Pharmacodynamics of Caspofungin in a Murine Model of Systemic Candidiasis: Importance of Persistence of Caspofungin in Tissues to Understanding Drug Activity

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Received 17 February 2005/Returned for modification 23 April 2005/Accepted 2 September 2005

Pharmacokinetic and pharmacodynamic studies were conducted in a murine model of systemic candidiasis to determine the pharmacodynamic parameter linked with caspofungin efficacy. Additional studies defined the importance of persistent tissue drug concentrations to treatment outcome. The pharmacokinetics of caspofungin were determined in the serum and kidneys of infected mice over 96 h. Population pharmacokinetic analysis demonstrated a serum terminal half-life (t½) for caspofungin of 20.2 h when only serum concentrations were considered, but the terminal t½ increased to 59.2 h when serum and kidney concentration-time data were comodeled. In dose-range studies, the dose-response effect was well described by an inhibitory sigmoid curve for the exposure-effect killing caused by the drug (r² > 0.96; P < 0.001). In dose-fractionation studies, fungal counts in kidneys were not statistically different for total doses given as one, two, or four equally divided doses over 96 h, indicating that the area under the concentration-time curve/MIC is the pharmacodynamic parameter that predicts caspofungin efficacy in our infection model. In a separate study, mice infected with Candida albicans 24 h after serum concentrations of caspofungin fell below the MIC for the fungal isolate had significant reductions in fungal densities in their kidneys compared with the growth of fungi in the kidneys of untreated controls (P = 0.005). This in vivo biological assay demonstrates that therapeutic concentrations of caspofungin persist at the site of infection in kidney tissue well after serum concentrations fall below the MIC, underscoring the primacy of caspofungin levels in tissues on determining treatment outcome.

Candida albicans is an important cause of invasive infection in people whose immune systems are compromised by antineoplastic chemotherapy, chronic steroid use, or infection due to human immunodeficiency virus and in people who require long-term intravenous catheters for parenteral administration of nutrition, drugs, or fluids (7, 10, 17, 34). The incidence of bloodstream infections caused by Candida species is increasing, such that Candida now ranks as the fourth most common cause of nosocomial bloodstream infections (5, 7). Despite treatment with the azole antifungal agent fluconazole or the polyene amphotericin B, substantial proportions of patients with candidemia fail to respond to therapy (26, 28, 29). The overall crude mortality rate in surgical patients with deep-seated Candida infections with resultant candidemia is 61%, with an attributable mortality rate of 49% (14).

Pharmacodynamic studies are used to determine whether a drug’s effect increases with drug exposure and, if so, whether the activity of the drug is best predicted by the time above the MIC (T > MIC), the area under the concentration-time curve (AUC)/MIC ratio, or the peak concentration/MIC (peak/MIC) ratio. Pharmacodynamic studies have been used to develop dosing strategies that optimize treatment outcomes for antibacterial and antiviral drugs (8, 16, 22, 27). Currently, the use of pharmacodynamic studies for the optimization of antifungal therapies is underutilized.

Caspofungin is an echinocandin antifungal agent that has potent activity against many fungal species, including Candida albicans (1, 4, 6, 18, 25). Clinical studies have shown that caspofungin is as active as amphotericin B and fluconazole in the treatment of invasive candidal disease (4, 23, 33). Caspofungin exerts its fungicidal activity by inhibiting the synthesis of (1,3)-β-glucan, which leads to cell wall damage (9, 19). The pharmacodynamic parameter linked with the efficacy of caspofungin has not been adequately defined.

In this study we conducted pharmacokinetic (PK), dose-range, and dose-fractionation studies in a murine model of systemic candidiasis to determine the pharmacodynamic parameter that predicts the efficacy of caspofungin. Unique to this study, we also measured the kinetics of caspofungin in kidney tissue and conducted studies to determine the importance of persistent tissue concentrations of caspofungin on the overall activity of this antifungal drug. A better understanding of the pharmacodynamics of caspofungin may lead to novel dosing strategies that may improve outcomes associated with systemic candidiasis.

(Materials and Methods

Candida isolate. C. albicans isolate ATCC 30602 (American Type Culture Collection, Manassas, VA) was maintained on Sabouraud dextrose agar (SDA;
BBL, Sparks, MD) at 4°C until use. For each study, two to three colonies of the fungus were subcultured onto fresh SDA and incubated at 35°C for 24 h. A fungal suspension was prepared by transferring several colonies to normal saline, which was diluted to the desired concentration with normal saline or growth medium. The concentration of the final fungal suspension was confirmed by quantitative culture.

MIC determination. The MIC of the fungal isolate for caspofungin was determined by using the test conditions specified by the CLSI (formerly the NCCLS) for antifungal susceptibility testing for yeast by using a broth macrodilution method in RPMI 1640 with morpholinopropanesulfonic acid (MOPS) (24). Serial twofold dilutions were used for concentrations below 0.2 mg/liter, and 0.2-ml increments of drug were studied for concentrations above this level. The MICs (approximately 80% reduction) were determined after 48 h of incubation. These MIC studies were repeated in parallel with MIC determinations in 80% mouse serum to characterize the effect of protein binding on drug activity.

