SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Efficacy of higher doses of caspofungin for treatment of invasive candidiasis caused by *Candida albicans* and *Candida tropicalis* in neutropenic murine models

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The Examination takes place at the Library of the Department of Pharmacology and Pharmacodynamics, Medical and Health Science Centre, University of Debrecen,
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Introduction

Candida species are yeasts which are normally present as individual cells and which predominantly replicate asexually by budding or fission. The term "*yeast fungus*" is most commonly applied to yeasts fall under the kingdom of the fungi. *Candida* species are comprised of around 200 species, although few are important for man. The most important of these are *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. guilliermondii, C. krusei, C. kefyr, C. dubliniensis,* and *C. lusitaniae*.

Candida species are the leading cause of invasive fungal infections in humans, producing infections that range from non–life-threatening mucocutaneous disorders to invasive disease that can involve any organ. Invasive candidiasis is largely a disorder of medical progress, reflecting the great advances in health care technology over the past decades.

Invasive candidiasis has a significant impact on patient outcomes, based on findings of Gudlaugsson et al. patients who develop candidemia are still very likely to die during hospitalization. In a study with a group of patients, the attributable mortality rate has been reported ranging from 5% to 71%, and crude mortality rates have been reported to be as high as 81%.

One of the major causes for the continuing high mortality rates despite the availability of active antifungal agents is the inability to recognize and diagnose early invasive fungal infection, resulting in inappropriate or delayed initiation of therapy, with increased costs of care and excess length of hospitalization. During recent years numerous studies and efforts have been directed toward a better understanding of the pathogenesis of invasive candidiasis and might aid the development of new treatment strategies that could reduce the high mortality associated with nosocomial candidiasis.

Despite recent advances in antifungal pharmacotherapy, the morbidity and mortality caused by invasive fungal infections remains unacceptably high. This thesis will describe therapeutic implementation of caspofungin doses in two different species of *Candida* in two different studies.

Candida species

Among clinically important *Candida* species, *C. albicans* is the leading agent and following *non-albicans* most frequent species distribution are varied in different geographical area and it is distributed through all ages. *C. tropicalis* is one of the more common *Candida* causing human disease in tropical areas. *C. tropicalis* is taxonomically close to *C. albicans* and it shares many pathogenic characteristics with *C. albicans*.

The epidemiology of species responsible for invasive candidiasis, both worldwide and on the local levels, has been changing, a shift to increased prevalence of infections caused by non-albicans *Candida* species, which can be resistant to fluconazole (*C. krusei* and *C. glabrata*) or difficult to eradicate because of biofilm production by *C. parapsilosis* isolates. These findings emphasize the importance of defining the epidemiology of candidemia in every setting.

A very dramatic increase in the relative proportion of ICU-associated candidemia episodes caused by *non-albicans Candida* species was observed in the United States through the 1990s. But other recent multicenter studies still suggest that *C. albicans* remains the predominant invasive *Candida* species among ICU patient cohorts, accounting for 40–60% of candidemia episodes.

A recent study has highlighted variation in *Candida* species causing bloodstream infection. The following were the most commonly isolated of 1239 *Candida* BSI isolates from 79 medical centers in 2008 to 2009: 50.0%, 17.4%, 17.4%, 9.8%, and 1.8% were *C. albicans, C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*, respectively.

Echinocandins

After the discovery of the penicillin which specifically inhibits bacterial cell wall synthesis, achieving similar drug to target the fungal cell wall has become the center of focus of the antifungal drug discovery. Given that the cell wall is uniquely vital for the fungal cell and considering that its components are lacking in the mammalian host, the fungal cell wall provides an excellent target for antifungal agents.

The second generation semisynthetic echinocandins with extended spectrum against *Candida* and *Aspergillus* species have been introduced during the last decade. Echinocandins are preferred because of their excellent safety profile, efficacy and preferred pharmacokinetic characteristics in clinical studies. These echinocandins include caspofungin (Cancidas™), micafungin (Mycamine™), and anidulafungin (Ecalta™ and Eraxis™). With specific exceptions, existing records show that these three compounds are not basically different in connection with pharmacodynamics, pharmacokinetics, spectrum, safety, and antifungal efficacy.

