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Pros and Cons of Extrapolating Animal Data on Antifungal Pharmacodynamics to Humans

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Abstract

Both the incidence of invasive fungal infections and the number of antifungal agents available to clinicians have expanded significantly over the past two decades. Successes with pharmacokinetic and pharmacodynamic evaluations of other antimicrobial agents in animal models and their clinical correlations with patient outcomes have led to an increased number of studies evaluating both old and new antifungal agents. Recently, animal models have successfully defined target pharmacodynamic indices for many antifungal agents and fungal infections, but validation of these targets in human studies is frequently lacking. This article evaluates the potential pros and cons of extrapolating to humans the animal data on antifungal pharmacodynamics.

Keywords

Murine; Rabbit; Animal model; Pharmacokinetic; Pharmacodynamic; Invasive fungal infection; Amphotericin B; 5-Flucytosine; Fluconazole; Isavuconazole; Voriconazole; Posaconazole; Caspofungin; Micafungin; Anidulafungin

Introduction

The incidence of invasive fungal infections and the number of available antifungal agents have increased substantially over the past two decades. Optimizing antifungal therapy in patients with invasive fungal infections creates a unique challenge because of a paucity of human studies evaluating antifungal pharmacokinetic (PK) and pharmacodynamic (PD) indices.

The use of animal models as a method to evaluate antimicrobial PK and PD has become common. In the case of invasive fungal infections, animal studies have been the cornerstone for determining relationships between antifungal drug concentrations and therapeutic efficacy, such as maximum concentration ($C_{\max}$)/minimum inhibitory concentration (MIC),

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area under the concentration-time curve (AUC)/MIC, or time greater than MIC. The reliance on animal studies has been due in part to the infrequent occurrence in humans of many fungal infections, which does not allow for the appropriate and timely enrollment of humans in the clinical studies necessary to make reliable conclusions. In these situations, animal models may serve as the only comparative studies of antifungals in monotherapy and in combinations.

Testing multiple dosages and intervals is commonly required to elucidate the most appropriate PK and PD indices that correlate with efficacy. Conducting the broad spectrum of evaluations required to define the dose-response relationship would not be ethical in humans. The optimal antifungal dosage strategies necessary to obtain target tissue concentrations at the infection site and reduce fungal burden are commonly elucidated in animal models, but the ability to reliably extrapolate this data to humans is frequently questioned. Many variables such as host immune function, organ dysfunction, and pathogen play a large role in outcome for animals and humans. Thus, both the animal model and the endpoint must be scrutinized when attempting to apply the resulting PD principles to humans. This review discusses some of the pros and cons of applying to humans the antifungal PK and PD indices obtained in animal models.

Pros of Extrapolating Animal Data to Humans

Animal models have proven useful in early assessment of antifungal drug discovery, delivery, activity, and distribution, and when they are carefully designed, they can provide valuable insight into potential synergism, antagonism, and PK/PD dosing parameters corresponding to a higher probability of improved outcomes for a given fungal infection. Additionally, animal models allow the investigation of known agents in treating rare fungal diseases, using large numbers and controlled designs that cannot be achieved in humans to provide data for rational drug selection and dosing.

The ultimate goal of the design of animal models is the development of a pathophysiologic representation reflective of human disease and outcomes. For a given organism and infection site, the general assumption is that similar drug exposure will lead to analogous decreases in fungal infection burden in animals and humans. Compared with human models, however, animal studies evaluate an in vivo homogeneous population, allowing for a clearer evaluation of the relationship of drug concentration versus pathogen. The result is better delineation of the appropriate PK/PD index predictive of efficacy and toxicity. For this reason, animal models may offer more comprehensive analysis of PD than currently available cell culture models or in vitro human studies.

Predicting PK/PD Indices

Murine models were instrumental in assessing fluconazole PD parameters associated with improved outcomes. For fluconazole in both nonneutropenic and neutropenic mice, an AUC/MIC ratio of 20 to 25 for *Candida albicans* was associated with improved efficacy (Table 1) [1, 2]. In humans, a retrospective analysis confirmed fluconazole dose/MIC greater than 50 as a predictor of clinical response ($P = 0.0003$) [3]. Although AUC was not addressed in this retrospective study, assuming similar drug distribution and clearance with a

consistent dosing interval, drug dose can serve as a surrogate for AUC. Further, using a regression equation approximating fluconazole clearance as a function of creatinine clearance, Pai et al. were able to estimate a fluconazole AUC in a large retrospective cohort of patients with candidemia. Higher dose/MIC and AUC/MIC ratios were again correlated with a trend towards decreased mortality ($P=0.0272$ and $P=0.09$, respectively) [4]. Specifically, survivors had a mean weight-normalized dose/MIC ratio of 13.3, compared with 7 in nonsurvivors ($P=0.03$). Classification and regression tree analysis established a weight-normalized dose/MIC breakpoint for mortality at 12 ($P=0.007$) and an AUC/MIC breakpoint at 55.2 ($P=0.008$).

