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Effect of Neutropenia and Treatment Delay on the Response to Antifungal Agents in Experimental Disseminated Candidiasis▽

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Disseminated candidiasis is associated with a high rate of morbidity and mortality. The presence of neutrophils and the timely administration of antifungal agents are likely to be critical factors for a favorable therapeutic outcome of this syndrome. The effect of neutropenia on the temporal profile of the burden of *Candida albicans* in untreated mice and those treated with amphotericin B was determined using a pharmacodynamic model of disseminated candidiasis. A mathematical model was developed to describe the rate and extent of the *C. albicans* killing attributable to neutrophils and to amphotericin B. The consequences of a delay in the administration of amphotericin B, flucytosine, or micafungin were studied by defining dose-response relationships. Neutrophils caused a logarithmic decline in fungal burden in treated and untreated mice. The combination of amphotericin B and neutrophils resulted in a high rate of *Candida* killing and a sustained anti-*C. albicans* effect. In neutropenic mice, 5 mg/kg of body weight of amphotericin B was required to prevent progressive logarithmic growth. An increased delay in drug administration resulted in a reduction in the maximum effect to a point at which no drug effect could be observed. Neutrophils and the timely initiation of antifungal agents are critical determinants in the treatment of experimental disseminated candidiasis.

Disseminated candidiasis is a syndrome associated with a high rate of morbidity and mortality. Despite an improved understanding of both the pharmacodynamics of antifungal agents against *Candida* spp. (2, 14) and the epidemiology of disseminated candidiasis, there has been no appreciable change in the attributable mortality of this syndrome in the past decade (7, 30). *Candida* spp. remain the fourth most cause of common bloodstream infections in the United States (31). *Candida albicans* is the dominant *Candida* pathogen, accounting for approximately 50 to 60% of all *Candida* blood culture isolates (31). There is an ongoing need to refine therapeutic strategies in order to improve the outcome of disseminated candidiasis.

Neutrophils play a key role in the prevention of fungal growth and the invasion of tissues. Defects in neutrophil number and function have been consistently implicated in the pathogenesis of disseminated candidiasis (6, 29). This, in part, has provided the clinical impetus to limit the depth and duration of the neutropenic period. Additional clinical and experimental approaches have included the augmentation of neutrophil function and the use of granulocyte infusions (20–23, 26). There remains, however, relatively little understanding of the magnitude of the in vivo anti-*C. albicans* effect which can be specifically attributed to neutrophils and a paucity of information regarding the in vivo interaction between neutrophils and antifungal agents.

The failure to administer an active anti-infective agent in a timely manner is associated with a suboptimal therapeutic outcome. This has been demonstrated for a range of pathogens including *Candida* spp. (12, 16). Although timely administration of antifungal therapy may be intuitively apparent to many clinicians, little is known about the period of time for which a therapeutic delay is detrimental to the host and the mechanisms by which delayed treatment is associated with a suboptimal outcome.

Herein, we characterize the effects of neutropenia and delay in the initiation of antifungal therapy on therapeutic response in a well-validated murine model of disseminated candidiasis with *C. albicans*. Our objectives were as follows: firstly, to develop a mathematical model to describe unrestrained fungal growth and the candidacidal effect of neutrophils and amphotericin B; secondly, to describe the exposure-response relationships following progressively longer delays in the administration of the antifungal agents amphotericin B, micafungin, and flucytosine (5FC); and finally, in order to place the foregoing in a pathological context, we sought to describe the histological changes in the kidneys of neutropenic and nonneutropenic mice with disseminated candidiasis in the first 24 h of infection.

(This work was presented, in part, at the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 30 October to 2 November 2004 [29a].)

MATERIALS AND METHODS

Drugs. Amphotericin B deoxycholate (Bristol-Myers Squibb Pharmaceuticals Ltd., Dublin, Ireland) and micafungin (Astellas Pharmaceuticals Ltd., Osaka, Japan) were reconstituted in 5% dextrose immediately prior to use. Flucytosine solution (10 mg/liter) (Valeant Pharmaceuticals, Basingstoke, United Kingdom) was stored at room temperature.

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TABLE 1. Inocula of *C. albicans* and dosages of antifungal agents used in the various experiments

Expt	Inoculum[a]	Antifungal dosages (mg/kg)[b]		
		Amphotericin B	Micafungin	Flucytosine
Neutrophil-related candidacidal effect				
Neutropenic mice	2×10^4	0, 0.63, 1, 2.5, 5		
Nonneutropenic mice	5×10^5	0, 0.63, 1, 2.5, 5		
Delay models				
5 h	2×10^4	0, 0.01, 0.06, 0.1, 0.5, 1	0, 0.2, 0.5, 1, 2.5, 5.4	0, 0.01, 0.03, 0.1, 0.4, 1.6, 6.25
14 h	2×10^4	0, 0.1, 0.5, 1, 2.5		
24 h	2×10^4	0, 0.63, 1, 2.5, 5	0, 0.2, 1, 2.5, 5.4	0, 6.25, 25, 100, 200
36 h	2×10^3	0, 0.63, 1, 2.5, 5		

[a] The various inocula were administered in 0.2 ml of PBS, via the lateral tail vein. The values refer to the absolute numbers of organisms administered to each mouse.
[b] Amphotericin B and micafungin were administered once within the 24-hour period; three dosages of flucytosine were administered at time zero and then 8 and 16 hours later.

Organism. A well-characterized clinical isolate of *Candida albicans* (F/6862) was used for all experiments (9, 10). The MICs of F/6862 to amphotericin B and flucytosine were 0.03 and 0.125 mg/liter, respectively, using CLSI M27-A2 methodology (17). The MIC of F/6862 to micafungin was 0.008 mg/liter, determined using a modification of CLSI M27-A2 methodology with antibiotic medium 3 plus 2% glucose. The experimental isolate was retrieved 24 h prior to use from beads stored at minus 70°C, placed in Sabouraud broth (Oxoid, Basingstoke, United Kingdom), and incubated at 35°C on a shaker. Immediately prior to use, the broth was centrifuged at 2,200 × *g* for 5 min and the pellet washed twice in phosphate-buffered saline (PBS). The final inoculum was obtained by serial dilution in PBS and verified by quantitative culture.

Models of invasive candidiasis. Male CD1 mice, 24 to 26 g in weight, were used. Food and water were provided ad libitum. An established and reproducible pharmacodynamic model of disseminated candidiasis was used (9, 10). Neutropenia was induced with cyclophosphamide (Pharmacia, Milton Keynes, United Kingdom), 200 mg/kg of body weight, administered intravenously (i.v.) in 0.2 ml 0.9% saline, via the lateral tail vein on day −3. The different inocula used in the experiments to study the effect of neutrophils and delay in drug administration are summarized in Table 1; in all cases, blastoconidia were administered in 0.2% PBS, i.v., via the lateral tail vein. The inoculum used in neutropenic mice was based upon previous studies (9, 10). Preliminary studies with nonneutropenic mice demonstrated that 5×10^5 organisms resulted in an established infection, without the induction of mortality within the study period. In the delay experiments, a lower inoculum in the 36-h-delay group was used to ensure that there was no mortality. Neutrophil counts were determined in both nonneutropenic ($n = 3$) and neutropenic ($n = 3$) mice. At the conclusion of all experiments, mice were sacrificed; both kidneys were dissected, weighed, homogenized together, and submitted for quantitative culture.