All three compounds possess poor oral bioavailability and are for intravenous use only. The potential disadvantages of this new class of antifungal agent include their higher cost than the other FDA approved antifungals, lack of oral formulations, and lack of *in vitro* activity against emerging pathogens like *Fusarium, Scedosporium,*, and *Zygomycetes*.

The echinocandins generally exhibit concentration dependent activity, non-competitive inhibitors of 1,3-β- and 1,6-β-D-glucan synthase, and is encoded by two homologous FKS1 and FKS2 genes.

Glucan is a major component of the fungal cell walls, and is consisting of 30 to 60 percent in *Candida* and *Saccharomyces* species cell walls. Changes in the components of the cell wall can cause osmotic fluctuations and cell lysis and death. Due to the lack of 1,3-β-D-glucan in human cells, the echinocandins avoid direct human cell toxicity.

Early studies confirmed that echinocandins show a species dependent mechanism of action. *In vitro* studies support this conclusion and reveal echinocandins fungicidal activity against a large number of *Candida* species and fungistatic activity against *Aspergillus* species.

The three echinocandins have potent *in vitro* activity against all clinically important isolates of *Candida* species. The three *Candida* including *C. parapsilosis, C. guilliermondii* and *C. lusitaniae* in comparison with remaining *Candida* species exhibit higher MICs for all echinocandins. The echinocandins also have potent and broad spectrum activity against *Aspergillus* species.

Generally, the echinocandins have MIC_{90} values of ≤2 mg/L against *Candida* species. A concise review of the existing data has analyzed the MIC values of the echinocandins, and

demonstrated that normally, anidulafungin displays the lowest MIC values against the vast majority of *Candida* species, follows by micafungin and caspofungin. However, the presence of human serum reduces the *in vitro* effectiveness of all the echinocandins and reduces the effects of the *in vitro* MIC superiority of micafungin over caspofungin.

Caspofungin

Caspofungin (Cancidas) was the first member of the echinocandins family, approved in 2001 by the FDA for the treatment of invasive fungal infections in adults and in 2008 for use in children ≥3 months of age.

Caspofungin (caspofungin acetate, MK-0991; L-743872) is a semi-synthetic, water soluble lipopeptide antifungal produced from a fermentation product from the fungus *Glarea lozoyensis*. It belongs to the echinocandin family, and is a derivative of the natural product pneumocandin B_0.

After single intravenous administration of 5 to 100 mg caspofungin dosages to healthy subjects, linear pharmacokinetics with a beta half-life of 9 to10 h and an average plasma clearance of 10 to 12 mL/min was demonstrated over the dose range. At higher dosages, an additional, prolonged gamma half-life from 40 to 50 h was evident. In plasma, caspofungin is bound to proteins (97.5 %).

Multiple dose studies at doses of 15, 35, and 70 mg daily for 2 and 3 weeks revealed dose-related accumulation of drug in plasma as high as 50%. A loading dose of 70 mg, followed by 50 mg daily, maintained plasma concentrations higher than 1 mg/mL from day 1 in advance; this is above the documented MIC values for the majority of susceptible fungi.

Data from higher dosages studies of caspofungin displayed constant pharmacokinetics following by single doses of 150 and 210 mg and keeping with twenty-one days of 100 mg; peak concentrations were 29.4 and 33.5 mg/L, respectively.

Tissue distribution experiments in murine models revealed preferential exposure of liver, kidney, and large intestine, but exposure for lung, spleen and small intestine was comparable to that relating to plasma. Organs having a lower intensity of exposure consisted of the heart, brain, and thigh.

Paradoxical growth

Paradoxical growth is a phenomenon initially described in vitro, though some reports suggest that it may also be detected in vivo. Paradoxical growth is defined by efficient inhibition or killing by echinocandins at the MIC and supra-MIC concentrations, but lack of activity at concentrations well above the MIC. Paradoxical growth at present is considered echinocandin-specific.

Cell wall content analysis detected increased chitin concentrations in strains surviving in very high echinocandin concentrations. This enhanced chitin synthesis is the most probable explanation of paradoxical growth. As the concentrations at which paradoxical growth is usually detected are far above those that occur in patients with the recommended clinical regimen, the clinical importance of this phenomenon is still unclear.

