Killing rates of caspofungin in 50 percent serum correlate with caspofungin efficacy against *Candida albicans* in a neutropenic murine model

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Running title: Serum-based susceptibility testing of echinocandins

Keywords: echinocandins, Etest, fungicidal effect, protein-binding, serum-based susceptibility testing, time-kill

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Abstract

Previous studies suggested that caspofungin dose escalation against *Candida* species is more beneficial than currently used lower daily doses. Thus, we determined *in vitro* and *in vivo* activity of caspofungin against six wild-type *C. albicans* clinical isolates, the ATCC 10231 strain and an echinocandin resistant strain. MIC ranges of clinical isolates in RPMI-1640 with and without 50% serum were 0.125-0.25 and 0.015-0.06 mg/L, respectively. Two and three isolates showed paradoxical growth in MIC and time-kill tests, respectively, in RPMI-1640 but not in 50% serum. Caspofungin killing rate (*k*) in RPMI-1640 at 1 mg/L was higher than at 16 and 32 mg/L for all isolates ($p<0.001$). Killing rates for five of six isolates were concentration independent between 1-32 mg/L in 50% serum ($p>0.05$ for all comparisons), but for one isolate *k* value at 32 mg/L was significantly lower that at 1-16 mg/L. Although *k* values at 1-32 mg/L showed a great variability in 50% serum (the lowest and highest *k* value ranges were 0.085-0.109 and 0.882-0.985 1/h, respectively), daily 3, 5 and 15 mg/kg caspofungin was effective in a neutropenic murine model against all isolates, without significant differences between the effective doses. This study confirms that paradoxical growth does not affect the *in vivo* efficacy of caspofungin. We demonstrated that dose escalation did not increase the efficacy of caspofungin against *C. albicans* either *in vitro* or *in vivo*. These results are in concordance with the clinical experience that efficacy of echinocandins does not increase at larger doses.

1. Introduction

Echinocandins show low MIC values and concentration-dependent fungicidal or fungistatic activity against the majority of *Candida* species in test media RPMI-1640 and antibiotic medium 3 [1-9]. Moreover, echinocandins proved to be highly effective in the treatment of invasive infections caused by *Candida* species both in animal models and in clinical situations [1-4, 10, 11].

Echinocandins are increasingly used as first line agents in candidemia and other types of deep-seated life-threatening infections [1-4, 10-12]. Unfortunately, mortality rate due to invasive *Candida* infections is still increasing (mean mortality rate around 40% at present) [10-13]. Although the basic illness is recognized as the most important risk factor of death among severely ill patients, appropriate antifungal treatment is a key factor for clinical success [2, 4, 10-13], therefore optimizing the echinocandin therapy is an urgent challenge.

As echinocandins are highly protein-bound antifungals (≥97.5%) serum or tissue proteins may alter their activity [1-5]. Both decreased and increased *in vitro* activities of echinocandins against *Candida* and *Aspergillus* species were demonstrated when the test medium was supplemented with 5-50% serum in the broth microdilution (BMD) MIC tests [14-18]. Time kill studies in 50% serum revealed decreased echinocandins activity against *Candida* species [19, 20].

In our previous studies we have compared the caspofungin killing rate in RPMI-1640 and RPMI-1640 plus 50% serum against *C. albicans*, *C. krusei* and *C. inconspicua* isolates [19, 20]. We noticed increased MIC values in RPMI-1640 plus 50% serum and concentration independent killing activity of caspofungin at 1-32 mg/L against the three species. Moreover, when the same isolates were tested with various caspofungin doses in a neutropenic murine model in cases of *C. krusei* and *C. inconspicua* no differences were

found between effective doses in decreasing the fungal kidney tissue burden [19]. Such comparisons have nor yet been performed against the most frequency isolated *C. albicans*.

The aim of this study was to test the *in vivo* activity of caspofungin in a neutropenic murine model against *C. albicans* clinical isolates. In order to compare *in vivo* activity to *in vitro* efficacy, caspofungin killing rate in RPMI-1640 and RPMI-1640 plus 50% serum were determined.

2. Materials and methods

2.1. Strains

We studied six randomly selected *C. albicans* bloodstream strains isolated in 2014 in our Laboratory (Table **1**). All isolates were unique and first isolates (isolated prior to antifungal administration). Isolates were identified with conventional methods and MALDI Biotyper (Bruker, Bremen, Germany) [20]. In the preliminary experiments two isolates (10781 and 34350) showed paradoxical growth (PG) in the BMD test. PG is defined as growth at supra-MICs [6, 7]. Two echinocandin resistant strains, DPL18 (F641S) and DPL20 (F645P) and three ATCC type strains (*C. albicans* ATCC 10231, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019) were also evaluated in the study [20, 21].

2.2 Susceptibility testing

Two test media were used, RPMI-1640 as recommended by CLSI (referred to as RPMI) and RPMI-1640 supplemented with 50% human serum from a human male, type AB, Sigma, Budapest, Hungary (referred to as 50% serum) [16-20, 22, 23]. Caspofungin (Sigma, Budapest, Hungary) MICs in RPMI and in 50% serum were determined simultaneously using the standard CLSI BMD method. Caspofungin final concentration

ranges were 0.015-32 mg/L. MICs were determined after 24 h according to the partial inhibition criterion [16-20, 23].

