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Pharmacokinetics and Pharmacodynamics of a Novel Triazole, Isavuconazole: Mathematical Modeling, Importance of Tissue Concentrations, and Impact of Immune Status on Antifungal Effect[▽]

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Isavuconazole is a triazole with broad-spectrum activity against medically important fungal pathogens. We investigated the pharmacokinetics and pharmacodynamics of isavuconazole in a murine model of disseminated candidiasis. We determined the pharmacokinetics in both plasma and kidney. The relationship between tissue concentrations and the resultant antifungal effect was described using a mathematical model. The pharmacodynamic parameter that optimally links drug exposure with the antifungal effect was determined using dose fractionation studies. The impact of the immune status of mice receiving isavuconazole was determined in persistently and temporarily neutropenic animals. The pharmacokinetics of 1.6 to 28 mg isavuconazole/kg of body weight were linear. Exposure-response relationships demonstrated near-maximal effect following the administration of >15 mg/kg. The mathematical model showed that exposures in the kidney were 5.77 times higher than those in plasma, and there was persistence of the drug at this site despite concentrations in plasma falling to undetectable levels. The in vitro and in vivo postantifungal effects were 2 to 5 and 8.41 h, respectively. The area under the concentration-time curve (AUC)/MIC ratio was the parameter that optimally linked drug exposure with the observed antifungal effect. The total drug AUC/MIC ratios associated with a 90% probability of survival in temporarily and persistently neutropenic mice were 270 and 670, respectively. Once corrected for protein binding, these values are similar to the magnitude of drug exposure associated with a high probability of a successful therapeutic outcome for other triazoles. This study provides the experimental foundation for the use of isavuconazole in patients with disseminated candidiasis.

Disseminated candidiasis is a leading cause of morbidity and mortality in patients with neutropenia and a wide range of critically ill nonneutropenic patients. *Candida* spp. are consistently the fourth most common cause of bloodstream infections in the United States (21). Despite advances in diagnostics and therapeutics, there has not been an appreciable change in attributable mortality in the last decade (7, 20). While new drugs and drug classes have become available, significant limitations remain in terms of cost, toxicity, limited aqueous solubility, and complex drug interactions. Thus, the development of new antifungal agents is warranted.

The triazoles have revolutionized the treatment of disseminated candidiasis. BAL8557 is a water-soluble prodrug that is completely converted to the active moiety, isavuconazole (BAL4815), and the pharmacologically inactive prodrug cleavage product BAL8728 under the action of plasma esterases (15, 16). Isavuconazole has broad in vitro activity against a range of medically important fungi, including *Candida* spp., *Aspergillus* spp., *Cryptococcus neoformans*, dermatophytes, and possibly zygomycetes (14, 17–19). Both an intravenous (i.v.) and an oral formulation are available. Data from healthy volunteers and neutropenic patients suggest that isavuconazole is extensively orally bioavailable, displays linear pharmacokinetics, and has a favorable safety profile. In humans, isavuconazole is >90% protein bound and has a long terminal half-life (circa 80 to 100 h) and a large volume of distribution (400 to 500 liters) (5, 15, 16). Phase III clinical trials examining the utility of this agent for the treatment of invasive fungal infections are currently being conducted.

Pharmacokinetics and pharmacodynamics are increasingly used to inform dosage and schedule selection and to provide decision support for the identification of antimicrobial susceptibility breakpoints. The aim of this study was to define the pharmacokinetics and pharmacodynamics of isavuconazole in a murine model of disseminated candidiasis. We measured the concentration of drug at the primary effect site and linked this to the antifungal effect using a mathematical model. We explored the impact of persistent neutropenia on survival and compared this with the outcome for mice for which the neutrophil count was allowed to recover during the treatment period.

(This work was presented in part at the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, October 2008 [5].)

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MATERIALS AND METHODS

Organism. *Candida albicans* FA/6862, a well-characterized clinical isolate (8, 10), was used for all experiments. The organism was subcultured from beads

stored at −80°C on Sabouraud dextrose agar (Oxoid, Basingstoke, United Kingdom) and incubated at 37°C for 48 h. Subsequently, the organism was subcultured in Sabouraud dextrose broth (Oxoid, Basingstoke, United Kingdom) and incubated at 37°C for 16 h on an orbital mixer. A fungal suspension was prepared by two washes in phosphate-buffered saline (PBS).

In vitro susceptibility testing and determination of the postantifungal effect (PAFE). Isavuconazole was provided as a pure powder by Basilea Pharmaceutica International Ltd. (Basel, Switzerland). The MIC was determined according to CLSI M27-A2 methodology (13). MICs were determined in duplicate on three separate occasions, and for the purposes of pharmacodynamics analyses, the modal value was used.

For the determination of the in vitro PAFE, the organism was grown overnight in Sabouraud dextrose broth, washed in PBS, and then resuspended in 250 ml of warmed RPMI 1640 supplemented with 2% glucose to achieve a final fungal density of 1×10^6 CFU/ml. The suspension was incubated at 37°C in a water bath for 30 min and then divided into 10-ml aliquots. Isavuconazole was added to tubes at 1/10×, 1/4×, 1/2×, 1×, 2×, 5×, 10×, 40×, and 100× the MIC. Separate flasks containing the *C. albicans* suspension in drug-free medium served as the controls. The suspension was incubated for 3 h prior to being centrifuged and washed twice. Twenty-five milliliters of warmed RPMI 1640 was then added, and quantitative subcultures were obtained immediately and hourly thereafter for 8 h and then at 14 and 24 h. Subcultures were incubated at 37°C for 48 h, and quantitative colony counts were determined. The PAFE was defined as the difference in the time required for treated fungi to increase in density by 1 $\log_{10}$ CFU/ml after drug removal relative to the time taken for untreated controls to grow 1 $\log_{10}$ CFU/ml.

Murine models of disseminated candidiasis. All experiments were performed under United Kingdom Home Office project license PPL40/2356 and approved by The University of Manchester Ethics Committee. Male CD1 mice (Charles River Ltd., Kent, United Kingdom) weighing 22 to 24 g were used. The mice were housed in vented HEPA-filtered cages, and food and water were provided ad libitum.

A persistently neutropenic model was used for the majority of experiments, but a temporarily neutropenic model was also used in survival experiments (see below). All mice received cyclophosphamide (Sigma, Poole, United Kingdom) at 200 mg/kg of body weight intraperitoneally on day −3 relative to infection. This resulted in profound neutropenia for 6 days (i.e., 3 days postinoculation). For the persistently neutropenic group, a second dose of cyclophosphamide was administered 5 days later (i.e., 48 h postinfection). Serial neutrophil counts were determined in both cohorts. For survival experiments, mice were treated for 5 days and then observed for 2 days. Mice surviving to the end of the experiment were sacrificed. The final fungal inoculum was prepared by dilution in PBS and verified by quantitative culture. Mice were infected i.v. via the lateral tail vein with 2×10^4 organisms in a 0.2-ml volume.