For voriconazole, a free AUC/MIC ratio of 24 (range, 11–58) has also been shown to be predictive of response in the neutropenic murine candidiasis model [5]. In humans, trough concentrations have been the most-used therapeutic drug monitoring parameter because of the ease of sampling. Trough concentrations less than 1 µg/mL were associated with clinical failure and concentrations greater than 2 µg/mL were associated with response [6, 7]. Patients in whom therapy failed with low trough concentrations demonstrated response at higher doses [6]. Although not directly corroborating the PD parameters elucidated in animal models, increasing doses to achieve higher trough concentrations by default increases drug exposure and AUC, potentially leading to a superior AUC/MIC ratio.

For posaconazole, free AUC/MIC ratio of 16.9 (range, 6.12–26.7) has been shown to be predictive of response in the neutropenic murine candidiasis model [8]. A total AUC/MIC ratio greater than 80 to 100 was predictive of success in *Aspergillus fumigatus* murine models and suggests that higher than licensed doses may be needed as the *A. fumigatus* MIC exceeds 0.5 mg/L [9, 10]. The mean serum concentration resulting in half-maximal suppression of fungal growth in the mice was 0.598 mg/L [10]. Similarly, human studies of *Aspergillus* infections demonstrated that patients with mean serum concentrations of 0.411 to 0.719 mg/L had a 53% response rate. Higher C_{max} and higher average serum concentrations were associated with a lower probability of failure, but well-defined correlations will require further study [11•, 12]. Similar to voriconazole, an optimal posaconazole AUC/MIC predictive of treatment success needs further elucidation in humans.

For echinocandins, PD targets in the neutropenic candidiasis murine model have been shown to be similar between caspofungin, anidulafungin, and micafungin. For *C. albicans*, a free AUC/MIC ratio of 20 predicted response, whereas in *Candida glabrata* and *Candida parapsilosis*, a lower free AUC/MIC of 7 predicted response [13•]. Validation of these PD targets in humans is currently lacking, but currently approved dosage strategies are likely to achieve these PD targets for most *Candida* species that cause invasive disease in humans.

Amphotericin B exhibits concentration-dependent fungicidal activity against a variety of invasive fungi. In animal models, the C_{max} /MIC ratio appears most predictive of reductions in fungal burden [14, 15]. A murine model comparing the PK of amphotericin B deoxycholate (AMB), amphotericin B lipid complex (ABLC), and liposomal amphotericin B (L-AMB) demonstrated that AMB is approximately 4.3-fold to 8-fold more potent than ABLC and L-AMB on a mg/kg basis. This difference relates well to common dosing

strategies used between the available amphotericin B products in clinical practice. Significant differences in drug distribution are also seen, with L-AMB yielding the highest serum concentrations and ABLC yielding the highest tissue concentrations. These differences in the pharmacokinetics of AMB, ABLC, and L-AMB have prompted numerous comparisons in animal models in an attempt to optimize dosing strategies and differentiate efficacy amongst these agents.

Recent murine models of invasive pulmonary aspergillosis and mucormycosis have demonstrated significant differences in lung concentrations achieved in the first 72 h of therapy with ABLC compared with L-AMB [16, 17•]. The results of these animal models suggest that ABLC may suppress pulmonary fungal burden earlier in treatment, and that higher initial doses of L-AMB may be required to achieve a similar effect. However, the largest human study to date evaluating an L-AMB loading regimen of 10 mg/kg per day times 14 days compared with a standard 3 mg/kg per day regimen failed to demonstrate any improvement in efficacy and increased the incidence of nephrotoxicity and hypokalemia [18].

