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Efficacy of single large doses of caspofungin in a neutropenic murine model against the "psilosis" group

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SUMMARY

We compared the *in vivo* efficacy of single large dose of caspofungin to that of daily smaller caspofungin doses (with same cumulative doses) against *C. albicans* (echinocandin susceptible and resistant isolates) and the "psilosis" group in a neutropenic murine model. Seven treatment groups were formed for *C. orthopsilosis*, *C. metapsilosis* and *C. albicans* (no treatment, 1, 2 and 3 mg/kg caspofungin daily for five days; single 5, 10 and 15 mg/kg caspofungin doses). For *C. parapsilosis* there were five treatment groups (no treatment, 3 and 4 mg/kg caspofungin daily for five days; single 15 and 20 mg/kg caspofungin). Tissue burdens of *C. orthopsilosis* and *C. parapsilosis* were significantly decreased by daily 3 mg/kg and 10 or 15 mg/kg single caspofungin doses (P<0.05-0.01) and daily 4 mg/kg and by single 15 and 20 mg/kg caspofungin doses (P<0.05-0.01), respectively. Against *C. metapsilosis* all treatment arms except the daily 1 mg/kg were effective (P<0.05-<0.001). Against *C. albicans* all treatment doses were effective. Neither daily 16 mg/kg nor single 80 mg/kg were effective against the resistant *C. albicans* strain. Higher doses and less frequent administration of caspofungin were comparable or sometimes superior to the lower, daily-dose regimen against the "psilosis" group supporting further studies with this therapeutic strategy.

KEY WORDS: Intermittent dosing regimen, Echinocandins, Candida parapsilosis, Candida albicans, Fungal tissue burden, Echinocandin-resistant.

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INTRODUCTION

Echinocandins (caspofungin, micafungin and anidulafungin) are lipopeptide antifungals with potent *in vitro* and *in vivo* activity against *Candida* species (Andes *et al.*, 2010; Chen *et al.*, 2011; Pfaller *et al.*, 2011). The vast majority of *Candida* species are inhibited at low echinocandin concentrations *in vitro*; the least susceptible *Candida* species to echinocandins are *C. parapsilosis* and *C. guilliermondii* (Andes *et al.*, 2010; Chen *et al.*, 2011; Pfaller *et al.*, 2011). *In vivo* efficacy of echinocandins is driven by both AUC/MIC and C_{max}/MIC (Andes *et al.*, 2010; Gumbo, 2007), suggesting that intermittent dosing regimens of echinocandins will promote effective therapy (Gumbo, 2007).

It has been demonstrated using immunocompetent as well as severely immunocompromised murine models that single larger doses of caspofungin and aminocandin are as effective as smaller daily doses in systemic infections caused by *C. albicans, C. glabrata* and *C. tropicalis* (Bayegan *et al.*, 2010; Bayegan *et al.*, 2011, Brzankalski *et al.*, 2008; Najvar *et al.*, 2008). Moreover, higher peak caspofungin concentra-

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tions with less frequently administered doses were beneficial in reducing the tissue fungal burden in a murine model of invasive aspergillosis (Wiederhold *et al.,* 2004). However, the efficacy of single larger caspofungin doses has not yet been determined against the medically important "psilosis" group.

This group is a target for intensive investigation regarding echinocandin susceptibility, because in spite of the measurably decreased *in vitro* susceptibility, *in vivo* efficacy against them is favorable both in experimental models and in clinical case studies (Andes *et al.*, 2010; Földi *et al.*, 2012; Pappas *et al.*, 2009; Pfaller *et al.*, 2011; Pfaller *et al.*, 2012). This is also demonstrated by the fact that more than 50% of invasive candidasis cases caused by *C. parapsilosis* were treated effectively with echinocandins in a large clinical trial yielding a survival rate comparable to that seen with *in vitro* more susceptible species (Pfaller *et al.*, 2012).

Therefore, the aim of our study was to determine the *in vivo* efficacy of single large caspofungin doses against two isolates each of *C. orthopsilosis*, *C. metapsilosis* and *C. parapsilosis sensu stricto* in a severely neutropenic murine model. For comparison, three *C. albicans* isolates (including one echinocandin-resistant) were also tested.

