

Assessment of the Paradoxical Effect of Caspofungin in Therapy of Candidiasis

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Paradoxical growth of some *Candida albicans* isolates in the presence of caspofungin (CAS) in vitro has been demonstrated previously. We sought to determine whether a similar phenomenon occurred in vivo. A systemic model of candidiasis was studied in CD-1 mice by intravenous inoculation of different isolates of *C. albicans*. Infected animals were treated with CAS at various dosages (0.01 to 20 mg/kg) and CFU remaining in the kidneys determined. Four clinical isolates that showed paradoxical growth in vitro and one that did not were tested. Recovery of CFU from the kidneys showed that dosages of CAS at 0.1 mg/kg and above were efficacious in the reduction of *C. albicans*, but were not curative. Against isolates that show paradoxical growth in vitro, CAS was efficacious, but lacked dose responsiveness above 0.5 mg/kg against three of the four. One isolate, 95-68, showed paradoxical growth in vivo with significantly higher CFU recovered from mice given CAS at 20 mg/kg than those given CAS at 5 mg/kg, but the effect was not reproducible in a subsequent experiment. When CAS was given prophylactically and therapeutically, improved efficacy and cure rate were observed. Overall, these data indicate that CAS is highly efficacious against systemic murine candidiasis and a paradoxical effect was not reproducibly demonstrated in vivo.

The echinocandin antifungal, caspofungin (CAS), has been demonstrated to have good activity against various species of *Candida* both in vitro and in vivo (4, 17). In addition, CAS has been shown to be routinely fungicidal against *Candida albicans*, but less so against other species of *Candida* (4, 17). Thus far, the development of resistance to CAS has been infrequent, which is encouraging for its future long-term use (4). However, a recent study has reported the possible correlation of clinical outcome with in vitro susceptibility. In that study, temporal isolates of *C. albicans* showed increasing resistance to CAS in vitro, which correlated with the outcome of experimental infection and treatment with CAS (9).

Our laboratory has recently reported observations made during in vitro studies on the paradoxical resistance of some isolates of *C. albicans* to high (supra-MIC) but not low concentrations of CAS (20). The capacity of these isolates to grow in the presence of CAS was reproducible and seen most often with *C. albicans* (20). The frequency of this phenomenon has been noted, using CLSI (formerly NCCLS) methodology (14), in 15/72 (21%) of *C. albicans* isolates and 16/88 (18%) of non-*albicans Candida* (20, 21; D. A. Stevens, C. Pujol, and D. R. Soll, Abstr. 43rd Meet. Infect. Dis. Soc. Am., abstr. 281, 2005; M. Espiritu, R. Parmar, and D. A. Stevens, unpublished data). Observations of this type from in vitro studies are potentially relevant to treatment outcome.

In experimental studies, several investigations have shown a lack of dose responsiveness and cure at doses of above 5 mg of CAS per kilogram of body weight in both murine and rabbit

models of aspergillosis, with higher CFU recovered from the tissues of animals given the higher dosages of CAS (10, 11, 15, 16). In models of candidiasis, CAS appears to be more effective, resulting in cure in some instances, although the dose responsiveness again appears somewhat flat at doses above 0.5 mg/kg (1, 2, 5, 6). This study was done to further examine the potential of an in vivo paradoxical effect using isolates of *C. albicans* that do, or do not, exhibit the paradoxical effect in vitro.

MATERIALS AND METHODS

Isolates. *Candida albicans* isolates 03-321, 98-144, 95-68, 03-178, and 03-202 were used in these studies. The patterns of growth in vitro in the presence of caspofungin have been reported previously (20). Isolate 98-144 displays no paradoxical growth in the presence of caspofungin, whereas each of the other isolates grows in the presence of high concentrations of caspofungin. All of the isolates studied had a MIC of ≤ 0.39 $\mu\text{g/ml}$ caspofungin by classical criteria (20), if the paradoxical growth of some isolates is ignored. Long-term storage of isolates was done by freezing suspensions of yeast in 40% glycerol at -80°C . Inocula for in vivo studies were prepared as previously described (3, 8).

Systemic murine model. A model of systemic candidiasis studied in CD-1 mice was used as described previously (3, 8). In brief, 5-week-old male mice were purchased from Charles River Laboratories and infected intravenously with the desired number of yeast. Treatment with caspofungin (CAS; Cancidas; Merck & Co., Inc., West Point, Pa.; commercial preparation purchased from SCVMC pharmacy) was initiated on day 4 postinfection and continued for 7 consecutive days. All treatment was given intraperitoneally once daily. CAS was delivered in saline. Control mice received no therapy. For all groups, $n = 10$.

