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Infection and Drug Resistance

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ORIGINAL RESEARCH

# Efficacy of humanized single large doses of caspofungin on the lethality and fungal tissue burden in a deeply neutropenic murine model against *Candida albicans* and *Candida dubliniensis*

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**Background:** Echinocandins are the first-line therapy for treatment of invasive *Candida* infections, but the mortality rate remains high, calling for novel strategies. Giving single larger echinocandin doses infrequently is an alternative regimen. Our aim was to test this novel approach in a neutropenic murine model.

**Materials and methods:** We compared the in vivo efficacy of single 10 and 40 mg/kg of caspofungin (2.5× and 10× the normal humanized dose) to that of the same cumulative doses of daily 2 and 8 mg/kg doses for 5 days against 2 each of wild-type *C. albicans* and *C. dubliniensis* as well as echinocandin resistant *C. albicans*. As a comparator, we tested daily 1 mg/kg amphotericin B.

**Results:** In lethality experiments, all caspofungin and amphotericin B regimens improved survival against wild-type *C. albicans* and *C. dubliniensis* clinical isolates ($P<0.0001$) and decreased the mean fungal kidney burdens of both species compared to controls. However, fungal kidney burden decreases were not always statistically significant, especially with single 10 or 40 mg/kg caspofungin doses. Amphotericin B was the least active drug against wild-type *C. albicans*. Against echinocandin-resistant strains, monodose 40 mg/kg caspofungin and 1 mg/kg of daily amphotericin B were effective in lethality experiments. Although, significant kidney CFU decreases were never found, except for amphotericin B against one of the isolates ($p<0.05$ at day 3 and $p<0.001$ at day 6).

**Conclusion:** Single 40 mg/kg caspofungin and 1 mg/kg amphotericin B proved to be effective in the lethality experiments against wild-type and echinocandin-resistant *C. albicans* and wild-type *C. dubliniensis*. This was not always shown regarding fungal tissue burdens. Single caspofungin doses used in mice in this study are attainable in humans as well, suggesting a potential place of this dosing strategy not only in prevention but also in curative treatment of evolved invasive *Candida* infections.

**Keywords:** humanized caspofungin doses, intermittent dosing regimen of echinocandins, *Candida albicans* complex, fungal tissue burden, echinocandin resistance

## Introduction

The frequency of invasive *Candida* infections has increased worldwide in the last decades. Although the proportion of non-*albicans Candida* species increased significantly, *C. albicans* is still the most frequently isolated *Candida* species from normally sterile body sites.[1–5] The mortality rate of invasive *Candida* infections is around 40%, but in intensive care units, 50–75% mortality rates were observed

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among critically ill patients even in case of *C. albicans* despite the introduction of echinocandins into the antifungal armamentarium.[2–4]

Echinocandins (caspofungin, micafungin, and anidulafungin) inhibit the β-1,3-glucan-synthase in *Candida* species.[6–8] This antifungal class shows excellent in vitro and in vivo activity against *Candida* species and has quickly become the first therapeutic choice for the treatment of candidemia and other forms of invasive *Candida* infections.[9] Currently, echinocandins are administered as single daily intravenous doses with minimal side effects.[8,9]

The unacceptably high mortality rate caused by *Candida* infections has inspired physicians to give higher daily doses to severely ill patients as efficacy of echinocandins is correlated with AUC/MIC or $C_{max}$/MIC parameters.[6–8,10–14] However, dose escalation studies did not reveal any advantage in survival rate over the traditional lower, daily echinocandin doses. Another possible dosing strategy is using single, large echinocandin doses infrequently (eg, weekly) with the same cumulative dose as the daily divided doses.[12,14] Such a dosing strategy was followed only for prophylaxis of esophageal candidiasis (300 mg micafungin given every other day versus daily 150 mg),[12] but clinical data are still lacking as to how the mortality among patients with disseminated candidiasis is influenced by single larger echinocandin doses.

Therefore, the aim of our study was to determine the in vivo efficacy of single large caspofungin doses one day post-infection against four *C. albicans* (including two echinocandin resistant) and two *C. dubliniensis* isolates in a severely neutropenic murine model.

# Materials and methods

## In vitro studies

Two wild-type *C. albicans* isolates were derived from blood samples. Strain 3666 was isolated in 2004 from a 51-year-old male patient with *C. albicans* mediastinitis, while strain 2606 was isolated in 2014 from a 1-year-old girl with acute lymphoid leukemia. Two echinocandin-resistant *C. albicans* strains, DPL18 (F641S) and DPL20 (F645P), were isolated from normally sterile body sites during echinocandins treatment. *C. dubliniensis* isolates originated from our previous studies.[15] MICs of caspofungin and amphotericin-B (both from Sigma, Budapest, Hungary) were determined using the standard CLSI method (M27-A3) in RPMI-1640.[16] MIC values for caspofungin and amphotericin B were read visually after 24 hrs using the partial and total inhibition criteria, respectively. *C. africana*, the third member of the *C. albicans* complex was not tested because of its poor replication ability in vivo, even in a neutropenic murine model.[17] *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 strains were used as quality control strains.[16] All isolates were tested three times.