MATERIAL AND METHODS

All *C. parapsilosis*, *C. orthopsilosis,* and *C. metapsilosis* isolates as well as two *C. albicans* (17433 and 10920) isolates were well characterized in our previous studies (Földi *et al.*, 2012; Varga *et al.*, 2008). The third *C. albicans* isolate was resistant to caspofungin (Fks1p-F645P). Caspofungin MIC values of these isolates were determined using the standard CLSI method in RPMI-1640 (2008). All isolates were tested three times.

BALB/c female mice (19-21 g) were given cyclophosphamide 4 days before infection (150 mg/kg), 1 day before infection (100 mg/kg), 2 and 5 days postinfection (100 mg/kg) (Andes *et al.*, 2010; Földi *et al.*, 2012).

The animals were maintained in accordance with the Guidelines for the Care and Use of Laboratory Animals; experiments were approved by the Animal Care Committee of the University of Debrecen, Debrecen, Hungary (permission no. 12/2008). Mice were infected intravenously through the lateral tail vein. The infectious dose for *C. albicans* was $8x10^4$ CFU/mouse. In case of the "psilosis" group mice were given $6x10^6$ CFU/mouse. Inoculum density was confirmed by plating serial dilutions on Sabouraud agar plates.

Mice were assigned to seven treatment groups (seven or eight mice for all groups) for *C. orthopsilosis, C. metapsilosis* and for the two wild type *C. albicans* isolates, (no treatment, 1, 2 and 3 mg/kg caspofungin daily for five days; and the corresponding single doses of 5, 10 and 15 mg/kg).

As the daily 1 and 2 mg/kg for five days and their corresponding single 5 and 10 mg/kg doses were not effective, we used five treatment arms for *C. parapsilosis sensu stricto* (no treatment, 3 and 4 mg/kg caspofungin daily for five days; and the corresponding single doses of 15 and 20 mg/kg). In case of the echinocandin-resistant DPL20 *C. albicans* isolate, five treatment groups (no treatment, 4 and 16 mg/kg caspofungin daily for five days; and the corresponding single doses of 20 and 80 mg/kg) were used.

These dose groups were chosen based on the data that one, 2 and 4 mg/kg daily caspofungin doses produce maximum concentrations (C_{max}s) 3.9-5.9, 7.6-11.6 and 16-18.4 mg/L, respectively, (Flattery *et al.*, 2011) and single 5, 20 and 80 mg/kg caspofungin doses produce C_{max}s 18, 26 and 58 mg/L, respectively (Andes *et al.*, 2010).

All regimens were started 24 hours postinoculation; the drug was administered intraperitoneally in a 0.5 ml volume.

At the beginning of the therapy, fungal kidney burden was determined after dissection of four untreated mice in case of each isolate (day 1 control burden).

At the end of treatment, all mice were sacrificed; kidneys were removed, weighed and homogenized aseptically. Fungal tissue burden was determined by quantitative culturing. The lower limit of detection was 50 CFU/g of tissue. Kidney burden at day six was analyzed using the Kruskal-Wallis test with Dunn's post-test (Földi *et al.*, 2012).

TABLE 1 - *Geometric means of MIC values of caspofungin against C. albicans, C. parapsilosis sensu stricto, C. orthopsilosis and C. metapsilosis isolates.*

Clinical isolates	*MIC (mg/L) (geometric mean)*
C. albicans 17433	0.019
C. albicans 10920	0.024
C. albicans DPL20	5.04
C. parapsilosis 896/1	1
C. parapsilosis 9150	1.26
C. orthopsilosis CP85	0.157
C. orthopsilosis CP125	0.198
C. metapsilosis CP5	0.25
C. metapsilosis CP86	0.198

RESULTS

Geometric means of MIC values of caspofungin against the seven isolates are shown in Table 1. According to the revised CLSI break-points, both *C. parapsilosis*, and two out of three *C. albicans* isolates (17433 and 10920) were susceptible to caspofungin (Pfaller *et al*., 2011). DPL20 *C. albicans* isolate was resistant to caspofungin; there is no formal clinical breakpoint for these species, although an MIC value ≥8 mg/L for caspofungin against *C. orthopsilosis* and *C. metapsilosis* is considered resistant (Pfaller *et al*., 2011).