On day 11 postinfection, all surviving mice were euthanized and the number of CFU remaining in the kidneys was determined by quantitative plating of organ homogenates on Sabouraud's dextrose agar-chloramphenicol (3, 8).

In vivo studies. Several in vivo studies were performed using different dosages of CAS and different isolates of *C. albicans*. Preliminary studies were done using 03-321 and 98-144 to determine inocula that did not cause deaths prior to day 5 or 6 postinfection and resulted in at least 5 to 6 \log_{10} CFU per kidney pair after 11 days of infection. Inocula of 1×10^5 to 5×10^5 of each isolate met these parameters. Thereafter, inocula were from 3.2×10^5 to 6×10^5 yeast per mouse, depending on the study.

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The design of these studies was that of inducing severe systemic infection, where all mice would, if left untreated, have $\geq 6 \log_{10}$ *C. albicans* CFU recovered from the kidneys on day 11 postinfection and that some animals would succumb to infection. Treatment with CAS was done as a dose escalation to determine whether efficacy would be proportional to higher dosages, as well as to have drug tissue concentrations in the range that had resulted in the paradoxical in vitro growth.

Two treatment experiments were done using isolates 03-321 (shows in vitro paradoxical effect) and 98-144 (does not show in vitro paradoxical effect). In the first experiment, CAS was dosed at 0.01, 0.5, 5, and 15 mg/kg. In the second experiment, CAS was dosed at 0.1, 5, or 20 mg/kg. An additional experiment was done using isolates 95-68, 03-178, and 03-202, all of which demonstrate the paradoxical effect in vitro, with CAS dosed at 0.1, 5, and 20 mg/kg. In the final experiment, only 95-68 (shows in vitro paradoxical effect) was used. In this experiment, CAS was dosed therapeutically beginning day 4 postinfection for 7 days at 0.5, 2.5, 5, 10, or 20 mg/kg. In addition, other groups received CAS prophylactically and therapeutically at 5 or 20 mg/kg beginning 1 day prior to infection and continuing through day 10 (total of 12 doses).

Statistical analysis. Statistical analyses were performed as described previously using a Mann-Whitney U test to compare burdens of *C. albicans* recovered from the kidneys and, in some instances, a log rank test for comparative survival (3, 8). All analyses were done using GraphPad Prism, version 3.02 (GraphPad Software, San Diego, Calif.). For the analyses of comparative organ burdens of *C. albicans*, samples missing due to death were assigned a value of $7.5 \log_{10}$ CFU (13, 19), which ensures that death is considered a worse outcome than is survival with any amount of fungal burden. This value approximates the fungal burden just prior to death.

RESULTS

The initial treatment experiment used isolates 03-321 and 98-144. No mice infected with 03-321 succumbed to infection during the course of the study. In contrast, for those infected with 98-144, 50% given no treatment or 60% given CAS at 0.01 mg/kg succumbed to infection; none given CAS at 0.5, 5, or 15 mg/kg died, and these doses were superior in efficacy to controls or CAS at 0.01 mg/kg ($P < 0.001$).

The recovery of CFU from the kidneys of surviving mice is presented in Fig. 1A. CAS at 0.01 mg/kg was ineffective compared with controls against both isolates ($P > 0.05$). CAS dosages at 0.5 mg/kg or more were efficacious against both isolates ($P < 0.001$), but were equivalent to each other ($P > 0.05$), indicating a flat dose response above 0.5 mg/kg of CAS. Although, for each isolate, the median values from mice given CAS at 15 mg/kg were higher than from mice given CAS at 5 mg/kg, this was a nonsignificant increase in CFU.

A second experiment was performed using the same isolates, but with slightly higher inocula (6×10^5 compared to 4×10^5 used initially) to make the disease somewhat more severe. CAS doses were changed to examine a dose higher than 15 mg/kg (i.e., 20 mg/kg) and also a dose between the previously ineffective 0.01 mg/kg and effective 0.5 mg/kg (i.e., 0.1 mg/kg). In this experiment, no control mice infected with 98-144 survived through 9 days of infection and all doses of CAS were efficacious in prolonging survival ($P < 0.0001$); however, two mice given CAS at 20 mg/kg and one given 5 mg/kg died. Similar to the first study, no mice infected with 03-321 succumbed to infection.