## In vivo studies

### Mice and immunosuppression

In the fungal tissue burden experiments BALB/c female mice (23–25 g) were given cyclophosphamide 4 days before infection (150 mg/kg), 1 day before infection (100 mg/kg), 2 and 5 days post-infection (100 mg/kg).[13,18,19] In the lethality experiments, this immunosuppression was continued by administration of 100 mg/kg cyclophosphamide every third day until the end of the experiment at the 21st day. The Guidelines for the Care and Use of Laboratory Animals were strictly followed during maintenance of the animals; experiments were approved by the Animal Care Committee of the University of Debrecen (permission no. 12/2014).

### Lethality experiments

Mice were assigned randomly to study groups (ten mice/group) and were infected intravenously through the lateral tail vein (day 0). The infectious dose for *C. albicans* and *C. dubliniensis* was $2\times10^4$ and $2\times10^5$ CFU/mouse, respectively, in volumes of 0.2 mL. Inoculum densities were confirmed by plating serial dilutions on Sabouraud agar plates.

Treatment with Caspofungin (Cancidas®, commercial preparation) and amphotericin B (Fungizone®, commercial preparation) began 24 hrs post-infection (day 1). Both drugs were purchased from our University Pharmacy and reconstituted according to the recommendation of the manufacturer. In case of wild-type *C. albicans* and *C. dubliniensis* isolates, six treatment groups were created (no treatment; 1 mg/kg amphotericin B, or 2 or 8 mg/kg caspofungin daily for 5 days; and the corresponding single caspofungin doses of 10 and 40 mg/kg). Higher single caspofungin doses (60 and 80 mg/kg) led to cca. 80% mortality within 1 day suggesting toxicity of Cancidas®. In case of the echinocandin-resistant *C. albicans* isolates, against which the daily 2 mg/kg for 5 days and its corresponding single 10 mg/kg doses were not effective (100% mortality within 4 days in preliminary experiments), we used 4 treatment arms (no treatment, 1 mg/kg amphotericin B daily for 5 days, 8 mg/kg caspofungin daily for

5 days and the corresponding single dose of 40 mg/kg). All treatments were given intraperitoneally in a 0.5 mL volume.

All groups were monitored twice daily for lethality.[18] After 21 days, survival rate was analyzed by Kaplan–Meier test; the effect of different caspofungin doses and amphotericin B was compared using the logrank test.[17–19]

### Fungal kidney tissue burden experiments

At the beginning of the therapy, fungal kidney burden was determined after dissection of three untreated mice in case of each isolate (day 1 control burden). Treatment groups were assigned as in the lethality experiment, 1 treatment group included 14–15 mice. The infectious doses for *C. albicans* and *C. dubliniensis* were $10^4$ and $10^5$ CFU/mouse, respectively.

On day 3 and 6, 7–8 mice were sacrificed; both kidneys from each animal were removed, weighed and homogenized aseptically in 1 mL of saline and serially diluted. Fungal tissue burden was determined by quantitative culturing. The lower limit of detection was 50 CFU/g of tissue. Kidney burden on day 3 and 6 was analyzed using the Kruskal–Wallis test with Dunn's post-test.[17–19]

### Pharmacokinetics of single 10 and 40 mg/kg caspofungin in mice

As the relationship of caspofungin doses (administered once) with $AUC_{0-\infty}$ as well as peak plasma concentration ($C_{max}$) values is linear in mice,[13] published pharmacokinetic data on single 5, 20 and 80 mg/kg caspofungin doses were used to calculate $AUC_{0-\infty}$ and $C_{max}$ values for single 10 and 40 mg/kg caspofungin doses using GraphPad Prism 8.0.1 for Windows (GraphPad Software Inc., La Jolla, CA, USA) as follows:

(a) $y=6.324x+170.7$; ($r^2=0.97$),
where $y$ is $AUC_{0-\infty}$ (mg·h/L), and $x$ is the dose of caspofungin (mg/kg),
(b) $y=0.5333x+15.33$; ($r^2=1$),
where $y$ is $C_{max}$ (mg/L), and $x$ is the caspofungin dose (mg/kg), with $r^2=1$.