MIC values were also determined using Etest method (AB Biodisk, Sweden) [24]. Etest was carried out using freshly prepared RPMI 1640 agar supplemented with 2% glucose with and without 50% human serum. Etest was carried out according to the instructions of the manufacturer and the results were read after 24 hours [3, 23, 24].

2.3. Time-kill studies

As results for ATCC 10231 and the echinocandin resistant strain DPL20 were reported in previous studies [20], in killing studies we tested only the six *C. albicans* clinical isolates in the present study. Caspofungin activity was determined in RPMI-1640 with and without 50% human serum at 0.25, 1, 4, 8, 16 and 32 mg/L concentrations using a starting inoculum of ~10^5 cells/ml in a final volume of 10 ml [16-20]. Aliquots of 100 μl were removed after 0, 4, 8, 12, 24 and 48 hours of incubation, tenfold serial dilutions were prepared, and samples of dilutions (4x30 μl) were plated onto a single Sabouraud dextrose agar (SDA) and incubated at 35 °C for 48 hours [16-20]. All experiments were performed in both media with two repetitions.

2.4. Analysis of in vitro *data*

Caspofungin activity was defined as fungicidal when at least 99.9% reduction in viable cell count was observed as compared to the starting inoculums [6-9, 16-20, 22].

Killing kinetics at the tested concentrations were analysed in both media (RPMI and 50% serum), as described previously [8, 9, 19, 20]. Briefly, an exponential equation was fitted to the mean data at each time point: $N_t = N_0 \times e^{-kt}$, where N_t is the number of viable yeasts

at time t, N_0 is the number of viable yeasts in the initial inoculum, k is the killing rate, and t is the incubation time. Thus killing rate represents the overall killing capability of the drug, taking into account of killing at each tested concentration. Negative k values indicate growth and positive k values indicate killing. The goodness of fit for each isolate was assessed by the r^2 value ($r^2 \geq \pm 0.8$) [19, 20]. The mean times to achieve the fungicidal endpoint ($T99.9=3/k$) were calculated from the k values for each isolate and concentrations in both media [8, 9, 19].

Killing kinetics among isolates was compared using one-way ANOVA with Tukey's post-testing in either RPMI-1640 or 50% serum. The effect of the same drug concentration in RPMI-1640 and 50% serum was analysed using *T* test (with Welch's correction, where appropriate) [19, 20].

2.5. In vivo studies

Groups of seven to eight female BALB/c mice (20-22 g) were immunosuppressed with four doses of cyclophosphamide, i.e. 4 days before infection (150 mg/kg), 1 day before infection (100 mg/kg), 2 days postinfection (100 mg/kg) and 5 days postinfection (100 mg/kg) [16, 19, 20]. The Guidelines for the Care and Use of Laboratory Animals was strictly followed during maintenance of the animals; experiments were approved by the Animal Care Committee of the University of Debrecen (permission no. 12/2008). All six clinical isolates as well as the ATCC 10231 and the DPL20 strains were tested in *in vivo* model. Mice were inoculated intravenously through the lateral tail vein with an infectious dose of 7.5×10^4 CFU/mouse. Confirmation of inoculum density was performed using plating serial dilutions onto SDA plates [16, 19, 21].

Five-day intraperitoneal treatment with daily 1, 2, 3, 5 and 15 mg/kg caspofungin (Cancidas, commercial preparation) was started after 24 hours [16, 19, 21]. This dosing strategy was based on previous pharmacokinetic studies and on previous results of our

study group [16, 19, 21, 25-28]. On day six after infection, all mice were sacrificed; the kidneys were removed (both), weighed and homogenized aseptically. Homogenates were diluted tenfold; aliquots of 0.1 ml of the undiluted and diluted (1:10) homogenates were plated onto SDA plates and incubated at 35 °C for 48 h. The lower limit of detection was 50 CFU/g of tissue. Statistical analysis of the kidney burden was performed using the Kruskal-Wallis test with Dunn's post-test for multiple comparisons [16, 19, 21].

3. Results

3.1. In vitro studies

3.1.1. MIC results in RPMI-1640

MIC values are presented in Table **1**. Clinical isolates as well as the ATCC type strains were susceptible to caspofungin according to the revised CLSI breakpoints in RPMI in the BMD tests [3, 23]. Confirming the results from the preliminary experiments, isolates 10781 and 34350 showed PG in RPMI. The DPL20 and DPL 18 strains were resistant to caspofungin [3, 23].

Etest MICs were 2-≥8 times higher than MICs observed in the BMD (Table **1**). PG was not observed, the inhibition zone was clear for all clinical isolates. *C. albicans* and *C. parapsilosis* ATCC strains were susceptible to caspofungin, while *C. krusei* ATCC strain was intermediate susceptible to caspofungin (Table **1** and Fig. **1**) [3, 23]. MIC values for the two echinocandin resistant isolates were 2 and 32 mg/L (Table **1** and Fig. **1**)

155 *3.1.2. MIC results in RPMI-1640+50% serum*

156 MIC values in the BMD and Etest methods were 2-16-fold higher in 50% serum when
157 compared to the MICs obtained in RPMI without serum. MIC ranges of clinical isolates in
158 50% serum in the BMD and Etest test were 0.125-0.25 and 0.25-0.5 mg/L, respectively.
159 PG was never observed regardless of the method used (Table **1** and Fig. **1**).

