Intermittent Dosing of Micafungin Is Effective for Treatment of Experimental Disseminated Candidiasis in Persistently Neutropenic Rabbits

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Background. The current standard of treatment of invasive candidiasis with echinocandins requires once-daily therapy. To improve quality of life, reduce costs, and improve outcome, we studied the pharmacokinetics (PK), efficacy, and safety of alternate dosing regimens of micafungin (MFG) for the treatment of experimental subacute disseminated candidiasis.

Methods. MFG was administered for 12 days starting 24 hours after intravenous inoculation of 1×10^3 *Candida albicans* blastoconidia. Study groups consisted of MFG at 1 mg/kg every 24 hours (MFG1), 2 mg/kg every 48 hours (MFG2), and 3 mg/kg every 72 hours (MFG3), and untreated controls. PK of MFG were determined on day 7 by high-performance liquid chromatography and modeled using nonparametric adaptive grid program. A 2-compartment PK model with volume of the central compartment (V_c), clearance (SCL), and the intercompartmental rate constants K_{cp} and K_{pc} was used. The fungal burden in 7 tissues was determined 312 hours after the initiation of therapy.

Results. PK of MFG were linear and the parameter means ± SD were $V_c = 0.41 \pm 0.18$ L, $K_{cp} = 2.80 \pm 1.55$/hour, $K_{pc} = 1.71 \pm 0.93$/hour, and SCL = 0.16 ± 0.003 L/hour ($r^2 = 0.99$). The area under the plasma drug concentration-time curve for MFG1, MFG2, and MFG3 was 198.7 ± 19.8, 166.3 ± 36.7, and 192.8 ± 46.2 mg × hour/L, respectively ($P = .24$). All treatment groups showed significant and comparable resolution of (1→3)-β-D-glucan levels and clearance of *C. albicans* from liver, spleen, kidney, brain, lung, vitreous humor, and vena cava in comparison to untreated controls ($P \le .05$). There were no differences in hepatic or renal function among study groups.

Conclusions. Less fractionated MFG regimens of every 48 and 72 hours are safe and as effective in experimental disseminated candidiasis as once-daily therapy in neutropenic hosts.

Keywords. micafungin; *Candida albicans*; disseminated candidiasis; neutropenia; serum (1→3)-β-D-glucan.

Disseminated candidiasis is an important cause of healthcare-associated fungal infections in immunocompromised patients [1–4]. Echinocandins (anidulafungin, caspofungin, and micafungin) inhibit the enzyme (1→3)-β-D-glucan synthase. Echinocandins are effective for the treatment of candidemia and disseminated candidiasis, and are usually administered once daily.

However, intermittent schedules may facilitate more flexible outpatient care, improve quality of life, and reduce hospital costs. We therefore studied the pharmacokinetics, efficacy, and safety of intermittent dosing schedules of micafungin for the treatment of experimental disseminated candidiasis in persistently neutropenic rabbits.

MATERIALS AND METHODS

Animals

Female New Zealand White rabbits (weighing 2.2–3.3 kg; n = 30; Covance Research Products, Inc, Denver, Pennsylvania) were used in this study. Animals were

monitored under humane care and use of standards in facilities, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and according to the guidelines of the National Research Council for the care and use of laboratory animals and under approval of an institutional Animal Care and Use Committee [5]. Rabbits were individually housed and maintained with water and standard rabbit feed ad libitum. Atraumatic vascular access was established in each rabbit by the surgical placement of a Silastic tunneled central venous catheter as previously described [6]. The Silastic catheter permitted nontraumatic venous access for repeated blood sampling for studies of biochemical and hematological parameters, plasma pharmacokinetics, and administration of parenteral agents. Blood samples were drawn from rabbits at the initiation of immunosuppression, during the course of disseminated candidiasis, and before euthanasia. Rabbits were euthanized according to Animal Care and Use Committee–approved prespecified humane endpoints by intravenous administration of pentobarbital (65 mg/kg of body weight of pentobarbital sodium in the form of 0.5 mL beuthanasia-D special [euthanasia solution; Schering-Plough Animal Health Corp, Union, New Jersey]) at the end of the study, 312 hours after study drug initiation.

Immunosuppression and Maintenance of Neutropenia

Cytarabine (AraC; Cytosar-U, Pharmacia, Kalamazoo, Michigan) was administered intravenously for induction and maintenance of neutropenia. Profound neutropenia (a neutrophil concentration of <100 neutrophils/μL) was achieved with an initial intravenous course of 440 mg/m^2 of AraC daily for 5 days before inoculation of the rabbits. A maintenance dose of 440 mg/m^2 of AraC was administered at 2-day intervals during the study.