As human single caspofungin doses between 5 and 210 mg also show similar pharmacokinetics,[20,21] the single human doses corresponding to single mouse doses were determined using mouse $AUC_{0-\infty}$values and the following equation:

(c) $y=1.809x-9.614$; ($r^2=0.9847$), where $y$ is $AUC_{0-\infty}$ (mg·h/L) and $x$ is the single caspofungin dose in human (mg).

# Results

## MIC results

MIC values for the quality control strains (*C. krusei* ATCC 6258 and *C. parapsilosis* 22019 ATCC strains) were within the published acceptable ranges.[16] Caspofungin MICs for wild-type *C. albicans* clinical isolates were within the accepted clinical break-points (Table 1).[16] DPL18 and DPL20 *C. albicans* isolates were resistant to caspofungin.[16] Caspofungin MICs of *C. dubliniensis* isolates were never higher than the published epidemiological cut off value for *C. dubliniensis* (0.125 mg/L) (Table 1).[22] Amphotericin B MICs for the six isolates were within the published ECOFF values (2 mg/L) for the two species.[23]

## Pharmacokinetics of single 10 and 40 mg/kg caspofungin in mice

Single 10 and 40 mg/kg caspofungin produce 234 and 424 mg·h/L AUC, and 20.7 and 36.7 mg/L $C_{max}$ values in mice, respectively. These AUC values in humans correspond to single 135 mg and 241 mg calculated caspofungin doses, respectively.

## Lethality

All caspofungin and amphotericin B regimens improved the survival in case of the wild-type *C. albicans* and *C. dubliniensis* clinical isolates ($P<0.0001$, Figure 1). At day 7, the survival rates were ≥90% for all treatment arms. However, survival rates decreased to 10–30% and 20–30%, respectively, by day 21 with daily 2 mg/kg and the corresponding single 10 mg/kg caspofungin doses in cases of *C. albicans* isolate 3666 and *C. dubliniensis*

**Table 1** MIC values of caspofungin and amphotericin B against *C. albicans* and *C. dubliniensis* isolates

| Isolates | MIC (mg/L) | |
|---|---|---|
| | Caspofungin | Amphotericin B |
| *C. albicans* 3666 | 0.25 | 1 |
| *C. albicans* 2606 | 0.125 | 1 |
| *C. albicans* DPL18 (F641S) | 2 | 0.5 |
| *C. albicans* DPL20 (F645P) | 8 | 0.5 |
| *C. dubliniensis* 1081 | 0.125 | 0.25 |
| *C. dubliniensis* 2953 | 0.125 | 0.25 |

