Efficacy of Caspofungin in a Juvenile Mouse Model of Central Nervous System Candidiasis

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Neonatal candidiasis is an increasingly common occurrence causing significant morbidity and mortality and a higher risk of dissemination to the central nervous system (CNS) than that seen with older patients. The current understanding of optimal antifungal therapy in this setting is limited. We have developed a model of disseminated candidiasis with CNS involvement in juvenile mice to assess the efficacy of the echinocandin caspofungin relative to amphotericin B (AmB). Juvenile mice were inoculated intravenously with 5.64 × 10^4 CFU of Candida albicans MY1055. Treatment with caspofungin at 1, 2, 4, and 8 mg/kg of body weight/day, AmB at 1 mg/kg/day, or a vehicle control (VC) was initiated 30 h after infection and continued for 7 days. Pharmacokinetic parameters for caspofungin were also determined. Culture and histology showed evidence of disseminated candidiasis with multifocal encephalitis at the start of antifungal therapy. Survival was 100% in all treated groups, while mortality was 100% in the VC by day 11 after infection. By day 5, all mice in the caspofungin treatment (four doses) groups showed reductions in kidney and brain burden relative to the VC, while AmB treatment reduced kidney burden but gave no reduction of brain fungal burden. Systemic levels of caspofungin were similar in infected and uninfected mice, while brain levels were higher in infected animals. In this juvenile mouse model, caspofungin demonstrated dose-dependent activity, equivalent to or better than that of AmB at 1 mg/kg, against disseminated candidiasis with CNS involvement.

**MATERIALS AND METHODS**

**Drugs.** Caspofungin acetate (Merck Research Laboratories, Rahway, NJ) was solubilized and serially diluted in sterile distilled water. Amphotericin B deoxycholate was purchased as amphotericin B for injection, USP (X-GEN Pharmaceuticals, Inc., Northport, NY), reconstituted according to the manufacturer’s instructions, and further diluted in sterile distilled water.

**Animals.** Complement component 5-deficient DBA/2N mice (Taconic Farms, Germantown, NY) 8 to 10 weeks old and weighing approximately 18 g (16 to 20 g) at the start of antifungal therapy were used in these studies. The average weight was 18.0 g for infected mice and 18.5 g for uninfected mice used for pharmacokinetic analysis. Mice were housed in sterile microisolator cages (5 to 10 per cage) with sterilized bedding, feed, and acidified water. All procedures were performed in accordance with the highest standards for the humane handling, care, and treatment of research animals and were approved by the Merck Institutional Animal Care and Use Committee (IACUC).

**Organism and preparation of inoculum.** Candida albicans MY1055 (Merck Culture Collection; caspofungin and amphotericin B MIC = 0.25 μg/ml) was cultured on Sabouraud dextrose agar (SDA; BBL, Cockeysville, MD) plates at 35°C for 24 h. Yeast cells were washed from the surface of the agar plate and suspended in sterile physiological saline. Cell concentrations were quantitated by hemacytometer count, and viable counts were confirmed by serial dilution and by plating the inoculum on SDA.

**Procedure-disseminated candidiasis.** A disseminated Candida infection was induced in juvenile DBA/2N mice by intravenous (i.v.) injection of 0.2 ml of the above yeast suspension containing 5.64 × 10^6 CFU of C. albicans MY1055 into the lateral tail vein. This infectious dose was chosen in order to obtain high kidney and brain Candida burden and 100% mortality in vehicle-treated controls during the study. A slightly higher inoculum of 7.28 × 10^5 CFU/mouse resulted in a severe infection with substantial mortality beginning at 48 h after infectious challenge despite antifungal treatment (data not shown).

Antifungal therapy was initiated at 30 h after infectious challenge and was given by intraperitoneal (i.p.) injection once daily for 7 days. Mice were treated with caspofungin at 1, 2, 4, or 8 mg/kg/day, amphotericin B at 1 mg/kg/day, or vehicle control (VC; sterile distilled water). Efficacy in this model was assessed in 3 ways: by monitoring survival in a group of 10 animals in each treatment group, by monitoring Candida burden in kidney and brain tissues in a second set of...
treated animals, and by histologically evaluating the kidneys and brains in a third set of treated animals. Mice were euthanized by CO2 inhalation, and tissues for both culture and histology were sampled at 30 h (vehicle-treated control only) and at days 5 (24 h after 4th dose), 8 (24 h after last dose), 14, 21 (caspofungin-treated only), and 28 after challenge.