160

161 *3.1.3. Time kill results in RPMI-1640 and RPMI-1640+50% serum*

162 Maximum log decrease values are shown in Table **2**; representative time-kill plots are
163 shown in Fig. **2**. The mean time to achieve 99.9% (T99.9) growth reduction from the
164 starting inoculums at different caspofungin concentrations are shown in Table **3**.

165 CFU decreases were isolate, concentration and medium dependent. Some isolates showed
166 re-growth in both media (Table **2**).

167 All isolates grew similarly well in both media; the mean time ranges of controls to growth
168 1 log in RPMI and 50% serum were 8.66-9.77 and 8.9-10.89 hours, respectively.

169 In RPMI, caspofungin against 14171 and 35035 isolates produced fungistatic effect,
170 however, at lower (0.25 and 1 mg/L) concentrations CFU decrease were higher than at 16
171 and 32 mg/L concentrations (Table 2.). Against isolate 18799, caspofungin was fungicidal
172 at ≥4 mg/L (Tables **2** and **3**). In cases of 5265, 10781 and 34350 isolates typical PG was
173 observed; fungicidal activity of caspofungin at lower concentrations and fungistatic effect
174 at 16 and 32 mg/L. The T99.9 values for these three isolates at 1-8 mg/L were short (<12 h,
175 Table **3**).

176 Caspofungin in 50% serum produced fungistatic effect against isolates 14171, 18799 and
177 35035 (Fig. **2** and Table **2**). Caspofungin killing activity in 50% serum was increased in

case of all three isolates which showed PG in RPMI-1640; all of these were killed at 4-32 mg/L within 10 hours (Tables **2** and **3**).

3.1.4. Killing rates in RPMI-1640

Caspofungin killing rates (*k*) were isolate and concentration dependent (Fig. **3**). There was a trend producing higher *k* values at lower (0.25 or 1 mg/L) concentrations, while *k* values were very low at 32 mg/L. For all isolates *k* values were significantly higher at 1 than at 16 and 32 mg/L ($P<0.001$). This paradoxical effect was the most prominent in cases of 35035 and 5265 isolates where *k* values at 1 and 32 mg/L were 0.314 and 0.295 1/h, and 0.021 and 0.011 1/h, respectively. Numerically, the highest *k* value was observed in case of 10781 isolate, at 1 mg/L (0.961 1/h) (Fig. **3**).

3.1.5. Killing rates in RPMI-1640 plus 50% serum

Killing rate values at 0.25 mg/L were negative in cases of isolates 14171, 35035 and 10781, indicating that growth occurred. For the remaining three isolates *k* value ranges at 0.25 mg/L were 0.093-0.398 1/h (Fig.**3**).

Killing rates for 18799, 35035, 5265, 10781 and 34350 isolates were concentration independent at 1-32 mg/L ($p>0.05$ for all comparisons). The lowest *k* value range was noticed in cases of isolate 18799 (0.085-0.109 1/h), while the highest range was found in case of isolate 10781 (0.882-0.985 1/h) (Fig. **3**).

Isolate 14171 behaved differently. Killing rate values at 1, 4, 8 and 16 mg/L (*k* value range was 0.241-0.271 1/h) were significantly higher that at 32 mg/L (*k* value was 0.126 1/h) ($P<0.05$-0.001) (Fig. **3**).

3.1.6. Comparison of the killing kinetics at the same caspofungin concentrations in RPMI-1640 and 50% serum

Killing rates at 0.25 mg/L were higher in RPMI than in 50% serum for all isolates (P<0.05-0.001). In cases of isolates 14171 and 18799 *k* values were significantly higher at all tested concentrations in RPMI than in 50% serum, with the exception of 16 mg/L in case of 14171 and 32 mg/L in case of 18799 isolate (P<0.05-0.001).

Caspofungin killing activity at 4-32 mg/L against the remaining isolates increased in 50% serum when compared to RPMI (P<0.05-0.001) with the exception of the concentration of 8 mg/L in case of 5265, and of 4 and 32 mg/L in case of 34350 isolates.

3.2. *In vivo* experiments

All caspofungin doses decreased the fungal tissue burden for all tested clinical isolates (Fig. **4**). One mg/kg caspofungin did not decrease significantly the fungal tissue burden in cases of isolates 18799, 10781 and 34350; moreover 2 mg/kg also proved to be ineffective in case of isolate 10781. However, caspofungin doses of 3, 5 and 15 mg/kg proved to be effective for all isolates (P<0.05 to 0.001). The largest caspofungin dose produced the highest mean fungal tissue burden decreases in cases of isolates 18799, 5265 and 10781. Numerically, caspofungin doses of 2, 3 and 2 mg/kg produced the lowest mean fungal tissue burden in cases of isolates 14171, 35035 and 34350, respectively (Fig. **4**). However, statistically significant differences between the effective doses were never observed.