Determination of the effect of neutropenia on the time course of *Candida* kidney burden. The time course of *C. albicans* kidney burden in untreated neutropenic and nonneutropenic mice was determined in a single experiment. Both cohorts were comprised of 24 mice. Paired groups of three mice from each cohort were sacrificed at 24, 27, 30, 34, 38, 48, 52, and 56 h postinoculation, and the *C. albicans* kidney burdens were determined as described above.

Effect of neutropenia on the candidacidal effect of amphotericin B. The dose-response relationships of amphotericin B in groups of neutropenic and nonneutropenic mice were defined in two separate experiments. Three mice per dosage group were used. The *C. albicans* burden was determined in a control group ($n = 3$) of neutropenic and nonneutropenic mice just prior to drug administration (i.e., 24 h postinoculation). Amphotericin B was administered intraperitoneally (i.p.) (as described elsewhere [10, 13]) in 0.2 ml 5% dextrose and in dosages summarized in Table 1. The fungal kidney burden was determined for each dosage group after 24 h of therapy (i.e., 48 h postinoculation). The fungal kidney burden was also determined in both neutropenic and nonneutropenic mice receiving 0.63 and 5 mg/kg at 0.5, 6, 10, 14, and 24 h post-drug administration

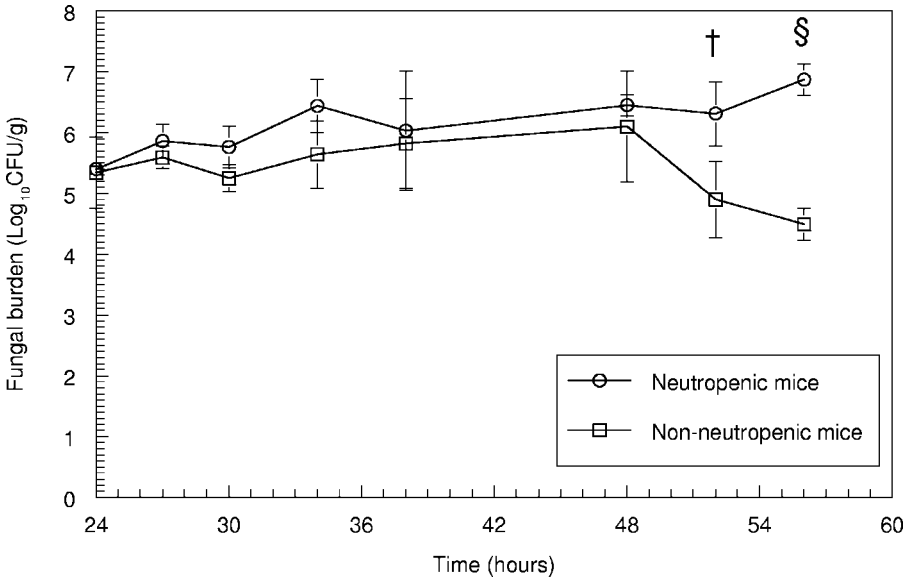

FIG. 1. Time course of the fungal burden of *Candida* in the kidneys of untreated neutropenic and nonneutropenic mice, showing the decrement in nonneutropenic mice 48 h postinoculation. Data are the means ± standard deviations of three mice. The overall fungal burden in neutropenic mice was greater than that in nonneutropenic mice as determined using analysis of variance ($P < 0.001$); †, $P = 0.039$; §, $P < 0.001$.

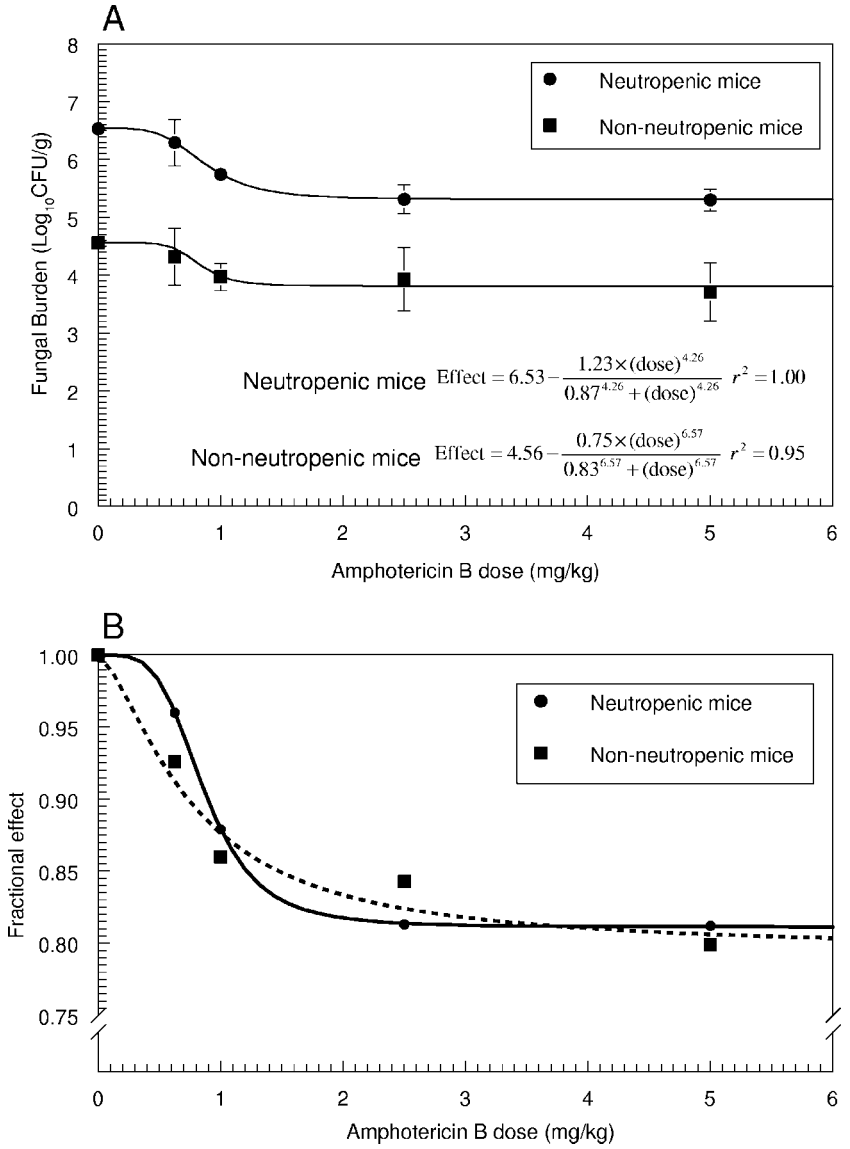

FIG. 2. Effect of neutropenia on the dose-response relationships of amphotericin B determined in two separate experiments. (A) Dose-response relationships of amphotericin B in neutropenic mice versus nonneutropenic mice. Data points represent the means ± standard deviations of three mice. The *C. albicans* burden is higher in neutropenic than in nonneutropenic mice treated with amphotericin B ($P < 0.001$). (B) The fractional candidacidal effects induced by amphotericin B in neutropenic and nonneutropenic mice are similar.