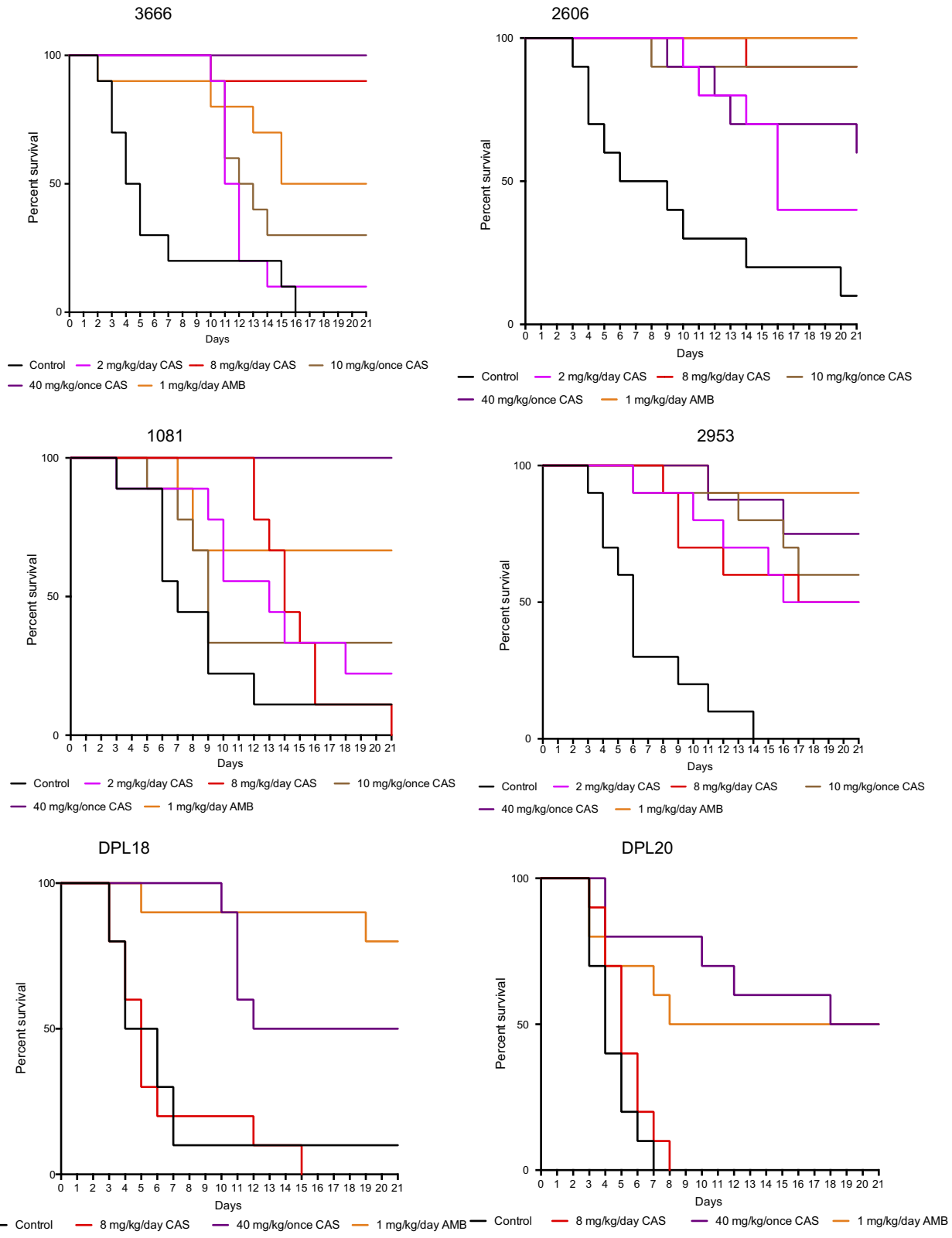


**Figure 1** Survival of mice infected with echinocandin-susceptible *C. albicans* (3666 and 2606), *C. dubliniensis* (1081 and 2953) and echinocandin-resistant *C. albicans* (DPL18 and DPL20). The infectious dose for echinocandin-susceptible and echinocandin-resistant *C. albicans* and echinocandin-susceptible *C. dubliniensis* were $2\times10^4$, $2\times10^4$ and $2\times10^5$ CFU/mouse, respectively. Intraperitoneal caspofungin and amphotericin B treatment were started 24 hrs after postinfection. After 21 days, the survival rate was analyzed by Kaplan–Meier test.

isolate 1081. In contrast, daily 8 mg/kg led to no survival by day 21, while the corresponding 40 mg/kg single dose resulted in 100% survival for these two isolates. Single dose 40 mg/kg was not inferior to the corresponding daily 8 mg/kg in case of the other two echinocandin susceptible isolates (2606 and 2953) either.

Survival rates of untreated controls at day 7 were lower in cases of echinocandin-resistant *C. albicans* isolates (0–10%) compared to wild-type isolates (20–50%). Daily 8 mg/kg of caspofungin did not improve the survival rates compared to untreated controls (10–20% within 7 days). In, contrast single 40 mg/kg caspofungin and 1 mg/kg amphotericin B increased the survival rates significantly (to 80–100% and to 70–90% at day 7, respectively; $P<0.0001$, Figure 1).

## Fungal kidney tissue burden

At the beginning of therapy, the mean fungal tissue burden ranges were $2.6\times10^5$–$3.1\times10^5$, $9.8\times10^4$–$1.1\times10^5$ and $4.8\times10^4$–$1.1\times10^5$ cells per mouse for wild-type *C. albicans*, echinocandin-resistant *C. albicans* and wild-type *C. dubliniensis*, respectively. Yeasts grew to 1.1–2.8, 1.2–1.4 and 2.8–3.3 log units in cases of wild-type *C. albicans*, echinocandin-resistant *C. albicans* and wild-type *C. dubliniensis*, respectively, after 6 days in untreated control mice.

With wild-type *C. albicans* and *C. dubliniensis*, all caspofungin treatment arms decreased the mean fungal kidney burdens compared to controls on day 3 or 6, but the fungal kidney burden decreases were not always statistically significant (Figure 2), especially for single 10 or 40 mg/kg doses. Fungicidal activity of daily 2 and 8 mg/kg of caspofungin (>3 log decreases) was frequently observed for *C. dubliniensis* at day 6. In contrast, with *C. dubliniensis* isolate 1081 the fungal tissue burden was higher on days 3 and 6 compared to day 1 burden with single 10 and 40 mg/kg caspofungin. Amphotericin B treatment yielded higher fungal kidney burdens on day 6 (but not on day 3) in case of *C. albicans* than in the case of *C. dubliniensis*.

