [66,70]. Tenfold less drug was needed for efficacy when administered using the most fractionated dosing strategy by prolonging the time of the antifungal exposure. The time course and dose fractionation results in therapy against *C albicans* suggest the %T > MIC would be the most predictive parameter [66]. Recent study of flucytosine in an in vivo *Aspergillus* model also suggests that the most fractionated regimen (every 6 as opposed to every 12 or 24 hours) was most effective [69].
Predictive pharmacodynamic magnitude

Maximal efficacy from in vivo candidiasis and aspergillosis models has been observed when flucytosine levels exceeded the MIC for only 20% to 40% of the dosing interval [66,69]. These data also suggest a concordance of pharmacodynamic relationships among fungal species.

Clinical impact

Although no clinical studies have examined the relationship between flucytosine pharmacokinetics, MIC, and efficacy, there are several investigations that demonstrate a strong relationship between flucytosine kinetics and toxicity [71]. These studies have shown that bone marrow toxicity is observed when levels in serum exceed 50 to 60 mg/L. If one were to consider the human kinetics of the most frequently recommended flucytosine dosing of 150 mg/kg/d divided into four doses, each dose of 37.5 mg/kg would remain higher than the MIC for 90% of C albicans isolates tested for 12 to 14 hours [72]. Use of significantly smaller amounts of drug would allow flucytosine administration with much less concern about related toxicities. Whether higher concentrations would be optimal for cryptococcal CNS infection remains an important unanswered question.

Echinocandin pharmacodynamics

Predictive pharmacodynamic parameter

In vitro time course studies with each of the available echinocandin drugs have demonstrated concentration-dependent killing and prolonged PAFEs similar to those observed with the polyenes [18,73]. In vivo studies have confirmed these pharmacodynamic characteristics [74,75]. Following single escalating doses of the new echinocandin, aminocandin, marked killing of C albicans was observed when drug levels in serum were more than four times the MIC. The extent of killing increased as concentrations relative to the MIC approached a factor of 10. Early dose fractionation studies with the first echinocandin derivative, cilofungin, also demonstrated enhanced efficacy by maximizing serum and tissue concentrations [75]. Subsequent investigations in vivo with newer derivatives against C albicans and A fumigatus found that efficacy was maximized by providing large, infrequently administered doses [74,76,77]. The total amount of drug necessary to achieve various microbiologic outcomes over the treatment period was 4.8- to 7.6-fold smaller when the dosing schedule called for large single doses than when the same amount of total drug was administered in two to six doses [74]. The concentration-dependent killing pattern and results from dose fractionation studies would suggest that either the C_{max}/MIC or AUC/MIC would best represent the driving pharmacodynamic parameter [1]. In vivo studies using serum kinetics suggest that the C_{max}/MIC was
better predictive of efficacy [74]. A tissue kinetic study, however, also demonstrated the importance of the AUC/MIC parameter [76].

**Predictive pharmacodynamic magnitude**

Study against multiple *C. albicans* strains in a murine model demonstrated maximal efficacy when the total drug C<sub>max</sub>/MIC of aminocandin approached a value of 10 (net inhibitory outcomes were observed with values near 3) [74]. In a pulmonary aspergillosis model, caspofungin efficacy was similarly maximized at a C<sub>max</sub>/MEC ratio in the range of 10 to 20 [77]. These data support the principle that pharmacodynamic relationships are similar for drugs within the same mechanistic drug class (echinocandins) and for different fungal species.

**Clinical impact**

Most clinical studies with echinocandins have not been extensively examined from the pharmacodynamic standpoint. Several observations, however, can be gleaned from the dose/effect data evident from the group of clinical studies as a whole [78–81]. Accumulating evidence with several of the echinocandins in trials of esophageal candidiasis and candidemia suggest that increasing drug concentrations improves efficacy. A recently presented study with micafungin for esophageal candidiasis is the first to examine not only dose escalation but alternative dosing intervals [82]. The results suggest efficacy can be optimized with a dosing strategy that maximizes the C<sub>max</sub> and allows dosing less frequently than daily. It will be interesting to see if this strategy can be used in treatment of systemic fungal infections.