The observation of the paradoxical effect of caspofungin *in vitro* at supra-MIC concentrations has been linked to upregulation of FKS1, GSL2, MKC1, and GSC1 gene expression, as a possible cause of phenotypic drug resistance.

To date, the paradoxical effect could not be demonstrated reproducibly in animal models. But, there has been no signal in subjects treated with high doses of these three antifungals. The *in vitro* paradoxical effects tend to occur at concentrations greatly above those that are safely achieved in plasma may describe the lack of an *in vivo* correlation.

Aims of the study

The therapy of invasive fungal infections, though having advanced enormously in the past decade, is still a therapeutic challenge. Only three major group of antifungal agents can be used against the most frequent infections candidiasis and aspergillosis. For these reasons, understanding the ins and outs of antifungal chemotherapy is one of the most important field of research at present in clinical mycology. This is especially true for the newest class of antifungals, echinocandins, which, as a consequence of the novelty, are the presently less understood group of antifungals.

Specific aims of this work are:

1. To test the efficacy of various clinically relevant caspofungin doses (single 6 mg/kg, two times 3 mg/kg and 1 mg/kg) on a mouse model of infection and their therapeutic efficacy on tissue burden in disseminated candidiasis due to *C. albicans* infection.
2. To find an effective drug concentration of caspofungin for treating an isolate of *C. tropicalis* showing paradoxical growth *in vivo* in an intraperitoneal abscess of model of infection and its potential implication in clinical use for treating the disseminated candidiasis due to infection to this *Candida* species.

Materials and Methods

Animals

We have used female BALB/c mice weighing from 26 to 28 g and 18 to 20 g in the experiments with *C. albicans* and *C. tropicalis*, respectively. In the lethality experiments mice received ceftazidime (5 mg/day subcutaneously) during the experiments to prevent bacterial superinfection and its potential confounding effect. The experiments were approved by the local Animal Care Committee (permission no. 12/2008).

Immunosuppression of mice

All BALB/c mice were immunosuppressed intraperitoneally using three doses of 200 mg/kg of cyclophosphamide at four days prior to infection, one day after receiving the infectious organisms and finally four days after the initiation of therapy in the experiment with *C. albicans*. In the second set of studies with *C. tropicalis*, the mice were immunosuppressed intraperitoneally with two doses of 200 mg/kg of cyclophosphamide four days prior to and one day after the infection.

Models

Intravenous infection with *C. albicans*:

We used three *C. albicans* bloodstream isolates (10920, 4780 and 17471), derived from our previous study, with caspofungin MICs uniformly 0.03 mg/L. Isolate 17471 was resistant to fluconazole (MIC=64 mg/L).

In the lethality experiments the infectious dose was set at 10^5 CFU/mouse (in a 0.2-ml volume) based on preliminary studies.

Tissue burden experiments were performed with isolates 10920 and 17471. To ensure 100% survival in the control groups, we used $4x10^4$ CFU/mouse for the tissue burden determination. Inoculum density was confirmed by plating serial dilutions on Sabouraud agar plates. At this stage, the kidneys were removed in a sterile manner, then homogenized. The homogenates were diluted by 1 ml sterile saline, and serial tenfold dilutions were prepared in saline. Aliquots of 100 µl of these dilutions were plated onto Saboraud

dextrose agar plates. After incubation of the plates for 48 h at 30 ºC the resulting colonies were counted and used to determine the CFUs/kidney pairs.

Intraperitoneal abscess model of study with *C. tropicalis*:

The *C. tropicalis* isolate tested showed a caspofungin MIC of 0.024 mg/L; grew at both 6.25 and 12.5 mg/L caspofungin concentrations in the time-kill experiment, but was killed at concentrations from 0.048 to 3.12 mg/L of caspofungin within 24 hours (paradoxical growth).

We used the intraperitoneal abscess model described by Ninomiya et al. in 2005. Autoclaved caecal content from mice were mixed with equal amount of fungal suspension (0.25-0.25 ml) and inoculated into mice intraperitoneally (final inoculum 10^7 CFU/mouse). Inoculum density was confirmed by plating serial dilutions on Sabouraud agar plates (see above).