(i.e., 24.5, 30, 40, 44, and 48 h postinoculation) to investigate the rate of drug-induced killing.

Combined mathematical model to describe the candidacidal activity of neutrophils and amphotericin B. In order to obtain an estimate of the rate and extent of the candidacidal effect of neutrophils and amphotericin B, the following data sets were comodeled: (i) the time course of the *C. albicans* kidney burden in untreated neutropenic and nonneutropenic mice (as above), (ii) the *C. albicans* kidney burden in neutropenic and nonneutropenic mice observed following a 24-h delay in the administration of amphotericin B (as above), and (iii) the serum amphotericin B levels following the administration of a single dose of 0.63, 1.0, 2.5, and 5.0 mg/kg, i.p., with measurement of serum concentrations at 0.5, 1, 3, 6, 10, 14, and 24 h post-drug administration (details regarding the measurement of amphotericin B and a pharmacokinetic model describing these data have been previously published [10]). Fifty-five data points were analyzed using the Big Nonparametric Adaptive Grid program of Leary, Jelliffe, Schumitzky, and van Guilder (11). The structural pharmacokinetic and pharmacodynamic model is contained within the Appendix. The Bayesian parameter estimates for individual data points were obtained using the mean population parameter values. The fit and predictive performance of candidate models for the data were assessed using a regression of the observed-versus-predicted values after the Bayesian step, log-likelihood values, and measures of precision and bias.

Effect of treatment delay on the dose-response relationships. The effect of a delay in the initiation of treatment for amphotericin B, micafungin, and 5FC was further determined in neutropenic mice. The effects of 5-, 14-, 24-, and 36-h delays in the commencement of treatment from the time of the initiation of infection were determined for amphotericin B, and the effects of 5- and 24-h delays were determined for micafungin and 5FC. Each delay experiment was performed once. Groups of three mice per analysis point were used. Amphotericin B and 5FC were both administered i.p. in their respective diluents; micafungin was administered i.v. in 0.2 ml 5% dextrose.

Pharmacodynamic and statistical analyses. All dose-response relationships were modeled using an inhibitory sigmoid-E_{max} model which took the form

$$\text{Effect } (\log_{10} \text{CFU/g}) = E_{con} - \frac{E_{max} \times \text{dose}^H}{\text{EC}_{50}^H + \text{dose}^H}$$

TABLE 2. Estimates for the mean and median population parameter values and their standard deviations

Parameter[a]	Mean	Median	SD
K_a (h^{-1})	18.30	19.297	1.97
Vol of central compartment (liters)	0.0274	0.0262	0.0183
SCL (liters/h)	0.0029	0.0031	0.00039
K_{cp} (h^{-1})	16.007	18.076	3.054
K_{pc} (h^{-1})	10.611	9.183	5.008
K_{gmax} ($\log_{10}$ CFU/g/h)	4.064	4.263	0.378
$C_{50}g$ (mg/liter)	2.481	2.752	0.578
H_g	9.162	5.031	5.89
POPMAX (CFU/g)	4.759×10^6	5.541×10^6	3.87×10^6
K_{kmax} ($\log_{10}$ CFU/g/h)	3.315	3.309	0.411
$C_{50}k$ (mg/liter)	0.099	0.099	0.012
H_k	7.630	4.252	4.838
$WBCKILL_{max}$ ($\log_{10}$ CFU/g/h)	1.043	0.1373	1.34
$WBCKILL_{50}$ (CFU/g)	3.525×10^5	4.313×10^4	4.61×10^5
Initial condition (CFU/g)	2.516×10^5	2.504×10^5	2.283×10^3

[a] K_a is the first-order rate connecting the peritoneum with the central compartment; SCL is the clearance from the central compartment; K_{cp} and K_{pc} are the first-order rate constants connecting the central and peripheral compartments; K_{gmax} is the growth constant describing maximal growth; $C_{50}g$ is the concentration of amphotericin B 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; K_{kmax} is the rate constant for maximal amphotericin-induced killing; $C_{50}k$ is the drug concentration needed to effect 50% maximal killing; H_k is the sigmoidocity constant for *Candida* killing; $WBCKILL_{max}$ is the rate constant for the maximal neutrophil-induced killing; $WBCKILL_{50}$ is the *C. albicans* burden at which killing is half maximal. The initial condition is the fungal burden at the time of amphotericin B administration.

where effect refers to the residual effect after drug exposure, E_{con} is the fungal burden in the absence of therapy, E_{max} is the asymptotic reduction in fungal burden from E_{con} induced by antifungal therapy, EC_{50} is the exposure at which the antifungal effect is half maximal, and H is the slope function (Hill constant). The model was implemented in the identification module of the software program ADAPT II of D'Argenio and Schumitsky (5). The data were weighted by the inverse of the observed variance. Differences were determined using analysis of variance (one- and two-way analysis of variance) and the Bonferroni correction for post hoc comparisons, as appropriate. Statistical significance was determined at the 0.05 level.

Histological appearances. The histological features of the kidneys of neutropenic and nonneutropenic mice not exposed to antifungal agents were assessed at the 5-, 14-, and 24-h time points. The histological sections were stained with periodic acid-Schiff stain.

RESULTS

Models of disseminated candidiasis. Infection of neutropenic and nonneutropenic mice with *C. albicans* F/6862 produced an established but sublethal infection. The mean (± standard deviation) neutrophil counts in neutropenic and nonneutropenic mice at the time of inoculation were $(0.127 \pm 0.02) \times 10^9$/liter and $(4.20 \pm 0.99) \times 10^9$/liter, respectively. In neutropenic and nonneutropenic mice, the neutrophil counts 48 h postinoculation were $(1.03 \pm 0.45) \times 10^9$/liter and $(2.58 \pm 0.08) \times 10^9$/liter, respectively.

Effect of neutropenia on tissue burden. The fungal burdens in the kidneys of untreated neutropenic and nonneutropenic mice at 24 h postinfection were similar ($P = 0.682$). At time points beyond 48 h, there was progressive logarithmic growth in the kidneys of neutropenic mice and a decline in the fungal kidney burden in nonneutropenic mice which was statistically different at 52 and 56 h ($P = 0.039$ and $P < 0.001$, respectively) (Fig. 1).