Preparation of isavuconazole for in vivo use. The prodrug, BAL8557, was obtained from the manufacturer (Basilea Pharmaceutica International Ltd., Basel, Switzerland) as pure powder and reconstituted in distilled water to produce a stock solution of 5 mg/ml. This was further diluted in 5% glucose to obtain the final desired concentrations. All dosages were administered to mice in a 0.1-ml volume. Due to toxicity in laboratory animals associated with rapid i.v. administration, the subcutaneous (s.c.) route was used for all experiments. Dosages of isavuconazole were expressed in terms of active compound by multiplying the amount of the prodrug by 0.55.

Pharmacokinetics of isavuconazole in infected immunosuppressed mice. Both single- and multiple-dose pharmacokinetics of isavuconazole were determined in infected immunosuppressed mice. For single-dose studies, 1.6, 5, 13, and 28 mg/kg of isavuconazole was administered s.c., and blood samples were obtained at 0.5, 1, 2, 4, 8, and 24 h posttreatment via cardiac puncture under isofluorane anesthesia. For multiple-dose pharmacokinetics, mice received 1.6 and 28 mg/kg every 24 h. Samples were obtained on day 5 to ensure that a steady state had been achieved. Blood and kidney samples were obtained at 96.5, 97.5, 100, and 104 h after initiation of therapy. Three mice were used for each dose-time point combination. Blood samples were centrifuged and then stabilized with 10 μl 2 M citric acid per ml plasma and stored at minus 80°C prior to analysis.

Measurement of the active moiety, isavuconazole, in plasma and kidney. Concentrations of isavuconazole in plasma and kidney were determined using a validated liquid chromatography-tandem mass spectrometry method as previously described (15, 16). Briefly, protein precipitation was achieved with the addition of 150 μl of acetonitrile/water (8:2) also containing 0.1% formic acid to 50 μl of plasma. Samples were centrifuged at 40,000 × *g* for 20 min, and 100 μl of the supernatant was mixed with 350 μl of 1 M ammonium acetate (pH 5). Samples were analyzed using a Shimadzu liquid chromatograph coupled with an ABI Sciex QTrap 2000 mass spectrometer; a Phenomenex Synergi 4-μm Polar-RP 80A analytical column; and a Haipeek Clipeus, Phenyl, 3-μm trapping column. The injection volume was 30 μl. The dynamic ranges for plasma and kidney were 0.01 to 10 mg/liter and 0.005 to 5 mg/liter, respectively. The limits of quantification were 0.008 mg/liter and 0.004 mg/liter in plasma and kidney, respectively.

Exposure-response relationships. Exposure-response relationships were determined over the course of multiple experiments. An understanding of the exposure-response relationship informed the design of dose fractionation studies and additional pharmacodynamics studies (see below). Multiple dosages of isavuconazole in the range 1.6 to 28 mg/kg were administered once, s.c., 5 h postinoculation, and all mice were sacrificed 24 h later (i.e., 29 h postinoculation). Both kidneys were dissected, weighed, and homogenized in 2 ml PBS. Subsequently, 100 μl was plated on Sabouraud dextrose agar and incubated at 37°C for 48 h. Quantitative colony counts were obtained and expressed as $\log_{10}$ CFU/g kidney.

The data were described using an inhibitory sigmoid E_{max} model that took the following form: effect ($\log_{10}$ CFU/g) = $E_{con} - [(E_{max} \times dose^H)/(E_{50}{}^H + dose^H)]$, where E_{con} is the fungal burden in the absence of therapy, E_{max} is the asymptotic reduction in the fungal burden induced by fungal therapy, E_{50} is the dose associated with half-maximal effect, and H is the slope (or Hill) function. The model was implemented within the identification module of the program ADAPT II (6), and the data were weighted by the inverse of the observed variance.

Time course of antifungal effect, mathematical modeling, and PAFE. To determine the time course of the antifungal effect induced by isavuconazole, 1.6 to 28 mg/kg was administered to groups of three or four persistently neutropenic mice once daily. These relationships were defined in two separate experiments. In the first, mice received 0, 1.6, 4, 14, and 28 mg/kg and were serially sacrificed at 24, 48, 72, 96, and 120 h. In the second experiment, the course of antifungal effect between dosages was defined in detail, and groups of mice receiving 28 mg/kg/day were serially sacrificed in the first (at 2, 4, 8, 12, 18, and 24 h) and third (at 48, 50, 56, 64, and 72 h) dosing intervals.

The data were described using a mathematical model in which all drug concentrations (plasma and kidney) and kidney fungal burden data were comodeled (details are presented in the appendix). The input of drug into the system was calculated in terms of the active drug isavuconazole (i.e., all dosages of the prodrug were multiplied by 0.55 to ensure mg-mg equivalence and accurate estimates of V_c and SCL [see the appendix]). Importantly, it was not possible to directly link plasma concentrations with the antifungal effect at the primary effect site given the very rapid clearance of drug from the central compartment; rather, the concentrations of isavuconazole in the kidney were allowed to directly influence fungal growth. Since a drug-induced decline in the fungal burden was not observed even with the administration of the highest dosage of drug, concentrations of isavuconazole in the kidney were allowed only to impede fungal growth rather than to induce net killing (i.e., a fungistatic term alone was employed rather than a combination of fungistatic and fungicidal terms, as in our previous work with amphotericin B deoxycholate [8]). The weighting terms were determined using the maximum-likelihood estimator available in the identification module of ADAPT II (6). The model was fitted using the population pharmacokinetics program Big Non Parametric Adaptive Grid (BIG NPAG) (11).

The ability of the mathematical model to describe the data was assessed in terms of the log-likelihood value, as well as visual inspection and the r^2 value for the observed-predicted plots for the three output equations describing concentrations in plasma and kidney and colony counts.

The areas under the concentration-time curve (AUC) in plasma and kidney were determined by integration of the respective concentration-time profiles using ADAPT II, and the ratio of the two was calculated. The mathematical model was used to calculate the duration of the in vivo PAFE; this was defined as the difference between the time taken for a 1 $\log_{10}$-CFU/g increase in the fungal burden in the kidney once total plasma drug concentrations had fallen beneath the MIC of 0.004 mg/liter and for the growth of 1 $\log_{10}$ CFU/g in controls.