Treatment

To assess the therapeutic efficacy of caspofungin against the *Candida* species, the commercial preparation of caspofungin (Cancidas) was used. Caspofungin was dissolved in sterile saline for the *in vivo* experiments.

Intravenous infection with *C. albicans*:

Mice were assigned randomly into the study groups (ten mice/group) as follows; no treatment, 1 mg/kg daily dose for 6 days, 3 mg/kg two times and a single dose of 6 mg/kg of caspofungin (Cancidas, commercial preparation). Intraperitoneal treatment (0.5 ml of caspofungin) was started 10 hours postinfection. Mice were followed up for six days to observe the effect of treatment on early lethality.

Survival rate was analyzed by Kaplan-Meier test; the effect of different caspofungin doses was compared using logrank test.

In tissue burden experiments, treatment groups were assigned as in the lethality experiment, one treatment group included 45-60 mice. Kinetics of drug efficacy was monitored by determining CFU average per kidney pair in seven to ten mice on each study

day for six days postinfection. Two mice from each group were sacrificed and analyzed at the start of therapy to verify the fungal replication initiation in the kidneys.

Drug efficacy was compared by determination of the CFU numbers in every kidney pair among the treatment groups every day using Kruskal-Wallis test (with Dunn's post-testing). P values of <0.05 were regarded as significant. For statistical analysis GraphPad Prism (Windows version 4.03) was used.

Intraperitoneal abscess model of study with *C. tropicalis*:

In the first set of the *in vivo* experiments, we have used seven treatment groups with single doses of caspofungin treatment (0.12, 0.25, 1, 2, 3, 5 and 15 mg/kg) besides their control group. Treatment groups consisted of 5 mice. Caspofungin treatment was started one hour after the inoculation. We have followed the experiment for seven days, and the survival rate was also measured.

In the second set of experiments, we administered caspofungin with daily doses for 5 days, using the same doses 0.12, 0.25, 1, 2, 3, 5 and 15 mg/kg. Treatment groups consisted of 5 to 8 mice. This experiment was performed twice.

All mice were monitored twice daily and those who became immobile and showed signs of severe illness were euthanized and recorded as death on the same day. Those mice who survived till the end of the experiment were sacrificed and the colony numbers of viable fungi were determined from an abdominal lavage sample taken by washing the peritoneal cavity with 1 ml of sterile saline. Additionally, the total number of abdominal abscesses and the fungal colony numbers in each abscess were also determined.

To analyze the relationship between treatment and survival of mice, we have used chi-square test. Colony forming unit of peritoneal lavage and peritoneal abscesses were compared by Kruskal-Wallis test with Dunn's post-testing. Values of $p<0.05$ is considered to be significant. For statistical analysis GraphPad Prism (Windows version 4.03) was used.

Results

Experiments with *C. albicans*

Lethality

BALB/C mice have been approved to have disseminated candidiasis after intravenous infection with all three strains of *C. albicans*. All caspofungin regimens used in this study improved the survival of mice infected with isolates 17471, 10920 and 4780 (P values were <0.0001, p=0.0014, and p=0.0003, respectively). 50% was the lowest survival rate among the treated groups, found in the 2x3 mg/kg dose group with the 10920 isolate. However, differences between groups in efficacy of caspofungin were not statistically significant (p>0.05).

Fungal tissue burden

The activity of the three *C. albicans* isolates in the tissue burden experiment were 3.32 ± 2.53 and 3.37 ± 2.46 log10 CFU/kidney at the beginning of therapy for isolates 10920 and 17471. All treatment regimens decreased the tissue burden in comparison to the control group. We did not observe any paradoxical growth at the higher doses.

All treatment regimens except the 1 mg/kg on the second day (p>0.05) and 2x3 mg/kg on the third day (p>0.05), were found to be beneficial in reducing the tissue burden for each day (p<0.05-<0.001) in case of isolate 10920. The single 6 mg/kg dose significantly decreased the fungal tissue burden on each day in comparison to the all days results of the control group (p<0.05-0.001). Between different treatment groups there were no statistically significant differences.