Quantitative culture. The brains and both kidneys from 5 mice per treatment group were aseptically removed, placed in sterile WhirlPak bags, weighed, and then homogenized in 2 ml of sterile physiological saline. Tissue homogenates were then serially 10-fold diluted in sterile saline, and 100 ml was plated on SDA. A 1 ml aliquot of each homogenate was also plated onto a 150-mm SDA plate to lower the limit of detection and to rule out potential drug carryover effects. Plates were incubated at 35°C, and colonies enumerated after 30 to 48 h. Mean numbers of CFU per gram of tissue in caspofungin-treated groups were compared to those in amphotericin B- and vehicle-treated groups. Percent clearance was used to adjust values for multiple testing. Multiplicity adjustments were applied separately to comparisons of caspofungin to amphotericin B (32 comparisons across all doses, time points, and tissues), comparisons of both antifungals at the 7th (final) dose of caspofungin. Three mice per dose level per time point were euthanized by CO2 inhalation, and tissues for Pharmacokinetics. Plasma and brain levels of caspofungin were determined on solid-phase extraction of caspofungin and its internal standard L-000733560. The standard at 500 nM was added to the samples just prior to extraction. The extracted drug and the internal standard were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Zic HILIC 5-μm by 2-mm column. A gradient high-performance LC (HPLC) separation using 0.1% formic acid in water and 0.1% formic acid in acetonitrile as the mobile phases was used. The run time was around 8 min. The analytes were ionized by electrospray in positive-ion mode. The doubly charged ions of caspofungin and its internal standard were measured by the use of appropriate ion transitions in multiple reaction monitoring (MRM) mode. For quantitation of caspofungin in brain, mouse brains were homogenized in water (3 ml H2O/g brain). Protein precipitation using acetonitrile was used to caspofungin and its internal standard at 500 nM were measured by the use of appropriate ion transitions in multiple reaction monitoring (MRM) mode.

Histopathology. The brains and kidneys from 3 mice per treatment group were aseptically removed and placed into vials containing Prefer fixative (Anatech Ltd., Battle Creek, MI). Fixed tissues were embedded in paraffin, sliced, and stained with hematoxylin and eosin (H&E) for visualization of inflammation or periodic acid-Schiff (PAS) for fungus visualization. Three or four kidney sections and two brain sections (cerebrum and cerebellum) with each stain were examined by a pathologist blinded to sample identity and were assigned inflammation and fungal burden severity scores of 0 (normal/no fungus), 1 (minimal/trace), 2 (mild), 3 (moderate), 4 (marked), or 5 (severe).

Pharmacokinetics. Plasma and brain levels of caspofungin were determined for infected (as described above) and age-matched uninfected DBA/2N mice. The antifungal treatment regimens were identical for the two groups of mice. Pharmacokinetic samples were taken at 1 and 24 h after the 1st and 4th doses of caspofungin and just prior to and at 30 and 30 min and 1, 2, 4, 6, and 24 h after the 7th (final) dose of caspofungin. Three mice per dose level per time point were euthanized by CO2 inhalation. Mice were bled via cardiac puncture, and brains were aseptically collected and weighed. Blood was collected into EDTA Microtainer tubes (Becton Dickinson, Franklin Lakes, NJ), and plasma was separated by centrifugation. Plasma and brain samples were stored at −70°C until analysis.

The assay for quantitation of caspofungin in mouse plasma samples was based on solid-phase extraction of caspofungin and its internal standard L-000733560. Briefly, 50 μl of mouse plasma was extracted for each sample; 10 μl of internal standard at 500 nM was added to the samples just prior to extraction. The

RESULTS

Vehicle-treated controls. Histological analysis of kidney and brain samples taken at the initiation of antifungal therapy (30 h after infection) showed evidence of marked to severe kidney infection and minimal to mild multifocal encephalitis with little meningitis (Fig. 1 and 2). This was confirmed microbiologically, with recovery of 5.79 and 3.92 CFU Candida/g kidney and brain, respectively (Table 1; Fig. 3), at the initiation of therapy. Vehicle-treated mice had a progression of infection, and brain samples taken at the initiation of antifungal therapy (30 h after infection) resulted in 100% survival

FIG. 1. Mean fungal burden histology score after 30-h-delayed therapy with caspofungin or amphotericin B in a DBA/2N mouse disseminated Candida albicans MY1055 infection model.
through day 28 relative to vehicle control treatment, which resulted in 100% mortality by day 11 after infectious challenge.