Evaluating PK/PD at the Infection Site

The ability to sample tissue and target sites for drug concentration in healthy and infected animals provides immensely useful insights into designing appropriate human therapy strategies. For example, when treating fungal endophthalmitis, drug penetration to the site of infection (e.g., the choroid, retina, or vitreous) may be beneficial. Because of clinical experience, amphotericin is still considered a first-line agent for *Candida* endophthalmitis, but amphotericin distribution into the vitreous is minimal and probably inflammation-dependent. Intravenous and oral fluconazole produces adequate vitreal concentrations in rabbit models and has been used successfully as an alternative to amphotericin in susceptible *Candida* endophthalmitis [19–22]. Similarly, adequate voriconazole penetration into the vitreous of 38% plasma concentration after oral administration is supported by human data [23]. Additionally, animal studies evaluating vitreal administration of voriconazole, assessing PK and toxicity, were conducted to explore an adjunctive route of administration [22], further underscoring the usefulness of animal data in assessing target-site PK of a potential therapy for refractory infections when human data are lacking. The distribution of micafungin and fluconazole in the choroid-retina and vitreous was assessed in a rabbit model. For micafungin, investigators found adequate retina-choroid concentrations that were 66% of the plasma concentration, and undetectable vitreous concentrations; fluconazole demonstrated retina-choroid concentrations equal to or above plasma concentrations and a vitreous $C_{\max}$ that was 46% of plasma concentrations [24]. All echinocandins exhibit poor penetration into the vitreous and therefore are not a preferred therapy [22].

For analysis of central nervous system (CNS) penetration of antifungals, infected animal models can provide both cerebrospinal fluid (CSF) and brain tissue concentrations. In healthy guinea pigs, voriconazole showed adequate distribution into CSF, with concentrations 68% of those found in the serum; brain tissue concentrations were 6.8 µg/g 15 min after oral gavage administration and 2.7 µg/g 1 h after the administration [25]. Adequate penetration of voriconazole has also been reported in humans [26]. Echinocandins

in general show very low, dose-dependent CNS penetration in infected animal models and should not be considered first-line monotherapy [26]. Amphotericin B is considered an effective and primary therapy option for cryptococcal meningitis [27], but CNS penetration of amphotericin B is poor and both human and animal studies have demonstrated low CSF and brain tissue concentrations [28–30]. Similarly, fluconazole, but not amphotericin, was detected in the nucleus pulposus of healthy rabbits [31]. The limitations of noninfected animal models should be noted in these cases, as inflammation may affect drug distribution to the site of infection. Although animal model data does not always exactly predict the extent of tissue penetration in humans, knowledge of drug distribution in animals provide enough qualitative data to make rational drug choices when treating an infection focus.

Evaluating Synergy and Antagonism

Extrapolating animal data concerning synergistic combinations of antifungals to human practice is very tempting, especially when prognosis is poor. Animal models provide reasonably large, homogeneous populations for studying synergistic effects. Additionally, one or multiple infection sites and antifungals can be investigated in an animal model. Louie et al. were able to compare fluconazole, amphotericin B, or 5-fluorocytosine as monotherapy and in combinations for *C. albicans* endocarditis, endophthalmitis, and pyelonephritis. Investigators assessed outcomes in multiple infections and measured target site and tissue concentrations of each drug to relate to efficacy. Combination therapies showed beneficial outcomes compared with some monotherapies in select infection foci. Of note, amphotericin B with or without 5-fluorocytosine was more effective than fluconazole alone for *C. albicans* endocarditis, whereas fluconazole in combination with amphotericin B showed antagonism for fungal endocarditis [32]. Amphotericin B is still a drug of choice in *C. albicans* endocarditis. Other animal models evaluating synergy show mixed results. Anidulafungin and voriconazole as monotherapy and in combination for pulmonary aspergillosis were assessed in neutropenic rabbit and rat models. In the rat model, antifungal dosage and concentrations were conducted to simulate human exposures. Voriconazole monotherapy was superior to anidulafungin. Survival was similar between the voriconazole monotherapy group and the anidulafungin/voriconazole combination group, failing to show synergy [33•]. In the rabbit model, some synergy was noted with standard voriconazole doses at lower anidulafungin doses, but loss of synergy and potential antagonism occurred in the group receiving a higher anidulafungin dose [34]. The results of these studies are disappointing, given that in vitro synergy between echinocandins and voriconazole has been demonstrated. This highlights the importance of conducting in vivo studies in animals and humans to confirm synergistic benefit. Until further data are produced in humans, animal studies do not support a combination of voriconazole and an echinocandin for synergy against *Aspergillus*.

Predicting Antifungal Resistance

Animal models allow for the investigation of factors that drive in vivo resistance. In mice, *C. albicans* resistance to fluconazole developed as drug concentrations fell below the MIC for extended periods of time. An extended dosing interval was a predictor of resistance formation [35]. To extrapolate to humans needing dosage adjustments because of organ dysfunction, clinicians may consider safely maintaining a target 24-hour to 48-hour AUC/MIC without overextending the dosing interval. Extrapolating animal PK/PD efficacy

parameters, such as dose or AUC/MIC ratio, in triazoles may improve response to therapy in humans while minimizing the risk of developing resistance.