For the *C. albicans* isolate 17471, all doses except 2x3 mg/kg caspofungin on its first day proved to be effective in clearing the tissues (p<0.05-0.001). Three mice in the 2x3 mg/kg caspofungin group on day 4-6 showed higher than 1000 CFU/kidney, indicating an incomplete eradication of infection. Differences between groups treated with different regimens were not significant statistically.

Experiments with *C. tropicalis*

Lethality

After the intraperitoneal infection with *C. tropicalis*, all mice in the control group succumbed to infection within five days. Treatment with single caspofungin doses of 0.12, 0.25, 1, 2, and 3 mg/kg did not decrease mortality (100% mortality within five days), however, 100 % survival rate was observed at 5 and 15 mg/kg doses.

Five days of caspofungin treatment significantly decreased the mortality rate when compared to the control group regardless of the dose ($p<0.0001$); differences among the caspofungin-treated groups were statistically not significant ($p>0.05$).

Peritoneal lavage

Caspofungin treatment significantly decreased the number of viable yeasts in the peritoneal lavage samples in case of all groups as compared to the control ($p<0.001$ in each case), with the exception of the 0.12 mg/kg dose group ($p>0.05$).

Intraperitoneal abscess model

The vast majority of abscesses were found in the liver. The number of abscesses containing viable yeasts as well as yeast CFU numbers in the abscesses decreased significantly in the groups treated with 1, 2, 3, 5, and 15 mg/kg caspofungin ($p<0.001$ in case of 1, 3, 5 and 15 mg/kg and $p<0.01$ in case of 2 mg/kg), in comparison to the control group. There was no difference between the groups treated with the two lowest doses of 0.12 and 0.25 mg/kg of caspofungin and the control group ($p\geq0.05$ in all cases). Viable yeast CFU numbers significantly decreased in groups treated with 1, 3, 5 and 15 mg/kg of caspofungin doses ($p<0.05$) as compared to the 0.12 mg/kg caspofungin treated group. These data were reproducible in the second independent experiments.

In the two experiments using 2 mg/kg of caspofungin, one and two out of five and eight mice, respectively, yielded viable yeasts in the abscesses after treatment, i.e. sterilization of the abscesses was not achieved (20000 CFU/mL in the first and 180 and 560 in the second

experiment in the single liver abscesses, respectively,). The yeast CFU numbers in case of the 0.12 and 2 mg/kg caspofungin treated groups did not differ significantly from each other. To test the reproducibility of these results found in the 2 mg/kg of caspofungin group, we repeated the experiment with this dose for third time. The third experiment has led to similar results (one of eight mice remained infected, carrying 6300 CFU/ml in a single abscess). The results of this third experiment were statistically comparable to the former ones.

Discussion

Due to the high level of reduced fluconazole susceptibility of *Candida* species, the echinocandin antifungals are now considered first line for the treatment of infections caused by these pathogens. All three agents (caspofungin, micafungin, and anidulafungin) have been approved by the U. S. Food and Drug Administration for the treatment of esophageal candidiasis and invasive candidiasis, including candidemia. Echinocandins are fungicidal against *Candida* species and actively growing tip of *Aspergillus* hyphae and have been proved to be highly effective both in animal models and in clinical trials.

Dose escalation is one of the echinocandins treatment strategies for invasive *Candida* infection. The maximum tolerated dose of caspofungin is unknown, but caspofungin at two and three times the standard 50 mg/day of dosing regimen were well tolerated in adult non-candidemic and candidemic patients. High daily doses of caspofungin have produced a higher favorable overall response rate in the patients infected with *C. albicans* and *C. parapsilosis* when compared to the standard dosing regimen.

In a study with a murine model, caspofungin concentrations have reached in a range of cca. 6 and 9 mg/L in the liver in the subjects received 1 mg/kg of caspofungin. In another study with healthy adult participants following multiple 100-mg doses of caspofungin, on day 21 geometric mean $AUC_{0\text{-}24}$ was 227.4 mg·h/L, peak concentration was 20.9 mg/L, and trough concentration was 4.7 mg/L. These results confirm that higher daily dose of echinocandins lead to higher serum and probably tissue concentration at the infected site of body, therefore a better treatment and tissue clearance can be expected.