Against echinocandin-resistant *C. albicans* strain DPL18 (Fks-F641S), single 40 mg/kg caspofungin and 1 mg/kg amphotericin B led to 0.7 and 1.2, and 0.8 and 2.0 log CFU decrease comparing to day 3 and 6 controls, respectively (Figure 3), of which only amphotericin B produced significant decrease ($P<0.05$ and 0.001 on day 3 and 6, respectively). Against the DPL20 (Fks-F645P) isolate daily 8 mg/kg caspofungin, single 40 mg/kg caspofungin and 1 mg/kg amphotericin B produced 0.08, 0.25 and 0.33 log CFU decrease only on day 6, respectively (on day 3 no CFU decrease was detected in any treatment arm), but these were not statistically significant ($P>0.05$ for all doses).

# Discussion

Increasing amounts of data suggest that the currently used standard echinocandin doses (caspofungin: 70 mg loading dose, then 50 mg daily; anidulafungin: 200-mg loading dose and then 100 mg daily; or micafungin: 100 mg daily) may be insufficient to cure invasive *Candida* infections among certain severely ill patients.[10,11] The standard echinocandin dosing regimen may result in low echinocandin exposure in intensive care units, among patients with obesity, with severe burn injuries and patients with complicated intra-abdominal infections.[24–28] In order to increase the probability of attaining therapeutic concentrations in infected body sites, several studies experimented with higher daily doses in treating invasive *Candida* infections. However, elevated daily doses of caspofungin and micafungin did not increase cure rates significantly.[10,11] Our previous studies in a neutropenic murine model also confirmed that there were no significant differences in fungal tissue burden decreases between daily 3 mg/kg, 5 mg/kg and 15 or 20 mg/kg caspofungin against echinocandin-susceptible *C. albicans, C. glabrata* and *C. krusei*, respectively.[29–31] Against echinocandin-resistant *C. albican*s and *C. glabrata* even 16 or 20 mg/kg caspofungin were ineffective in reducing the fungal kidneys burden.[19,29,30]

Administering single larger echinocandin doses infrequently is another therapeutic option compared to divided smaller daily doses with the same cumulative doses. Fungal tissue burden experiments showed that single larger echinocandin doses were not inferior compared to daily treatment with the same cumulative dose for prophylaxis and treatment of invasive candidiases.[19,32–35] Moreover, single larger aminocandin (an early echinocandin that stuck in phase I clinical trial) doses against *C. albicans* postinfection significantly increased the survival rates in a disseminated immunocompetent murine model.[35] Infrequently given large doses are the recommended treatment regimen for a next-generation echinocandin agent, rezafungin (CD101); in phase 3 for treatment of candidaemia and invasive candidiasis. This dosing recommendation is based on findings that infrequent larger doses showed better fungal elimination (lethality data were not obtained) than daily doses against *C. albicans*.[36]