Figure 1B shows the results of the recovery of CFU from the kidneys of surviving mice. All doses of CAS were efficacious against both isolates ($P = 0.004$ to 0.0001 , dependent on comparison). Against isolate 03-321, CAS at 5 or 20 mg/kg was superior to 0.1 mg of CAS per mg ($P = 0.0001$), whereas against isolate 98-144, only CAS at 5 mg/kg was superior ($P = 0.015$) and CAS at 20 mg/kg was equivalent to the 0.1-mg/kg

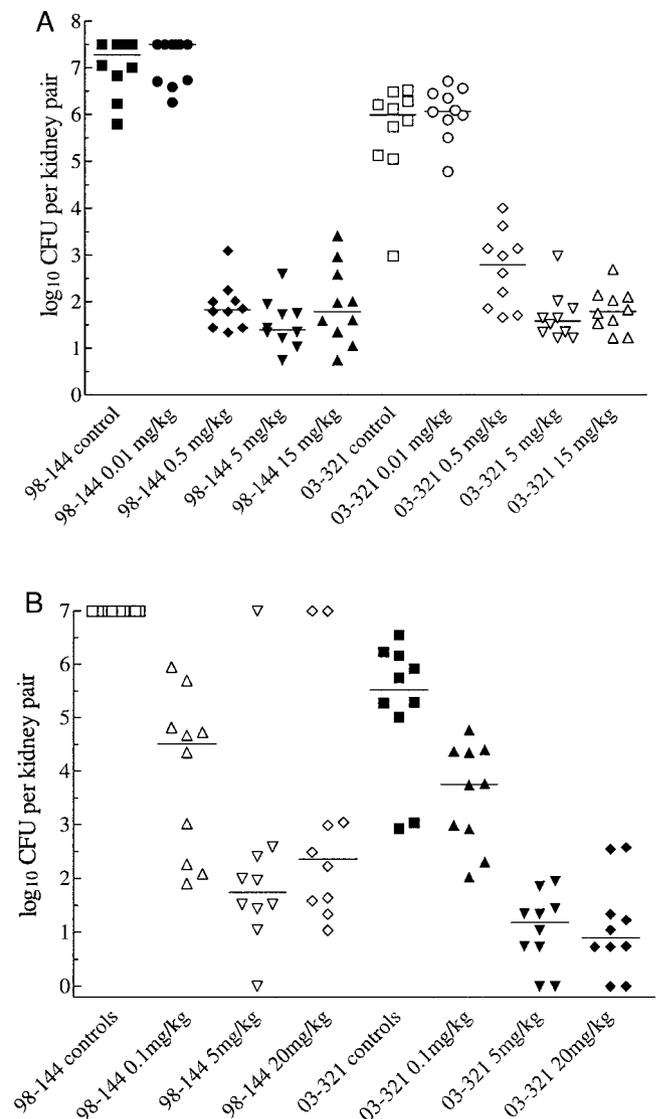


FIG. 1. Recovery of *C. albicans* isolates 98-144 and 03-321 from the kidneys of mice given (A) no treatment (controls) or treated with CAS at 0.01, 5, or 15 mg/kg or (B) no treatment or treated with CAS at 0.1, 5, or 20 mg/kg. Bars represent the median group value. Isolates 98-144 and 03-321 do not and do, respectively, demonstrate paradoxical growth in the presence of caspofungin in vitro.

dose ($P > 0.05$). Against isolate 03-321, CAS at 5 or 20 mg/kg cured two animals of detectable infection; against isolate 98-144, one mouse given CAS at 5 mg/kg was free of infection. No increase in efficacy was observed when the dose of CAS was increased from 5 to 20 mg/kg.

To further explore the in vivo response of *C. albicans* to CAS, we studied three additional isolates. As shown in Fig. 2, the isolates exhibited differences in virulence. Isolate 95-68 was the least virulent (70% of controls survived) and 03-202 the most virulent (no controls survived) (Fig. 2). All dosages of CAS significantly prolonged survival regardless of isolate ($P < 0.02$ to 0.0001 , dependent on comparison).