In our work, caspofungin proved to be highly effective in an immunocompromised murine model of infection with a *C. tropicalis* strain with a proof of showing paradoxical growth *in vitro* in our preliminary studies [second study]. In this work, the subtherapeutic daily doses of 0.12 and 0.25 mg/kg did not improve the survival significantly, and were not able to eradicate the infection in the tissue sites. Among the evaluated dosages, the caspofungin standard daily dose of 1 mg/kg, cleared and eradicated the infection from the peritoneal cavity and has led to 100% survival in the lethality experiment. Other supratherapeutic caspofungin doses (including 2, 3, 5 and 15 mg/kg/day) were also effective in decreasing lethality and in preventing abscess development. These results are in concordance with previous studies

which produced excellent therapeutic outcome using higher echinocandins daily dosages. But, in our *C. tropicalis* experiment, three out of 13 mice which were receiving the 2 mg/kg of daily dose did not recover from the infection. These results were confirmed in a third repeated experiment.

Candida exposure to high concentration of caspofungin leads the organisms to induce the chitin synthesis. This stress-induced chitin synthesis is thought to be the mechanism for paradoxical growth. Clinical relevance of paradoxical growth is unknown, but a number of reports suggest that paradoxical growth may be associated with therapeutic failure in clinical situations.

High daily doses of caspofungin used in clinics have produced lower favorable overall response rate in patients infected with *C. tropicalis* in contrast to the standard daily dose of caspofungin regimen. These differences were not statistically significant and were not considered to be associated with the phenomenon of paradoxical growth.

In the experiment with *C. tropicalis*, in contrast to 1 mg/kg in sterilizing the abscesses, the 2 mg/kg of caspofungin dose did not eradicate the infection in 3 out of 13 mice; which the paradoxical growth may speak in these cases. The reduction in infection burden was statistically significant at both 1 and 2 mg/kg of body weight per day and there was not a significant difference among the two groups.

Other researchers have also found a paradoxical growth with *C. albicans* strains *in vivo* at 20 mg/kg of caspofungin dose in examination the kidneys of the infected mice. But, they could not reproduce their results and they have concluded that paradoxical growth had role limited in an *in vivo* murine model.

These results suggest that supratherapeutic doses can result in incomplete sterilization in some animals and it could be the source of relapse after the discontinuation of therapy. Therefore, the role of paradoxical growth in the late clinical failure cannot be excluded.

Although, high daily doses of caspofungin did not lead to statistically decreased therapeutic effect against immunocompromised mice infected with *C. tropicalis* isolate, the most important finding in this work was the excellent survival rate (100 %) elicited by a single dose

(5 and 15 mg/kg) of caspofungin. This result shows that these dosing regimens can be useful for further evaluation in treating the infections caused by other *Candida* species as well.

Pharmacokinetic studies indicated that caspofungin distributes to and accumulates in tissues. These tissues act as slow release of drug reservoirs which may explain the good efficacy of larger single and divided echinocandin doses in animal models. These facts raised the possibility that larger echinocandin doses used infrequently may be beneficial in certain clinical situations.

Other observations also support that less frequent large doses of echinocandins may be effective for the treatment of invasive candidiasis. The only human data with once every two days dosing of an echinocandin was published by Buell et al. The authors have conducted a multicenter, multinational, double-blind, randomized, parallel group, noninferiority study of 452 patients with confirmed esophageal candidiasis. Patients were randomized (1:1:1) to intravenous micafungin 300 mg/every other day, intravenous micafungin 150 mg/day and intravenous caspofungin 50 mg/day for a minimum 14 days and for 7 days after resolution of clinical symptoms of esophageal candidiasis (maximum 28 days). The vast majority of the identified *Candida* was *C. albicans* (91.4-94 %) followed by *C. glabrata* (2.6-5.3). Their results suggest that micafungin 300 mg administered intravenously every other day was as safe and effective as intravenous daily micafungin 150 mg and caspofungin 50 mg in treating esophageal candidiasis. Moreover, micafungin 300 mg resulted in less frequent relapse at 2 and 4 weeks post-treatment in comparison to micafunngin 150 mg and caspofungin 50 mg.