Ceftazidime (75 mg/kg intravenous twice daily; Glaxo Pharmaceuticals, Division of Glaxo Inc, Research Triangle Park, North Carolina), gentamicin (5 mg/kg intravenous every other day; Elkins-Sinn, Inc, Cherry Hill, New Jersey), and vancomycin (15 mg/kg intravenous daily; Abbott Laboratories, North Chicago, Illinois) were administered from day 4 of immunosuppression for the prevention of opportunistic bacterial infections during neutropenia. To prevent antibiotic-associated diarrhea due to *Clostridium spiroforme*, all rabbits received 50 mg of vancomycin per liter of drinking water. Total leukocyte counts and the percentages of neutrophils were monitored twice weekly with a Coulter Counter (Coulter Corporation, Miami, Florida) and by use of peripheral blood smears and differential counts, respectively.

Organism and Inoculation

A well-characterized clinical isolate of *Candida albicans* NIH-8621 (American Type Culture Collection strain MYA-1237), obtained from a neutropenic patient with autopsy-proven disseminated candidiasis, was used for the study. The minimum inhibitory concentration (MIC) for *C. albicans* was performed according to Clinical and Laboratory Standards Institute (CLSI) criteria [7]. The MIC of micafungin (Astellas Pharma US, Inc, Deerfield, Illinois) was 0.125 μg/mL.

For preparation of the inoculum, *C. albicans* isolate was subcultured from a frozen stock culture stored at −80°C on potato dextrose agar slants (K-D Medical, Inc, Columbia, Maryland) on Sabouraud dextrose agar (SGA) plates (K-D Medical, Inc), incubated at 37°C for 24 hours. Three- to 5-well–isolated colonies were sampled from freshly grown culture plates and suspended into 50 mL of Emmon's modified Sabouraud glucose broth (K-D Medical, Inc) (pH 7.0) in a 250-mL Erlenmeyer flask. The suspension was incubated in a gyratory water bath at 80 oscillations/minute at 37°C for 18 hours. The *Candida* suspension was then centrifuged at 1600g for 10 minutes, and washed 3 times with sterile 0.9% normal saline (K-D Medical, Inc). The concentration was adjusted by use of a hemacytometer and was confirmed by quantitative cultures of a 10-fold serial dilution. The final inoculum of 1×10^3 blastoconidia (suspended in a 5-mL volume of 0.9% sterile normal saline) was slowly administered to each rabbit via the indwelling Silastic central venous catheter on day 6 of immunosuppression to establish the model of subacute disseminated candidiasis [8]. The inoculum size was confirmed by plating serial dilutions onto SGA plates.

Antifungal Compound, Treatment Groups, and Pharmacokinetic Design

Micafungin powder was reconstituted and diluted further in sterile 0.9% normal saline to achieve the desired concentration of 1 mg/mL. Treatment groups consisted of untreated control animals (n = 6) and animals treated with micafungin (n = 24) at dosages of 1 mg/kg every 24 hours (n = 8), 2 mg/kg every 48 hours (n = 8), and 3 mg/kg every 72 hours (n = 8) administered intravenously. Therapy was initiated 24 hours postinoculation, and continued for 12 days.

Assessment of In Vivo Antifungal Efficacy

Antifungal activity in the model of disseminated candidiasis was determined by quantitative clearance of *C. albicans* from tissues. Representative sections of liver, spleen, kidney, lung, cerebrum, and anterior vena cava were weighed, and each tissue sample was then homogenized (Stomacher 80; Tekmar Corp, Cincinnati, Ohio) in sterile reinforced polyethylene bags (Tekmar Corp) with sterile 0.9% saline for 30 seconds.

Antifungal activity in treatment of *Candida* infection of the eyes was also assessed postmortem. The globes of the eyes were carefully dissected using aseptic technique. The removed globe was transferred to a sterile Petri dish (Falcon, Becton Dickinson

Labware, Becton Dickinson and Co, Franklin Lakes, New Jersey). The sclera was incised with sharp scissors at the posterior pole, and 0.3–0.4 mL of vitreous humor was slowly aspirated into a sterile tuberculin syringe (Tyco Healthcare Group LP, Mansfield, Massachusetts). The specimens of vitreous humor from both globes were pooled into 2-mL Sarstedt microtubes (Sarstedt Ag & Co, Numbrecht, Germany), and processed together.

Each tissue homogenate or vitreous humor specimen was serially diluted 10^{-1} to 10^{-4} in sterile 0.9% normal saline. The aliquots of 100 μL of undiluted tissue homogenate or vitreous humor and of each dilution were separately plated onto Emmon's modified SGA containing chloramphenicol and gentamicin. Culture plates were incubated at 37°C for 24 hours, after which colony-forming units (CFU) were counted and the number of CFU/g of tissue was calculated for each organ. Potential carryover of the drug was minimized by serial dilution and by streaking a small-volume (100 μL) aliquot onto a large volume of agar (1 full agar plate/100-μL aliquot). The limit of detection was ≥ 10 CFU/g or ≥ 10 CFU/mL, respectively. The culture-negative plates were counted as 0 CFU/g or 0 CFU/mL. Data were graphed as the mean of $\log_{10}$ (CFU/g or CFU/mL) ± standard error of the mean (SEM).