Cons of Extrapolating Animal Models to Humans

Although experimental animal models evaluating antifungal PK and PD have many benefits, several limitations must be considered when applying their results to fungal infections in humans. As mentioned previously, validation of animal models with antifungal PD in humans has been best elucidated with fluconazole. The correlation of PD effects with other antifungals in animals versus humans is less clear, and discrepancies between animal and human models have been described (Table 1).

Pharmacokinetic variances in absorption, distribution, protein binding, and elimination may occur in different animal species and must be accounted for in a PD analysis and interpretation [36]. Delivery of the drug to the systemic circulation may occur differently depending on the size of the animal and the model used. In addition, compared with animals, human cells and proteins may interact differently with antimicrobial agents, though such differences have not yet been demonstrated with antifungal agents. Antifungal binding to receptor sites, tissues, albumin, or other proteins may differ significantly between animal species and humans, but the impact of these alterations in humans on highly protein-bound drugs such as posaconazole and the echinocandins has not been fully elucidated. It is expected that PK/PD indices will produce more reliable results if free drug concentrations are evaluated, but this approach has not been the expectation of published studies [36]. Elimination half-lives in smaller animals are significantly shorter, so evaluation of high-dose, extended-interval dosing strategies may not adequately demonstrate differences in efficacy and safety that would be realized in humans. Experimental models of antimicrobials have used induction of organ dysfunction to produce elimination rates similar to those in humans and thereby have yielded more reliable and reproducible PK/PD results [37]. However, inducing organ dysfunction may limit the interpretation of clinical endpoints such as survival.

As mentioned previously, in animal studies, the AUC/ MIC ratio appears most predictive of voriconazole efficacy. In humans, it is important to note that high patient PK variability in voriconazole clearance and saturable hepatic metabolism significantly complicates the PK/PD relationship. As such, the desired human AUC/MIC has not been well established, and direct measuring or calculation of AUC is not practical in the clinical setting [11•].

Interestingly, with increasing drug concentrations above a certain threshold, a paradoxical reduction in antifungal activity resulting in increased tissue fungal burden has been demonstrated in some animal models of echinocandins [38, 39]. However, this paradoxical effect has not been validated in humans. Studies evaluating higher-dose echinocandin regimens have not demonstrated reduced efficacy or increased mortality with escalating dosage [40, 41].

Another point to consider is the substantial interpatient PK variability seen in humans with most of the currently available antifungal agents. Extrapolation from animal models to

humans assumes that the drug concentration achieved at the animal infection site is predictive of human concentrations and provides adequate exposure to combat the organism. Population PK models and Monte Carlo simulations have attempted to account for variations and important covariates, but each of these methods is limited by the patient population chosen for the model. Except for the echinocandins and perhaps fluconazole, using PK parameters from a healthy population will provide an unreliable model for most patients who develop severe invasive fungal disease.

Serum and tissue concentrations are frequently evaluated in animal studies, and PK/PD models are developed based upon the correlation. Tissue homogenates used in animal studies do not differentiate intracellular and interstitial compartments, both of which can be sites of fungal invasion. In humans, serum concentrations are commonly the only measurement that can be routinely obtained, requiring the assumption that serum and tissue concentrations are proportionally related. However, it is known that antifungal agents have differing concentrations and an increased half-life in the tissues compared with the serum [42]. The proportion of antifungal drug concentrations in the serum versus tissues may be an important consideration for echinocandins and triazoles, as drug accumulation may occur in tissues, altering the reliability of serum PD analyses. In these antifungals, tissue concentrations are not consistently reflective of serum concentrations and can persist even after concentrations in serum are below the MIC of the organism. Evaluation of serum concentrations alone may not fully elucidate drug exposure at the site of infection [42, 43]. Assuming that similar compartmental distribution occurs between animal and human models thus may lead to inappropriate antifungal selection and dosage.

Concomitant medication use and drug-drug interactions are rarely accounted for in animal models, but they are frequently encountered when managing patients with invasive fungal infections. Altered expression or activities of metabolizing enzymes, especially induction or inhibition of the cytochrome P450 enzymes, may lead to significant antifungal PK alterations. Genetic alterations in metabolic activity in humans also may be important for some antifungals, such as voriconazole. The importance of these interactions in animal models is not known, and it is difficult to account for them. They are likely to be most important for the triazole antifungals.

The acuity of the fungal infection in animal models may not represent the typical pathogenesis in humans and may result in differences in antifungal PD response. The inflammatory response in humans may significantly alter antifungal distribution and concentrations at the infection site. Commonly, one strain of an infecting pathogen is used in animal models, and therefore it is unlikely to represent the heterogeneity of clinical isolates causing infections in humans. Also, the timing of antifungal therapy is often consistent amongst animals treated and does not adequately represent the high variability in time from fungal infection to antifungal therapy in clinical practice.