All doses but 1 mg/kg of caspofungin was effective against the ATCC type strain (P<0.05 to 0.001); there was no significant differences between the effective doses. Against the echinocandin resistant strain caspofungin proved to be ineffective regardless of the doses (data not shown).

4. Discussion

Frequency of invasive *Candida* infections is still increasing; though the non-albicans *Candida* species cause increasingly higher proportion of such infections, the most frequently isolated species is still *C. albicans* [3, 10-12]. Mean mortality rate is species dependent, the highest for *C. krusei* and *C. glabrata* (50-70%) and the lowest in case of *C. parapsilosis* (20-30%) [10-12]. The trend in the mortality did not change radically in the last decade, despite echinocandins were introduced into the antifungal armamentarium. Moreover, Lortholary and coworkers [13] noticed that the incidence and mortality of candidemia significantly increased in intensive care units (ICU), especially in case of *C. albicans* and *C. glabrata* infections. This trend is alarming because echinocandins usage increased from 4.6% to 48.5% between 2002 and 2010 among ICU patients in France [13]. Fortunately, the rate of resistance to echinocandins is low worldwide [2-4, 13]. These data strongly suggest that optimization of antifungal therapy is crucial not only in cases of fluconazole or echinocandin resistant *Candida* isolates [2, 3, 10-12] but also in cases of drug-naïve (wild-type) isolates, which are probably highly susceptible to antifungals [2, 3, 10-13].

Efficacy of echinocandins correlate with AUC/MIC (area under the concentration curve per MIC) or C_{max}/MIC (peak concentration per MIC), thus larger single or daily doses may lead to better clinical outcome [1, 2, 4, 5, 21, 28]. Betts and coworkers [29] used three times higher daily dose (150 mg) than the currently recommended (70 mg on day first followed by daily 50 mg) caspofungin doses for the treatment of adult patients with invasive candidasis [2-4, 11, 29]. The daily 150 mg caspofungin produced numerically but not statistically higher cure rates among patients suffering from invasive *C. albicans* and *C. parapsilosis* infections when compared to the currently recommended dosage strategy,

without causing severe side effects [29]. As echinocandins are not the first choice in case of invasive *C. parapsilosis* infections our attention was drawn to the examination of the efficacy of higher caspofungin doses against *C. albicans* [1-4, 11, 16, 21, 29].

In our study we used six clinical isolates which showed diverse behavior in killing studies using RPMI as test medium; caspofungin was fungicidal against one isolate, was fungistatic against two isolates and three isolates showed PG. Decreased activity of caspofungin at higher (16-32 mg/L) concentrations was confirmed by the lower *k* values for all isolates in RPMI. Paradoxical growth is associated with the induction of cell wall salvage mechanisms by high caspofungin concentrations, which leads to increased amount of chitin in the cell wall compensating for the decreased β-glucan level [22, 30]. The detected PG of caspofungin against *C. albicans* is consistent with the results from other studies [6, 17, 22, 30].

In concordance with previous results [17, 22, 30], paradoxically decreased activity of higher concentration of caspofungin was eliminated by 50% serum both in MIC and in time-kill tests in cases of isolates 5265, 10781 and 34350. Fifty percent serum restored the killing activity of caspofungin at higher concentrations as revealed by *k* values. On the contrary, in case of isolate 14171 the killing rate was higher at 32 mg/L in RPMI than in 50% serum.

The most notable finding of this study is that caspofungin showed concentration-independent activity in 50% serum at 1-32 mg/L against five out of six isolates as determined by the *k* values (the exception was again isolate 14171). However, the mean *k* value ranges were isolate dependent. We expected that isolates showing higher *k* values of caspofungin either in RPMI or in 50% serum will produce better efficacy in a severely neutropenic murine model, especially at the largest dose [5, 28, 29]. Caspofungin produced concentration-dependent *in vivo* efficacy against the clinical isolates and the ATCC type

strain, i.e. concentrations 3, 5 and 15 mg/kg, but not 1 and 2 mg/kg were uniformly effective against all tested strains. Moreover, the largest (15 mg/kg) caspofungin dose not only did not produce significantly better fungal tissue burden decrease than the lower effective doses 3 and 5 mg/kg, but sometimes the highest numerical decrease was found in case of lower doses. These *in vivo* results also confirm that PG has no effect on the *in vivo* efficacy of caspofungin against *C. albicans* [2, 3, 17, 30]. The comparable efficacy of the safely effective doses is in line with the killing rate results in 50% serum. We have found similar correlation of *in vitro* and *in vivo* efficacy against *C. krusei* and *C. inconspicua* [19]. In agreement with previous findings, caspofungin was ineffective against the homozygous *fks1* mutant strain DPL20 [21, 31]. Thus, our *in vivo* experiments support the currently recommended caspofungin dosing strategy (70 mg on day first followed by daily 50 mg), and indicate that dose-escalation has limited benefits against susceptible *C. albicans* in the clinic [2-4, 11, 29].