Quantitative culture of *Candida* burden in kidney and brain tissues. Caspofungin reduced recovery of viable *Candida* from kidney and brain tissues compared to vehicle control treatment on day 5, when control burden peaked (Table 1 and Fig. 3). These reductions of 2.72 to 3.28 log CFU/g kidney and 1.68 to 2.47 log CFU/g brain were significantly different from those for vehicle-treated controls, compared without multiplicity adjustments, but only kidney reduction at doses of 2 to 8 mg/kg remained statistically significant when adjusted for multiple comparisons (16 comparisons). Kidney and brain burden in all caspofungin-treated mice at day 5 was also significantly reduced relative to the burden at the start of therapy (30 h after inoculation). At day 5, amphotericin B-treated mice had 2.2- and 1.58-log-CFU/g reductions in kidney fungal burden relative to vehicle-treated controls at day 5 and at the start of therapy (30 h after inoculation), respectively, but no reduction in brain burden. Caspofungin-treated mice dosed with 2 mg/kg or greater had significantly lower brain burden than amphotericin-B-treated mice at day 5, using the multiplicity-adjusted *t* test (32 comparisons).

At day 8 only two vehicle-treated controls in the tissue burden group survived for quantitative kidney and brain culture. One had substantially lower kidney *Candida* burden than the other (3.71 and 5.71 log CFU/g), which resulted in decreased average kidney burden relative to day 5. Amphotericin B and caspofungin treatment reduced kidney fungal burden by 1.7 log CFU/g and 2.46 to 3.64 log CFU/g, respectively, relative to the vehicle control treatment, but these reductions were not significant due to the large variation in the vehicle control kidney burden. By day 8, fungal burden in the brains of control mice had decreased to 2.57 log CFU/g, as is typical in this model in which brain burden peaks at around day 4 or 5 and then decreases over time. Treatment with caspofungin at 8 mg/kg resulted in a 1.55-log-CFU/g reduction in brain fungal burden at day 8 relative to vehicle control treatment, which was significantly reduced by the standard *t* test but did not remain significant by the multiplicity-adjusted *t* test (16 comparisons). At day 8 the multiplicity-adjusted *t* test (32 comparisons) showed that caspofungin treatment at 4 and 8 mg/kg resulted in significantly lower kidney *Candida* burden than that from amphotericin B, while brain burden was significantly lower at 1 and 8 mg/kg caspofungin. No vehicle-treated mice survived for sampling at later time points, but kidney and brain fungal burden continued to decrease in all antifungal-treated mice through day 28 after infectious challenge.

Histological analysis of *Candida* burden. Culture results were confirmed by histopathology, which showed reduced fungal burden and inflammation in both the kidneys and brains in all antifungal-treated groups relative to vehicle-treated controls at day 5 (Fig. 1 and 2) and continued low severity scores for both inflammation and fungal burden in kidneys and brains from all antifungal-treated mice at later time points (Fig. 4). In addition, the inflammation changed from an acute granulomatous type in all vehicle-treated controls and in antifungal-treated mice on day 5 to a more chronic inflammation (lymphocytes, macrophages, and fibrotic reaction) from day 8 through day 28. This was accompanied by a change from predominantly mycelial forms of yeast in all vehicle-treated controls and in antifungal-treated mice on day 5, to less mycelia over time, with only intracellular remnants of fungi in clusters of macrophages from day 14 and thereafter. Figure 4A shows fungi and acute inflammation in both kidneys and brains of vehicle-treated controls at day 5. The continued presence of yeast, mycelia, and granulomatous inflammation in the kidneys
and brains of caspofungin- and amphotericin B-treated mice is seen at day 5 (Fig. 4B and C). By day 14, caspofungin- and amphotericin B-treated mice have more chronic inflammation (lymphocytes and macrophages) in the kidneys and brains, with only remnants of fungi in macrophages in the kidneys (Fig. 4D and E).