Dose fractionation studies. Dose fractionation studies were performed to identify the pharmacodynamic variable that best linked drug exposure with the observed antifungal effect. The isavuconazole dose-response relationship was used to identify total dosages that produced 20, 40, 60, and 80% of the maximum effect, thus encompassing the most informative portion of this relationship. These total dosages were administered once daily and as fractionated schedules in which two half-doses were administered every 12 h and four-quarter doses were administered every 6 h to groups of six mice. All mice were sacrificed 24 h following the initiation of treatment, and quantitative kidney counts were determined as described above.

Each of the dosages was converted to AUC/MIC, peak plasma concentration/

TABLE 1. Means, medians, and standard deviations for parameter values from the mathematical model

Parameter[a]	Mean	Median	SD
K_a (h^{-1})	26.50	26.97	1.40
V_c (liter)	0.075	0.085	0.027
SCL (liter/h)	0.037	0.032	0.017
K_{cp} (h^{-1})	15.01	16.34	8.08
K_{pc} (h^{-1})	18.84	19.68	2.7
K_{ck} (h^{-1})	0.169	0.23	0.007
K_{kc} (h^{-1})	18.29	17.95	0.84
V_{kidney} (liter)	0.00019	0.00019	0.00007
K_{gmax} ($\log_{10}$ CFU/g/h)	0.17	0.16	0.07
H_g	9.17	9.39	0.84
C_{50g} (mg/liter)	0.86	0.37	0.83
POPMAX (CFU/g)	39,443,500	35,591,644	66,770,500
Initial condition (CFU/g)	1,684	2,217	800

[a] K_a is the first-order rate connecting the s.c. injection site with the central compartment; V_c and SCL are the volumes of distribution and clearance from the central compartment, respectively; K_{cp} and K_{pc} are the first-order rate constants connecting the central and peripheral compartments; K_{ck} and K_{kc} are the first-order rate constants connecting the central compartment and kidney; V_{kidney} is the volume of the kidney; K_{gmax} is the growth constant describing maximal growth; C_{50g} is the concentration of isavuconazole required to produce 50% effect on the maximal rate of growth; H_g is the sigmoidocity constant for the drug effect on *C. albicans* growth; POPMAX is the theoretical maximum *C. albicans* burden in the kidney. The initial condition is the fungal burden at the time of systemic drug administration.

MIC (peak/MIC), and the fraction of the dosing interval in which total drug concentrations were more than the MIC ($T > MIC$); this was achieved using the median parameter values from the mathematical model and with the simulation module of ADAPT II (see below and Table 1). The AUC was determined by integration. An inhibitory sigmoid E_{max} model was fitted to the AUC/MIC-effect, peak/MIC-effect, and $T >$ MIC-effect relationships, and the extent to which these models accounted for the individual data sets was assessed using the coefficient of determination (r^2).

Relationship between drug exposure and survival in persistently versus temporarily neutropenic mice. To explore the impact of drug exposure and immunological status on survival, the outcome for a cohort of persistently neutropenic mice was compared with that for a temporarily neutropenic group. Dosages of isavuconazole in the range of 1.6 to 28 mg/kg were administered daily to groups of six mice. Therapy continued for 5 days, and the mice were then observed for a further 2 days.

Data were described using a Cox proportional-hazard model. The AUC/MIC for each dosage was calculated using the pharmacokinetic-pharmacodynamic mathematical model described above. The impacts of both the AUC/MIC and immune status on survival were determined. Mice surviving to the end of the experiment were right censored. The Cox model was used to calculate the probability of survival for various AUC/MICs for both persistently and temporarily neutropenic mice.

RESULTS

Susceptibility testing and in vitro PAFE. The MIC was 0.004 mg/liter. An in vitro PAFE for isavuconazole was not apparent at concentrations of ≤1× MIC. At concentrations of 2× MIC, the PAFE was 2 h, and at concentrations of 5×, 10×, 40×, and 100× MIC, the PAFE was 5 h.

Pharmacokinetics. The pharmacokinetics of BAL4815 following the administration of isavuconazole at 1.6, 5, and 13 mg/kg s.c. once to immunocompromised infected mice were linear (Fig. 1). After five doses of 1.6 and 28 mg/kg, the pharmacokinetics of isavuconazole were also linear. Concentrations of isavuconazole in the kidney were five to six times higher than those observed in plasma and underwent relatively rapid exponential decline (Fig. 1). Estimates of the pharmacokinetics parameters were obtained from the linked population pharmacokinetics model (see below) and are summarized in Table 1. The terminal half-life of isavuconazole in plasma was 3.41 h (calculated using the mathematical model discussed below).

Exposure-response relationships. The exposure-response relationship for isavuconazole is shown in Fig. 2. The fungal burden in the kidney at the time of treatment initiation was 2.44 ± 0.16 $\log_{10}$ CFU/g and increased to 5.5 to 6 $\log_{10}$ CFU/g at the end of the study (i.e., 29 h postinoculation). The fit of the inhibitory sigmoid E_{max} model to the data was acceptable, with a coefficient of determination of 0.84. The exposure-response relationship was relatively shallow, with near-maximal reduction in the fungal burden observed with dosages of >15 mg/kg.

Time course of antifungal effect. The fit of the linked mathematical model to the data was highly acceptable, with a coefficient of determination for the observed-predicted plasma drug concentrations, kidney drug concentrations, and kidney colony counts of 0.96, 0.98, and 0.96, respectively (Fig. 3). The mean and median estimates for the model parameter values and their standard deviations are summarized in Table 1. Both measures of central tendency adequately described the data, but the medians gave slightly better predictions for the plasma pharmacokinetics.

Model simulations (Fig. 4) following the administration of isavuconazole show the rapid clearance of drug from the plasma, the relatively higher concentrations achieved in the kidney, and the logarithmic growth of *Candida* in the latter portion of the dosing interval. The ratio of the AUC in the plasma to the AUC in the kidney was 1:5.77, suggesting moderate accumulation of drug at the primary effect site. Simulations demonstrated progressive fungal growth when plasma and kidney concentrations fell below ~0.14 and 0.8 mg/liter, respectively, which are approximately 2 orders of magnitude higher than the MIC of 0.004 mg/liter. The mathematical model was used to determine the duration of the PAFE. The time taken for an increase of 1 $\log_{10}$ CFU/g kidney in the initial logarithmic growth phase of controls was 5.98 h (determined directly from the data), whereas the time taken for a 1 $\log_{10}$-CFU/g increase in the fungal burden in the kidneys of mice receiving isavuconazole once the plasma concentrations had dropped beneath the MIC was 14.39 h. Therefore, the in vivo PAFE was 8.41 h for this strain of *C. albicans*.

Dose fractionation studies. The total dosages of isavuconazole associated with 20, 40, 60, and 80% maximal effect were 3.3, 5.4, 8.1, and 13.5 mg/kg, respectively. The mathematical model was used to convert total dosages to AUC/MIC, peak/MIC, and $T >$ MIC, and these estimates are summarized in Table 2. The regressions for the three variables versus effect are shown in Fig. 5 and demonstrate that the best fit of the inhibitory sigmoid E_{max} model to the data was achieved using the AUC/MIC as the independent variable. An exposure-response relationship was not apparent for $T >$ MIC versus effect.