Currently, routine testing or reporting of CLSI and EUCAST MICs of caspofungin for different *Candida* species is not recommended, because of the unacceptably high interlaboratory variability in caspofungin MIC distribution in RPMI (wide MIC ranges coupled with bimodal MIC distributions) [2, 32]. Caspofungin MIC determination by Etest is not recommended either. Instead of caspofungin MIC determination both CLSI and EUCAST recommend testing for micafungin or anidulafungin MIC and these results should be interpreted for caspofungin susceptibility as well [2, 32].

In our study we used two types of serum-based susceptibility methods (BMD and Etest) to determine the MIC values for wild-type and echinocandin resistant strains. In order to detect PG by Etest, we used RPMI with and without 50% serum [24]; the serum-based Etest showed good correlation with serum-based BMD, hence, it may be applicable to determine caspofungin MICs both in case of susceptible and resistant isolates of *C.*

albicans (Fig. **1**). Although we used low number of wide-type isolates for MIC testing, it is notable that the MIC range was narrower than in RPMI (Table **1**). As serum-based susceptibility methods have not yet been standardized, they are not recommended currently for routine susceptibility testing of fungi [14, 23, 33]. The issues to be solved in the future include the optimal concentration of serum used in the tests, the origin (human or animal source), as well as the high cost of serum [33]. However, serum from animals (i.e. bovine serum) may replace human serum in the laboratories, decreasing the cost of serum-based MIC determinations [14]. Testing higher number of clinical *Candida* isolates with serum may provide a sufficient database to determine new epidemiological cutoff values and possible new clinical break-points for caspofungin against *Candida* species.

In spite of the extensive work on these antifungal agents, the mortality caused by invasive fungal infections is still increasing [10, 11, 12], highlighting the need for either new therapeutic agents, or other novel approaches. In parallel, the increasing resistance to antibacterial drugs induced research on enhancing the efficacy by modern delivery methods, e.g. linking the drugs with proteins [34] or incorporating them into nanoparticles or liposomes [35]. The latter approach also exists in antifungal therapy; three lipid formulations of amphotericin B are licensed. Research on lipid formulations of other antimycotics to improve efficacy [36] or to trap drugs at the site of infection [37] as well as on combination therapy [38] is also available.

5. Conclusion

Caspofungin activity was uniformly higher at lower than at higher concentrations in RPMI, with visible PG in MIC test or with prominent fungistatic activity at higher concentrations in time-kill tests. Fifty percent serum decreased the activity of caspofungin at 0.25 mg/L, but at 1-32 mg/L we noticed concentration independent killing activity. In a neutropenic murine model we demonstrated that caspofungin is equally effective against isolates showing and not showing PG *in vitro*, and dose escalation did not increase the efficacy of caspofungin against *C. albicans*. As serum-based susceptibility testing showed better predictive value of *in vivo* results than the currently recommended method, which is in accordance with the high protein binding of the echinocandin drugs, standardization of a serum-based protocol for susceptibility testing may be useful.

6. Conflict of interests

L. Majoros received conference travel grants from MSD, Astellas and Pfizer.

7. Acknowledgements

The authors are grateful to Dr. David S. Perlin for kindly providing the echinocandin resistant isolates. We thank Katalin Orosz-Tóth for the expert technical assistance.

R. Kovács was supported by the TÁMOP 4.2.4. A/2-11-1-2012-0001 ”National excellence Program-Elaborating and operating an inland student and researcher personal support system”. The project was subsidized by European Union and co-fianced by the European Social Fund.

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Figure legends

Figure **1**

Caspofungin MIC values in case of isolate 10781 as determined by Etest agar diffusion test. MIC values in RPMI-1640 (left) and RPMI-1640 supplemented with 50% serum (right) were 0.125 and 0.25 mg/L, respectively. Numbers on the Etest strip refer to caspofungin concentration in mg/L.

Figure **2**

Time-kill curves of caspofungin against *Candida albicans* isolate 14171 in RPMI-1640 (A) and 50% serum (C), as well as 10781 isolate in RPMI-1640 (B) and 50% serum (D). The broken lines represent the fungicidal limit (3-log decrease).

Figure **3**

Killing rates of caspofungin and the corresponding adjusted regression lines (dashed lines) against six *Candida albicans* clinical isolates in RPMI-1640 (RPMI) and RPMI-1640 plus 50% serum (Serum). Positive and negative *k* values indicate the decreases and increases, respectively, in viable cell numbers.

Figure **4**

Kidney tissue burden of severely neutropenic BALB/c mice infected intravenously with six *C. albicans* isolates. Intraperitoneal caspofungin (CAS) (1, 2, 3, 5 and 15 mg/kg) daily treatment was started 24 hours after the infection. The bars represent the medians. Level of statistical significance compared to the control population is indicated at $P<0.05$ (*), $P<0.01$ (**) and $P<0.001$ (***).