Pharmacokinetics. Caspofungin exposure in terms of area under the concentration-time curve (AUC) and maximum concentration of drug (C\text{max}) was relatively dose proportional in both plasma and the brain after the 7th daily dose in infected and uninfected animals, with brain exposure being significantly lower than plasma exposure (Fig. 5). The lowest dose of caspofungin in this study, 1 mg/kg/day, corresponded to a day 7 caspofungin exposure AUC from 0 to 24 h (AUC\text{0–24}) of 35.5 μM · h in plasma and of 7.84 μM · h in the brain tissue of infected animals. Plasma pharmacokinetics indicate that the exposure levels and half-lives of caspofungin were similar in both infected and uninfected mice (Table 2). However, brain exposure and thus the brain/plasma ratio were higher in infected caspofungin-treated mice (brain/plasma AUC\text{0–24} = 0.14 to 0.22) than in uninfected mice (brain/plasma AUC\text{0–24} = 0.08 to 0.12). The concentration of caspofungin in brain tissue was relatively constant over 24 h after the 7th dose, indicating that the half-life in brain tissue is longer than in plasma. However, the half-life of caspofungin in brain tissue could not be calculated due to the flat nature of the brain concentration-time curve. Some accumulation of caspofungin in both the plasma and brains of infected and healthy juvenile DBA/2N
mice was indicated by the increasing minimum concentrations of drug ($C_{\text{trough}}$) after repeat administration (data not shown).

**DISCUSSION**

Disseminated candidiasis is a serious medical issue in neonates, due to the associated morbidity and mortality and frequent CNS involvement. Preclinical studies suggest that echinocandins may be useful in the treatment of *Candida* infection of the CNS (7, 9). Caspofungin is well tolerated in neonates (15) and may be efficacious for the treatment of candidiasis in this population (12, 13). In the current study, a juvenile mouse model was used to examine the efficacy and pharmacokinetics of caspofungin in disseminated candidiasis with CNS involvement.

Caspofungin (1 to 8 mg/kg) or amphotericin B (1 mg/kg) was given i.p. once daily for 7 days beginning at 30 h after infection, when culture and histology showed evidence of disseminated candidiasis with multifocal encephalitis. All animals in the active treatment groups survived through day 28, whereas all vehicle-treated controls died by day 11. Caspofungin produced dose-dependent reductions in the recovery of viable *Candida* from kidney and brain relative to vehicle control treatment. Over the course of the infection, the reduction of kidney and brain *Candida* burden by caspofungin was equivalent to or better than that by amphotericin B, which reduced kidney but not brain *Candida* burden. Histopathology confirmed the culture results, with reductions in fungal burden and inflammation in all active treatment groups relative to vehicle-treated controls. In vehicle-treated controls and in treated groups at day 5, fungal mycelial growth was accompanied by acute granulomatous inflammation, with both more predominant in the kidneys than in the brains. In surviving antifungal-treated mice, this acute inflammation gradually changed over time to a more chronic inflammatory response of lymphocytes, macrophages, and fibrotic reaction, indicating an attempt of resolution and repair. By day 14, inflammation in the brain was associated predominantly with the choroid plexus in contrast to the multifocal encephalitis noted earlier in the infection. These inflammatory changes corresponded to a change from mycelia to intracellular remnants of fungi in clusters of macrophages in surviving antifungal-treated mice.

Pharmacokinetic parameters for caspofungin were studied in infected and age-matched uninfected mice. Caspofungin exposure was relatively dose proportional in both the plasma and brains of all groups. Caspofungin at 1 to 4 mg/kg/day resulted in a day 7 AUC exposure of 35.5 to 174.8 μM·h in plasma and 7.84 to 27.81 μM·h in brain. The plasma AUC levels observed with adult and pediatric patients were within the plasma exposure levels observed with the mouse model at the efficacious doses (16). The exposure to caspofungin at 1 mg/kg/day in the mouse was sufficient to provide equivalent or better efficacy than amphotericin B at 1 mg/kg in this model. The half-life of caspofungin appears to be longer in brain tissue than in plasma, and caspofungin appears to pen-

**TABLE 1—Continued**

| Kidney | Brain | Kidney | Brain | Kidney | Brain |
|--------|-------|--------|-------|--------|-------|
| NS | NS | 2.93 (0)$^a$ | 1.69 (0)$^a$ | 0.94 (40) (0.0052) | 0.94 (40) (0.0082) |
| 0.94 (40) (0.0052) | 0.77 (40) (0.0082) | 0.94 (40) (0.0052) | 0.94 (40) (0.0082) |
| 1.11 (20) (0.0083) | 1.57 (0) (0.6174) | 0.82 (75)$^a$ | 0.49 (100)$^a$ |

FIG. 4. Effect of caspofungin (B and D) and amphotericin B (C and E) on fungal burden and inflammation in kidney and brain. (A) Fungi and acute inflammation in both kidneys and brains of vehicle-treated controls at day 5.
etrate brain tissue more effectively in the presence of *Candida* infection. The latter finding has not been observed with mica-fungin, amphotericin B deoxycholate, or liposomal amphotericin B in rabbits (4, 7).