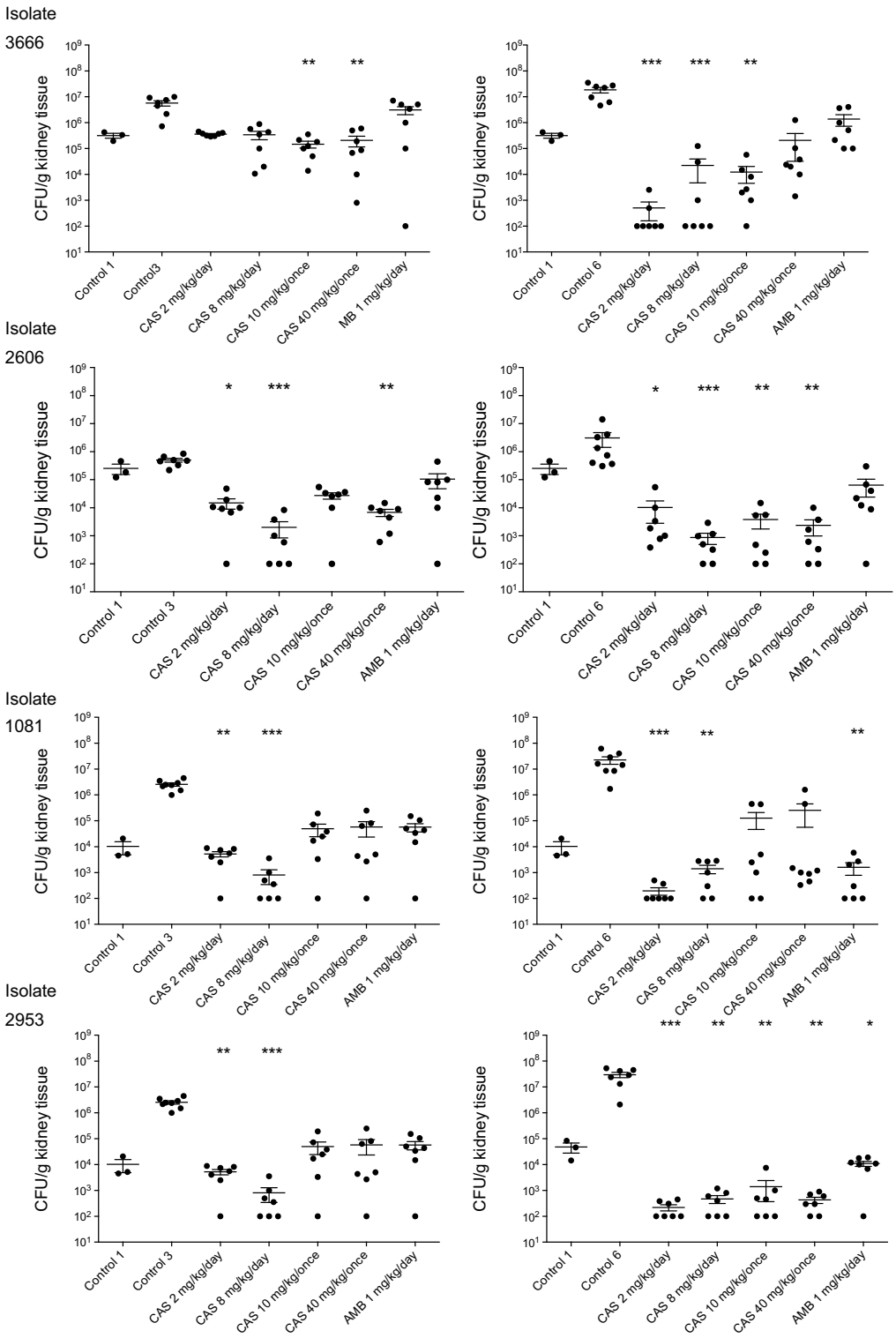


**Figure 2** Kidney tissue burden of profoundly neutropenic BALB/c mice infected intravenously with *C. albicans* 3666, *C. albicans* 2606, *C. dubliniensis* 1081 and *C. dubliniensis* 2953. Fungal kidney tissue burden was determined at day 3 (left panel) and at the end of experiments on day 6 (right panel). Day 1 tissue burden is shown in all graphs for comparison (Control 1). The bars represent the medians. Burdens of treated mice were compared to burdens of untreated controls measured on the same day of the experiment (Control 3 and Control 6 in the left and right panels, respectively). Level of statistical significance is indicated at $P<0.05$ (*), $P<0.01$ (**) and $P<0.001$ (***).