Effect of neutropenia on the candidacidal effect of amphotericin B. The presence of neutrophils was an important determinant of the fungal burden following the administration of amphotericin B. The overall *C. albicans* burden was significantly higher in neutropenic mice treated with amphotericin B than in nonneutropenic mice ($P < 0.001$) (Fig. 2A). As demonstrated in Fig. 2A, the shapes of the dose-response relationships in neutropenic and nonneutropenic mice were similar; the major difference, however, between the two dose-response relationships was the fungal burden in the absence of therapy (E_{con}). Once the antifungal effect was normalized, the exposure-response relationships were seen to overlie one another (Fig. 2B).

Mathematical model of the time course of *C. albicans* kidney burden in neutropenic and nonneutropenic mice treated with amphotericin B. The estimates for the model parameter values which described the entire data set including the time course of (i) amphotericin B concentrations following the administration of different dosages, (ii) the rate and extent of unrestrained *C. albicans* growth, and (iii) the rate and extent of the antifungal effect associated with amphotericin B in neutropenic and nonneutropenic mice are summarized in Table 2. The fit of the model to the data was highly acceptable, with coefficients of determination of 0.90 and 0.76 for the pharmacokinetics of amphotericin B and growth of *C. albicans* in the kidney, respectively (Fig. 3). Model simulations, shown in Fig. 4, were performed using the mean parameter values of the population. There was net growth of *C. albicans* following the administration of 0.63 mg/kg of amphotericin B to neutropenic mice (Fig. 4A). A dose of 5 mg/kg was required to overcome progressive growth of *C. albicans* and produce a fungal density which was comparable to that observed at the time of the initiation of therapy (Fig. 4C). In contrast, in nonneutropenic mice, the resultant antifungal effect of amphotericin B at 0.63 and 5 mg/kg in combination with neutrophils resulted in a high rate of killing and a net logarithmic decline in fungal burden (Fig. 4B and D). In nonneutropenic mice receiving 0.63 mg/kg of amphotericin B, there was limited regrowth of *C. albicans* as the concentrations of amphotericin B declined in the latter part of the dosing interval (Fig. 4B), while the administration of 5 mg/kg resulted in a sustained kill without fungal regrowth (Fig. 4D).

Effect of delay in initiation of therapy. The effect of a delay in initiation of therapy with amphotericin B, micafungin, and flucytosine is shown in Fig. 5 and 6. For all three drugs, the maximum decline in fungal kidney burden was observed with earlier therapy. A progressive delay in the administration of amphotericin B resulted in higher fungal densities within the kidney ($P = 0.011$). As shown in Fig. 5, the anti-*C. albicans* effect induced by amphotericin B administered 5 h postinfection was not significantly different from that observed when amphotericin B was administered after 14 h ($P = 0.054$), but 24- and 36-h delays in drug administration resulted in significantly higher fungal burden ($P = 0.002$ and 0.013, respectively). There was an exponential decline in the maximum effect as a function of the duration of delay in the initiation of amphotericin B therapy (Fig. 5E).

There was a significant difference between the antifungal effects following 5- and 24-h delays in the administration of

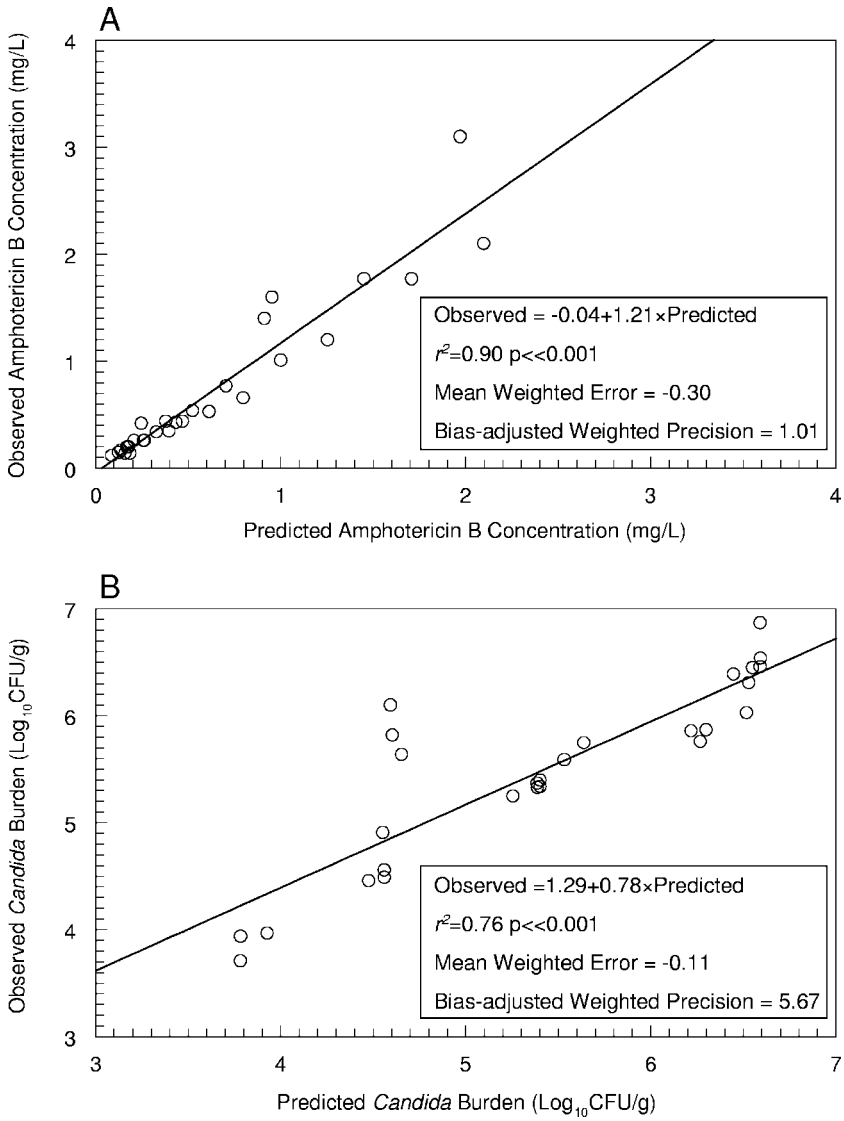

FIG. 3. Observed-versus-predicted plots after the Bayesian step. (A) Amphotericin B pharmacokinetics; (B) fungal density of *C. albicans* within the kidney.

both micafungin ($P = 0.002$) and flucytosine ($P < 0.001$) (Fig. 6A and B). Furthermore, for amphotericin B and flucytosine, no dose-response relationship was seen if antifungal therapy was delayed for 36 and 24 h, respectively (Fig. 5D and Fig. 6B).