At the beginning of therapy, fungal tissue burden ranges were $8.1x10^4$-$7.4x10^5$, $8.5x10^4$-

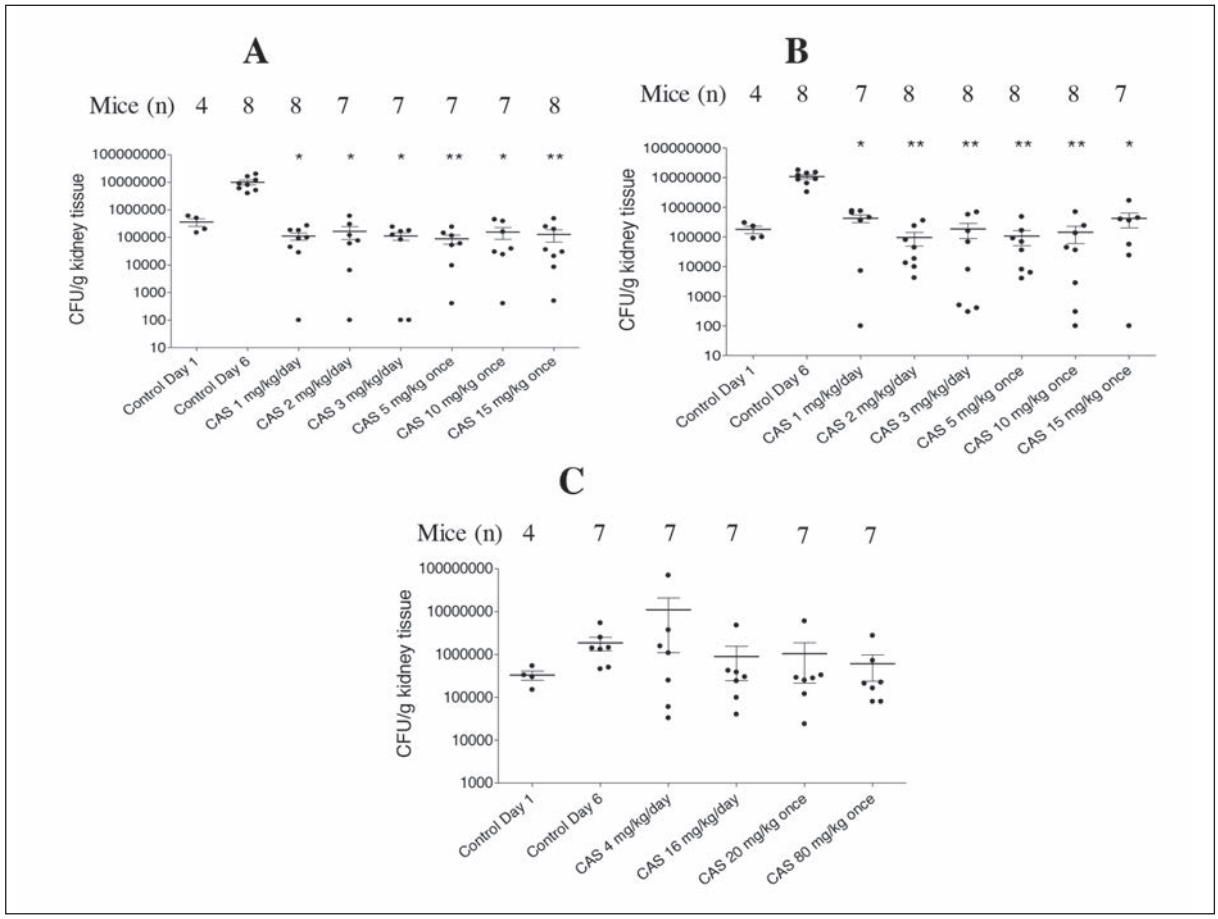

FIGURE 1 - *Kidney tissue burden of deeply neutropenic BALB/c mice infected intravenously with C. albicans 17433 (A), C. albicans 10920 (B), C. albicans 17433 C. albicans DPL20 (C) isolates. Fungal kidney tissue burden was determined at the beginning of therapy on day 1 to set the initial (day 1) control burden (control 1) and at the end of experiments on day 6 (control 6). The bars represent the medians. Level of statistical significance is indicated at P<0.05 (*), P<0.01 (**) and P<0.001 (***).*