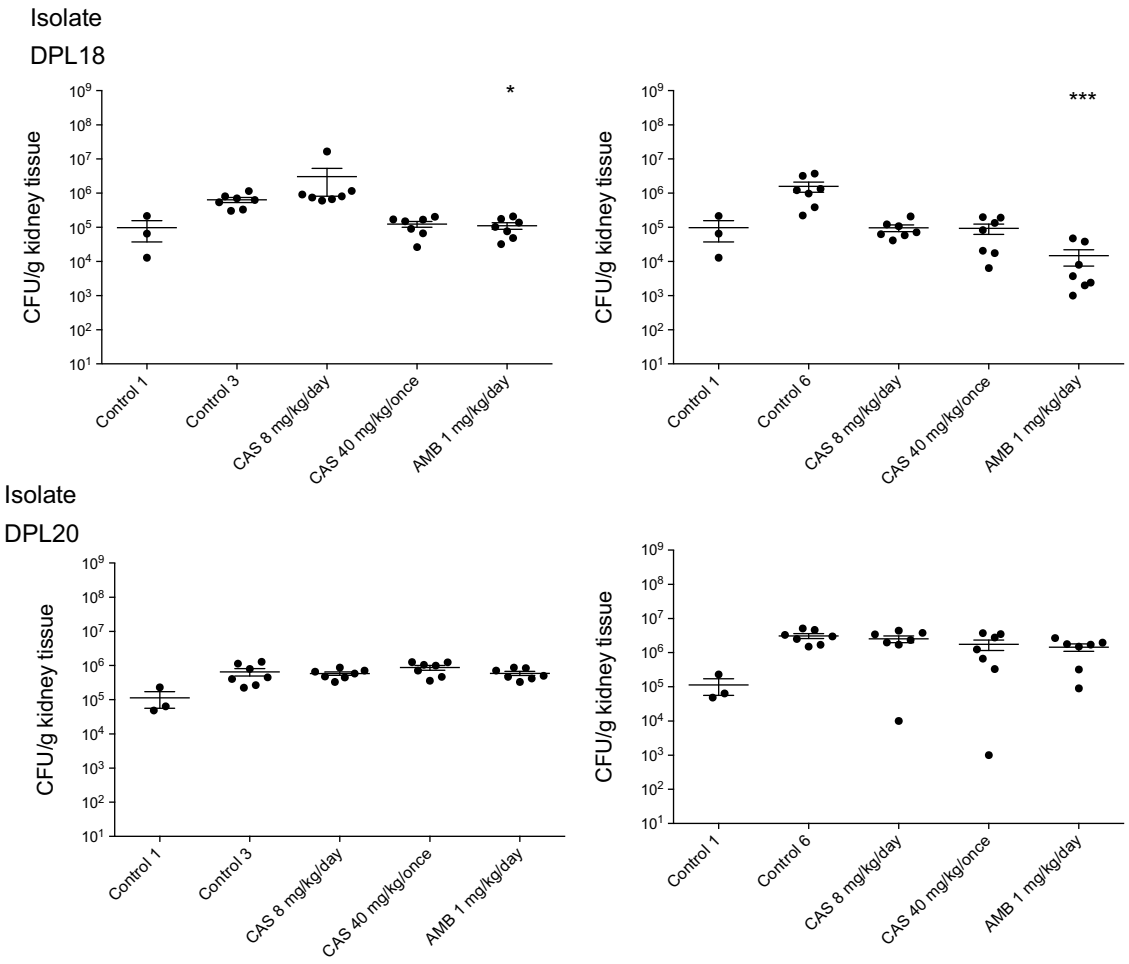


**Figure 3** Kidney tissue burden of profoundly neutropenic BALB/c mice infected intravenously with DPL18 and DPL20 echinocandin-resistant *C. albicans* isolates. Fungal kidney tissue burden was determined at day 3 (left panel) and at the end of experiments on day 6 (right panel). Day 1 tissue burden is shown in all graphs for comparison (Control 1). The bars represent the medians. Burdens of treated mice were compared to burdens of untreated controls measured on the same day of the experiment (Control 3 and Control 6 in the left and right panels, respectively). Level of statistical significance is indicated at $P<0.05$ (*) and $P<0.001$ (***).

In this study in the first 7 days, single 10 and 40 mg/kg caspofungin showed comparable efficacy on survival rates (90–100%) as the daily, 2 and 8 mg/kg doses against wild-type *C. albicans* and *C. dubliniensis* strains in a persistently neutropenic murine model. Moreover, the effect of single 40 mg/kg but not 10 mg/kg caspofungin on survival rate persisted in the next 14 days as well, increasing the survival rates up to 60–100% for both species. This dose-dependent effect is not surprising, as single 40 mg/kg caspofungin produced significantly higher AUC and $C_{max}$ values than single 10 mg/kg. Efficacy of echinocandins best correlates with a free-drug AUC/MIC (optimal ratio >10–20) or Cmax/MIC (optimal ratio >1) values.[6–9,13,14,37] AUC/MIC value ranges produced by 10 mg/kg single doses were 32.8–65.5 for wild-type *C. albicans* and *C. dubliniensis* isolates calculating with 3.5% free-caspofungin, while $C_{max}$/MIC values were 2.9–5.8. In case of single 40 mg/kg caspofungin the free-drug AUC/MIC and $C_{max}$/MIC values were as high as 59.4–118.7 and 5.2–10.3, respectively. Fungal tissue burdens on day 3 and 6 were concordant with our lethality results at day 7; all regimens produced at least 1.95 log decrease in day 6 tissue burden. However, in cases of echinocandin-resistant *C. albicans* strains, the free-caspofungin AUC/MIC and $C_{max}$/MIC values even with the 40 mg/kg dose only were 1.9–7.4 and 0.2–0.6, respectively, predicting poor outcome in vivo. This was applicable to the fungal burden data, as the single large dose produced lower tissue burden decreases, but not for lethality data, where 7-day lethality was comparable, while 21-day lethality was more favorable in the 40 mg/kg caspofungin arm.

This is in line with the widely accepted opinion that paradoxical growth may be confined to in vitro situations.[10,11,14,32]

Similar difference between lethality and fungal burden results was reported by Najvar et al,[35] comparing 5 and 30 mg/kg single-dose caspofungin in immunocompetent murine bloodstream infection model. Such short-term therapies as used in animal models of fungal infections are almost always reported deficient in sterilizing activity; indicating that for eradication a long-term therapy is crucial.[9,29–31] Interestingly, the calculated pharmacodynamic parameters predicted the weaker activity on the fungal burden well, while prognosticated poorly the clinical effect, ie, increased survival.