(1→3)-β-D-Glucan Levels

Antifungal efficacy also was assessed by (1→3)-β-D-glucan levels in sequential serum samples. Blood from each rabbit infected with *C. albicans* was collected every day, and (1→3)-β-D-glucan levels were determined according to the manufacturer's specifications. In brief, aliquots of 5 μL of serum were added to duplicate wells of a 96-well microtiter plate and pretreated for 10 minutes at 37°C with an alkaline reagent (20 μL; 0.125 M potassium hydroxide/0.6 M potassium chloride). Then, an aliquot of 25 μL of the standards (100 to 6.25 pg/mL pure pachyman, a linear β-glucan) was added to each well. An aliquot of 100 μL of Fungitell reagent (lyophilized (1→3)-β-D-glucan–specific *Limulus* amebocyte lysates) was reconstituted with 2.8 mL of glucan-free reagent grade water (RGW), followed by 2.8 mL of pyrosol reconstitution buffer (2 M Tris hydrochloric acid, pH 7.4), and 100 μL of this mixture was added to each sample. The plate was monitored at 405 nm (with 490 nm background subtraction) for 40 minutes at 37°C in a Bio-Tek ELx808 automated microplate reader (Bio-Tek Instruments, Inc, Winooski, Vermont) equipped with KC4 software (Bio-Tek Instruments, Inc). The mean rate of optical density change was determined for each well, and (1→3)-β-D-glucan concentration was determined by comparison to a standard curve. When absorbance was outside the range of the standard curve, the serum samples were serially diluted in RGW and tested again. The serum value of ≥ 80 pg/mL was considered positive. The correlation coefficient of the standard curve was $r \geq 0.992$ (range, 0.980–0.998).

Pharmacokinetic Studies

The plasma pharmacokinetics of micafungin were investigated in 6 infected rabbits in each dosage group using minimal plasma sampling and determined on postinoculation day 7 (144 hours after drug initiation), when the drug was administered to all groups. Micafungin was administered as a steady intravenous bolus over 4 minutes. Blood samples were collected into heparinized syringes before administration of drug at 0 (baseline) and then at the maximum concentration of drug (C_{max}), 0.07, 0.25, 0.5, 2, 4, 6, 8, and 24 hours after the drug administration. Plasma was immediately separated by centrifugation at 400g, and samples were stored in 2-mL Sarstedt microtubes at −80°C prior to analysis.

Micafungin was extracted from plasma using solid phase extraction with C_8 Bond Elut cartridges (100 mg, 1 mL) (Varian, Palo Alto, California) and Vac-Elut vacuum manifold (Analytichem International, Harbor City, California). Cartridges were conditioned by completely draining 500 μL of high-performance liquid chromatography (HPLC)–grade acetonitrile and then half-draining 1 mL of acetonitrile-ammonium acetate (phosphate buffer, pH 4.0) (10:90 vol/vol). An aliquot of 300 μL of plasma was applied to the half-filled cartridge, together with 25 μL of the internal standard (50 mg/L anidulafungin; Vicuron Pharmaceuticals Inc, King of Prussia, Pennsylvania). Acetonitrile-ammonium acetate (10:90 vol/vol) (pH 4.0) was then added to completely fill the cartridge (300 μL), and the sample was drained slowly under vacuum.

After drainage, C_8 cartridges were washed with 1 mL of acetonitrile-ammonium acetate (10:90 vol/vol), and dried under the vacuum. The cartridges remained at high vacuum for 30 seconds to dry. Subsequently, 1 mL of 100% chloroform (Mallinckrodt, Phillipsburg, New Jersey) was added, and the cartridges were drained and thoroughly dried under vacuum for 1 minute. Micafungin was eluted with 1 mL of acetonitrile-ammonium acetate (70:30 vol/vol) (50 mM, pH 4.0), briefly followed by full vacuum pressure to get the draining started, then vacuum pressure was reduced to allow material to drain slowly into 12- × 75-mm disposable culture test tubes. After all the eluent in the cartridge was pulled through into culture test tubes, vacuum was applied briefly (approximately 5 seconds) to deplete any remaining eluent remaining in the guide needles attached to the vacuum. The culture tubes containing eluent were then evaporated under nitrogen using a Zymark Turbo Vap LV evaporator (American Laboratory Trading LLC, Niantic, Connecticut), at 40°C for 90 minutes or until dry.