506 Figure **1**

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510 Figure **2**

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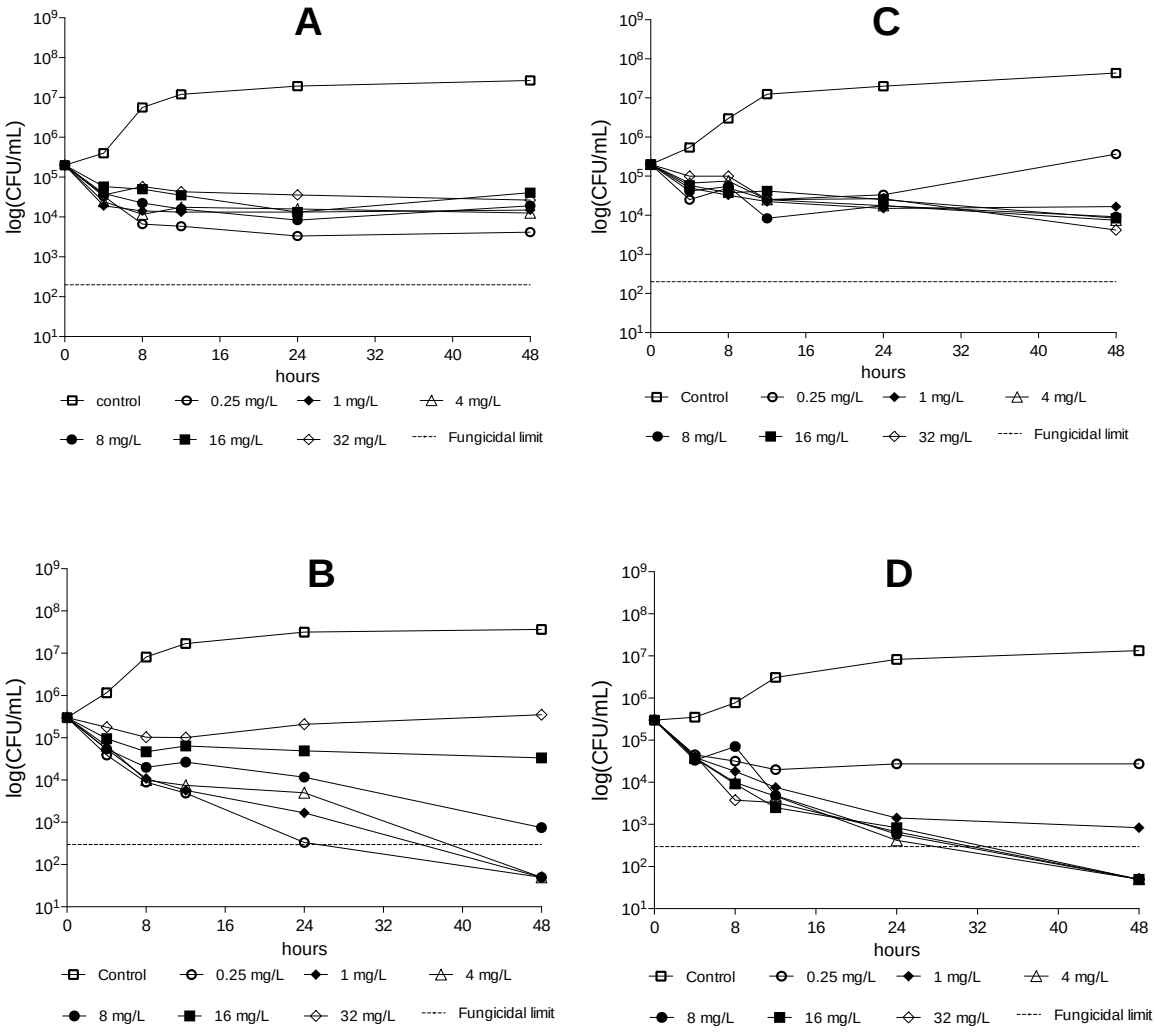