The use of fungal burden as a therapeutic endpoint may have some limitations. Some of the inoculated fungi may not actually cause disease in the infected organ, but can grow once plated, resulting in overestimation of the fungal burden. The addition of tests that evaluate the presence of fungal antigens may improve results [44]. The growth rate of the fungi also

may affect the rapidity of killing and therefore make it difficult to extrapolate effects to other pathogens.

The infection model and infection location must be considered when interpreting the results of animal studies. The invading fungus is localized to one area of the animal model in most cases. Differing primary inoculum sites in murine models (e.g., pulmonary vs bloodstream) may yield differing results. For example, murine models of L-AMB have demonstrated improved efficacy compared to ABLC treatment when intravenous injection is used to cause infection [45], but ABLC treatment had greater efficacy than L-AMB in a pulmonary inoculum murine model [17]. The commonly used kidney or meningoencephalitis models may not be easily extrapolated to infections of other organs.

One of the most difficult items to predict when extrapolating evidence is the difference in physiological response that may be seen in animals and humans. It has not been uncommon for animal models of infection to yield beneficial effects on morbidity and mortality that could not be duplicated in humans [46]. Patients with invasive fungal infections commonly have significant and heterogeneous comorbidities that cannot be routinely replicated or accounted for in animal models. This difference is clinically important, because human studies commonly find that comorbidities are the independent predictors of patient outcomes despite adequate antifungal therapy [47]. The impact of neutropenia on the variability in PD response is well illustrated in a recently published murine model of invasive candidiasis. This study found a significant association between immune status, AUC/MIC, and survival in mice treated with isavuconazole. In mice that only temporarily had neutropenia, an AUC/MIC of 270 was needed to achieve a 90% survival rate, but in persistently neutropenic mice, an AUC/MIC of 670 was required [43]. These data highlight the variability in PD targets that may be needed with varying comorbidities in humans, requiring further studies beyond neutropenic and nonneutropenic models.

The initial evidence regarding mechanisms of fungal resistance to antifungal agents is elucidated via in vitro methods. Animal models can be useful for simulating PK/PD relationships that may select for antifungal resistance, but these models cannot account for the variety of fungal pathogens, resistance mechanisms, and selective pressures that occur with the use of antifungal agents at any given institution. Nevertheless, extrapolation to humans of animal-model data on antifungal resistance mechanisms, in addition to other drivers for fungal resistance, needs to be considered when designing both prophylactic and treatment dosage regimens, so that potential detriment to long-term antifungal activity can be avoided.

Conclusions

Animal models of invasive fungal infections and antifungal pharmacodynamics have provided considerable knowledge to researchers and clinicians managing patients with invasive fungal infections. Understanding the pros and cons of extrapolating animal data to humans is imperative to ensure optimal pharmacodynamic target attainment and improved patient outcomes. Experimental animal models reflecting a variety of infection and drug-therapy designs that provide reproducible results predictive of human disease are needed to

better arm clinicians treating patients with invasive fungal infections. Ultimately, validation of animal models via phase III or IV studies in humans would be optimal, but these initiatives are extremely costly and time intensive.

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Table 1

Pharmacodynamic parameters predictive of antifungal response

Antifungal agent	Animal model target attainment	Human model target attainment or clinically relevant data
Fluconazole [3, 4]	AUC: MIC >12–25	Efficacy: Dose: MIC >50 AUC: MIC 55–500
Voriconazole [6, 48]	Free AUC: MIC >24	Prophylaxis efficacy: Trough >0.5 µg/mL Treatment efficacy: Trough >1–2 µg/mL Toxicity: Trough >5.5 µg/mL
Posaconazole [12, 48]	Free AUC: MIC >17	Prophylaxis efficacy: C_{ave} <0.7 µg/mL associated with failure Treatment efficacy: Trough >0.5–1.5 µg/mL Salvage therapy: eC_{max} and C_{ave} = 1.48 and 1.25 µg/mL associated with success
Echinocandin [38, 42, 49, 50]	C_{max} : MEC >10 AUC: MIC >250	Further elucidation required
Amphotericin B deoxycholate [14, 15]	<i>Candida</i> : C_{max} : MIC >10 <i>Aspergillus</i> : C_{max} : MIC >2.4	Further elucidation required

AUC area under the concentration-time curve, C_{ave} average concentration, C_{max} maximum concentration, *MEC* mean effective concentration, *MIC* minimum inhibitory concentration