Dried eluent was reconstituted in 150 μL of methanol-ammonium acetate (50:50 vol/vol; 50 mM, pH 4.0; methanol was from J.T. Baker, Phillipsburg, New Jersey) in culture test tube, vortexing the tube for 1 minute. The reconstituted eluent was transferred to Eppendorf tubes and centrifuged for 5 minutes at 2600g before being transferred into a microvial insert for HPLC injection and placed in the autosampler at 9°C.
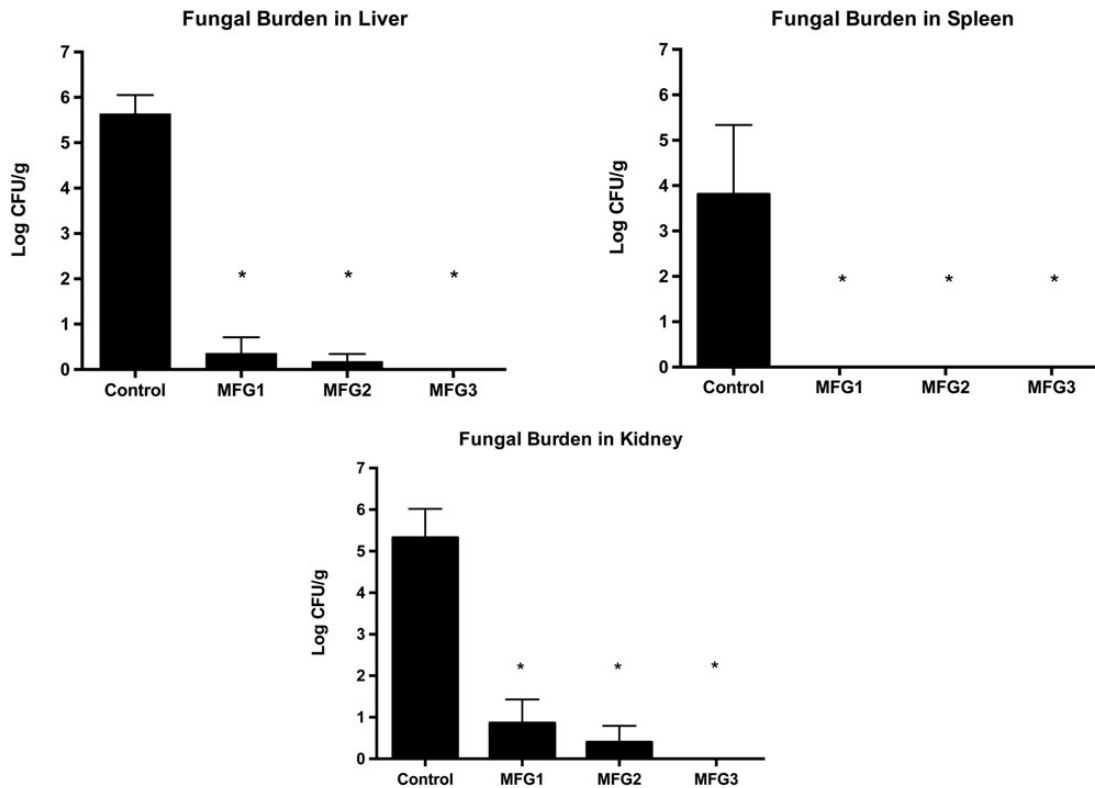
Fungal Burden in Liver

Fungal Burden in Spleen

Fungal Burden in Kidney

Figure 1. All treatment groups showed significant and comparable clearance of *Candida albicans* across the micafungin dosage groups of 1 mg/kg every 24 hours (MFG1), 2 mg/kg every 48 hours (MFG2), and 3 mg/kg every 72 hours (MFG3) from liver, spleen, and kidney and in comparison to untreated control rabbits (*$P < .01$). Abbreviation: CFU, colony-forming units.

Concentrations of micafungin were determined using a reversed-phase HPLC (Waters 2695 separation module). Acetonitrile-ammonium acetate (50:50, vol/vol; 50 mM, pH 4.0) was used as the mobile phase, with an isocratic flow rate of 0.5 mL/minute. A C_8 analytical column (150 × 4.6 mm, 5 μm) (Alltech Inertsil; Alltech Associates, Deerfield, Illinois) maintained at 50°C was preceded by a C_8 column guard (7.5 × 4.6 mm, 5 μm) (Alltech Inertsil). The injection volume was 75 μL. Micafungin and the internal standard, anidulafungin, were detected using ultraviolet light (wavelength of 271 nm) and eluted between 7.6 and 8.1 and between 12.7 and 13.1 minutes, respectively. Quantitation was based on the ratio of peak area of micafungin relative to that of the internal standard.

Standard curves that encompassed the expected experimental range of micafungin concentrations were constructed in their respective matrix. The lower limit of quantification was ≤0.075 mg/L. The coefficient of determination was $r^2 \geq 0.996$. The intra- and interday coefficients of variation were both <14%.

The pharmacokinetic data from individual rabbits were modeled using a population methodology by means of the NPAG (nonparametric adaptive grid) program with adaptive γ [9].

An open 2-compartment model with zero-order time-delimited input and first-order elimination from the central compartment was used. The data were weighted by the inverse of the estimated variance of the drug assay. Models were discriminated on the basis of the log-likelihood value, mean weighted error, bias-adjusted weighted mean squared error, and a visual inspection of the regression of observed-vs-predicted values obtained after the Bayesian step. The entire data set (ie, 1, 2, 3 mg/kg groups) was co-modeled. The Bayesian estimates of the PK parameters for each individual rabbit were determined by using the "population of one" utility within NPAG. For each dosage group, these Bayesian estimates were collected and the average obtained. The total area under the plasma drug concentration-time curve (AUC_{0-312h}) at steady state was calculated for each rabbit by integration, and compared for rabbits within the 1, 2, 3 mg/kg dosage groups using analysis of variance (ANOVA).

