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Evaluation of aminocandin and caspofungin against *Candida glabrata* including isolates with reduced caspofungin susceptibility

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Background: Aminocandin is an investigational echinocandin with excellent activity against *Candida* species, including *Candida albicans* and *Candida tropicalis*. However, few data are available for this agent versus *Candida glabrata*. We compared the *in vitro* potency and *in vivo* efficacy of aminocandin and caspofungin against clinical isolates of *C. glabrata* including those with reduced caspofungin susceptibility (MIC > 2 mg/L).

Methods: *In vitro* activity was assessed using microdilution broth susceptibility testing. Three isolates, one with a low and two with elevated caspofungin MICs, were chosen and mice were infected with *C. glabrata* followed by a single dose of aminocandin or caspofungin (0.5–100 mg/kg), or daily doses of caspofungin (0.07–14.3 mg/kg) begun 1 day after inoculation. Reduction in fungal burden, assessed in kidney tissue on day 8 post-inoculation, was the marker of antifungal response.

Results: Aminocandin was more potent than caspofungin against each isolate with reduced caspofungin susceptibility. Mice infected with the caspofungin-susceptible isolate had significant decreases in tissue burden with low doses of either drug. Higher single doses of aminocandin ($\geq$10 mg/kg) were required to reduce fungal burden against the two isolates with elevated caspofungin MICs. Single dose administration of caspofungin was ineffective against one of these isolates, and higher daily doses were required to reduce fungal burden.

Conclusions: These studies suggest that aminocandin has the potential for extended interval dosing in the treatment of *C. glabrata* infections caused by susceptible isolates. However, higher doses may be required against isolates with reduced caspofungin susceptibility.

Keywords: echinocandin, candidiasis, murine model

Introduction

Candida species are the most common causes of invasive fungal infections, ranging from oesophageal candidiasis to fulminating life-threatening candidiasis. Invasive candidiasis caused by *Candida glabrata* is of increasing concern due to the increased incidence and high mortality rates in patients with multiple comorbidities.[1–3] Furthermore, concern has also been raised about the utility of the azoles in the treatment of invasive infections caused by this species, as an increasing proportion of *C. glabrata* clinical isolates within the USA is resistant to fluconazole.[4–6] For serious

C. glabrata infections, the echinocandins are alternative first-line antifungal agents. *In vitro* and *in vivo* studies have demonstrated excellent potency and efficacy of these agents against the *Candida* species, including *C. glabrata*,[5] findings that appear to be supported by results from clinical trials.[7–10] However, concern over the development of resistance to these agents exists as 2% of *C. glabrata* isolates submitted to the Fungus Testing Laboratory of the University of Texas Health Science Center at San Antonio have caspofungin MIC values of >2 mg/L (A. Fothergill, Department of Pathology, University of Texas Health Science Center at San Antonio, personal communication).

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Aminocandin (IP-960, formerly HMR3270, Indevus, Lexington, MA, USA, now NXL201, Novexel, Romainville, France) is an investigational echinocandin with good *in vitro* potency and *in vivo* efficacy against many *Candida* species, including fluconazole-resistant *Candida albicans* and *Candida tropicalis*.[11,12] Like the other echinocandins, aminocandin is thought to act by non-competitive inhibition of the 1,3-β-D-glucan synthase enzyme complex, resulting in decreased synthesis of the major cell wall component 1,3-β-D-glucan. By inhibiting this fungus-specific target, echinocandins avoid many of the toxicities associated with other classes of antifungal agents.[13] Because of the long half-lives of these agents and the excellent tolerability observed in clinical trials and dosage escalation studies,[7–10,14–17] extended interval dosing has been proposed as a potential means of avoiding the requirement of daily intravenous therapy. Data from our group and other investigators have demonstrated this to be an effective strategy in murine models of invasive candidiasis.[18,19] However, this strategy has not been evaluated for aminocandin against *C. glabrata*, and data are lacking regarding its utility against strains with reduced susceptibility to this agent and caspofungin. Therefore, we sought to compare the *in vitro* potency and *in vivo* efficacy of aminocandin and caspofungin against clinical isolates of *C. glabrata*, including isolates with reduced susceptibility to these agents.