The recovery of CFU from the kidneys of surviving mice is presented in Fig. 3. All doses of CAS were effective in com-

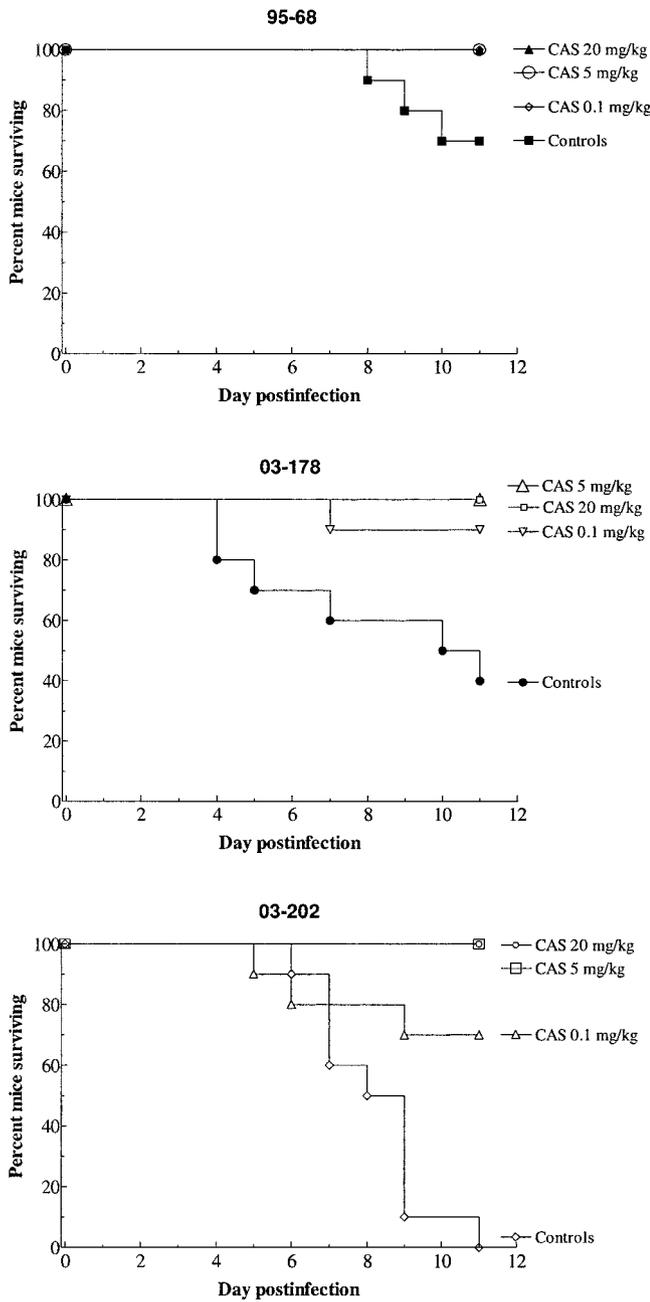


FIG. 2. Cumulative mortality of mice infected systemically with *C. albicans* 95-68, 03-178, or 03-202 and given no treatment (controls) or treated with CAS at 0.1, 5, or 20 mg/kg. All three isolates demonstrate paradoxical growth in the presence of high concentrations of CAS in vitro.

parison with controls for all three isolates tested ($P = 0.0001$ to 0.0005 , dependent on comparison). However, each isolate gave slightly different results with respect to the comparative efficacy of the different dosages. For isolate 03-202, all doses of CAS were effective; CAS doses of 20 or 5 mg/kg were equivalently effective and superior to 0.1 mg/kg ($P = 0.0001$). Against isolate 03-178, CAS showed a dose-responsive efficacy: 20 mg/kg was superior to CAS at 5 or 0.1 mg/kg and CAS at 5