Histological appearances. The histological appearances of renal candidiasis in untreated neutropenic and nonneutropenic mice at time points relevant to the current study are shown in Fig. 7. At 5 hours postinoculation, blastoconidia and hyphae were predominantly confined to the glomeruli. There was evidence at this time point of invasion from the glomeruli across Bowman's space and into contiguous structures, such as the proximal convoluted tubule (Fig. 7A and B). Blastoconidia in the kidneys of nonneutropenic mice 5 h postinoculation were extremely scant (data not shown). In the kidneys of both neutropenic and nonneutropenic mice 14 h postinoculation, hyphae were predominantly found within the tubules (Fig. 7C and D). In nonneutropenic mice, a peritubular neutrophil infiltrate was evident (Fig. 7D). In neutropenic mice 24 h postinoculation, there were progressive growth and invasion of *C. albicans* with evidence of tubular destruction and infarction (Fig. 7E). In nonneutropenic mice, there were progressive recruitment of neutrophils and evidence of peritubular abscess formation (Fig. 7F).

DISCUSSION

The current study addresses two well-accepted but critically important clinical concepts: firstly, neutrophils are one of the most important immunological effectors required for optimal killing of *C. albicans*, and, secondly, even a relatively short delay in the administration of an otherwise active antifungal agent results in a diminished antifungal effect to levels which are potentially detrimental to the host. The methodology that we employed enabled an estimate of the extent of killing contributed by neutrophils and an approximate window in which an optimal

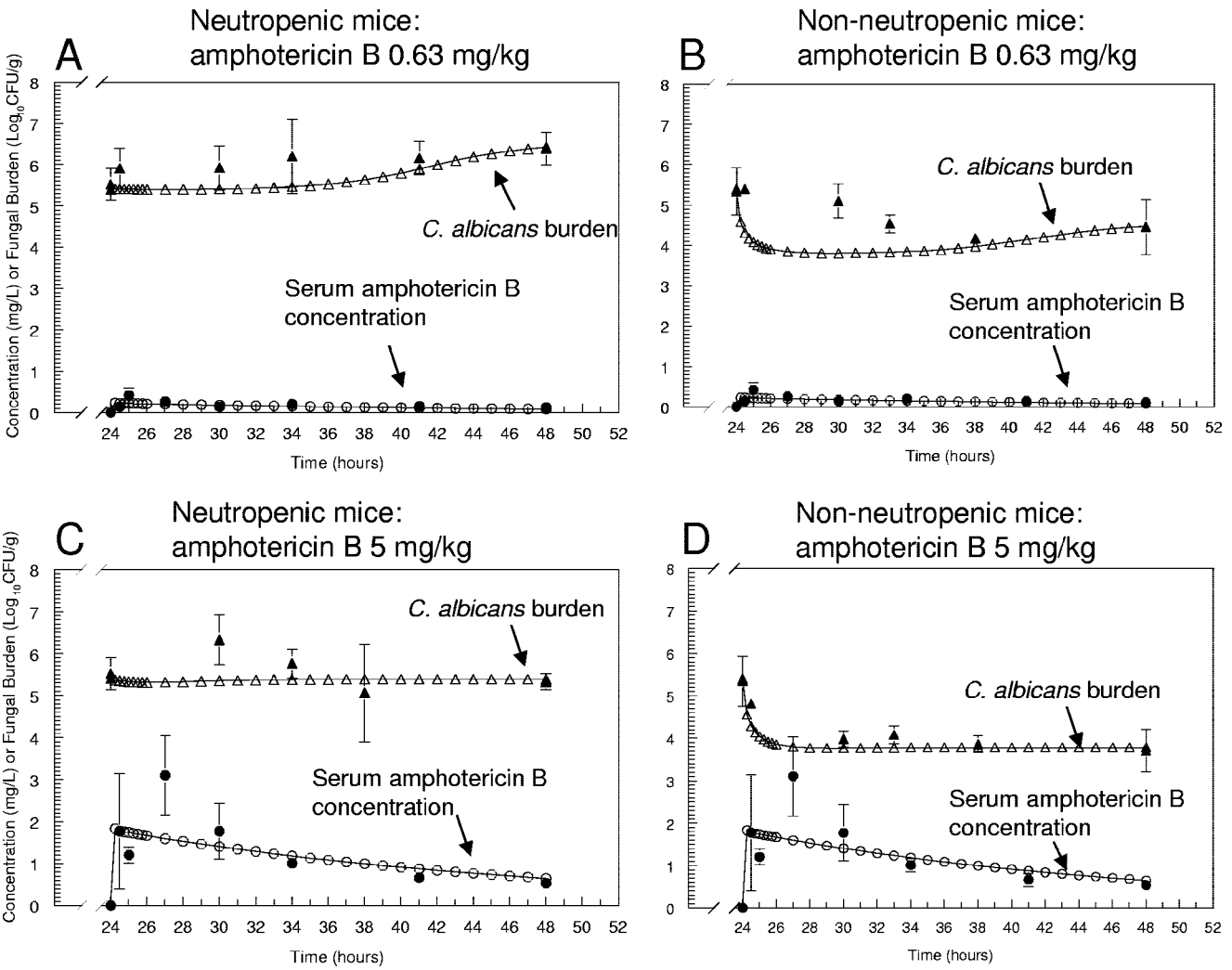

FIG. 4. Model simulations and raw data depicting (i) the time course of the serum concentrations of amphotericin B following the intraperitoneal administration of 0.63 and 5 mg/kg of amphotericin B and (ii) the associated anti-*C. albicans* effect in the kidney. Raw data (means ± standard deviations of three mice) from the following experiments are presented: (i) amphotericin B pharmacokinetics (solid circles) and (ii) growth of *C. albicans* (solid triangles). Model predictions for the pharmacokinetic and fungal burden values are represented by the open circles and triangles, respectively. In the absence of neutrophils, the administration of amphotericin B results only in the suppression of growth rather than effecting net kill (A and C). In nonneutropenic mice, the combination of amphotericin B and neutrophils leads to logarithmic killing and a sustained antifungal effect (B and D).

therapeutic response can be obtained. These analyses highlight some of the potential reasons for therapeutic failure in neutropenic hosts who are infected with strains of *C. albicans*, which otherwise demonstrate exquisite in vitro susceptibility to antifungal agents, and provide an experimental foundation for early initiation of antifungal therapy in this vulnerable population suspected to have invasive fungal infection.