Safety

Chemical determinations of creatinine, urea nitrogen, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and potassium in serum were tested in the penultimate sample drawn from each rabbit.
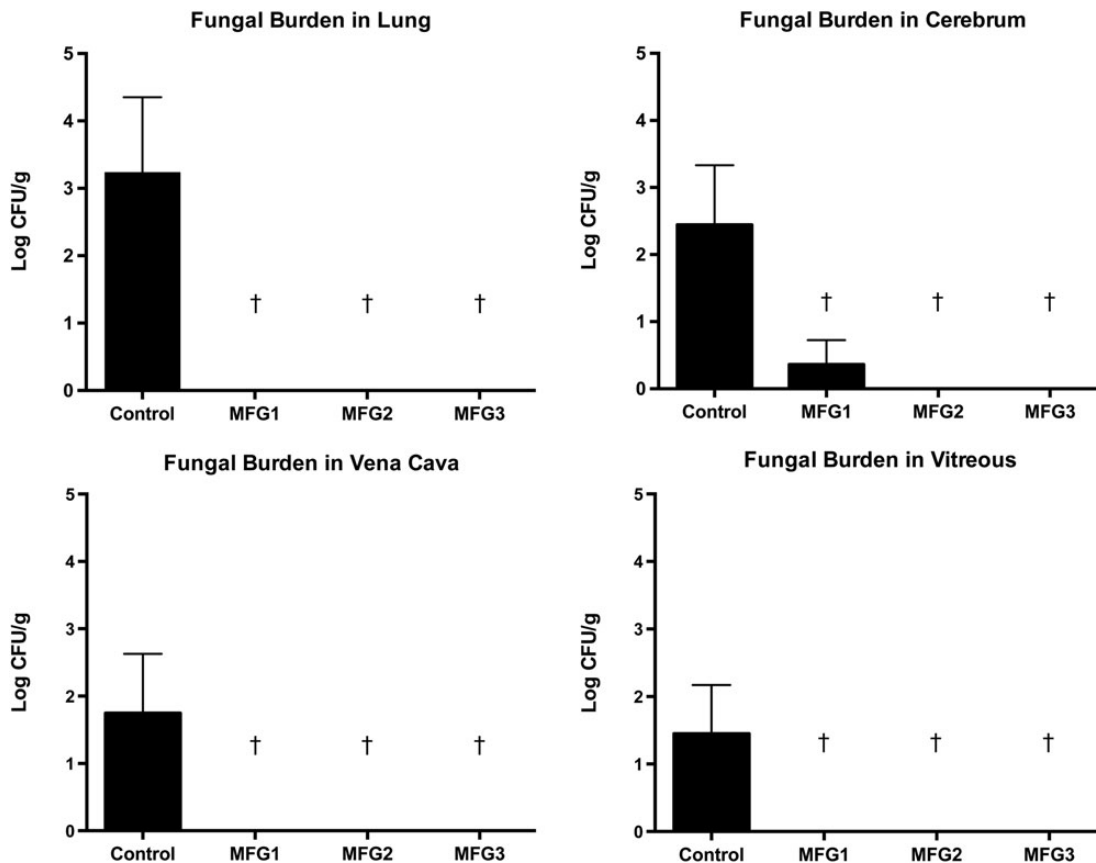
Figure 2. All treatment groups showed significant and comparable clearance of *Candida albicans* across the micafungin dosage groups of 1 mg/kg every 24 hours (MFG1), 2 mg/kg every 48 hours (MFG2), and 3 mg/kg every 72 hours (MFG3) from lung tissue, cerebrum, vena cava, and vitreous humor and in comparison to untreated control rabbits ([†]*P* < .05). Abbreviation: CFU, colony-forming units.

Statistical Analysis

Comparisons between groups were performed using the Kruskal–Wallis test (nonparametric ANOVA) or Mann–Whitney *U* test, as appropriate. A 2-tailed *P* value ≤.05 was considered to be statistically significant. Values are expressed as mean and SEMs. Statistical comparisons of pharmacokinetic parameters across dosage cohorts were made using ANOVA.

RESULTS

Antifungal Therapy

All treatment groups showed significant and comparable clearance of *C. albicans* from liver, spleen, and kidney in comparison to untreated controls (*P* < .01; Figure 1). There were no significant differences between treatment groups. There also was a significant decrease and comparable clearance of *C. albicans* across the dosage groups of mean fungal burden in lung tissue, cerebrum, vena cava, and vitreous humor in micafungin-treated rabbits compared with untreated controls

(*P* ≤ .05; Figure 2). There were no significant differences between treatment groups receiving micafungin.