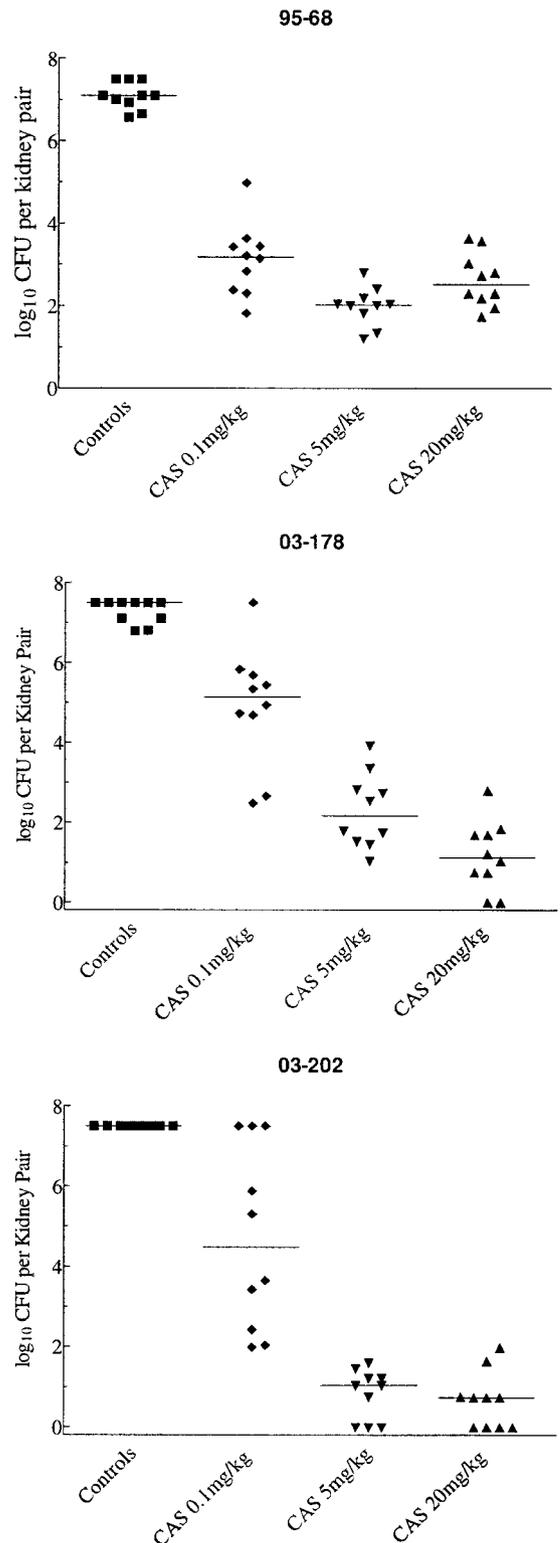


FIG. 3. Recovery of *C. albicans* isolates 95-68, 03-178, or 03-202 from the kidneys of mice given no treatment (controls) or treated with CAS at 0.1, 5, or 20 mg/kg. Bars represent the median group value.

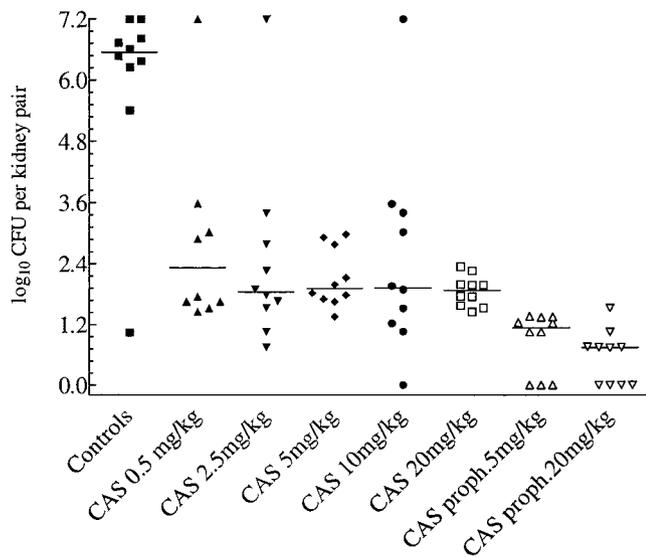


FIG. 4. Recovery of *C. albicans* isolate 95-68 from the kidneys of mice given no treatment (controls) or treated with CAS at 0.5, 2.5, 5, 10, or 20 mg/kg therapeutically or CAS at 5 or 20 mg/kg prophylactically (proph.) and therapeutically. Bars represent the median group value ($n = 10$).

mg/kg was better than 0.1 mg/kg ($P = 0.02$ to 0.0001 , dependent on comparison).

The efficacy of CAS against isolate 95-68 was of interest in that CAS at 5 mg/kg was superior to CAS at 0.1 or 20 mg/kg ($P = 0.002$ or 0.04 , respectively). Furthermore, CAS at 20 was equivalent to CAS at 0.1 mg/kg. Thus, isolate 95-68 appeared to have exhibited paradoxical growth in vivo.