Similarity of the lethality rates of untreated controls suggests that there are no significant differences in virulence between wild-type and echinocandin-resistant isolates in bloodstream infection of neutropenic mice. Our finding is different from the results by Ben-Ami et al who found that fitness of the homozygous fks-1 mutant (S645F) strains is significantly lower than for wild-type strain.[38] However, they used non-immunosuppressed BALB/c mice in their experiments. In accordance with the results of Wiederhold et al,[39] daily 8 mg/kg of caspofungin at day 7 yielded only 20% survival rates both for DPL 18 and DPL 20 isolates indicating that elevated daily doses of caspofungin did not result in survival benefit compared to untreated controls. In this study, single 40 mg/kg caspofungin and daily 1 mg/kg of amphotericin B produced 80–100% and 70–90% survival against echinocandin-resistant isolates at day 7, respectively. Moreover, single 40 mg/kg caspofungin prolonged the survival during the next 14 days as well, in spite of the low free-caspofungin AUC/MIC and $C_{max}$/MIC values.

The possible explanation for the efficacy of single 40 mg/kg of caspofungin against echinocandin-resistant isolates in the lethality experiments may be related to the protein-binding. Though in case of caspofungin the percent of the free fraction among healthy persons is 3.5% this value could be increased to 7.6% among persons with invasive *Candida* infections.[37,40] Calculating with 7.6% free-caspofungin the AUC/MIC and $C_{max}$/MIC values for isolate DPL18 (MIC=2 mg/L) were 15.9 and 1.38 which are higher than the recommended 10 and 1 ratios, respectively. In case of isolate DPL 18, the fungal tissue burden experiments were in accordance with the lethality experiments (the CFU values were not increased compared to day 1 control; fungistatic effect). However, for isolate DPL20 (MIC=8 mg/L) AUC/MIC and $C_{max}$/MIC were 3.9 and 0.34, respectively, confirming that echinocandin efficacy is weak in decreasing the fungal tissue burden against isolates very high MICs (ie, 8 mg/L) containing a prominent *FKS1* hot-spot amino acid substitution (S645F). Another potential explanation is that a dose larger even than loading doses leads to more rapid increase in tissue drug concentration, ie, the peak concentration will be higher and reached faster.[14,34,36,37]

Notably, survival rates in mice treated with single 40 mg/kg caspofungin were comparable in case of DPL20 isolate, in case of isolate DPL18 as well as in case of the echinocandin wild-type isolates.

Results obtained from our mice experiments can be translated to human as in earlier comparison outcomes in mice correlated well with treatment success in patients.[13,14,41] The basis for this similarity is that the drug target is within the organism and is independent of the host; thus, free-drug AUC/MIC or $C_{max}$/MIC needed for efficacy in a human must be the same as in a mouse. Single 10 and 40 mg/kg caspofungin produce 234 and 424 mg·h/L AUCs which correspond to single 135 and 241 mg caspofungin in humanized doses. The largest single doses of caspofungin used in healthy volunteers were 150 and 210 mg with minimal side effects suggesting that the maximum tolerated human dose still is not reached.[20,21] Inadvertent administration of up to 400 mg of caspofungin in one day did not result in clinically important adverse reactions.[40] Moreover, daily 200 mg of caspofungin was well tolerated in phase II dose escalation study for the treatment of probable aspergillosis.[42] Thus, elevated single dose of caspofungin (ie, 250 or 300 mg) probably can be used safely for the treatment of invasive *Candida* infections, though this assumption awaits confirmation.

In this study, we have found that humanized, single larger doses of caspofungin showed excellent in vivo efficacy both on the lethality as well as in fungal kidney burden experiments for the treatment of disseminated candidiasis caused by wild-type *C. albicans* and the closely related *C. dubliniensis* in a neutropenic murine model. It is noteworthy that not only 1 mg/kg of daily amphotericin B but single 40 mg/kg of caspofungin as well proved to be effective in survival studies with wild type and echinocandin-resistant *C. albicans* isolates with prominent mutations in the *fks* genes. However, this effect was not seen in the fungal tissue burden experiments. More importantly, single caspofungin doses for mice used in this study are attainable in humans as well, suggesting a potential place of this dosing strategy not only in prevention but also in treatment against evolved invasive *Candida* infections.

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## Disclosure

Mr Zoltán Tóth reports grants from the Ministry of Human Capacities, Government of Hungary, during the conduct of the study. Prof. Dr David Perlin reports a patent Pat # 9657334 issued, a patent Publ #20150125856 pending; and David Perlin serves on SABs and/or receives financial support from Merck, Astellas, Cidara, Scynexis, Amplyx, Martinas and N8. Dr Gábor Kardos reports grants from Hungarian Academy of Sciences, outside the submitted work. Ms Fruzsina Nagy reports grants from Ministry of Human Capacities, Government of Hungary, during the conduct of the study. Dr László Majoros reports grants from MSD, grants from Astellas, grants from Pfizer, outside the submitted work, and is currently part of an ongoing research project together with Cidara. The authors report no other conflicts of interest in this work.

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