Both amphotericin B and mica-fungin have been evaluated in the rabbit model of hematogenous *Candida* meningoencephalitis in which both were shown to penetrate the blood-brain barrier and reduce burden in brain tissue (4, 7). In contrast to caspofungin in mice, brain levels of amphotericin B were not increased in the presence of infection in rabbits, while mica-

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**FIG. 5.** Concentrations of caspofungin in plasma and brains of healthy and infected mice on day 7 after once-daily treatment for 7 days.

**TABLE 2.** Mean caspofungin pharmacokinetic parameters for healthy and infected mice on day 7 after once-daily i.p. treatment for 7 days

| Dose (mg/kg/day) | Animal | Plasma AUC$_{0-24}$ (μM·h) | $C_0$ (μM) | $C_{max}$ (μM) | $t_{1/2}$ (h) | Brain AUC$_{0-24}$ (μM·h) | $C_0$ (μM) | $C_{max}$ (μM) | $t_{1/2}$ (h) | Brain/plasma ratio (AUC$_{0-24}$) |
|----------------|--------|-----------------------------|-----------|----------------|-------------|-----------------------------|-----------|----------------|-------------|-----------------------------|
| 1              | Healthy | 53.59                       | 0.587     | 5.035          | 8.4         | 4.23                       | 0.139     | 0.205          | NC          | 0.08                        |
|                | Infected| 35.49                       | 0.577     | 3.576          | 7.6         | 7.84                       | 0.269     | 0.413          | NC          | 0.22                        |
| 2              | Healthy | 108.35                      | 1.013     | 10.612         | 7.1         | 10.45                      | 0.292     | 0.611          | NC          | 0.10                        |
|                | Infected| 73.89                       | 0.732     | 6.985          | 6.8         | 14.61                      | 0.519     | 0.762          | NC          | 0.20                        |
| 4              | Healthy | 150.24                      | 1.334     | 16.822         | 7.1         | 17.52                      | 0.547     | 0.871          | NC          | 0.12                        |
|                | Infected| 174.82                      | 1.359     | 14.596         | 7.6         | 27.81                      | 0.919     | 2.460          | NC          | 0.16                        |
| 8              | Healthy | 311.28                      | 3.926     | 37.873         | 6.9         | 33.73                      | 1.275     | 1.584          | NC          | 0.11                        |
|                | Infected| 369.45                      | 3.858     | 38.890         | 6.4         | 52.39                      | 2.257     | 2.694          | NC          | 0.14                        |

* NC, not calculated.
fungin levels were increased in the cerebellum tissues of infected rabbits, but this increase could not be entirely attributed to infection. Rather, the authors proposed that increased levels of micafungin in brain tissue may be due to the increasing magnitude of the plasma-to-brain concentration gradient at increasing doses, which may drive drug from plasma to the brain. The nonlethal rabbit model of hematogenous Candida meningoencephalitis represents an infection less severe than the juvenile mouse model in which only 20% of vehicle-treated mice survived through day 8 and 100% mortality was seen by day 11 after infectious challenge. The progression of infection and any accompanying histological evidence of inflammation have not been described for the rabbit model. Inflammation in the CNS could be more pronounced in the more severe mouse infection model relative to the rabbit model, and this increase could contribute to the caspofungin levels achieved in the brains of infected mice, which were higher than those of uninfected mice despite similar plasma exposures. Pharmacokinetics were not determined for amphotericin B in the mouse model, resulting in high levels of kidney and brain colonization and 100% mortality in vehicle-treated mouse mice. A slightly higher inoculum resulted in a severe infection, with substantial mortality despite antifungal therapy (data not shown). This method of infection was used to approximate the hematogenous method of Candida dissemination to the CNS often seen with neonatal patients. As in the adult mouse model, kidney burden in this severe juvenile infection model peaks early and remains high, while brain burden peaks at day 4 or 5 and then slowly decreases over time until animals succumb to candidiasis. While this may not mimic the exact progression of infection in neonatal patients, it does provide a similar route of dissemination with high enough Candida burden in the kidneys and the brain to characterize the effects of antifungal therapy in the setting of this severe infection. Caspofungin exposure was determined for plasma and brain in both infected and uninfected cohorts to gain an understanding of the effect of infection on distribution to the CNS in this model. Amphotericin B was included as a comparator because it is considered the standard of care for treatment of neonatal candidiasis. Animal models of disease will always have limitations and may not precisely represent the target patient population; however, they continue to play an important role in understanding the potential therapeutic utility of drugs.