To determine whether this effect was reproducible, a follow-up experiment was done using only isolate 95-68. In this study, an expanded range of doses was used to determine where the paradoxical effect, if any, would appear. Groups were also given prophylaxis with CAS, as well as therapeutic treatment to determine whether an effect might be more pronounced when tissue drug concentrations were present prior to infection. Isolate 95-68 again exhibited low virulence, with only 20% of control mice succumbing to infection; no mice given any regimen of CAS died. The recovery of CFU from the kidneys of surviving mice showed that CAS, except at 0.5 mg/kg given therapeutically, significantly reduced the CFU as compared with controls ($P < 0.05$ to 0.0001 , dependent on comparison) (4). However, all doses of CAS given therapeutically were equivalent to each other, showing no dose responsiveness. CAS doses of both 5 and 20 mg/kg given prophylactically and therapeutically were superior to all doses given only therapeutically ($P < 0.04$ to 0.0001 , dependent on comparison), and these regimens cleared three or four mice, respectively, of detectable infection in the kidneys, whereas therapeutic regimens cleared 1 mouse or 0 mice of detectable infection. Although dose escalation did not improve efficacy over a range of 0.5 to 20 mg/kg, the significant reduction of efficacy by high-dose CAS observed in the prior experiment was not observed in this experiment.

DISCUSSION

The basis for the current set of studies was the observation that some clinical isolates of *C. albicans* exhibited a reproducible pattern of paradoxical growth in the presence of CAS in vitro (20). In that study, CAS had reduced activity at higher concentrations, allowing for growth, whereas some lower concentrations completely inhibited growth (20). The concentrations at which the paradoxical effect was observed varied somewhat by isolate but covered a range of clinically relevant concentrations (20). This is similar to the “Eagle effect” of some antibacterial agents, which has been demonstrated in vitro and in vivo (7, 12, 18, 22–25). For penicillin in particular the induction of beta-lactamases has been implicated as a mechanism (18, 22, 25). As yet, a mechanism for this observation with CAS against *C. albicans* has not been elucidated (21).

The results of our current study indicate that it does not appear that paradoxical growth in vivo after treatment with CAS can be reproducibly demonstrated. In only a single instance with one isolate, 95-68, were we able to demonstrate a significantly higher number of CFU from the kidneys of mice given a high dosage (20 mg/kg) of CAS. Although this proved inconsistent in a replicate experiment, what did remain consistent were the lack of a significant improvement in clearance of fungal burden with dose escalation above 0.5 mg/kg (over a 40-fold dose range) and the cure of a significant number of animals. These results are similar to those reported by others (1, 2, 5, 6).

The lack of dose responsiveness appeared true for four of the five clinical isolates tested in vivo, with only isolate 03-178 responding better with each higher dosage. Interestingly, 03-178 shows paradoxical growth in vitro, whereas isolate 98-144 does not show paradoxical growth in vitro and responded equally to dosages of CAS above 0.5 mg/kg. Whether the lack of dose responsiveness to CAS is related to the pharmacodynamics of CAS or represents a type of paradoxical effect in vivo remains to be determined.

Of interest were the results from mice given CAS prophylactically and therapeutically. These regimens were superior to therapeutic regimens at the same dosage against isolate 95-68 and were the only regimens to effect cure of more than a single animal. These results are reassuring for the use of CAS as a preemptive or preventative treatment for patients that run a risk of acquiring candidiasis because of immunosuppression or long-term catheterizations. However, additional studies would be necessary to ensure the reproducibility of those data.

Overall, we were unable to demonstrate a reproducible paradoxical effect in vivo with CAS against *C. albicans*, which may be indicative that this is only an in vitro phenomenon or is somehow offset by in vivo conditions. Moreover, for most isolates we found no dose responsiveness at doses that result in clinically relevant concentrations. Thus, persistence or relapses of infection while therapy continues, or failures after discontinuation of therapy, might also be a result of incomplete clearance of the organism from the tissues. Our results indicate that the behavior of isolates of *Candida albicans* in vitro does not necessarily reflect how efficacious CAS will be against the same isolates in vivo.

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ERRATUM

Assessment of the Paradoxical Effect of Caspofungin in Therapy of Candidiasis

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Volume 50, no. 4, p. 1293–1297, 2006. Page 1296, column 1, line 21: “(4)” should read “(Fig. 4).”