Murine model of systemic candidiasis. Immune-normal female outbred Swiss-Webster mice (weight, 24 to 26 g; Taconic Farms, Taconic, NY) were injected intravenously (i.v.) with 8 × 105 CFU of Candida via a lateral tail vein in 0.2 ml of saline. This fungal inoculum resulted in an infection that was slowly progressive in untreated mice for at least 96 h. Between 96 and 120 h, untreated mice had to be humanely euthanized per our Institutional Animal Care and Use Committee protocol and Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines because they stopped eating and drinking, lost greater than 20% of their preinfection weight, or had become immobile. Infected mice were given intraperitoneal (i.p.) doses of caspofungin or saline (control group) beginning 5 h after fungal inoculation. Quantitative cultures were taken from kidneys collected from four mice that were killed immediately after fungal inoculation and another four mice that were killed just prior to drug administration to verify that the fungi were replicating before therapy was initiated. All animal procedures used were approved by the Institutional Animal Care and Use Committee.

HPLC analytical method. HPLC analysis was performed with an Alltech Alltima C18 rocket column (53 by 7 mm). A mobile phase consisting of 60% 0.1% methanol, 0.1% trifluoroacetic acid, and 0.1% trifluoroacetic acid–20% acetonitrile was delivered at 2 ml/min. The analytes activity. (24). Serial twofold dilutions were used for concentrations below 0.2 mg/liter, and the internal standard eluted at 3.4 min. For determinations in serum, the assay was linear over a range of 0.05 to 5 mg/liter (r2 > 0.995). The lower limit of quantitation was 0.01 mg/liter. Comparison of samples assayed on three separate days yielded coefficients of variation that ranged from 3.3 to 15%. For kidney samples, linearity over a range of 0.2 to 10 μg/g of tissue was observed (r2 > 0.98). The lower limit of quantitation was 0.1 μg/g of tissue.

Pharmacokinetic data analysis. All serum and kidney concentrations were simultaneously modeled by population pharmacokinetic analysis. The Non-Parametric Adaptive Grid (NPAG) with adaptive γ software program of Leary et al. was employed (20). The choice of weights was such that the total observation variance was assumed to be proportional to the assay variance. The adaptive γ was then optimized with each cycle to provide an estimate of the observation variance, so that inverse variance weighting, which approximated the homoscedastic assumption, would be employed. Models were evaluated by using Akaike’s information criterion (2).

A four-compartment open model with first-order input into and first-order clearance from the central compartment was employed. The four compartments were the intraperitoneal space (the absorption compartment), the serum (the central compartment), the kidney (the site of infection and the site of the drug effect), and the peripheral space (the rest of the tissues).

Pilot single-dose, dose-range study for caspofungin. In one dose-range study, 40 mice were infected i.v. with Candida and then separated into five groups; each group consisted of eight mice. Five hours later, the groups were given a single dose of 0.01, 0.1, 1.0, or 10 mg/kg of caspofungin i.p. In a second dose-range study, each group consisted of eight mice. Five hours later, the groups were given a single dose of 0.01, 0.1, 1.0, or 10 mg/kg of caspofungin i.p. Ninety-six hours later, the right kidneys were collected from mice that had been killed. Each kidney was weighed and homogenized. The homogenates were washed twice with normal saline and quantitatively cultured onto SDA. After 48 h of incubation at 35°C, the colonies were counted and the relationship between the drug dose and the reduction of the fungal density in the kidneys was determined. Dosages located on the steep portion of the dose-response curve were identified and were used in the design of a subsequent combined dose-range study. The data were analyzed by using the quantitative culture procedure, the entire volume of the most concentrated of the serial dilutions was inoculated onto multiple agar plates. The cultures reproducibly detected greater than 20 organisms/g of tissue.

Effect of dose fractionation of caspofungin on the fungal densities in kidneys after 96 h of drug exposure. A combined, expanded dose-range and dose-fractionation study was conducted in duplicate to identify the pharmacodynamic parameter that predicts the efficacy of caspofungin. The expanded dose-range studies were combined with the dose-fractionation experiments to validate that the total doses of drug examined in the latter studies were on the steep portion of the dose-response curve. Beginning at 0 h, cohorts of infected mice received saline or caspofungin as one or multiple doses predicted to fall on the steep part of the exposure-response curve. Each caspofungin dose was administered on three different schedules: (i) the whole dose administered at 0 h; (ii) one-half of the total dose administered at 0 and 48 h; or (iii) one-quarter of the total dose administered at 0, 24, 48 and 72 h. Each dose and schedule of administration was given to at least eight mice. The animals were killed at 96 h. The kidneys were aseptically collected, homogenized, and washed twice with normal saline. Then, the fungal burden in the kidney was determined by quantitative culture. The results for groups that received the same total dose of caspofungin by using different schedules of administration were compared.

Detailed dose-fractionation study. To fully characterize the effect of fractionating a dose of caspofungin on the fungal burden over time, six groups of mice were infected i.v. with 8 × 106 CFU of C. albicans. Five hours later the first three groups of mice were given 0.4 mg/kg of caspofungin i.p. as either the total dose at 0 h; two equally divided doses at 0 and 48 h; or four equally divided doses at
0, 24, 48, and 72 h. The fourth group of infected mice received saline instead of caspofungin and served as controls. The fifth and sixth groups were treated with 0.08 and 1 mg/kg of caspofungin, respectively, as a single dose administered at 0 h. The last two groups were used to confirm that the dose of caspofungin chosen for fractionation was on the steep portion of the inhibitory sigmoid dose-response curve for the maximal killing caused by the drug (E_{max}).