(1→3)-β-D-Glucan Levels

Levels of (1→3)-β-D-glucan in serum collected from micafungin-treated and untreated control rabbits are displayed in Figure 3. A significant increase of the mean concentration of (1→3)-β-D-glucan was observed in serum of untreated control rabbits, whereas serum (1→3)-β-D-glucan levels in micafungin-treated rabbits across all dosage groups remained <80 pg/mL through the entire study (*P* < .001). The (1→3)-β-D-glucan levels in serum obtained from all rabbits before inoculation with *C. albicans* ranged from 12 pg/mL to 18 pg/mL. After inoculation of *C. albicans*, the serum (1→3)-β-D-glucan levels in untreated rabbits reached their highest levels of 1593 ± 570 pg/mL (range, 409 to >969 pg/mL) on day 14 postinoculation. The serum (1→3)-β-D-glucan levels in micafungin-treated rabbits ranged from 12 pg/mL to 46 pg/mL through the entire study, and from 17 pg/mL to 20 pg/mL on postinoculation day 14.
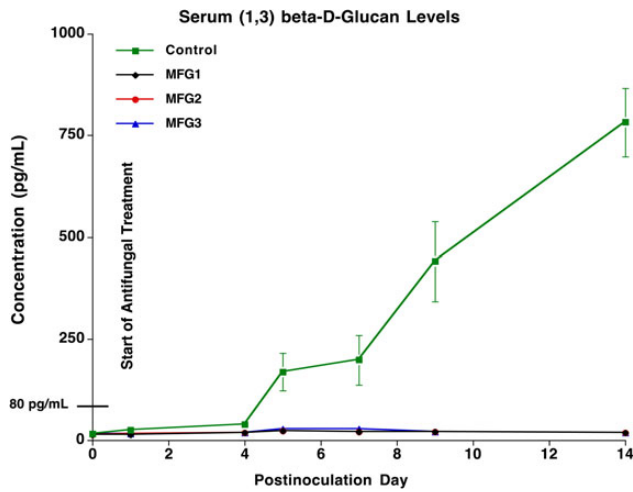
Figure 3. Serial levels of (1→3)-β-D-glucan in serum collected from micafungin-treated and untreated control (UC) rabbits. A significant increase of the mean concentrations of (1→3)-β-D-glucan was observed in serum of UC rabbits, whereas the concentrations of (1→3)-β-D-glucan in serum of micafungin-treated rabbits across all dosage groups remained <80 pg/mL through the entire study (*P* < .001). Abbreviations: MFG1, micafungin 1 mg/kg every 24 hours; MFG2, micafungin 2 mg/kg every 48 hours; MFG3, micafungin 3 mg/kg every 72 hours.

Pharmacokinetics of Micafungin

Micafungin exhibited linear plasma pharmacokinetics over the dose range used in the study. The fit of the pharmacokinetic model to the data was excellent, with a coefficient of determination of 0.95 after the Bayesian step. The estimates for the means and standard deviations of the pharmacokinetic model parameters are summarized in Table 1, which include volume of central compartment, first-order rate constants connecting the central and peripheral compartments, and clearance from central compartment. The fit of the PK model to the data was highly acceptable, with an r^2 of 0.99 (*P* < .01).

The mean AUC_{0-312h} values over the 13 days of dosing were similar for the 3 dosage groups (Figures 4 and 5). The mean AUC_{0-312h} in the 1 mg/kg dosage group after 12 administered
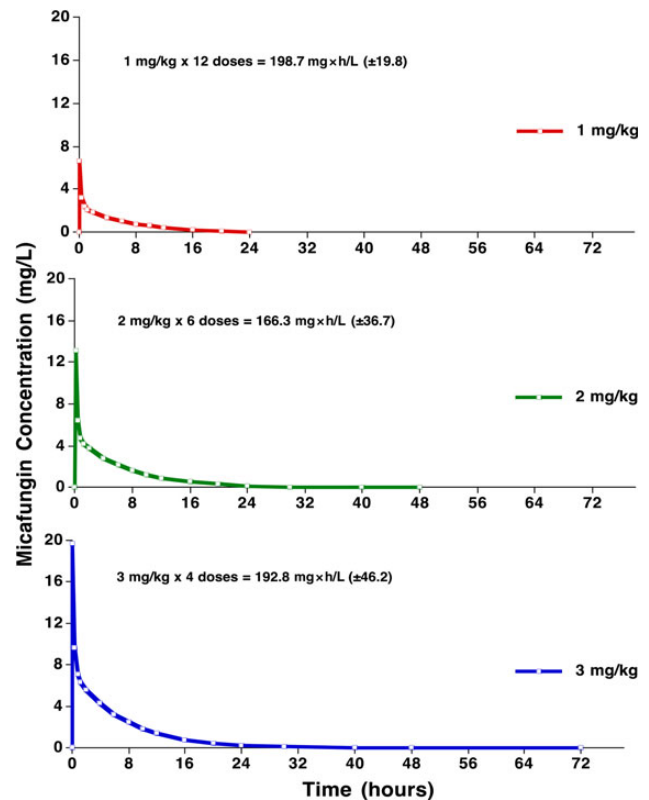

Figure 4. Plasma concentration-time curves of the 3 dosages of 1 mg/kg, 2 mg/kg, and 3 mg/kg micafungin administered intravenously on day 7.

dosages was 198.7 mg × hour/L (±19.8). Mean AUC_{0-312h} in the 2 mg/kg dosage group after 6 administered dosages was 166.3 mg × hour/L (±36.7). Mean AUC_{0-312h} in the 3 mg/kg dosage group after 4 administered dosages is 192.8 mg × hour/L (±46.2) (*P* = .235; ANOVA).