Survival studies. The Cox model suggested the significant impact of immune status and the AUC/MIC on survival (P = 0.004 and 0.002, respectively). As demonstrated in Fig. 6, temporarily neutropenic mice had a higher probability of survival without the administration of active drug, and 100% survival could be induced with smaller AUC/MIC exposures than was the case for persistently neutropenic animals. AUC/MICs of 270 and 670 were associated with a 90% probability of survival

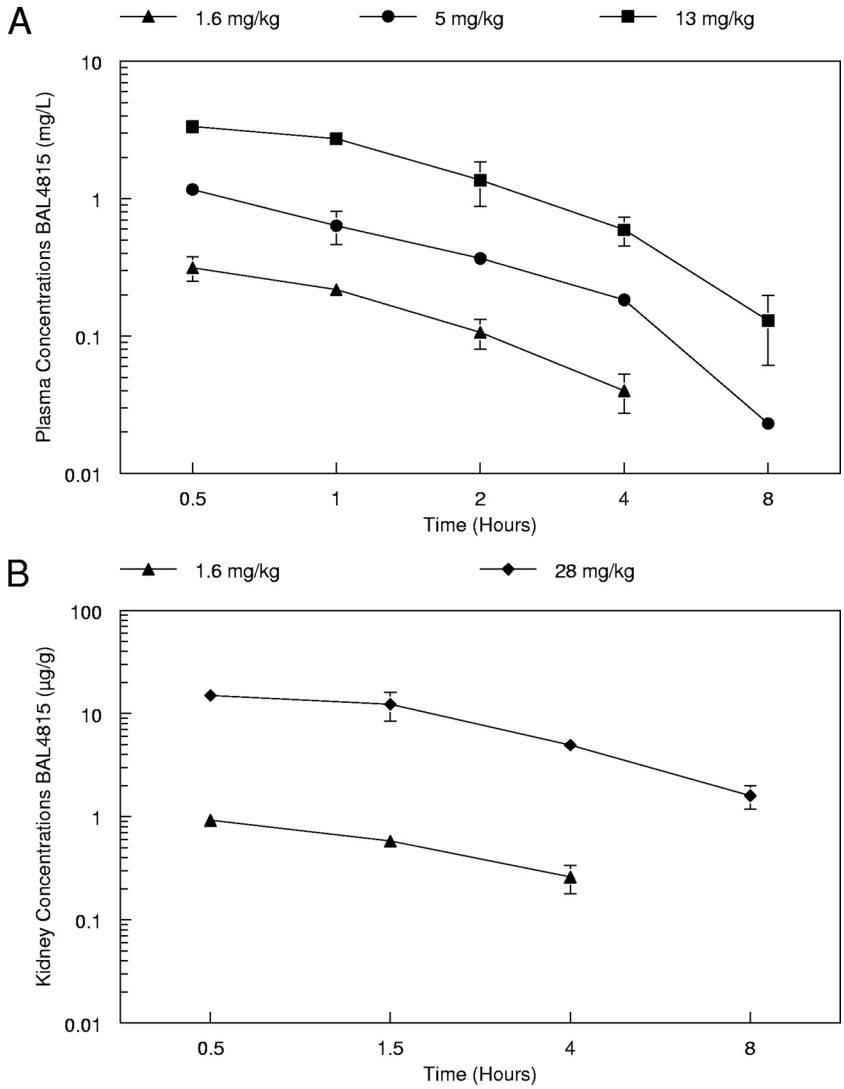

FIG. 1. (A) Single-dose pharmacokinetics of isavuconazole in plasma following administration of the prodrug. (B) Pharmacokinetics of isavuconazole in the kidney following five doses of the prodrug. The data are means ± standard deviations for three mice at each sampling time.

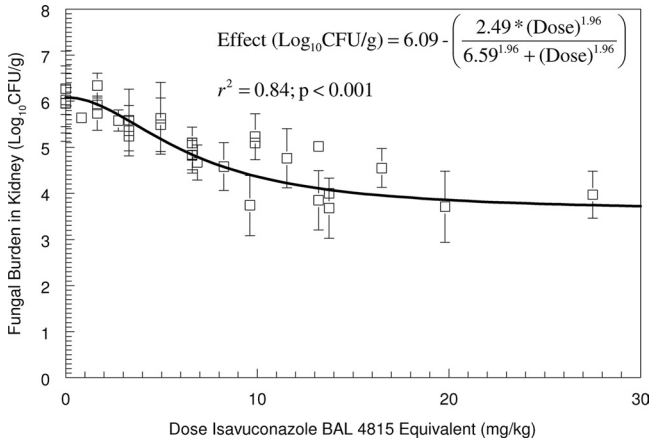

FIG. 2. Exposure-response relationship following the administration of isavuconazole. The data are means ± standard deviations of three or four mice. The solid line is the fit of the inhibitory sigmoid E_{max} model to the data.

in temporarily and persistently neutropenic mice, respectively (Fig. 6).

DISCUSSION

Despite the advent of new drugs and a new antifungal drug class, the mortality from disseminated candidiasis remains 30 to 50% (7). Isavuconazole is a novel triazole with potential clinical utility for the treatment of invasive fungal infections. This study provides the experimental foundation for the optimal use of this agent for patients with disseminated candidiasis.

There was clearly a relationship between plasma concentrations and the observed effect; this was exploited in the dose fractionation studies to define the pharmacodynamic index that best accounted for the antifungal effect of isavuconazole. Importantly, however, attempts to mathematically (i.e., directly) link plasma concentrations with the antifungal effect in the kidney repeatedly failed because of the juxtaposition of