In vitro PAE determination. C. albicans was prepared to a concentration of 1 x 10^7 CFU/ml in 50 ml of prewarmed yeast nitrogen base (Difco, Becton Dickinson Microbiology Systems, Sparks, MD) supplemented with 1% dextrose (YNB-D). After 30 min of incubation at 35°C in a water shaker bath, caspofungin was added to flasks to achieve final concentrations of 1/4, 1, 2, and 4 mg/kg of caspofungin as one, two, or four equally divided doses over 96 h and 96 h of incubation at 35°C in a water shaker bath. At the 24-h time point, the caspofungin-treated and control fungal suspensions were diluted with fresh, prewarmed YNB-D. Quantitative subcultures from the diluted samples were obtained immediately and every hour thereafter for 18 h and then every 2 to 3 h for up to 72 h. After the cultures were incubated at 35°C for 48 h, the colonies were enumerated. The postantibiostic effect (PAE) was defined as the difference in the time required for caspofungin-treated fungi to increase in density by a factor of 10^6 CFU/ml relative to the density of the untreated controls after the fungistatic concentration of drug was added. Pilot studies demonstrated that the 1:200 dilution of the caspofungin concentrations used in these studies eliminated drug carryover.

To test for drug carryover, an additional group of uninfected mice was treated at 0.4 mg/kg of caspofungin as one, two, or four equally divided doses over 96 h. After 30 min of incubation at 35°C in a water shaker bath, caspofungin was added to the fungal suspension to replicate the exposure (drug) and was diluted in fresh medium. Pilot studies demonstrated that the 1:200 dilution of the caspofungin concentrations used in these studies eliminated drug carryover.

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TABLE 1. Parameter values and their dispersions for the Non-Parametric Adaptive Grid population pharmacokinetic analysis for caspofungin administered to mice as a single i.p. dose

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_c$ (liter)</td>
<td>0.00846</td>
<td>0.00171</td>
<td>0.0180</td>
</tr>
<tr>
<td>SCL (liter/h)</td>
<td>0.000652</td>
<td>0.000561</td>
<td>0.000428</td>
</tr>
<tr>
<td>$k_{12}$ (h$^{-1}$)</td>
<td>1.764</td>
<td>1.407</td>
<td>1.178</td>
</tr>
<tr>
<td>$k_{31}$ (h$^{-1}$)</td>
<td>28.47</td>
<td>29.03</td>
<td>1.603</td>
</tr>
<tr>
<td>$k_{13}$ (h$^{-1}$)</td>
<td>18.05</td>
<td>18.82</td>
<td>2.230</td>
</tr>
<tr>
<td>$k_{32}$ (h$^{-1}$)</td>
<td>3.284</td>
<td>1.003</td>
<td>5.334</td>
</tr>
<tr>
<td>$V_{kid}$ (liter)</td>
<td>0.00489</td>
<td>0.0386</td>
<td>0.00312</td>
</tr>
<tr>
<td>$k_a$ (h$^{-1}$)</td>
<td>32.12</td>
<td>31.88</td>
<td>0.897</td>
</tr>
</tbody>
</table>

* $V_c$, volume of the central compartment; SCL, serum clearance; $k_{12}$, $k_{31}$, $k_{13}$, and $k_{32}$, first-order intercompartmental transfer rate constants, where $k_{12}$ and $k_{31}$ are the transfer rate constants between the central compartment and the extracellular and tissue sites of the mouse (excluding the kidneys) and $k_{13}$ and $k_{32}$ are the transfer rate constants between the central compartment and the kidneys; $V_{kid}$, volume of the kidney compartment; $k_a$, first-order absorption rate constant for the i.p. injection.

Caspofungin concentrations in serum or kidney were measured by the Bayesian step. The parameter means were used as Bayesian prior values.

### RESULTS

**MIC determination.** The caspofungin MIC was determined six times. The MIC was 0.2 mg/liter in RPMI 1640 plus MOPS and did not change when susceptibility studies were conducted in 80% mouse serum.

**Pharmacokinetics of caspofungin in mice.** The pharmacokinetics of caspofungin were assessed in the sera and kidneys of infected mice over 96 h for dosages between 0.25 and 1.5 mg/kg, given as a single i.p. dose. Pharmacokinetic analysis was performed with the NPAG with adaptive $\gamma$ software program of Leary et al. (20). A total of 130 datum points for the mice were analyzed. There were 111 serum samples and 128 kidney concentrations available for analysis. The data for caspofungin were best described by an open four-compartment model. The outcome of the NPAG analysis (means, medians, and standard deviations for the parameter estimates) is presented in Table 1. The Bayesian estimates for each of the dosing groups are presented in Table 2. The fit of the model to the data after the Bayesian step was good, with an $r^2$ value of 0.99 for serum concentrations and an $r^2$ value of 0.87 for kidney concentrations. The observed-predicted plots are presented in Fig. 2A and B.

Simulations from the parameter values in Table 1 indicate that the drug persists in the kidneys much longer than it does in serum. For population pharmacokinetic analysis with only the data for the serum samples, the calculated terminal half-life ($t_{1/2}$) was 20.2 h. However, when serum and kidney concentration-time data were comodeled, the terminal half-life increased to 59.2 h, indicating that the drug resides in the

![Fig. 2. Predicted-observed plot of caspofungin serum (A) and kidney (B) concentrations after the Bayesian step. The parameter means were used as Bayesian prior values.](http://aac.asm.org/links/figure/Figure2.jpg)
doses of caspofungin associated with stasis and 1-log and 2-log CFU/g decreases in fungal densities in kidneys 96 h after the administration of caspofungin were 0.13, 0.43, and 1.63 mg/kg, respectively.