Safety

Micafungin-treated rabbits had no significant increases in levels of serum creatinine, serum urea nitrogen, or serum hepatic aminotransferases, and no decreases in serum potassium (Table 2). There were no differences in renal or hepatic safety parameters

Table 1. Estimates of the Means and Standard Deviations for Each Parameter From the Population Pharmacokinetic Models of Micafungin in Rabbits

Treatment Regimen of Micafungin	V_c, L	K_{cp}, h^{-1}	K_{pc}, h^{-1}	SCL, L/h	AUC_{0-312h}, mg × h/L
1 mg/kg	0.435 (0.065)	0.552 (0.124)	0.396 (0.036)	0.118 (0.024)	198.7 (19.8)
2 mg/kg	0.450 (0.098)	0.664 (0.230)	0.350 (0.210)	0.115 (0.018)	166.3 (36.7)
3 mg/kg	0.533 (0.126)	0.584 (0.182)	0.514 (0.144)	0.134 (0.024)	192.8 (46.2)

Data are presented as mean (standard deviation).

Abbreviations: AUC_{0-312h}, the area under the plasma drug concentration - time curve; K_{cp} and K_{pc}, intercompartmental rate constants; SCL, clearance from central compartment; V_c, volume of central compartment.
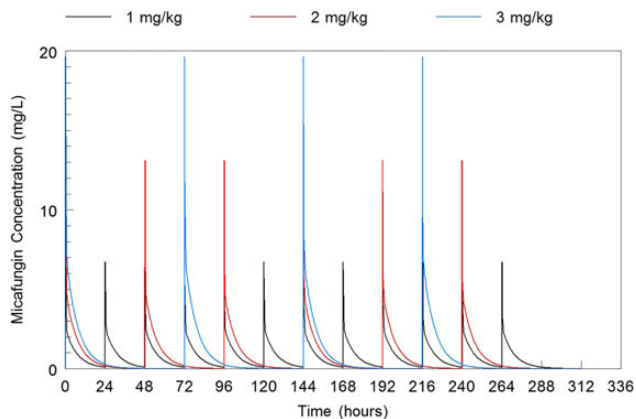
Figure 5. The mean AUC $_{0-312h}$ values over the 13 days of dosing were similar for the 3 micafungin dosage groups of 1 mg/kg every 24 hours, 2 mg/kg every 48 hours, and 3 mg/kg every 72 hours administered intravenously. Abbreviation: AUC$_{0-312h}$, the area under the plasma drug concentration - time curve.

in micafungin-treated rabbits compared with untreated controls and across dosage groups.

DISCUSSION

These data demonstrate that less fractionated micafungin regimens of as long as once every 3 days are as effective and as safe as a once-daily regimen against experimental disseminated candidiasis in persistently neutropenic hosts. These findings provide a pharmacokinetic and pharmacodynamic foundation for clinical studies to investigate intermittent dosing strategies.

While standard treatment of invasive candidiasis with micafungin requires once-daily therapy, longer dosing intervals of several days may allow for ambulatory management, earlier discharge, reduced costs, and improved quality of life. Reduced access to intravenous lines also may reduce healthcare-associated catheter-related bloodstream infections.

The comparable outcomes on tissue clearance at multiple sites of 1 mg/kg every 24 hours, 2 mg/kg every 48 hours, and 3 mg/kg every 72 hours is compatible with the known concentration-dependent pharmacodynamics of echinocandins where either the peak:MIC or AUC:MIC ratio is the relevant dynamically linked variable [10, 11].

The role of micafungin in treatment of central nervous system (CNS) candidiasis merits further discussion. As a large N-acyl–substituted cyclic hexapeptide, micafungin does not penetrate significantly through an intact blood–brain barrier. However, our studies of micafungin in treatment of CNS candidiasis demonstrated efficacy and penetration through a disrupted blood brain barrier. As reported by Hope et al in 2008 in the rabbit model of experimental hematogenous *Candida* meningoencephalitis (HCME), micafungin penetrated most CNS compartments, associated with an AUC of dosage with the highest concentrations of micafungin found in the meninges and choroid [12]. The antifungal efficacy of micafungin followed a sigmoidal C$_{max}$ response relationship with an approximate 99% reduction of *C. albicans* in cerebral tissue corresponding to a dosage of 8 mg/kg and an AUC:MIC ratio of approximately 10 000. These data predicted the dosage of 10 mg/kg/day of micafungin for treatment of infants with candidemia potentially complicated by HCME [13, 14].