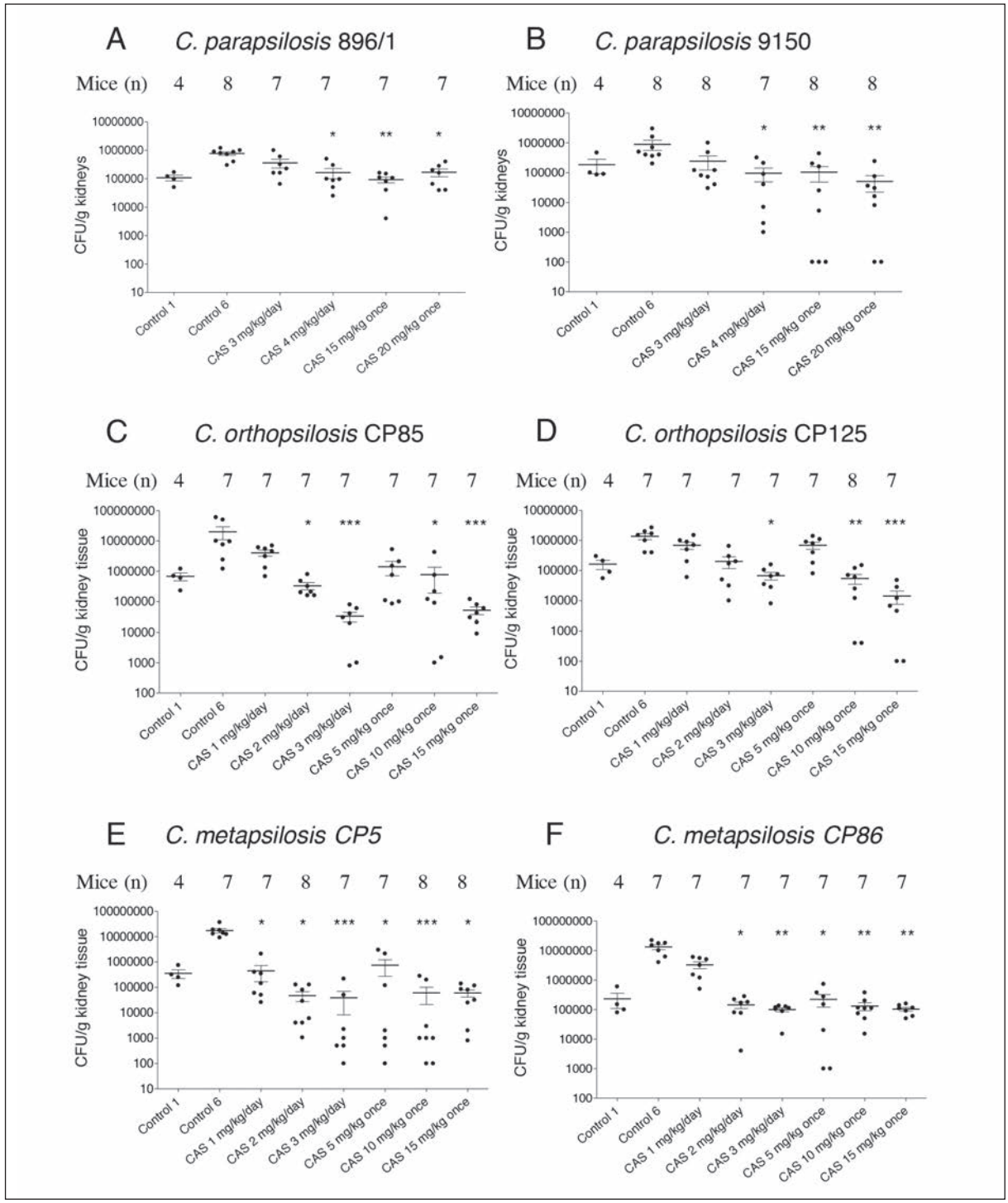

FIGURE 2 - *Kidney tissue burden of deeply neutropenic BALB/c mice infected intravenously with C. parapsilosis 896/1 (A) and C. parapsilosis 9150 (B), C. orthopsilosis CP85 (C) and C. orthopsilosis CP125 (D) and C. metapsilosis CP5 (E) and C. metapsilosis CP86 (F) isolates. Fungal kidney tissue burden was determined at the beginning of therapy on day 1 to set the initial (day 1) control burden (control 1) and at the end of experiments on day 6 (control 6). The bars represent the medians. Level of statistical significance is indicated at P<0.05 (*), P<0.01 (**) and P<0.001 (***).*

$4.7x10^5$, $3.9x10^5$-$1.5x10^6$ and $9.1x10^4$-$7.7x10^5$ cells per mouse for *C. albicans*, *C. parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis*, respectively. Yeasts grew to <2, <1, <2 and <2 log units in cases of *C. albicans*, *C. parapsilosis sensu stricto, C. orthopsilosis* and *C. metapsilosis*, respectively, after six days in untreated control mice.