Materials and methods

In vitro susceptibility testing

Twelve clinical isolates of *C. glabrata* were obtained from the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio, USA. Eleven of the isolates had previously been identified as having markedly decreased susceptibility to caspofungin (MIC $\geq$ 4 mg/L), while the susceptible isolate, A3 (CAS-S), had a low MIC ($\leq$0.5 mg/L) to both aminocandin and caspofungin. Aminocandin (IP-960, Indevus) and caspofungin (Merck & Co., Inc., Whitehouse Station, NJ, USA) stock solutions were prepared in sterile water and serial 2-fold dilutions were prepared for each antifungal agent, according to the CLSI M27-A3 methodology in RPMI 1640.[20] Drug concentrations in the microdilution trays of both antifungal agents ranged from 0.015 to 16 mg/L. Yeast inocula were measured spectrophotometrically and diluted in sterile water to obtain final inocula concentrations between 0.5×10^3 and 2.5×10^3 cells/mL. *Candida parapsilosis* ATCC 22019 (caspofungin MIC 1 mg/L) and *Candida krusei* ATCC 6258 (caspofungin MIC 0.5 mg/L) were used as control organisms in this study. Trays were incubated at 35°C, and the MIC endpoints were defined as the first concentration of the antifungal agent at which the turbidity in the well was at least 50% less than in the drug-free growth control well.

Animals

Outbred male ICR mice with an average weight of 28 g were maintained five per cage and given food and water *ad libitum*. Studies were conducted using an isolate with a low MIC to both agents (A3; CAS-S isolate), an isolate with a low aminocandin MIC and an elevated caspofungin MIC (05-62; CAS-R1 isolate), and an isolate with an increased MIC to both agents (04-1748; CAS-R2 isolate). At least eight mice were included in each dose group against each isolate tested. This study was approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio (Approval No. 98094-34-C), and all animals were maintained in accordance with the American Association for Accreditation of Laboratory Animal Care.[21]

Test organisms for in vivo studies

The caspofungin-susceptible isolate of *C. glabrata* A3 (caspofungin MIC 0.25 mg/L; aminocandin MIC 0.5 mg/L) was obtained from the Fred Hutchinson Cancer Research Center in Seattle. Isolates with reduced caspofungin susceptibility, 05-62 (CAS-R1) and 04-1748 (CAS-R2), were obtained from the University of Texas Health Science Center at San Antonio Fungus Testing Laboratory. Each isolate was subcultured onto Sabouraud dextrose agar from frozen milk stock and grown for 2–3 days prior to animal inoculation. Colony-forming units (cfu) from each isolate were collected and suspended separately in physiological saline with 0.1% Tween 20. Each inocula was washed three times, final inocula concentrations were determined with a haemocytometer, and viability was confirmed by plating dilutions of each inoculum and enumerating the cfu.

Infection model and dosing regimens

On the day prior to infection, mice were made neutropenic by intravenous administration of 5-fluorouracil at 150 mg/kg of body weight. On day 0, mice were infected intravenously with *C. glabrata* isolates at 10^8 cfu/mouse. Caspofungin was obtained commercially as lyophilized powder and was reconstituted with sterile distilled water according to the manufacturer's instructions. Aminocandin powder was reconstituted with a 5% weight/volume mannitol (pH 6–7) as per the instructions from the manufacturer. Mice inoculated with the CAS-S isolate received 0.2 mL of caspofungin intraperitoneally (ip) or aminocandin intravenously (iv) at single doses of 2.5, 10, 40, 60 or 100 mg/kg. Another group of animals received daily ip doses of caspofungin 0.36, 1.4, 5.7, 8.6 or 14.3 mg/kg on days 1 through 7 (cumulative dose corresponding to single doses of 2.5, 10, 40, 60 and 100 mg/kg, respectively). Animals in the control group received a single 0.2 mL dose of 5% mannitol solution iv on day 1. Animals inoculated with the CAS-R1 and CAS-R2 isolates received single doses of caspofungin ip or aminocandin iv at 0.5, 1.0, 2.5, 10, 25, 40, 60 or 100 mg/kg beginning on day 1. Similar to animals infected with the CAS-S isolate, other groups of mice inoculated with the CAS-R1 isolate were treated with daily doses of caspofungin ip at 0.07, 0.14, 0.36, 1.4, 3.6, 5.7, 8.6 or 14.3 mg/kg on days 1 through 7 (cumulative dose corresponding to single doses of 0.5, 1.0, 2.5, 10, 40, 60 or 100 mg/kg, respectively).

Tissue burden

To assess tissue fungal burden, mice were euthanized on day 8 post-inoculation. Kidneys were removed, weighed and homogenized using a tissue homogenizer (Plytron dispensing and mixing technology PT 2100, Kinematica, Cincinnati, OH, USA) in 2 mL of sterile saline containing piperacillin and amikacin at 60 mg/L to suppress bacterial growth. Serial dilutions of homogenate were prepared and plated on Sabouraud dextrose agar. After 24–48 h of incubation at 37°C, colonies were counted and the number of cfu/g kidney tissue was calculated.