The association between neutropenia and disseminated candidiasis was recognized nearly 40 years ago (3). Subsequently, neutropenia has been consistently implicated as a risk factor for the development of disseminated candidiasis and, more recently, has been demonstrated to be an important determinant of the likelihood of breakthrough infections, relapsed disease, chronic dissemination, and a poor prognostic marker for patients with candidemia (1, 4, 8, 19, 24, 27). There is a progressive understanding of critical events in the interaction between *Candida* and neutrophils including signaling, recruitment, phagocytosis, and intracellular killing (6). The combined pharmacokinetic and pharmacodynamic model used in the current study provides a number of clinically relevant insights. Firstly, the experimental data and the combined mathematical model provide a basis for a further understanding of the clinical adage that antifungal agents merely prevent progressive infection. Although only the antifungal effect following the administration of a single dose of amphotericin B was studied, the data and model simulations support the notion that a critical factor in terms of the elimination of the infection is neutrophil recovery. The model simulations demonstrate the relatively modest antifungal effect of amphotericin B when administered to neutropenic mice compared with the significantly greater killing which results when the drug is administered in the presence of neutrophils. In neutropenic mice, the

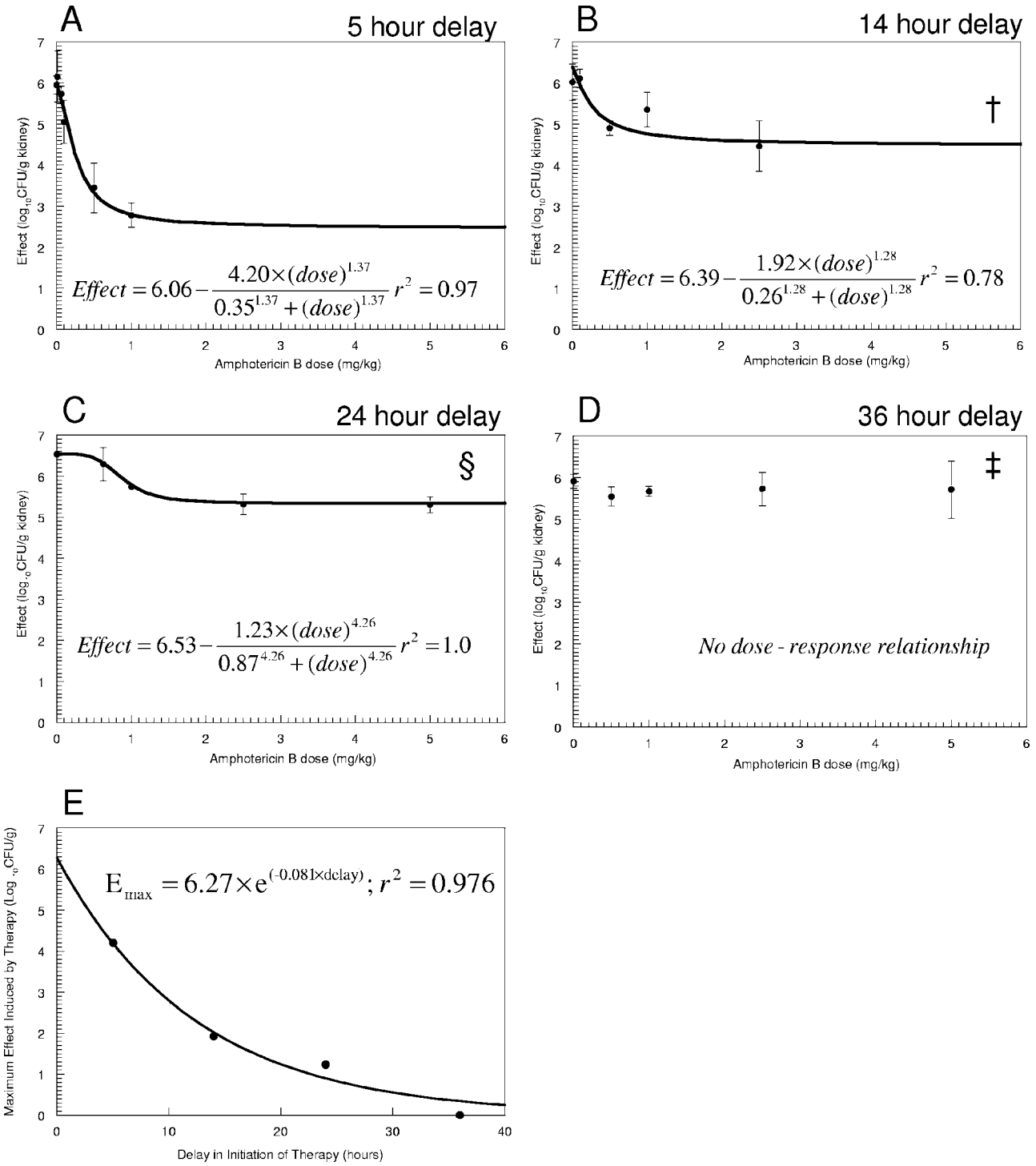

FIG. 5. Effect of an increasing delay in the administration of amphotericin B in neutropenic mice. An increased delay in drug administration results in diminished antifungal effect. (A to D) The dosages of amphotericin B in each of these experiments were as follows: (A) 5-h delay, 0, 0.01, 0.06, 0.10, 0.50, and 1.00 mg/kg; (B) 14-h delay, 0, 0.10, 0.50, 1.00, and 2.50 mg/kg; (C) 24-h delay, 0, 0.63, 1.00, 2.50, and 5.00 mg/kg; (D) 36-h delay, 0, 0.63, 1.00, 2.50, and 5.00 mg/kg. Data are the means ± standard deviations of three mice. †, $P = 0.054$; §, $P = 0.002$; ‡, $P = 0.013$ (all compared with a 5-h delay). (E) Relationship between the maximum effect and a delay in the administration of amphotericin B. Half of the maximal effect of amphotericin B is lost for every 8.6 h of delay.

administration of amphotericin B serves only to prevent progressive growth, while in nonneutropenic mice there is a sustained decrement in the fungal burden within the kidney. The mathematical model enables a further appreciation of the fact that the treatment of *C. albicans* is a dynamic process in which the various components affecting killing are continuously pitted against the growth of the organism. If killing is inadequate, for whatever reason, then growth dominates and a poor outcome for the host ensues. The model also highlights the fact that neutrophil killing is a saturable process. The reason for