For the dose-fractionation studies, analysis of variance demonstrated that the reductions in the fungal densities in the kidneys were similar for any total dose when it was given as one, two, or four equally divided doses over 96 h (Table 3). This indicates that the AUC/MIC ratio is the pharmacodynamic parameter that determines the efficacy of caspofungin in our infection model.

The results of the dose-fractionation study in Fig. 3 and Table 3 demonstrate that there was a small, statistically non-significant bias in favor of the two less fractionated groups (administration of the whole dose once and half the dose twice over 96 h) when the data were presented as dose (mg/kg) versus fungal density (log CFU/g). Although this bias is not statistically significant, it can be explained by the fact that even though a full day had elapsed between the administration of the last one quarter dose at hour 72 and the time that the mice were killed, this group had not developed the full AUC exposure by the time that they were killed (96 h). As shown in Table 4, for any total dose of caspofungin that was administered as four equally divided doses over 96 h, the AUC0–96 was 16.1 to 24.7% less than the AUC0–96 generated when the same total dose was given as a single dose at the beginning of the 96-h study. Therefore, we calculated the AUC to the point of killing for each of the fractionated groups, used these results to recalculate the AUC/MIC ratios, and reanalyzed the data. As shown in Fig. 4A, there was no demonstrable bias among the fractionated regimens when the exposures were corrected for the amount of AUC that actually developed to the time of killing. This demonstrates that AUC/MIC is the dynamically linked variable for caspofungin in our infection model. It should be noted that the AUC/MIC ratio in serum was used as the independent variable. However, the AUC/MIC ratio in the kidney could also be used as the independent variable. Since these values map one-to-one, the overall $r^2$ of the relationship would not change, but the actual values for the parameters (e.g., EC50, exposure at which the effect is 50% of the maximum) would change. We chose to present the serum data, as serum is the much easier compartment to sample, but the importance of drug exposures at the site of effect is demonstrated below.

![Graph](Image)

**FIG. 3.** Combined dose-range and dose-fractionation study showing (i) the relationships between increasing total doses of caspofungin and the reduction of fungal densities in the kidneys and (ii) the impact of fractionating each total dose of caspofungin on the antifungal drug effect. The equation represents the inhibitory sigmoid $E_{max}$ relationship with the dose (mg/kg) of caspofungin and the density of *C. albicans* (log CFU/g) in cultures of kidney tissue. Representation of the drug dose in mg/kg is synonymous with the assumption that the total drug exposure that develops between 0 h and the killing time point at 96 h are identical for a total dosage that is given as one, two, or four fractionated regimens over the experiment.

Peripheral tissues for a very long time. There were doses (including the 0.4-mg/kg dosage) where the serum concentration-time profile dropped below 0.2 mg/liter (the MIC for the fungal strain) by 24 h but persisted above this value significantly longer in kidneys.

**Results of dose-range and dose-fractionation studies.** Tenfold dose increments of caspofungin were examined in the pilot dose-range study to define the steep part of the dose-response curve. The doses ranged from 0.01 to 10 mg/kg, given as a single intraperitoneal dose. This study demonstrated that a dose exceeding 0.01 mg/kg was required to cause measurable activity. Activity was near maximal by 1 mg/kg (data not shown). These data were used to design the subsequent studies.

Expanded dose-range studies were conducted in conjunction with dose-fractionation studies. The results are displayed in Fig. 3. In this mouse infection model, the concentration of *Candida* in the kidneys at the start of therapy was $4.51 \pm 0.17 \log$ CFU/g. At 96 h, the fungal density in the kidneys of the controls was $4.98 \pm 0.34 \log$ CFU/g. All mice survived the observation period. The dose-effect relationship was well described by the inhibitory sigmoid $E_{max}$ equation: log $AUC_{96}$ (CFU/g) = $4.83 - (2.39 \times (dose)^{1.92}/[(dose)^{1.92} + 0.413^{1.92}]}$ ($r^2 = 0.963; P < 0.001$). In this equation the dose is given in mg/kg.

There were four total doses on the steep part of the exposure-response curve (0.1, 0.2, 0.4, and 1.0 mg/kg). These doses corresponded to 6.2, 19.9, 48.4, and 84.5% of the maximal effect, respectively. The cohort receiving 5 mg/kg of caspofungin had the drug administered only as the whole dose given at 0 h and was included to validate that $E_{max}$ was attained. This dose decreased the fungal density to $2.47 \pm 0.57 \log$ CFU/g, producing 99.2% of the calculated maximal effect. The total

![Graph](Image)

**TABLE 3.** Analysis of variance for the effect of dose fractionation (mg/kg) on *Candida* colony counts determined from the kidney after 96 h of caspofungin therapy