**Combination**

Despite the recent boom in antifungal drug development, patient outcome associated with invasive fungal infections remains less than acceptable. It has been theorized that combination of two or more antifungal compounds with different mechanisms of action could improve efficacy. The success of the combination of amphotericin and flucytosine for cryptococcal meningitis serves as a critical proof of principle [83]. Numerous in vitro and in vivo infection models have been used to investigate various combinations against *Candida* and *Aspergillus* [84–86]. The results have been variable, ranging from reduced to an enhanced effect. Prior study with antibacterial combination studies has demonstrated that consideration of pharmacodynamics can help to decipher these often complex relationships [87]. Even if two drugs together can enhance outcome, it is possible or even likely that this positive interaction is not evident at all drug concentration combinations. Recent in vitro antifungal combination studies using
pharmacodynamic analysis have shown this to be the case [67,88,89]. Examination of a wide variety of concentration combinations in these studies provides a means to determine not only if drug A and drug B interact in a helpful way, but they allow estimation of the optimal concentrations of each compound. In vivo pharmacodynamic studies should be useful to design clinical trials investigating antifungal drug combination therapy.

Summary

Application of pharmacodynamic principles to antifungal drug therapy of Candida and Aspergillus infections has provided an understanding of the relationship between drug dosing and treatment efficacy. Observations of the pharmacodynamics of triazoles and AmB have correlated with the results of clinical trials and have proven useful for validation of in vitro susceptibility breakpoints. Although there remain many unanswered questions regarding antifungal pharmacodynamics, available data suggest usefulness in the application of pharmacodynamics to antifungal clinical development. Future application of these principles should aid in the design of optimal combination antifungal therapies.

References


[70] Karyotakis NC, Anaissie EJ. Efficacy of continuous flucytosine infusion against Candida ha-
sitaniae in experimental hematogenous murine candidiasis. Antimicrob Agents Chemother
[71] Francis P, Walsh TJ. Evolving role of flucytosine in immunocompromised patients: new in-
sights into safety, pharmacokinetics, and antifungal therapy. Clin Infect Dis 1992;15:
1003–18.
[73] Ernst EJ, Roling EE, Petzold CR, et al. In vitro activity of micafungin (FK-463) against Can-
dida spp.: microdilution, time-kill, and postantifungal-effect studies. Antimicrob Agents
[74] Andes D, Marchillo K, Lowther J, et al. In vivo pharmacodynamics of HMR 3270, a gluca-
n synthase inhibitor, in a murine candidiasis model. Antimicrob Agents Chemother 2003;47:
1187–92.
ofungin, a 1, 3–3-glucan synthetase inhibitor, during continuous and intermittent intra-
venous infusions in treatment of experimental disseminated candidiasis. Antimicrob Agents
systemic candidiasis: importance of persistence of caspofungin in tissues to understanding
murine model of invasive pulmonary aspergillosis: evidence of concentration-dependent
[78] Pflicker MA, Diekema DJ, Boyken L, et al. Effectiveness of anidulafungin in eradicating Can-
dose-response study of micafungin compared with fluconazole for the treatment of esophag-
[80] Krause DS, Reinhardt J, Vazquez JA, et al. Phase 2, randomized, dose-ranging study evalu-
ating the safety and efficacy of anidulafungin in invasive candidiasis and candididemia. Anti-
parative, clinical trial of micafungin alone and in combination for treatment of newly diag-
[82] Buell D, Kovanda L, Drake T, et al. Alternate day dosing of micafungin in treatment of
esophageal candidiasis. ICAAC 2006;M719:419.
[83] Bennett JE, Dismukes WE, Duma RJ, et al. A comparison of amphotericin B alone and com-
bined with flucytosine in the treatment of cryptococcal meningitis. N Engl J Med 1979;301:
[84] MacCallum DM, Whyte JA, Odds FC. Efficacy of caspofungin and voriconazole combina-
tions in experimental aspergillosis. Antimicrob Agents Chemother 2005;49:
3697–701.
with voriconazole in a guinea pig model of invasive aspergillosis. Antimicrob Agents Chem-
[86] Warn PA, Sharp A, Morrissey G, et al. Activity of aminocandin (IP960) compared with am-
photericin B and fluconazole in a neutropenic murine model of disseminated infection caused
by a fluconazole-resistant strain of Candida tropicalis. J Antimicrob Chemother 2005;56:
590–3.
[87] Mouton JW, Van Ogrop JW, Andes D, et al. Use of pharmacodynamic indices to pre-
dict efficacy of combination therapy in vivo. Antimicrob Agents Chemother 1999;43:
2473–8.