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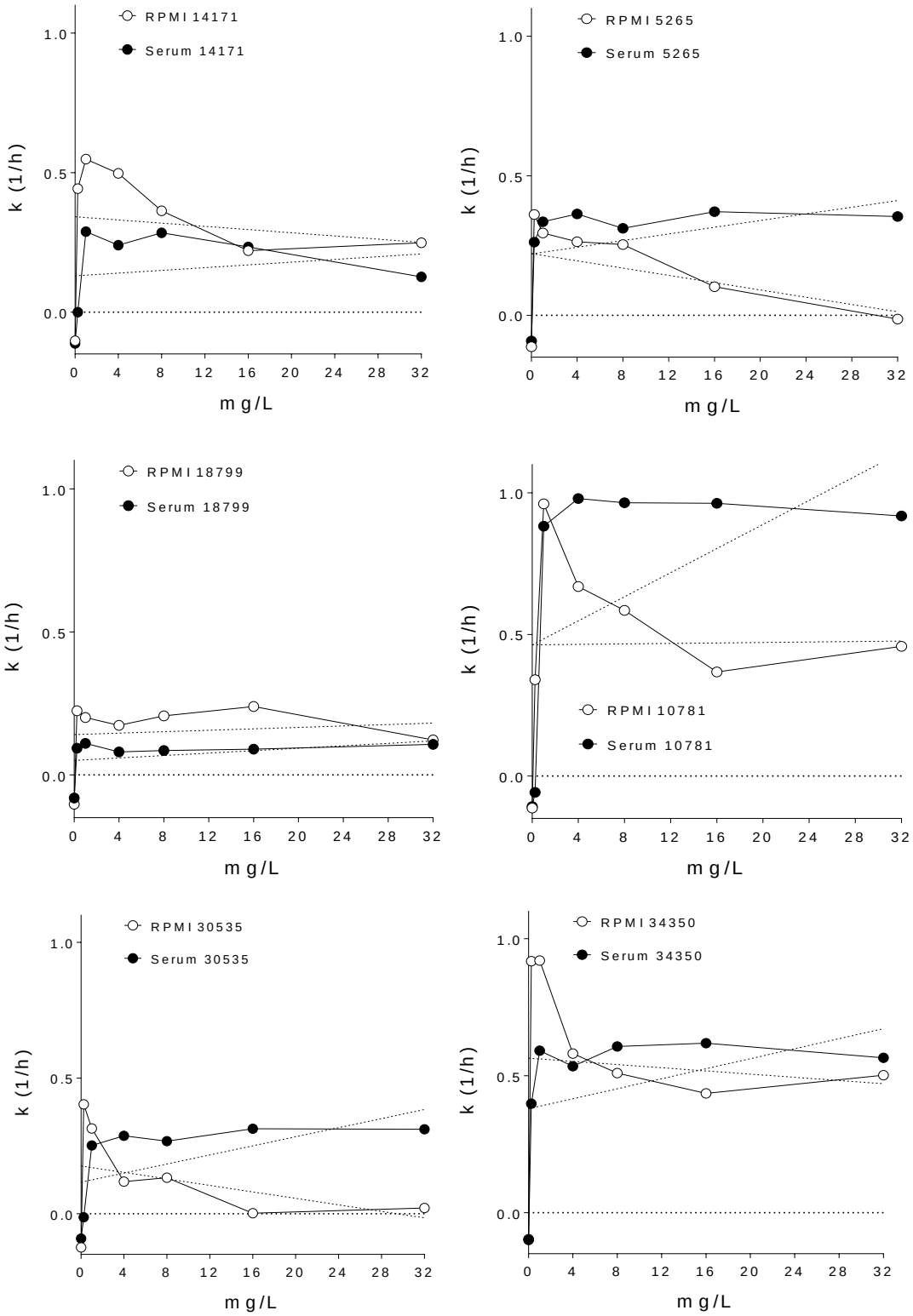
515 Figure **3**



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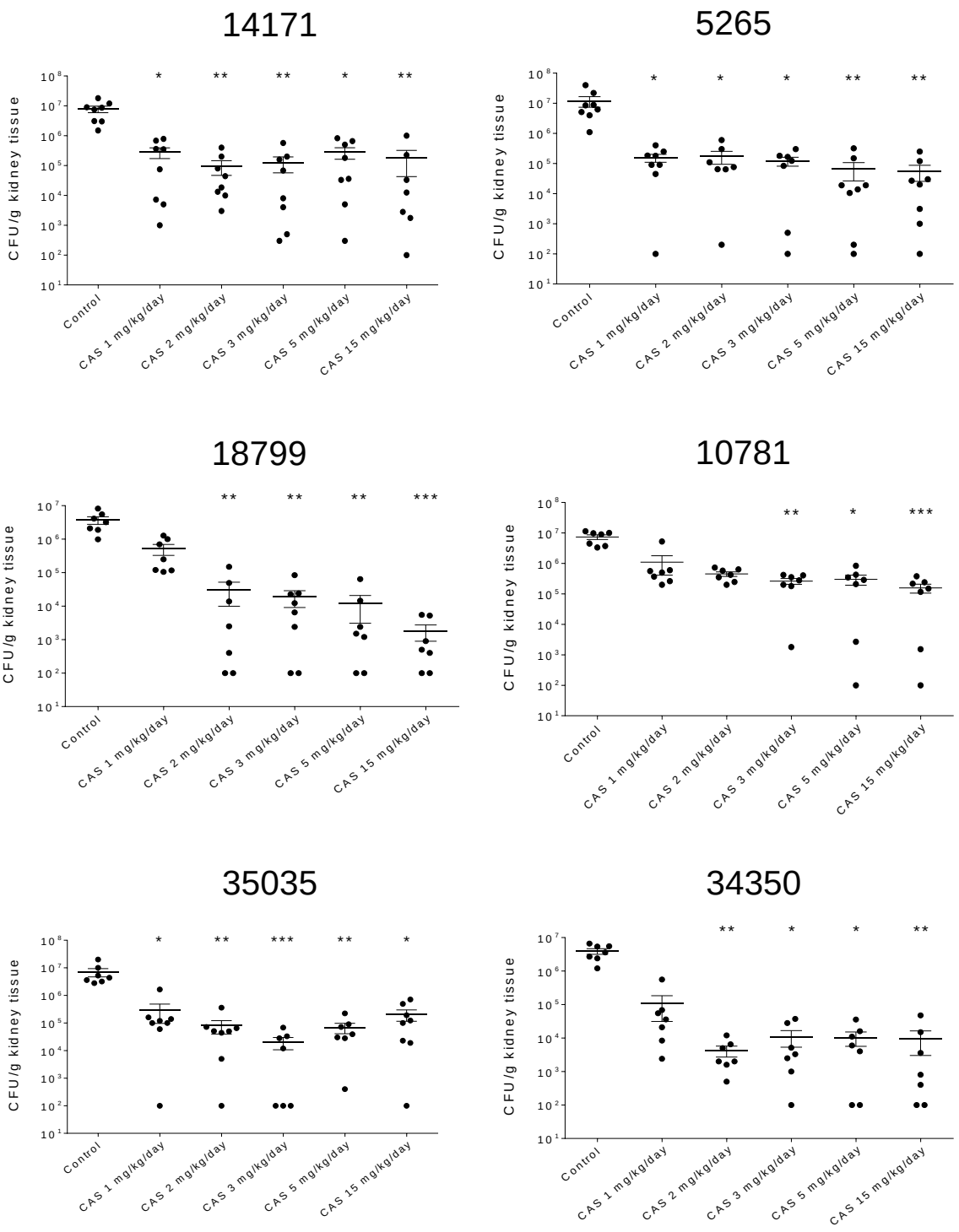