<table>
<thead>
<tr>
<th>Total dose (mg/kg)</th>
<th>Colony count (log CFU/g ± 1 SD)</th>
<th>$P$ valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One dose (given at 0 h)</td>
<td>Two half doses (given at 0 and 48 h)</td>
</tr>
<tr>
<td>0</td>
<td>4.97 ± 0.34</td>
<td>4.71 ± 0.27</td>
</tr>
<tr>
<td>0.1</td>
<td>4.58 ± 0.22</td>
<td>4.37 ± 0.21</td>
</tr>
<tr>
<td>0.2</td>
<td>4.05 ± 0.43</td>
<td>3.50 ± 0.44</td>
</tr>
<tr>
<td>1.0</td>
<td>2.83 ± 0.47</td>
<td>2.77 ± 0.34</td>
</tr>
<tr>
<td>5.0</td>
<td>2.47 ± 0.57</td>
<td></td>
</tr>
</tbody>
</table>

a A $P$ value of <0.05 was statistically significant.
For completeness, the relationship between peak/MIC and $T > $ MIC and their effects on fungal density are shown in Fig. 4B and C, respectively. The coefficients of determination ($r^2$ values) derived from nonlinear weighted least-squares regression analyses also show that the AUC/MIC ratio is the pharmacodynamic parameter that best predicts the efficacy of caspofungin in our infection model.

**Detailed dose-fractionation studies.** In our infection model, untreated controls were humanely killed at the 96-h time point (in accordance with our Institutional Animal Care and Use Protocol and AAALAC guidelines) because they had lost ≥20% of their preinfection body weight, had stopped eating and drinking, or had become immobile. Thus, we could not characterize the growth of Candida in the control group after the 96-h time point. However, animals treated with a single low dose of 0.08 mg/kg of caspofungin at 0 h showed progressive growth of fungi in the kidneys for at least 134 h (Fig. 5). The detailed dose-fractionation studies were functionally in vivo time-kill studies. These studies showed that the administration of 0.4 mg/kg of caspofungin as one, two, or four equally divided doses over 96 h resulted in a progressive decline in the fungal loads in the kidneys of infected mice. The fungal densities in the kidneys of mice for each fractionated regimen began to decrease after the 96 h time point and converged shortly thereafter (Fig. 5).

For animals given 0.4 mg/kg of caspofungin as two or four equally divided doses over 96 h, we calculated that it would take 118 and 134 h, respectively, for these regimens to develop the same AUC that would develop at 96 h for mice given 0.4 mg/kg of caspofungin as a single dose at 0 h. The quantitative culture results taken at these time points for the groups given 0.4 mg/kg of caspofungin as one, two, or four equally divided doses were 3.44 ± 0.44, 3.38 ± 0.23, and 3.40 ± 0.35 log CFU/g of kidney, respectively.

**Importance of persistent kidney concentrations of caspofungin for microbiological effect: results of the in vivo bioassay.** Administration of 0.4 mg/kg of caspofungin to mice resulted in mean serum caspofungin concentrations of 0.17 mg/liter at 24 h, 0.05 mg/liter at 48 h, and 0.03 mg/liter at 72 h. Therefore, the serum concentration declined below 0.2 mg/liter (which is the MIC for the C. albicans isolate studied) by the 24-h time point (Fig. 6). The mean concentrations of caspofungin in the kidneys of the same mice were 1.26, 0.58, and 0.13 μg/liter at 24, 48, and 72 h, respectively. Forty-eight hours after caspofungin or saline was administered, the mice in both groups were infected intravenously with C. albicans. At that time point the concentration of caspofungin in the serum of mice treated with this antifungal drug was 1/4 the MIC of the Candida strain used in the project, but it was 2.9× the MIC in the kidneys. As shown in Fig. 6, quantitative cultures of the kidneys of mice killed 30 min after fungal challenge were similar for caspofungin and saline recipients (5.01 ± 1.3 and 4.99 ± 0.14 log CFU/g, respectively), showing that the concentration of drug in the kidneys at the time of killing did not affect the quantitative culture results (i.e., there was no drug carryover). Twenty-four hours after fungal inoculation, the densities of Candida in the kidneys of caspofungin-treated mice decreased to 4.32 ± 0.26 log CFU/g but increased to 5.78 ± 0.48 log CFU/g in the saline recipients ($P = 0.005$). This in vivo biological assay demonstrates that bioactive concentrations of drug persisted at the site in the kidney where the fungi reside well after the serum concentrations fell below the MIC.

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**TABLE 4. Values of AUC$_{0–96}$/MIC ratio, peak/MIC ratio, and $T > $ MIC obtained for the caspofungin doses and their different schedules of administration**

<table>
<thead>
<tr>
<th>Total daily dose (mg/kg)</th>
<th>One dose (0 h)</th>
<th>Two doses (0 and 48 h)</th>
<th>Four doses (0, 24, 48, and 72 h)</th>
<th>Difference in AUCs (%) generated by giving one vs four divided doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{0–96}$/MIC ratio$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>23.52</td>
<td>21.54</td>
<td>19.74</td>
<td>16.1</td>
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<tr>
<td>0.2</td>
<td>47.04</td>
<td>43.07</td>
<td>39.47</td>
<td>16.1</td>
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<tr>
<td>0.4</td>
<td>79.8</td>
<td>67.58</td>
<td>60.05</td>
<td>24.7</td>
</tr>
<tr>
<td>1.0</td>
<td>227.85</td>
<td>205.20</td>
<td>186.45</td>
<td>18.2</td>
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<td>Peak/MIC ratio$^b$</td>
<td></td>
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<tr>
<td>0.1</td>
<td>0.77</td>
<td>0.39, 0.46</td>
<td>0.19, 0.28, 0.32, 0.34</td>
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</tr>
<tr>
<td>0.2</td>
<td>1.5</td>
<td>0.77, 0.93</td>
<td>0.39, 0.56, 0.64, 0.68</td>
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<tr>
<td>0.4</td>
<td>1.7</td>
<td>0.84, 1.2</td>
<td>0.42, 0.69, 0.87, 0.99</td>
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</tr>
<tr>
<td>1.0</td>
<td>6.7</td>
<td>3.40, 4.2</td>
<td>1.70, 2.50, 2.90, 3.10</td>
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<tr>
<td>Time &gt; MIC (% of 96 h)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>13.5</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
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<td>30.2</td>
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<tr>
<td>1.0</td>
<td>69.8</td>
<td>94.8</td>
<td>93.8</td>
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$^a$ The AUC values are those that actually developed from 0 to 96 h. The MIC for caspofungin for the C. albicans isolate was 0.2 mg/liter.