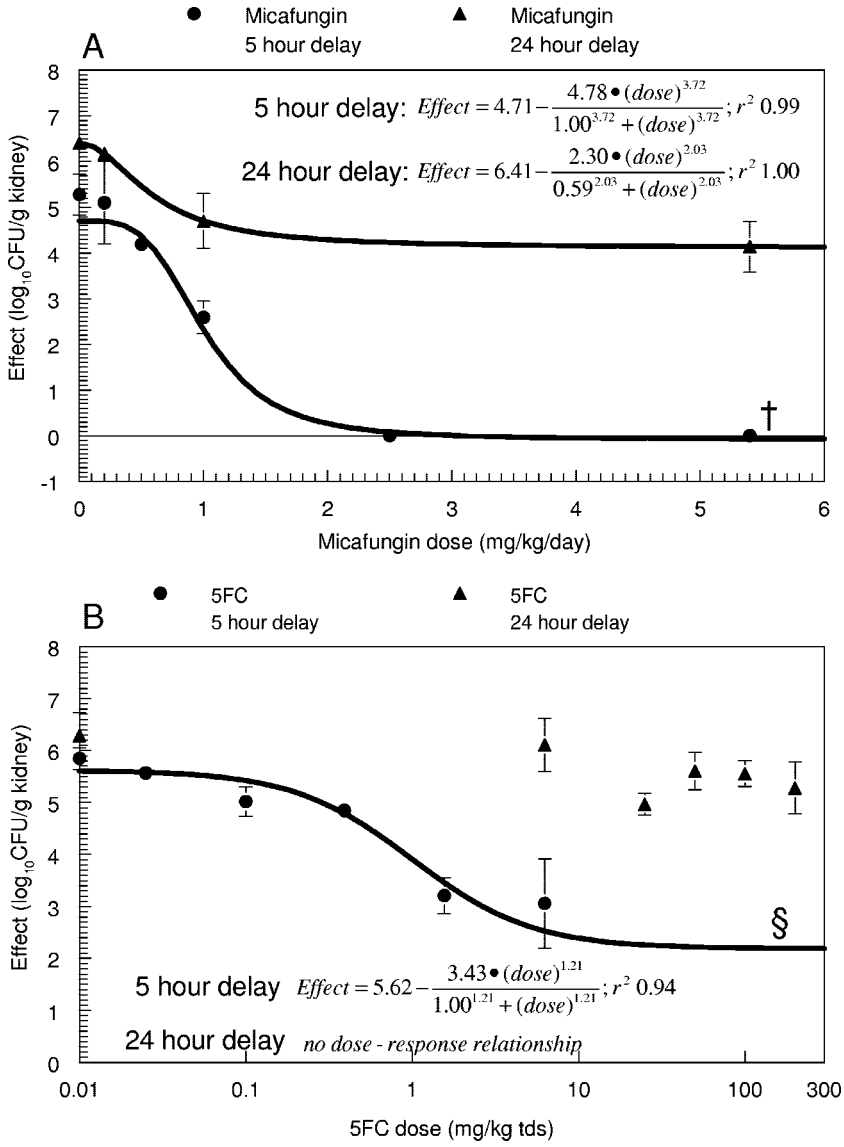

FIG. 6. Dose-response relationships with micafungin (A) and flucytosine (B) in neutropenic mice following 5- and 24-h delays in the administration of the respective drugs. Data are the means ± standard deviations of three mice. There was a significant difference between the antifungal effects following a 5-h delay and a 24-h delay in the administration of both micafungin and flucytosine. A delay in the administration of flucytosine by 24 h resulted in no discernible dose-response relationship. †, $P = 0.002$; §, $P < 0.001$ (both compared with a 5-h delay).

the poor outcome of neutropenic mice is that the killing capacity of the relatively fewer neutrophils becomes quickly saturated.

The advantages of treating disseminated candidiasis in neutropenic hosts at the earliest possible time seem intuitively obvious and are supported by both clinical and experimental data (15, 16, 18, 28, 29); the findings of our study are consistent with these publications. We extend these previous observations by demonstrating that there is only a narrow therapeutic window in which a significant decline in fungal tissue burden following antifungal exposure can be achieved—this time period appears to be within the first 24 h of infection. While our data do suggest that there are inherent differences in terms of the efficacy of the three antifungal agents that were studied, there does appear to be a clear trend of diminishing effect following a progressive delay in drug administration.

A remaining question relates to the underlying biological mechanisms by which progressive delays in therapy are associated with a diminished response to antifungal agents. The most obvious explanation is that a therapeutic delay allows for a larger fungal burden, which is less amenable to treatment. While this is undoubtedly true, the infectious burdens in the various delay models were designed to be comparable, suggesting that other factors may also be important. In this regard, the histological appearances provide some valuable insights. Clearly, the smallest drug effect occurs in the setting of more established and mature pathological appearances; thus, suboptimal drug penetration into hyphal masses, abscesses, infarcted