The results of this study demonstrate that caspofungin administered once daily by intraperitoneal injection at doses of 1, 2, 4, or 8 mg/kg/day for 7 days was effective in treating established disseminated candidiasis with CNS involvement in juvenile mice. Caspofungin showed dose-dependent activity in brain and kidney tissues by histopathological and microbiological endpoints. Overall, the efficacy of caspofungin at 1 to 8 mg/kg was equivalent to or better than amphotericin B at 1 mg/kg in this model. These results suggest that further studies of caspofungin for the treatment of neonatal candidiasis are warranted.

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REFERENCES

1. Benjamin, D. K. J., et al. 2006. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics 117:84
2. Fernandez, M., E. H. Moylett, D. E. Noyola, and C. J. Baker. 2000. Candidal meningitis in neonates: a 10-year review. Clin. Infect. Dis. 31:458–463.
3. Friedman, S. E., S. E. Richardson, S. E. Jacobs, and K. O’Brien. 2000. Systemic Candida infection in extremely low birth weight infants: short term morbidity and long term neurodevelopmental outcome. Pediatr. Infect. Dis. J. 19:499–504.
4. Groll, A. H., et al. 2000. Comparative efficacy and distribution of lipid formulations of amphotericin B in experimental Candida albicans infection of the central nervous system. J. Infect. Dis. 182:274–282.
5. Groll, A. H., E. Roilides, and T. J. Walsh. 2009. Fungal infections in pediatric patients. p. 481–499. In E. J. Anaissie, M. R. McGinnis, and M. A. Pfaffer (ed.), Clinical mycology, 2nd ed. Elsevier, Philadelphia, PA.
6. Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6:65–70.
7. Hope, W., et al. 2008. The pharmacokinetics and pharmacodynamics of micafungin in experimental hematogenous Candida meningoencephalitis: implications for echinocandin therapy in neonates. J. Infect. Dis. 197:163–171.
8. Hope, W., S. Shokham, and T. Walsh. 2007. The pharmacology and clinical use of caspofungin. Expert Opin. Drug Metab. Toxicol 3:263–274.
9. Kang, C., M. Rouse, J. Mandrekar, J. Steelberg, and R. Patel. 2009. Anidulafungin treatment of candidal central nervous system infection in a murine model. Antimicrob. Agents Chemother. 53:3576–3578.
10. Kim, R., D. Khachikian, and A. Reboli. 2007. A comparative evaluation of properties and clinical efficacy of the echinocandins. Expert Opin. Pharmacother. 8:1479–1492.
11. Merck & Co., Inc. 2009. Cancidas (caspofungin acetate) prescribing information. Merck & Co., Inc., Rahway, NJ.
12. Natarajan, G., M. Lalic-Botica, C. Rongkavilit, A. Pappas, and M. Bedard. 2005. Experience with caspofungin in the treatment of persistent fungemia in neonates. J. Perinatol. 25:770–777.
13. Odoo, D., et al. 2004. Caspofungin therapy of neonates with invasive candidiasis. Pediatr. Infect. Dis. J. 23:1093–1097.
14. Pappas, P., et al. 2009. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin. Infect. Dis. 48:5503–535.
15. Sáez-Llorente, X., et al. 2009. Pharmacokinetics and safety of caspofungin in neonates and infants less than 3 months of age. Antimicrob. Agents Chemother. 53:869–875.
16. Walsh, T., et al. 2005. Pharmacokinetics, safety, and tolerability of caspofungin in children and adolescents. Antimicrob. Agents Chemother. 49: 4536–4545.