$^b$ Multiple ratios are achieved for fractionated schedules.
In vitro time-kill studies.

In the in vivo biological assay described above, the concentration of caspofungin in the serum of mice at the time of fungal inoculation was 0.05 mg/liter. In the in vitro time-kill studies, incubation of *C. albicans* in 80% mouse serum and 0.05 mg/liter of caspofungin resulted in fungal densities of 3.34 log CFU/ml at 0, 24, and 48 h, respectively. The fungal densities for *C. albicans* cultured in 80% mouse serum (without caspofungin) were 3.41 log CFU/ml at 0, 24, and 48 h, respectively. The time-kill curve for caspofungin at 0.10 mg/liter (1/2 the MIC) was similar to that for the controls (data not shown), while 0.2 mg/liter of caspofungin had a fungistatic effect at 24 and 48 h, with fungal densities in quantitative cultures of 3.01 and 3.42 log CFU/ml, respectively. Thus, for our in vivo bioassay, the concentration of caspofungin in the serum of mice at the time of fungal inoculation had no effect on the growth of *Candida*.

In vitro PAE.

The PAE induced by caspofungin was determined for 1/4, 1/2, 1 x, 5 x, and 10 x the MIC (2 mg/liter). The PAEs for 1/4, 1/2, and 1 x the MIC were 0, 4, and 7 h, respectively. Caspofungin at 5 x the MIC produced a PAE of 14 h. The PAE associated with 10 x the MIC was between 36 and 48 h.

**DISCUSSION**

In this study we aimed to characterize the pharmacokinetics of caspofungin in a murine model of systemic candidiasis and to conduct dose-range and dose-fractionation studies to determine the pharmacodynamic parameter linked with caspofungin efficacy. Caspofungin induced a greater than 2.4-log CFU/g...
decline in the density of *Candida albicans* in the kidneys of immune competent mice. This demonstrates potent activity that exceeded that seen with theazole fluconazole in a similar mouse infection model (21).

We found that the concentration-time profile of caspofungin in serum was best described by a four-compartment open model in which the terminal half-life of this drug was heavily influenced by the accumulation of caspofungin in tissues. In our mouse infection model, the terminal \(t_{1/2}\) was 20.2 h when only serum pharmacokinetic data were used in population PK analysis. However, the terminal \(t_{1/2}\) increased to 59.2 h when both the serum and kidney PK data were modeled. This tripling of the terminal half-life suggests that some of the parent drug returns to the serum from tissues. This finding also implies that the prolonged residence time of caspofungin in the effect compartment (kidney) may be critical to understanding the effect seen.

To test this hypothesis, we measured the concentration of caspofungin in the serum and kidneys of mice treated with 0.4 mg/kg of caspofungin and found that the concentrations in the serum at 24, 48, and 72 h after drug administration were 0.17, 0.05, and 0.03 mg/liter, respectively. It should be noted that the last two values are circa one-fourth to one-sixth the MIC of 0.2 mg/liter for the *C. albicans* strain studied. At these time points, the concentrations of caspofungin in the kidneys of these mice were 1.26, 0.58, and 0.13 mg/liter, respectively. Mice inoculated with *Candida* at the 48-h time point (>24 h after the serum concentrations fell below the MIC) had a significant reduction in fungal densities in kidneys 24 h later, the fungi grew in the untreated controls (\(P = 0.005\)). Importantly, similar fungal densities were documented in the quantitative cultures of kidney tissues obtained from caspofungin and saline recipients that were killed 30 min after they were infected with *Candida*. This shows that the reduction in the *Candida* burden in kidneys collected from caspofungin-treated mice 24 h after fungal inoculation could not be attributed to drug carryover. Our in vitro time-kill studies conducted with mouse serum demonstrated that the concentration of caspofungin measured in the serum of these mice at 48 h had no antifungal effect. This was consistent with the findings of Ernst and colleagues (12), who found that caspofungin had no effect against two strains of *C. albicans* at concentrations ≥0.5× the MIC in their time-kill studies, which were conducted in RPMI 1640 plus MOPS. Taken together, the results of our in vitro time-kill study and in vivo biological assay show that therapeutic concentrations of caspofungin persist at the site of infection in the kidney well after serum concentrations fall below the MIC, underscoring the primacy of caspofungin levels in tissues in determining treatment outcome.