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523 **Table 1** *Candida albicans* isolates and MICs of caspofungin in RPMI-1640 (RPMI) and

524 RPMI-1640 plus 50 % serum (50% serum)

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Isolates	Broth microdilution MICs (mg/L)		Ratio (MIC_{RPMI}/ MIC_{serum})	Etest MICs (mg/L)		Ratio (MIC_{RPMI}/ MIC_{serum})
	RPMI	50% serum		RPMI	50% serum	
C. albicans ATCC 10231	0.03	0.5	16x	0.125	0.5	4x
C. albicans 14171	0.03	0.12	4x	0.125	0.25	2x
C. albicans 18799	0.06	0.12	2x	0.125	0.5	4x
C. albicans 35035	0.03	0.25	8x	0.125	0.5	4x
C. albicans 5265	0.015	0.25	16x	0.03	0.5	16x
C. albicans 10781	0.03^{PG}	0.12	4x	0.12	0.25	2x
C. albicans 34350	0.015^{PG}	0.25	16x	0.12	0.25	2x
C. albicans DPL20	4	>32	>8x	>32	>32	1x
C. albicans DPL18	2	16	8x	2	32	16x
C. krusei ATCC 6258	0.25	2	8x	0.5	8	16x
C. parapsilosis ATCC 22019	0.5	1	2x	1	2	2x

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528 PG: paradoxical growth

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Table **2** Maximum log changes in log CFU/mL compared to starting inoculum in time-kill studies in RPMI-1640 and RPMI-1640 plus 50 % serum (50% serum)

Isolate	Media	Maximum log decreases in CFU in time-killing experiments at the indicated caspofungin concentration (mg/L)					
		0.25	1	4	8	16	32
14171	RPMI-1640	-1.68	-1.12	-1.20	-1.38*	-0.69*	-0.88
	50% serum	-0.90*	-1.13	-1.43	-1.38*	-1.38	-1.68
18799	RPMI-1640	-2.60	-2.78	-3.00	-3.00	-3.00	-3.00
	50% serum	-0.89	-1.48	-1.71	-1.27	-1.88	-1.81
35035	RPMI-1640	-1.48	-2.68	-2.15	-1.15	-0.02	-0.38
	50% serum	-0.83*	-1.13	-2.10	-2.60	-1.38	-2.78
5265	RPMI-1640	-1.97*	-3.60	-3.60	-3.60	-1.68*	-2.08
	50% serum	-1.42*	-3.60	-3.60	-3.60	-3.60	-3.60
10781	RPMI-1640	-3.78	-3.78	-3.78	-2.60	-0.95	-0.16*
	50% serum	-1.18*	-2.65	-3.78	-3.78	-3.78	-3.78
34350	RPMI-1640	-3.52	-3.52	-3.52	-3.02	-2.09	-0.39*
	50% serum	-1.00	-3.52	-3.52	-3.52	-3.52	-3.52

*Re-growth occurred

Table **3** Time (hours, h) to reach 99.9% (T99.9) growth reduction from the starting inocula at different caspofungin concentrations (mg/L) in RPMI-1640 and RPMI-1640 plus 50 % serum (50% serum). Subtraction signs (-) mean that fungicidal effect was not achieved.

Isolate number	Media	Time (h)					
		0.25	1	4	8	16	32
14171	RPMI-1640	-	-	-	-	-	-
	50% serum	-	-	-	-	-	-
18799	RPMI-1640	-	-	17.31	14.54	12.56	24.53
	50% serum	-	-	-	-	-	-
30535	RPMI-1640	-	-	-	-	-	-
	50% serum	-	-	-	-	-	-
5265	RPMI-1640	-	10.18	11.38	11.83	-	-
	50% serum	-	8.96	8.27	9.68	8.08	8.48
10781	RPMI-1640	8.82	3.12	4.48	-	-	-
	50% serum	-	-	3.06	3.11	3.11	3.27
34350	RPMI-1640	3.27	3.26	5.17	5.87	-	-
	50% serum	-	5.08	5.61	4.94	4.85	5.31