Dose escalation have also been proved to be well tolerated; moreover, in an open-label study, up to 8 mg/kg of micafungin was well tolerated for seven days. Because all echinocandins show dose-dependent postantifungal inhibition, this postantifungal effect may also be enhanced by increasing the concentration of the echinocandins.

We have evaluated the efficacy of alterative dosing regimens of caspofungin against the three *C. albicans* isolates in preventing early (first six days postinfection) lethality in a deeply neutropenic murine model [first study]. The single 6 mg/kg and 2x3 mg/kg doses of caspofungin provided comparable but not superior results in comparison to the standard 1 mg/kg daily dose of caspofungin regarding the lethality and tissue fungal burden experiments. The isolates 17471 and 10920 relatively less responded to 2x3 in both lethality and tissue burden experiments. But the single six mg/kg of the caspofungin dose in the first three days of

experiment have produced significant decrease during the study period ($p<0.05$-0.001). However, differences between the three treatment arms disappeared by 4-6 days postinfection ($p<0.05$ for all isolates during the experiment). These results strongly suggest that antifungal therapy should be started as soon as possible and the higher echinocandin doses at beginning of therapy may provide a better outcome.

These results, together with those of numerous other authors, prove that echinocandins are excellent for the treatment of *Candida* infections; early therapeutic failure is rare and usually is associated with non-susceptibility. Recommended daily dosing may be supplanted by administering infrequent large doses, but the utility of the latter approach should be further examined in future experiments.

Summary

Candida species are the leading cause of invasive fungal infections in humans, producing infections that range from non–life-threatening mucocutaneous disorders to invasive disease that can involve any organ. In recent years novel antifungal agents have been released, significantly increasing options for the treatment of most serious fungal infections. The most recent approved antifungal drugs include those in the echinocandin class (caspofungin, micafungin, and anidulafungin), as well as the newer generation triazoles voriconazole and posaconazole.

In this respect we have conducted studies in defining the efficacy of caspofungin in treating systemic infections with two *Candida* species. First the *in vivo* efficacy against *C. albicans* was investigated. In a study with three *C. albicans* isolates single dose of six mg/kg, two times three mg/kg, six doses of one mg/kg efficacy have been examined in the two sub-studies including lethality and tissue burden in neutropenic murine models. In lethality experiments, all treatment regimens improved survival ($p<0.0014$ for all three isolates); differences among the treated groups were not statistically significant. The kidney fungal burdens for the two of isolates were counted in every day for the six-day study period continuously. The six mg/kg dose on the first three days made a significant change in decreasing the colony numbers ($p<0.05$-0.001), but in a longer period from forth to sixth day of study there was no difference among the treated groups ($p<0.05$).

The second study investigated the efficacy of caspofungin against *C. tropicalis* using an intraperitoneal infection model using a *C. tropicalis* isolate showing paradoxical growth *in vitro*. In this study a variety of caspofungin doses (0.12, 0.25, 1, 2, 3, 5, and 15 mg/kg) were used in one and daily doses for five-day of studies. The single doses of caspofungin were effective only at 5 and 15 mg/kg concentrations (100% survival). Five-day caspofungin treatment led to 100 % survival at 1 mg/kg and higher doses. Caspofungin treatment significantly decreased the number of viable yeasts in the peritoneal lavage samples as well as in the infected abscesses at 1 mg/kg as well as higher doses in comparison to the untreated control group ($p<0.001$ in all cases), and even to the group treated with 0.12 mg/kg of caspofungin ($p<0.05$ in all cases).

These results strongly suggest that antifungal therapy should be started at earliest time possible. More studies are required to examine the beneficial and therapeutic role of infrequently larger echinocandins doses for using in the clinical routine and determining a better dosing regimen using the echinocandins specific charateristics.

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Candidate: Sedigh Bayegan
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List of publications related to the dissertation

1. **Bayegan, S.**, Szilágyi, J., Kemény-Beke, Á., Földi, R., Kardos, G., Gesztelyi, R., Juhász, B., Adnan, A., Majoros, L.: Efficacy of a single 6 mg/kg versus two 3 mg/kg caspofungin doses for treatment of disseminated candidiasis caused by Candida albicans in a neutropenic mouse model.
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