The long terminal half-life calculated in our study is consistent with that in the work of Sandhu et al. (30), who reported that the serum PK in mice, rats, and monkeys consisted of a relatively rapid distribution \(t_{1/2} (t_{1/2u})\) of 4.4 to 5.5 h, followed by a slower elimination \(t_{1/2} (t_{1/2s})\) of 44.7 to 59.7 h. Slightly faster \(t_{1/2u}\) and \(t_{1/2b}\) values were reported for rabbits (13, 30), Hajdu et al. (15) and Wiederhold and colleagues (35) reported plasma half-lives in mice of 7.6 and 4.5 h, respectively. These values likely represent the distribution-phase value \( (t_{1/2u} )\), since these half-lives were calculated from data derived from 24-h pharmacokinetic studies. Of note, Hajdu and colleagues (15) also measured the concentration of caspofungin in tissues in their 24-h study and concluded from these data that the actual terminal \(t_{1/2}\) was >24 h since caspofungin was cleared from the liver, kidney, and large intestines more slowly than from plasma. Recently, Stone and colleagues (32) reported that the \(t_{1/2u}\) and \(t_{1/2b}\) of \(^{3}H\)caspofungin in human volunteers were 7.95 to 8.94 and 26.68 to 27.28 h, respectively. \(t_{1/2u}\) was best explained by the distribution of serum caspofungin into tissues and binding of drug to serum proteins, with peak tissue concentrations noted between 1.5 and 2 days after caspofungin administration. The long terminal phase was characterized by a slow decline in radioactivity in tissues and plasma due to the slow excretion of radioactivity into urine and feces (30, 32). Stone and colleagues (32) found that a portion of the radioactivity was irreversibly bound to plasma proteins. Since the percentage of irreversible binding in plasma was generally constant over the 8-week study, those investigators (32) concluded that this finding suggested that “the release of radioactivity from tissues is the rate-controlling step in the elimination of radioactivity.” They also state that “the slow decline of radioactivity in plasma appears to be due to a slow release of radioactivity from the tissues, rather than slow clearance of irreversibly bound radioactivity” (32).

In humans, little biotransformation or metabolism of caspofungin occurs in the first 24 to 30 h after the drug is administered, and the concentrations of parent drug and radioactivity are similar (15, 32). Thereafter, the concentration of caspofungin in plasma declines at a faster rate than the concentration of radioactivity does: the parent drug concentration falls below the limit of quantification of the HPLC assay (0.025 μg/ml) by 6.3 days, while the radioactivity concentration falls below the
limit of quantification for its assay (0.002 μg eq/ml) by 22.3 days (32). This suggests that in humans a large part of the radioactivity at the later times consisted of inactive metabolites or degradation products. Similarly, in pharmacokinetic studies in which mice were treated with a single dose of \(^{3}H\)caspofungin, Hajdu et al. (15) demonstrated that little metabolism or biotransformation of caspofungin occurred during their 24-h study. The parent drug distributed to tissues during the alpha (distribution) phase and continued to accumulate in the liver over 24 h, while the concentrations in the kidneys and other tissues remained essentially constant. In contrast, the concentration of caspofungin in plasma decreased with a half-life of 7.6 h. In a separate study, Sandhu et al. (30) reported that the concentration-time profile for the parent drug and radioactivity in mice treated with \(^{3}H\)caspofungin were similar throughout their 8-day experiment. After a steep decline in the level of the parent drug during the distribution phase, low levels of the parent drug were present in plasma for the remainder of the study. Based on the similar radioactivity profiles in the tissues of mice and other mammals, Sandhu et al. (30) concluded that “the slow decline in radioactivity in plasma can thus be attributed to slow release [of parent drug] from the tissues.” Although the caspofungin concentration in tissues does not appear to freely equilibrate with that in serum (30, 32), the tissues act as a reservoir from which biologically active drug slowly returns to the bloodstream, contributing to the long terminal half-life of caspofungin in plasma and serum.

The long mean residence time in the peripheral tissues helps explain the pharmacodynamic behavior of caspofungin. This is true even if only a small amount of drug is released from the organs over time, since the tripling of the serum half-life in our pharmacokinetic model when we comodeled the serum and kidney concentrations of caspofungin was dependent on the rate of decrease of caspofungin in this organ and not on the absolute concentrations of caspofungin measured at this site.

The compartmentalization of caspofungin into tissues, such as the kidneys, so that these tissues act as slow drug-release reservoirs heavily influences the pharmacodynamic evaluation of caspofungin. Without an appreciation of this pharmacokinetic characteristic, it is possible that the pharmacodynamic parameter linked with efficacy would be misidentified. In our murine model of systemic candidiasis, for example, presentation of the results of the dose-fractionation studies in mg/kg versus fungal density (log CFU/g) reveals a statistically non-significant bias toward a better outcome when the total dose was administered as a single dose at the start of therapy. Inherent with the reporting of the drug exposure in mg/kg is the assumption that the entire AUC that develops between the start of therapy and the time of killing is similar regardless of whether the total dose is given as one, two, or more divided doses over the duration of the experiment.

The bias seen in our study is readily explained by the fact that even though a full day had elapsed between the time that the fourth one-quarter dose was administered at hour 72 and the time of killing, this group had not yet developed the full AUC exposure. The \(\text{AUC}_{0-96}\) that developed over the duration of the experiment was the highest in animals that received the total dose as a single injection and was the lowest for mice that received the same total mg/kg of drug by using the most-fractionated schedule of administration (Table 4).