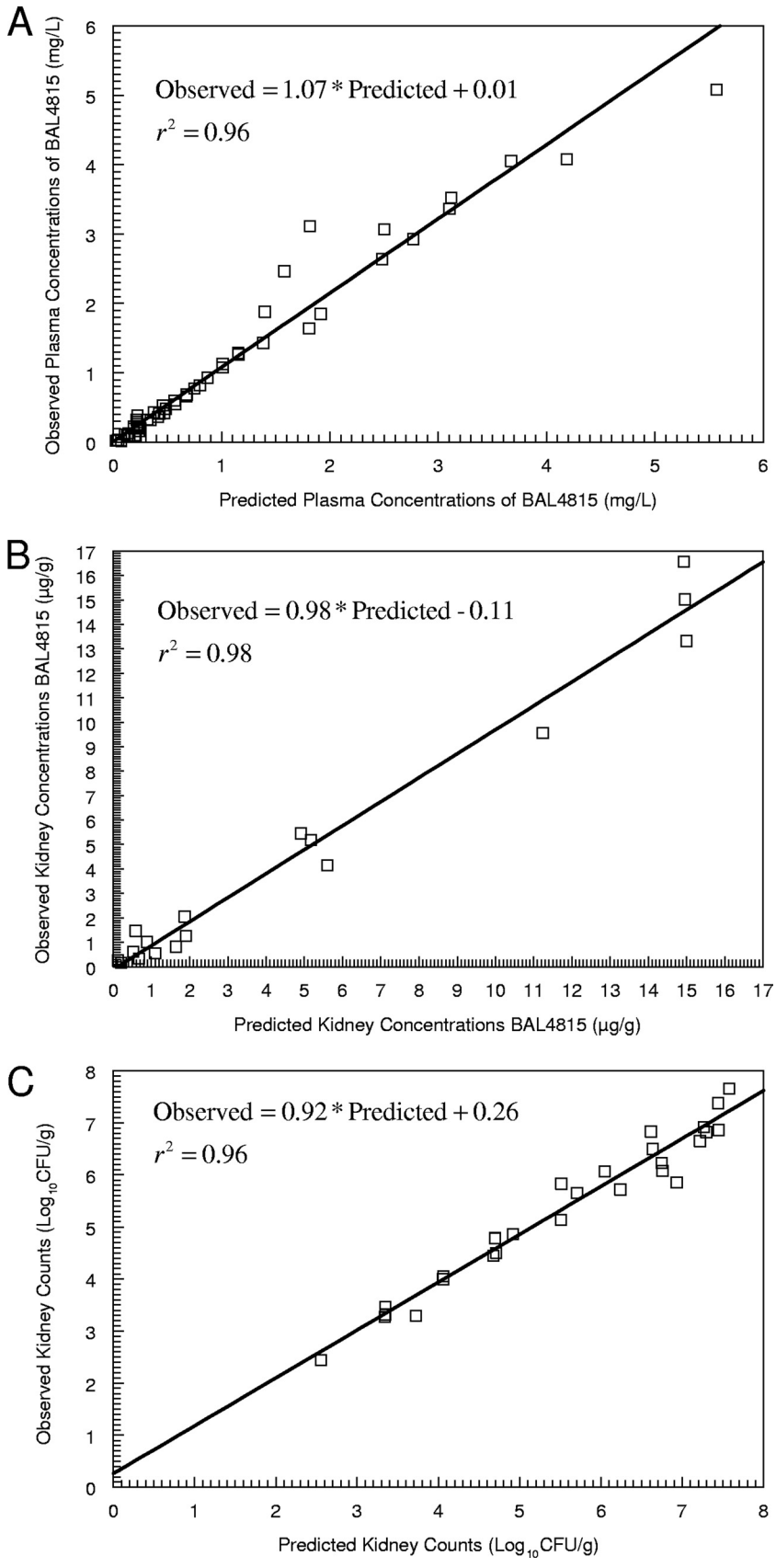

FIG. 3. (A) Observed-predicted plots after the Bayesian step for the concentrations of isavuconazole in plasma. (B) Concentrations of isavuconazole in the kidney. (C) Fungal density in the kidney.

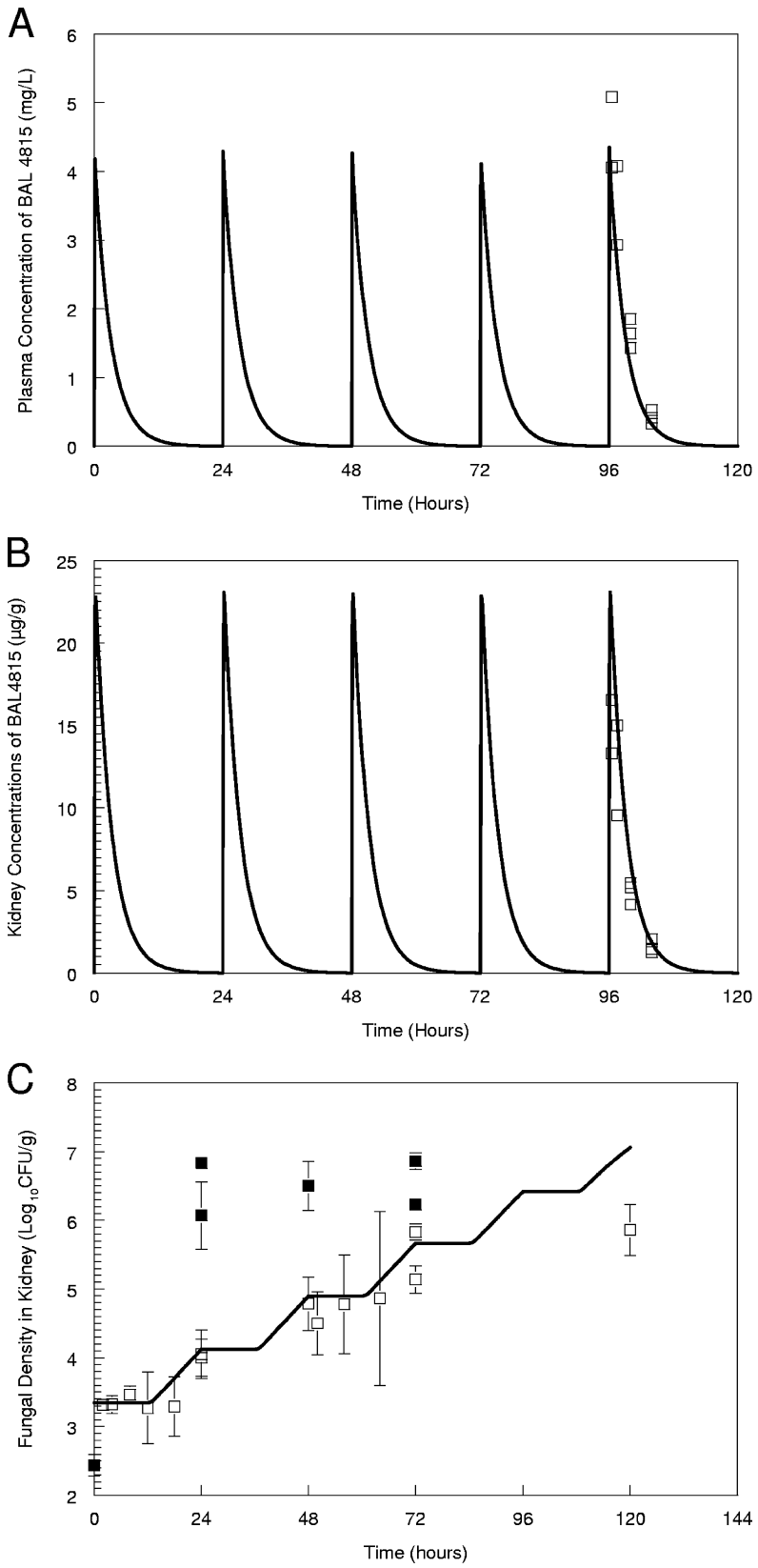

FIG. 4. Simulations of the time course of isavuconazole in plasma (A) and kidney (B) and the growth of *Candida* in the kidney (C) in mice receiving 28 mg/kg isavuconazole as the active drug. The open squares represent raw data from individual mice (A and B) or the means of three or four mice (C) receiving 28 mg/kg isavuconazole as the active drug. The closed squares in panel C represent the fungal burden in vehicle-treated controls. The solid line represents the fit of the mathematical model to the pharmacokinetic and pharmacodynamic data.