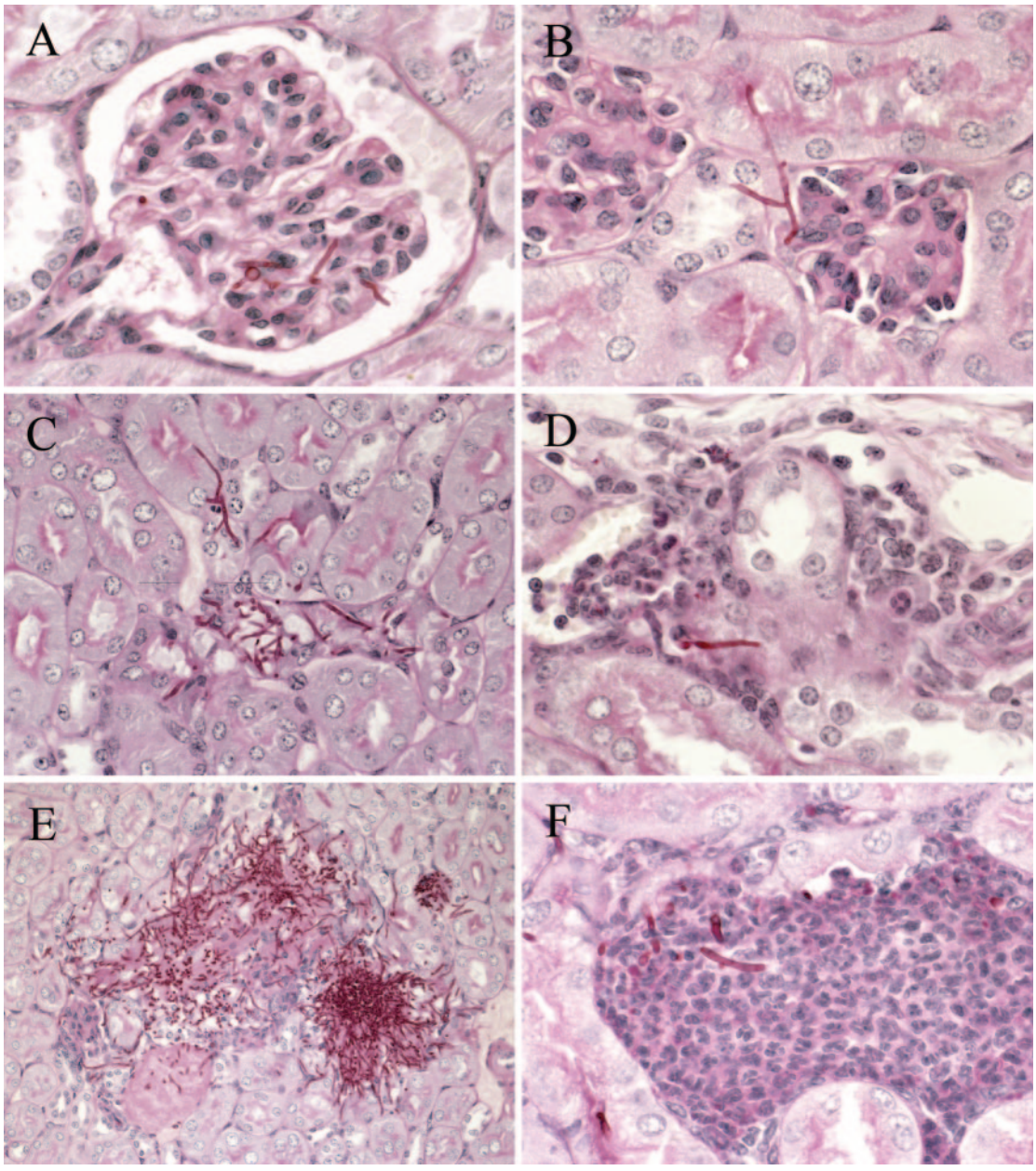

FIG. 7. Temporal sequence of histological appearances within the kidneys of both neutropenic and nonneutropenic mice infected with *C. albicans*. (A) Neutropenic mouse 5 h postinfection showing the transgression of hyphae across Bowman's space (magnification, ×630); (B) neutropenic mouse 5 h postinfection showing invasion into periglomerular structures (magnification, ×630); (C) neutropenic mouse 14 h postinfection showing tubular invasion (magnification, ×400); (D) nonneutropenic mouse 14 h postinfection showing a peritubular neutrophil infiltrate (magnification, ×400); (E) neutropenic mouse 24 h postinfection showing progressive hyphal extension and infarction (magnification, ×200); (F) nonneutropenic mouse 24 h postinfection showing the formation of a microabscess containing hyphae (magnification, ×200).

tissue, and biofilms may be the reason for the diminished effect (28). The relationship between underlying pathology and the antifungal effect has been previously documented by Walsh et al. (28), who observed a superior drug effect in acute compared with chronic disseminated candidiasis. In acute disseminated candidiasis, in which the largest magnitude of drug effect was observed, infection was not associated with an inflammatory infiltrate or fibrosis. In contrast, reduced drug activity was observed in chronic disseminated candidiasis, where the pathological features included fibrosis, central necrosis, and calcification. These pathological processes may have hindered the diffusion of drug to its microbiological target. The rate of fungal growth is also likely to be an important determinant of antifungal efficacy (9). Organisms which are rapidly growing, as is the case immediately following inoculation, may be more sensitive to antifungal agents than are organisms at later time points, which are likely to be replicating more slowly.

This mathematical model provides a way of (i) further understanding system behavior, (ii) estimating the relative contribution to the overall candidacidal effect of each of the system compo-

nents, and (iii) identifying broad principles related to the growth and killing of *C. albicans* which are also likely to be operational in clinical contexts. We must stress, however, that the structural model and the specific parameter estimates obtained in the fitting process apply only to the experimental design and conditions described in this study and cannot be directly extrapolated to other models or contexts.

We used a single strain of *C. albicans*; ideally, multiple *Candida* strains would be studied to examine if there are significant strain-to-strain differences in terms of the combined effect of neutrophils and amphotericin B. We determined fungal density within the kidney using $\log_{10}$ CFU/g and assumed that this biomarker is intricately linked with outcomes of clinical relevance such as disease resolution and survival. Despite the fact that only three mice were used for each sampling point, the degree of observed variability was acceptable and comparable to that in previous studies of disseminated murine candidiasis (10, 13). More importantly, however, the variability in the data was explicitly acknowledged via the weighting scheme, in which the point estimates of the means were weighted by the inverse of the observed variance.

In conclusion, these analyses support the concept that factors which are extraneous to the inherent activity of antifungal agents may be equally, if not more, important in determining the ultimate therapeutic outcome. Thus, strategies to optimize the number and function of neutrophils (25), as well as delivering appropriate antifungal agents at the earliest possible time in the infectious process, are critical issues in order to improve the therapeutic outcome of patients with disseminated candidiasis.

APPENDIX

The murine pharmacokinetics of amphotericin B and the candidacidal effect induced by the administration of amphotericin B in neutropenic and nonneutropenic mice were described using the following four simultaneous inhomogeneous differential equations:

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

$$\frac{dX(2)}{dt} = -\left(\frac{\mathrm{SCL}}{Vc}\right) \times X(2) - K_{\mathrm{cp}} \times X(2) + K_{\mathrm{pc}} \times X(3) \quad (2)$$

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

$$\frac{dX(4)}{dt} = K_{\mathrm{gmax}} \times \left(1 - \frac{X(4)}{\mathrm{POPMAX}}\right) \times X(4) \quad (4a)$$

$$\times 1 - \left(\frac{[\mathrm{AmB}]^{H_g}}{C_{50}g^{H_g} + [\mathrm{AmB}]^{H_g}}\right) \quad (4b)$$

$$- K_{\mathrm{kmax}} \times \frac{[\mathrm{AmB}]^{H_k}}{C_{50}k^{H_k} + [\mathrm{AmB}]^{H_k}} \times X(4) \quad (4c)$$

$$- \mathrm{WBCKILL}_{\mathrm{max}} \times \frac{X(4)}{\mathrm{WBCKILL}_{50} + X(4)}$$

$$\times R(1) \times X(4) \quad (4d)$$

Equations 1, 2, and 3 describe the pharmacokinetics of amphotericin B, using a standard open three-compartment pharmacokinetic model, with first-order elimination from the central compartment (compartment 2); zero-order, time-delimited input of amphotericin B [$B(1)$] into the peritoneal cavity (compartment 1), and the movement of drug into and out of the peripheral compartment (compartment 3). $X(1)$, $X(2)$, and $X(3)$ are the amounts of the drug (in milligrams) in compartments 1, 2, and 3, respectively. Vc is the volume of the central compartment (in liters), SCL is the clearance from the central compartment (liters/hour), and K represents the various first-order intercompartmental rate constants.

Equation 4 describes the rate of change of the number of *C. albicans* organisms within the kidneys of neutropenic and nonneutropenic mice. $X(4)$ represents the number of *C. albicans* organisms in the kidney. The term 4a 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. Most of the information enabling an estimate for POPMAX is derived from the growth of *C. albicans* in control mice. As the number of organisms within the kidney approaches POPMAX, the rate of *C.albicans* growth decreases and approaches zero. The term 4b in equation 4 provides a way of allowing amphotericin B 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 *C. albicans* grows in an unrestrained manner. Following a progressively higher concentration of amphotericin B, the growth rate of *C. albicans* is constrained to approach zero. $C_{50}g$ is the concentration of amphotericin B at which the effect on growth is half maximal, and H_g is the associated slope function. The term 4c describes the killing (fungicidal effect) associated with amphotericin B. K_{kmax} represents the maximum rate of amphotericin B-induced killing, H_k is the slope constant, and $C_{50}k$ is the concentration of amphotericin B at which the rate of *C. albicans* killing is half maximal. The term 4d describes the rate of saturable neutrophil-induced *C. albicans* killing. The maximum rate of neutrophil-induced killing is represented by $\mathrm{WBCKILL}_{\mathrm{max}}$, and $\mathrm{WBCKILL}_{50}$ is the *C. albicans* fungal burden at which the neutrophil-induced killing is half maximal.

The neutrophil counts obtained from both healthy and cyclophosphamide-treated mice were allowed to influence the *C. albicans* tissue burden via a discrete input function represented by $R(1)$ in equation 4d. In this regard the neutrophils were allowed to influence the growth rate of *C. albicans* in a manner analogous to the continuous infusion of a drug.

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