For example, for the 0.2-mg/kg group, mice that received this total dose as two equally divided doses at 0 and 48 h developed 91.6% of the AUC exposure that developed in animals that received the total dose as a single injection at the beginning of therapy. The most-fractionated regimen developed only 83.9% of the drug exposure by 96 h. When the actual \(\text{AUC}_{0-96}\) that developed at the time of killing was considered, the fit of the data to the inhibitory sigmoid \(E_{\text{max}}\) exposure-effect relationship improved and the bias toward a greater effect with administration of the total dose all at once had vanished (Fig. 4). Furthermore, in the detailed dose-fractionation study we extended our investigation from 96 to 134 h in order to more fully characterize the effect on kidney fungal loads of giving 0.4 mg/kg of caspofungin as different schedules of administration. These studies demonstrate that the fungal densities associated with the different dosing schedules converge at 118 and 134 h, the durations of time needed for the kidneys of mice given 0.4 mg/kg of caspofungin as two and four equally divided doses, respectively, to develop the same AUC as the kidneys in mice given this dose of caspofungin as a single dose at 0 h. The quantitative culture results conducted at these time points for the groups given 0.4 mg/kg of caspofungin as one, two, or four equally divided doses were 3.44 ± 0.44, 3.38 ± 0.23, and 3.40 ± 0.35 log CFU/g of kidney, respectively. These data show that the AUC/MIC ratio is the pharmacodynamic parameter that predicts the efficacy of caspofungin in our infection model.

Of note, Andes et al. (3) suggested that the \(C_{\text{max}}/\text{MIC}\) ratio was the pharmacodynamic parameter linked with efficacy for the echinocandin HMR 3270 in a neutropenic model of systemic candidiasis. However, the tissue concentrations of HMR 3270 were not measured. Therefore, it is unknown whether the kidney concentration-time profile for HMR 3270 was different from that for caspofungin. Similarly, Wiederhold et al. (35) also reported that for each of the total doses of caspofungin evaluated, the 96-h plasma AUCs generated were similar for the different schedules of administration. However, this conclusion was based on their calculated terminal half-life for caspofungin of 4.5 h, which most likely represents a distribution half-life. We and others reported significantly longer terminal half-lives for caspofungin in mice (30). Based on the longer terminal half-life identified in our study, we calculated that the 96-h AUCs do differ with different schedules of administration. Furthermore, Wiederhold et al. (35) did not account for the concentration of echinocandin at the infection site when calculating the caspofungin exposures that developed against the fungal pathogen. Hajdu et al. (15) showed that the peak levels of caspofungin in the lungs of mice were approximately one-third of those measured in the kidneys. However, over the 24-h study of these investigators, the concentrations of caspofungin in these tissues were relatively constant, while the concentrations of caspofungin in plasma declined with a \(t_{1/2}\) of 7.5 h. Thus, the pharmacokinetic
profiles of caspofungin in the lungs and the kidneys were different from the profile in plasma. Of note, since *Aspergillus* reacts to echinocandin therapy differently from *Candida*, it may be possible that different pharmacodynamic parameters are linked with efficacy for treatment of infections due to *Candida* and *Aspergillus*. Finally, Andes et al. (3) and Wiederhold et al. (35) used neutropenic animal models in their investigations, while we used an immune intact mouse infection model. In dose-fractionation studies, the impact of different immune states of the infected host on outcomes is unknown.

We can speculate on the reasons that the AUC/MIC ratio is the pharmacodynamically linked variable for caspofungin. It is likely that there are at least two reasons for this link. First, the long residence time of caspofungin in kidney tissue contributes to the AUC/MIC being the dynamically linked variable. In addition, caspofungin produces a profound and concentration-dependent PAE in vitro. The presence of both properties makes it difficult to impossible to sort out the cause of the in vivo PAE. It is likely that both phenomena play a role in the treatment outcome.

Currently, caspofungin is administered once daily as an intravenous formulation for the treatment of suspected or documented fungal infections or as prophylaxis against fungal infections. Our data show that the AUC/MIC ratio is the pharmacodynamic parameter linked with caspofungin efficacy (for the treatment of candidiasis) and that caspofungin has a long residence time in kidney tissue. This suggests that administration of the total cumulative dose once weekly or as two equally divided doses each week may be as efficacious as current dosing guidelines. A similar dosing regimen may be possible for drugs for which the Cₘₐₓ/MIC ratio is the pharmacodynamic parameter linked with efficacy, as was reported by Wiederhold et al. (35) for the treatment of aspergillosis with caspofungin and Andes et al. (3) for the treatment of candidiasis with HMR 3270. The less frequent schedule of administration would decrease the personnel needed for drug administration, hence, reducing health care costs; may decrease the need for long-term central venous access, since a temporary peripheral intravenous catheter may be sufficient for an infrequent dosing regimen; and if a long-term intravenous catheter is needed for other purposes, a less frequent dosing regimen for caspofungin may decrease the incidence of catheter-related infections. However, further studies on the efficacy of intermittent dosing regimens for the treatment of infections due to a variety of fungal pathogens localized to different sites need to be conducted to verify our findings. This is particularly critical, given the differences in the rates of metabolism of caspofungin between mice and humans. The tolerability and safety of such regimens will also need to be characterized.

**ACKNOWLEDGMENT**

This study was supported by an educational grant by Merck & Company.

**REFERENCES**