TABLE 2. Estimates for the various pharmacodynamics variables for dosage fractionation regimens

Dose of isavuconazole[a]	AUC/MIC[b]	Peak/MIC[b,c]	Fraction of dosing interval more than MIC[b]
3.27 once	387.75	128.75	0.62
1.63 b.i.d	384.25	64.5	1
0.82 q.i.d	372.25	32.25	1
5.35 once	630	209.25	0.68
2.67 b.i.d	633.75	106.25	1
1.34 q.i.d	614.25	48.25	1
8.12 once	969.5	322.5	0.73
4.06 b.i.d	960.25	161	1
2.03 q.i.d	930.5	80	1
13.48 once	1599.75	532.5	0.79
6.74 b.i.d	1584.5	265	1
3.37 q.i.d	1544.75	128.75	1

[a] Total active drug equivalent (mg/kg) administered s.c. b.i.d., twice a day; q.i.d., four times a day.
[b] The MIC was 0.004 mg/liter.
[c] The initial peak concentration is shown.

rapid clearance of the drug from the central compartment and an antifungal effect that persisted beyond the time drug concentrations in plasma were undetectable. When kidney concentrations were comodeled and the antifungal effect was linked to this compartment, a solution was obtained (see the appendix for details of the mathematical model). The triazoles are known to undergo extensive distribution to target tissues. Our data suggest that isavuconazole achieves ~6-fold-higher exposures in the kidney than in plasma and that levels within the kidney persist long after levels in plasma become undetectable. Whether this also occurs in humans is not known, and the penetration of isavuconazole into other relevant tissue compartments, such as the epithelial lining fluid of the lung, has not been studied. We readily accept that tissue homogenates provide only a crude estimate of the concentration of drug at the effect site and that all organs have subcompartments that have different properties in terms of fungal pathogenesis and drug penetration. Nevertheless, this study demonstrates that homogenates represent a useful, albeit imperfect, surrogate for tissue concentrations and that they may aid in further understanding antifungal effect.

The triazoles are widely regarded as having a fungistatic mode of action against *Candida* spp., and our data are entirely consistent with this notion. Both fluconazole and posaconazole do not induce overt fungal killing in experimental models of disseminated candidiasis but retard fungal growth (1, 4). Due to the relatively short terminal plasma half-life and short PAFE of isavuconazole, we observed progressive fungal growth in the latter portion of the dosing interval as both plasma and kidney levels of drug declined beneath a threshold level. Importantly, this threshold was not the MIC of isavuconazole but rather a concentration approximately 2 orders of magnitude higher. This is probably a reflection of the relatively high levels of protein binding exhibited by the compound, since only a small fraction of the total measured drug is free to engage with its microbiological target.

The classically designed dose fractionation studies clearly suggest that the AUC/MIC is the variable that best links isavuconazole exposure with the observed effect, and this is consistent with findings for other triazoles (1–4, 12). The mathematical model, however, suggests that the progressive growth seen in the latter portion of the dosing interval can be prevented by fractionated dosing to ensure that levels in plasma (or tissue) remain above a threshold. This was especially apparent in this study because of the short half-life of isavucon-

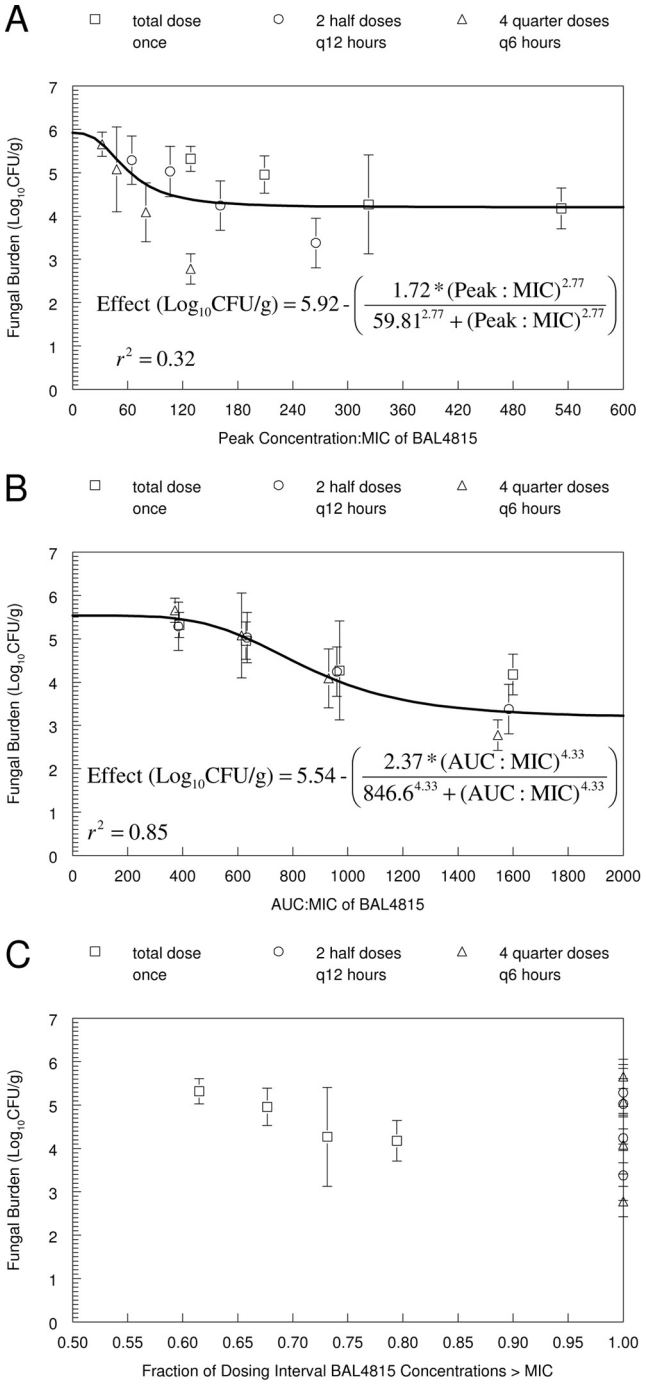

FIG. 5. Regressions from the dose fractionation experiments. (A) Total drug peak concentration/MIC versus effect. (B) Total drug AUC/MIC versus effect. (C) Fraction of the dosing interval in which total drug concentrations are more than the MIC. The data are means ± standard deviations for six mice. The solid line is the fit of the inhibitory sigmoid E_{max} model to the data.