Results of the *in vivo* experiments are shown in Figures 1 and 2. For both caspofungin susceptible *C. albicans* isolates (Figure 1), all six treatment arms proved to be effective (P<0.05-0.01) compared to the untreated control on day six. However, the mean decrease of tissue burden by all treatment arms was less than 1 log unit when compared to day 1 control for both isolates. Against the fks-resistant DPL20 *C. albicans* isolate, there were no effective caspofungin doses (P>0.05 for all doses).

For *C. parapsilosis* isolates, the kidney tissue fungal burdens were significantly decreased by a daily 4 mg/kg caspofungin dose and 15 and 20 mg/kg single caspofungin doses when compared to the untreated group on day six (P<0.05-0.01). Against these isolates, the daily 3 mg/kg caspofungin was ineffective. The mean decrease of tissue burden by all treatment arms was less than 1 log units when compared to day 1 control for both isolates.

Against *C. orthopsilosis* isolates, the highest daily dose (3 mg/kg) and 10 and 15 mg/kg single caspofungin doses were effective in reducing the fungal burden (P<0.05-0.01). In the case of *C. orthopsilosis* CP85 isolate, 2 mg/kg daily dose of caspofungin was also effective in reducing tissue burdens (P<0.05). A decrease in the mean tissue burden was noted only for the *C. orthopsilosis* CP85 isolate in groups treated with 3 mg/kg daily and with 15 mg/kg single dose of caspofungin when compared to day 1 control (less than 1 log unit).

All treatment arms, with the exception of a daily 1 mg/kg caspofungin in the CP86 isolate were effective against both *C. metapsilosis* isolates (P<0.05-<0.001). However, we noticed less than a 1 log unit mean tissue burden decrease when compared to day 1 control for both isolates.

In multiple comparisons with *C. orthopsilosis* CP85, caspofungin 3 mg/kg daily dose and 15 mg/kg single dose were superior to caspofungin 1 mg/kg daily (P<0.01 in both cases). For *C. orthopsilosis* CP125, caspofungin at a 15 mg/kg single dose was superior to caspofungin at a 5 mg/kg single dose and caspofungin at a 1 mg/kg daily dose (P<0.01 in both cases). For *C. parapsilosis* and *C. metapsilosis* isolates, no major differences between caspofungin doses were found.

DISCUSSION

Pharmacokinetic studies have shown that echinocandins distribute to and accumulate in target organ tissues. The maximum serum echinocandin concentration develops within 2 hours, while the peak tissue concentration is delayed with 1.5-2 days as noted in animal models and human studies (Louie *et al.*, 2005). Tissues serve as drug reservoirs from which the drug is released slowly, therefore strongly influencing the terminal half-life of echinocandins (Louie *et al.*, 2005). Importantly, the measured echinocandin concentrations in tissues were higher than the MIC_{90} for most *Candida* species (Andes *et al.*, 2010; Louie *et al.*, 2005). Although echinocandins are highly protein-bound antifungals, tissue echinocandin concentrations exert an antifungal effect against *Candida* species. This fact is important, because primarily tissue not serum echinocandin levels determine the outcome (Gumbo, 2007; Louie *et al.*, 2005).

Although a good tolerability of echinocandins was observed in dose escalation trials (Cornely *et al.*, 2011; Migoya *et al.*, 2011; Stone *et al.*, 2002), studies with alternate-day dosing of echinocandins are scant (Andes *et al.*, 2013; Buell *et al.*, 2005; Mehta *et al.*, 2010). However, all three studies with micafungin suggest that echinocandin therapy administered 1-3 times per week is a viable option.