These studies also provide insight into the pharmacodynamics of micafungin in the treatment of *Candida* endophthalmitis. A recent case report noted inadequate vitreal concentrations of systemically administered caspofungin in treatment of *Candida* endophthalmitis [15]. Our data from series of studies of *Candida* endophthalmitis demonstrate that *C. albicans* can be eradicated from the vitreous by micafungin at 2 mg/kg in persistently neutropenic rabbits. These findings were confirmed in the current experiments with a finding of comparable activity at 1 mg/kg, also in persistently neutropenic rabbits. By comparison, the nonneutropenic rabbit model of HCME showed incomplete reduction of *C. albicans* in the vitreous at dosages ≥16 mg/kg/day [16], suggesting that the inflammatory vitreal opacities may impair diffusion of the echinocandin. These data clearly demonstrate a

Table 2. Effects of Micafungin on Renal and Hepatic Safety Parameters in Persistently Neutropenic Rabbits With Subacute Disseminated Candidiasis

Treatment Group	Serum Creatinine, mg/dL	Serum Urea Nitrogen, mg/dL	Serum ALT, U/L	Serum AST, U/L	Serum Potassium, mmol/L
Controls (n = 6)	1.02 ± 0.12	18.50 ± 1.43	24.17 ± 6.04	35.83 ± 13.11	4.00 ± 0.17
MFG1 (n = 6)	0.92 ± 0.10	22.83 ± 5.13	21.33 ± 4.95	23.33 ± 7.98	4.05 ± 0.18
MFG2 (n = 6)	0.85 ± 0.03	19.50 ± 1.56	13.17 ± 1.08	15.17 ± 0.98	3.72 ± 0.65
MFG3 (n = 6)	0.96 ± 0.06	19.83 ± 1.88	15.00 ± 1.79	14.67 ± 2.86	4.56 ± 0.11

All values are expressed as mean ± standard error of the mean.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; MFG1, micafungin 1 mg/kg every 24 hours; MFG2, micafungin 2 mg/kg every 48 hours; MFG3, micafungin 3 mg/kg every 72 hours.

dose-response relationship of micafungin in treatment of *Candida* endophthalmitis. Similar findings of dose dependency and the need for higher-than-standard dosages were observed by Livermore et al for anidulafungin [17].

The linear kinetics of micafungin in our study permitted a predictable AUC that was dose-proportional, such that the sum of the AUCs of the less fractionated dosing regimen are similar to that of the daily regimen. This property of dose proportionality may predict safety as well as toxicity. The data in this study indicate that higher single dosages were not associated with hepatic or renal toxicity. Assuming that safety may be toxicokinetically linked to total exposure, then toxicity should be no greater in the 3 dosage regimens than that of the daily dosage of 1 mg/kg.

All echinocandins have a potential for infusion-related toxicity based on histamine release [18]; however, such events are uncommon. Despite the extensive global use of echinocandins, the number of well-documented cases of histamine-associated infusion-related toxicity is small and may be related more to relatively rapid infusion than to dosage. Within multiple, carefully monitored phase 1–2 clinical trials of the safety, tolerability, and pharmacokinetics of micafungin, anidulafungin, and caspofungin [13, 14, 19–21], only 1 episode of histamine-based infusion-related reaction was observed [14]. That patient, who was enrolled in the low-dosage cohort, was a 16-year-old male, who experienced moderate facial erythema and rash at the start of anidulafungin infusion that were not accompanied by additional adverse events of wheezing or hypotension. The symptoms observed with this patient were reminiscent of "red man syndrome," due to vancomycin-mediated systemic histamine release. The erythema and rash resolved within 1.5 hours after interruption of the study drug and administration of diphenhydramine. Subsequent resumption and 10 other infusions of anidulafungin occurred without recurrence of these adverse events in this patient. A review of literature reveals a paucity of well-characterized cases of infusion-related toxicity. One patient was reported to have developed flash pulmonary edema with a rapidly infused standard dose of anidulafungin [22].

The safety of high doses of 300 mg vs 150 mg was evaluated by Yamazaki et al in a retrospective study of adult patients with hematological malignancies [23]. These investigators found no significant differences in overall adverse events or in hepatobiliary adverse events. The safety of repeated doses of 300 mg was also demonstrated in an adult patient with CNS aspergillosis [24]. These findings lay the foundation for further exploration of once-weekly dosages for the treatment of invasive candidiasis.

Future pharmacokinetic and pharmacodynamic laboratory animal studies in the persistently neutropenic rabbit model of disseminated candidiasis would study the efficacy, safety, and tolerability of once-weekly micafungin in dosages of 7 mg/kg/day for 2 weeks for treatment and prevention against several medically important *Candida* species. The rabbit model's pharmacokinetics, intravenous route of administration, profound persistent neutropenia, presence of vascular catheter, duration of therapy, and distribution of infected tissues sites strongly correlates with those conditions in profoundly neutropenic patients. These data demonstrate that less fractionated micafungin regimens are safe and as effective as daily dosage of 1 mg/kg, and provide an experimental foundation for clinical trials to investigate alternate dosing strategies.

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