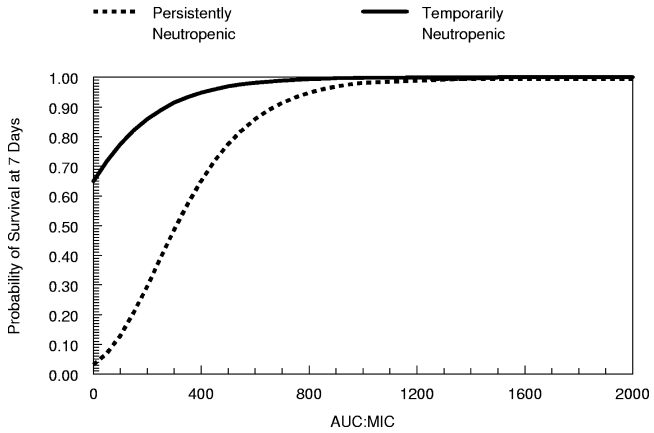

FIG. 6. Probability of survival for persistently versus temporarily neutropenic mice as a function of the total drug AUC/MIC at steady state.

azole (3.41 h) in relation to the extended dosing interval (24 h). This is unlikely to be clinically relevant given the much longer terminal half-life in humans. The in vivo PAFEs for fluconazole and posaconazole have been estimated to be 4 to 21 and 24 h, respectively, and this prolonged effect has been used to account for the observation that the AUC/MIC appears consistently as the dynamically linked variable for the triazoles. A number of factors may contribute to the in vivo PAFE, including sub-MIC effects, the time for organisms to recover from impaired ergosterol synthesis, and the persistence of the drug at the effect site (4). The mathematical model suggests that the prolonged mean residence time of isavuconazole in the kidney is an important determinant of the PAFE, and the same may also be true for other triazoles.

Several experimental studies suggest that immune function has a critical bearing on the antifungal drug effect (8, 9). This study highlights striking differences in the probabilities of survival for persistently and temporarily neutropenic mice receiving the same dosages of isavuconazole. While this is well known and intuitively obvious to many clinicians, there are important implications for the broad application of pharmacodynamics targets outside the setting in which they have been derived. The survival study and the Cox model suggest that a total drug AUC/MIC of 670, which is associated with 90% probability of survival after 5 days of therapy, is a potential pharmacodynamic target for isavuconazole for disseminated candidiasis in neutropenic hosts. Ideally this finding should be confirmed with several other strains. Interestingly, this value produces a submaximal decrement in the fungal burden when assessed using $\log_{10}$ CFU/g (Fig. 5). This is consistent with previous work that suggests that relatively small decrements in the fungal burden produce prolongation of survival (10). If a correction is made for protein binding, then the magnitude of this target is comparable to those identified for other triazoles. A lower drug exposure target may be appropriate for nonneutropenic hosts.

APPENDIX

The murine pharmacokinetics of isavuconazole and the antifungal effect induced by the administration of the prodrug to persistently neutropenic mice were described using the following five simultaneous inhomogeneous differential equations:

$$\frac{dX(1)}{dt} = B(1) - K_a * X(1) \quad (1)$$

$$\frac{dX(2)}{dt} = K_a * X(1) - K_{cp} * X(2) - K_{ck} * X(2) + K_{pc} * X(3) + K_{kc} * X(4) \quad (2)$$

$$\frac{dX(3)}{dt} = K_{cp} * X(2) - K_{pc} * X(3) \quad (3)$$

$$\frac{dX(4)}{dt} = K_{ck} * X(2) - K_{kc} * X(4) \quad (4)$$

$$\frac{dX(5)}{dt} = K_{gmax} * \left(1 - \frac{X(5)}{POPMAX}\right) * X(5) \quad (5a)$$

$$* \left(1 - \left(\frac{\left[\frac{X(4)}{V_{kidney}}\right]^H}{C_{50g}{}^H + \left[\frac{X(4)}{V_{kidney}}\right]^H}\right)\right) \quad (5b)$$

Equations 1, 2, 3, and 4 describe the pharmacokinetics of isavuconazole and its distribution to the kidney, which is the primary effect site. Compartments 1, 2, 3, and 4 represent the subcutaneous injection site, central compartment, peripheral compartment and kidney, respectively. $X(1)$, $X(2)$, $X(3)$, and $X(4)$ represent the amount of the drug (in milligrams) in these respective compartments. The prodrug was administered subcutaneously into compartment 1 as a bolus [$B(1)$], from where it equilibrated with the central compartment (compartment 2) and degraded completely to the active moiety isavuconazole. Drug in the central compartment equilibrated with the rest of the body (compartment 3) and the kidney (compartment 4), which has volume V_{kidney} (in liters). There was first-order elimination from the central compartment (SCL, in liters/h), which has volume V_c (in liters). K represents the various first-order intercompartmental rate constants.

Equation 5 describes the rate of change of the number of *Candida* organisms within the kidneys as influenced by the growth of *Candida* and the concentrations of isavuconazole in the kidney. $X(5)$ represents the number of *Candida* organisms in the kidney. The term 5a describes the capacity-limited growth of *C. albicans* within the kidney and contains the parameters K_{gmax} and POPMAX, which represent the maximum growth rate constant and the maximum theoretical fungal density achievable in the kidney, respectively. As the number of organisms within the kidney approaches POPMAX, the rate of *Candida* growth slows and approaches zero. The term 5b in equation 5 provides a way of allowing isavuconazole to exert a fungistatic as opposed to a fungicidal effect (i.e., growth suppression rather than explicit killing). In the absence of drug, this term defaults to 1, and *Candida* grows in an unrestrained manner. Following a progressively higher concentration of isavuconazole, the growth rate of *Candida* is constrained to approach zero. C_{50g} is the concentration of isavuconazole at which the effect on growth is half-maximal, and H_g is the associated slope function.