The "psilosis" group is the second or third most important among bloodstream fungal pathogens, especially among children (Andes *et al.*, 2010; De Luca *et al.*, 2012; Pfaller *et al.*, 2011). Echinocandins are not the first choice against *C. parapsilosis sensu lato* according to the current guideline of the Infectious Diseases Society of America (Pappas *et al.*, 2009). This recommendation is supported by animal studies, where only high caspofungin doses produced significant decreases in kidney tissue burden

when compared to the untreated controls (Földi *et al*., 2012; Spreghini *et al*., 2012). Moreover, micafungin and anidulafungin proved to be ineffective against *C. parapsilosis sensu stricto* even at 10 mg/kg daily doses which correspond to >100 mg daily human dose (Spreghini *et al*., 2012). This is not unique to the "psilosis" group, as Howard *et al*. (2011) found that only the highest 20 mg/kg dose led to 1 log or higher decrease in the kidney fungal burden in severely neutropenic mice in an invasive *C. glabrata* infection model. This low decrease found by Howard *et al.* (2011) as well as in the present study is probably due to the mainly fungistatic activity of echinocandins in such models, where the immune system also contributes relatively poorly to elimination of fungi.

However, there are clinical situations where echinocandins are the preferable therapy against the "psilosis" group. For patients with severe liver diseases, triazoles are not the first choice for the treatment of invasive candidasis (Pappas *et al*., 2009). Furthermore, in case of invasive candidasis the pathogen is frequently unknown at the beginning of therapy and echinocandins are the first-line antifungals. If the pathogen proves to be *C. parapsilosis* but the patient clinically improves, switching to another antifungal group is not always recommended (Pappas *et al*., 2009).

In accordance with previous studies (Andes *et al*., 2010; Bayegan *et al.*, 2011), the daily and the single higher doses of caspofungin with the same cumulative dose proved to be effective against *C. albicans* 17433 and 10920 isolates in our severely neutropenic murine model treated with different caspofungin regimens. However, in the case of the echinocandin-resistant *C. albicans* DPL20 strain even the highest 16 mg/kg daily and the single 80 mg/kg doses were totally ineffective. Similar results were obtained by Wiederhold *et al.*, (2011) and Slater *et al.*, (2011) who did not find reductions in kidney fungal burden even by 10 mg/kg daily caspofungin against two echinocandin-resistant *C. albicans* isolates (caspofungin MICs were 4 mg/L) in immunocompetent mice.

In the present study, in agreement with previous data (Földi *et al*., 2012; Spreghini *et al*., 2012), daily caspofungin doses were effective against the "psilosis" group; however, effective treatment of *C. parapsilosis sensu stricto* required the highest doses tested. Single high dose caspofungin regimens were not inferior when compared to the traditional daily treatment arms with same cumulative doses. Moreover, a single high dose of caspofungin showed better efficacy than the divided daily doses against *C. parapsilosis sensu stricto* (single 15 mg/kg of caspofungin versus 3 mg/kg daily doses for five days in case of both isolates tested), against *C. orthopsilosis* (single 10 mg/kg of caspofungin versus 2 mg/kg daily doses for five days in case of the CP125 isolate) and against *C. metapsilosis* (single 5 mg/kg of caspofungin versus 1 mg/kg daily doses for five days in case of the CP86 isolate). A probable explanation is that in the daily treatment arms the last one or two doses were at least partially lost for the therapy and could not fully contribute to the total AUC (Louie *et al*., 2005). Moreover, single higher caspofungin doses produce higher C_{max} than the smaller, daily doses with same cumulative dose. As echinocandin efficacy is correlated with C_{max}/MIC as well as AUC/MIC, higher echinocandin peak concentrations may potentially lead to a better outcome of invasive candidasis (Andes *et al.*, 2010; Flattery *et al*., 2011; Louie *et al*., 2005).

In summary, single large dose caspofungin regimens were comparable or sometimes superior to the traditional daily-dose regimen against the echinocandin-susceptible *C. albicans* and *C. parapsilosis* isolates as well against *C. orthopsilosis* and *C. metapsilosis* isolates. Single large caspofungin doses were not effective in overcoming resistance in the *C. albicans* isolate DPL20. However, it should be noted that caspofungin (and probably other echinocandins) may not be the best choice against the "psilosis" group, because even the most effective doses produced only weak or no kidney tissue burden decreases when compared to the day 1 control (modeling the tissue burden at the initiation of therapy). This suggests a potential fungistatic effect of caspofungin against the "psilosis" group as also predicted by *in vitro* data (Földi *et al*., 2012; Spreghini *et al*., 2012). Further studies are needed to validate the claim that single larger echinocandin doses possess therapeutic benefit over the traditional smaller daily doses.

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Transparency declaration

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