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REFERENCES

1. **Andes, D., K. Marchillo, R. Conklin, G. Krishna, F. Ezzet, A. Cacciapuoti, and D. Loebenberg.** 2004. Pharmacodynamics of a new triazole, posaconazole, in a murine model of disseminated candidiasis. Antimicrob. Agents Chemother. **48:**137–142.
2. **Andes, D., K. Marchillo, T. Stamstad, and R. Conklin.** 2003. In vivo pharmacodynamics of a new triazole, ravuconazole, in a murine candidiasis model. Antimicrob. Agents Chemother. **47:**1193–1199.
3. **Andes, D., K. Marchillo, T. Stamstad, and R. Conklin.** 2003. In vivo pharmacokinetics and pharmacodynamics of a new triazole, voriconazole, in a murine candidiasis model. Antimicrob. Agents Chemother. **47:**3165–3169.

4. **Andes, D., and M. van Ogtrop.** 1999. Characterization and quantitation of the pharmacodynamics of fluconazole in a neutropenic murine disseminated candidiasis infection model. Antimicrob. Agents Chemother. **43:**2116–2120.
5. **Cornely, O., A. Bohme, M. Heep, A. Schmidt-Hoffmann, and A. J. Ullmann.** 2008. Pharmacokinetics, safety and tolerability results of a dose escalation study of isavuconazole in neutropenic patients, abstr. M-2137. Abstr. 48th Annu. Intersci. Conf. Antimicrob. Agents Chemother. (ICAAC)-Infect. Dis. Soc. Am. (IDSA) 46th Annu. Meet. American Society for Microbiology and Infectious Diseases Society of America, Washington, DC.
6. **D'Argenio, D. Z., and A. Schumitzky.** 1997. ADAPT II. A program for simulation, identification, and optimal experimental design. User manual. Biomedical Simulations Resource, University of Southern California, Los Angeles, California. http://bmsr.esc.edu/.
7. **Gudlaugsson, O., S. Gillespie, K. Lee, J. Vande Berg, J. Hu, S. Messer, L. Herwaldt, M. Pfaller, and D. Diekema.** 2003. Attributable mortality of nosocomial candidemia, revisited. Clin. Infect. Dis. **37:**1172–1177.
8. **Hope, W. W., G. L. Drusano, C. B. Moore, A. Sharp, A. Louie, T. J. Walsh, D. W. Denning, and P. A. Warn.** 2007. Effect of neutropenia and treatment delay on the response to antifungal agents in experimental disseminated candidiasis. Antimicrob. Agents Chemother. **51:**285–295.
9. **Hope, W. W., M. J. Kruhlak, C. A. Lyman, R. Petraitiene, V. Petraitis, A. Francesconi, M. Kasai, D. Mickiene, T. Sein, J. Peter, A. M. Kelaher, J. E. Hughes, M. P. Cotton, C. J. Cotten, J. Bacher, S. Tripathi, L. Bermudez, T. K. Maugel, P. M. Zerfas, J. R. Wingard, G. L. Drusano, and T. J. Walsh.** 2007. Pathogenesis of *Aspergillus fumigatus* and the kinetics of galactomannan in an in vitro model of early invasive pulmonary aspergillosis: implications for antifungal therapy. J. Infect. Dis. **195:**455–466.
10. **Hope, W. W., P. A. Warn, A. Sharp, S. Howard, M. Kasai, A. Louie, T. J. Walsh, G. L. Drusano, and D. W. Denning.** 2006. Derivation of an in vivo drug exposure breakpoint for flucytosine against *Candida albicans* and impact of the MIC, growth rate, and resistance genotype on the antifungal effect. Antimicrob. Agents Chemother. **50:**3680–3688.
11. **Leary, R., R. Jelliffe, A. Schumitzky, and M. van Guilder.** 2001. An adaptive grid, non-parametric approach to pharmacokinetic and dynamic (PK/PD) models, p. 389-394. *In* Proceedings of the 14th IEEE Computer Society, Bethesda, MD.
12. **Louie, A., G. L. Drusano, P. Banerjee, Q. F. Liu, W. Liu, P. Kaw, M. Shayegani, H. Taber, and M. H. Miller.** 1998. Pharmacodynamics of fluconazole in a murine model of systemic candidiasis. Antimicrob. Agents Chemother. **42:**1105–1109.
13. **National Committee for Clinical Laboratory Standards.** 1997. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A2. National Committee for Clinical Laboratory Standards, Wayne, PA.
14. **Perkhofer, S., V. Lechner, and C. Lass-Florl.** 2009. In vitro activity of Isavuconazole against *Aspergillus* species and zygomycetes according to the methodology of the European Committee on Antimicrobial Susceptibility Testing. Antimicrob. Agents Chemother. **53:**1645–1647.
15. **Schmitt-Hoffmann, A., B. Roos, M. Heep, M. Schleimer, E. Weidekamm, T. Brown, M. Roehrle, and C. Beglinger.** 2006. Single-ascending-dose pharmacokinetics and safety of the novel broad-spectrum antifungal triazole BAL4815 after intravenous infusions (50, 100, and 200 milligrams) and oral administrations (100, 200, and 400 milligrams) of its prodrug, BAL8557, in healthy volunteers. Antimicrob. Agents Chemother. **50:**279–285.
16. **Schmitt-Hoffmann, A., B. Roos, J. Maares, M. Heep, J. Spickerman, E. Weidekamm, T. Brown, and M. Roehrle.** 2006. Multiple-dose pharmacokinetics and safety of the new antifungal triazole BAL4815 after intravenous infusion and oral administration of its prodrug, BAL8557, in healthy volunteers. Antimicrob. Agents Chemother. **50:**286–293.
17. **Seifert, H., U. Aurbach, D. Stefanik, and O. Cornely.** 2007. In vitro activities of isavuconazole and other antifungal agents against *Candida* bloodstream isolates. Antimicrob. Agents Chemother. **51:**1818–1821.
18. **Thompson, G. R., III, A. W. Fothergill, N. P. Wiederhold, A. C. Vallor, B. L. Wickes, and T. F. Patterson.** 2008. Evaluation of Etest method for determining isavuconazole MICs against *Cryptococcus gattii* and *Cryptococcus neoformans*. Antimicrob. Agents Chemother. **52:**2959–2961.
19. **Warn, P. A., A. Sharp, J. Mosquera, J. Spickermann, A. Schmitt-Hoffmann, M. Heep, and D. W. Denning.** 2006. Comparative in vivo activity of BAL4815, the active component of the prodrug BAL8557, in a neutropenic murine model of disseminated *Aspergillus flavus*. J. Antimicrob. Chemother. **58:**1198–1207.
20. **Wey, S. B., M. Mori, M. A. Pfaller, R. F. Woolson, and R. P. Wenzel.** 1988. Hospital-acquired candidemia. The attributable mortality and excess length of stay. Arch. Intern. Med. **148:**2642–2645.
21. **Wisplinghoff, H., T. Bischoff, S. M. Tallent, H. Seifert, R. P. Wenzel, and M. B. Edmond.** 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. **39:**309–317.