Data analysis

Differences in fungal burden endpoints (cfu/g) were assessed for significance using the Mann–Whitney test. A *P* value of $\leq$0.05 was

considered statistically significant for all comparisons. Mean and median tissue fungal burden values were plotted, and data were fitted to an inhibitory sigmoid model (modified Hill equation) using computer curve-fitting software (Prism 5; GraphPad Software, Inc., San Diego, CA, USA) to calculate the dose resulting in 50% reduction in colony-forming units (ED_{50}) compared with controls.

Table 1. MICs (mg/L) of aminocandin and caspofungin for *C. glabrata* isolates by microdilution methodology in RPMI

Isolate	Aminocandin	Caspofungin
A3 (CAS-S)	0.5	0.25
05-62 (CAS-R1)	0.5	4
04-1748 (CAS-R2)	2	>16
04-3525	0.125	>16
04-3144	2	>16
04-3477	4	>16
04-1026	4	>16
04-569	4	>16
04-2971	2	8
04-1400	4	8
04-2880	2	4
04-2596	1	>16

Results

Antifungal susceptibility testing

Aminocandin was more potent than caspofungin against each *C. glabrata* isolate tested. Against the isolates with reduced caspofungin susceptibility (MIC values ranged from 4 to >16 mg/L), aminocandin MICs ranged from 0.5 to 4 mg/L (Table 1).

Tissue burden with CAS-S

In the treatment of the isolate, A3, with low aminocandin and caspofungin MIC values, aminocandin single doses of 2.5–60 mg/kg were effective in reducing the tissue fungal burden compared with controls ($P < 0.05$; Table 2). Similarly, all doses of caspofungin, whether administered as a single dose or divided into daily doses, were effective in reducing tissue fungal burden compared with controls. In addition, the ED_{50} values predicted by the dose–response curves for single dose aminocandin (0.67 mg/kg) and caspofungin (2.18 mg/kg), and caspofungin administered daily (0.28 mg/kg) were less than the lowest doses tested (Figure 1a–c, respectively). These results are consistent with the observed data, which demonstrated that each of the tested doses for these regimens significantly lowered fungal burden compared with control. Acute toxicity with high-dose aminocandin and caspofungin appeared to occur as nine mice treated with a single dose of caspofungin at 100 mg/kg and two

Table 2. Kidney tissue fungal burden in mice inoculated intravenously with isolates of *C. glabrata* with varying susceptibility to aminocandin and caspofungin

Isolate and dose (mg/kg)	Single dose—median $\log_{10}$ cfu/g (range)		Daily dose caspofungin—median $\log_{10}$ cfu/g (range)	
	aminocandin	caspofungin	dose	fungal burden
A3 (CAS-S)—Control 7.3 (2.8–10)				
2.5	3.9 (1.6–5.9)*	5.2 (3.1–7.7)*	0.36	3.8 (3.3–4.2)*
10	3.8 (3.4–4.9)*	3.7 (3.5–7.8)*	1.4	3.7 (3.5–4.0)*
40	3.6 (3.4–4.2)*	3.9 (3.3–4.9)*	5.7	4.0 (3.1–4.3)*
60	3.8 (2.9–4.3)*	3.9 (2.9–4.2)*	8.6	3.9 (2.8–4.1)*
			14.3	1.8 (1.3–2.5)*
05-62 (CAS-R1)—Control 6.8 (4.8–8.5)				
0.5	7.1 (5.7–8.5)	7.7 (5.3–8.3)	0.07	7.4 (6.0–8.0)
1	8.2 (6.7–8.7)	7.2 (5.8–7.8)	0.14	7.8 (6.4–8.5)
2.5	7.5 (5.4–8.4)	7.3 (5.4–8.4)	0.36	7.4 (5.0–8.9)
10	5.0 (4.0–7.2)*	7.6 (6.8–8.8)	1.4	5.4 (5.2–5.9)*
40	4.9 (3.6–6.6)*	7.1 (4.7–8.7)	5.7	5.2 (4.5–5.5)*
60	5.4 (4.4–11)*	6.8 (3.1–8.3)	8.6	5.4 (5.1–6.0)*
			14.3	5.1 (4.8–5.6)*
04-1748 (CAS-R2)—Control 6.0 (4.9–7.3)				
0.5	5.9 (2.7–6.5)	6.3 (5.7–7.2)	—	—
1	5.9 (3.2–7.3)	6.3 (5.8–6.9)	—	—
2.5	6.1 (4.7–6.6)	5.9 (3.9–6.8)	—	—
10	6.3 (4.1–7.2)	5.8 (4.3–7.1)	—	—
40	5.2 (1.9–7.5)	5.8 (3.3–6.7)	—	—
60	3.8 (1.5–6.1)*	3.9 (3.3–5.9)*	—	—

cfu/g, colony-forming units per gram of kidney tissue.
*$P < 0.05$.
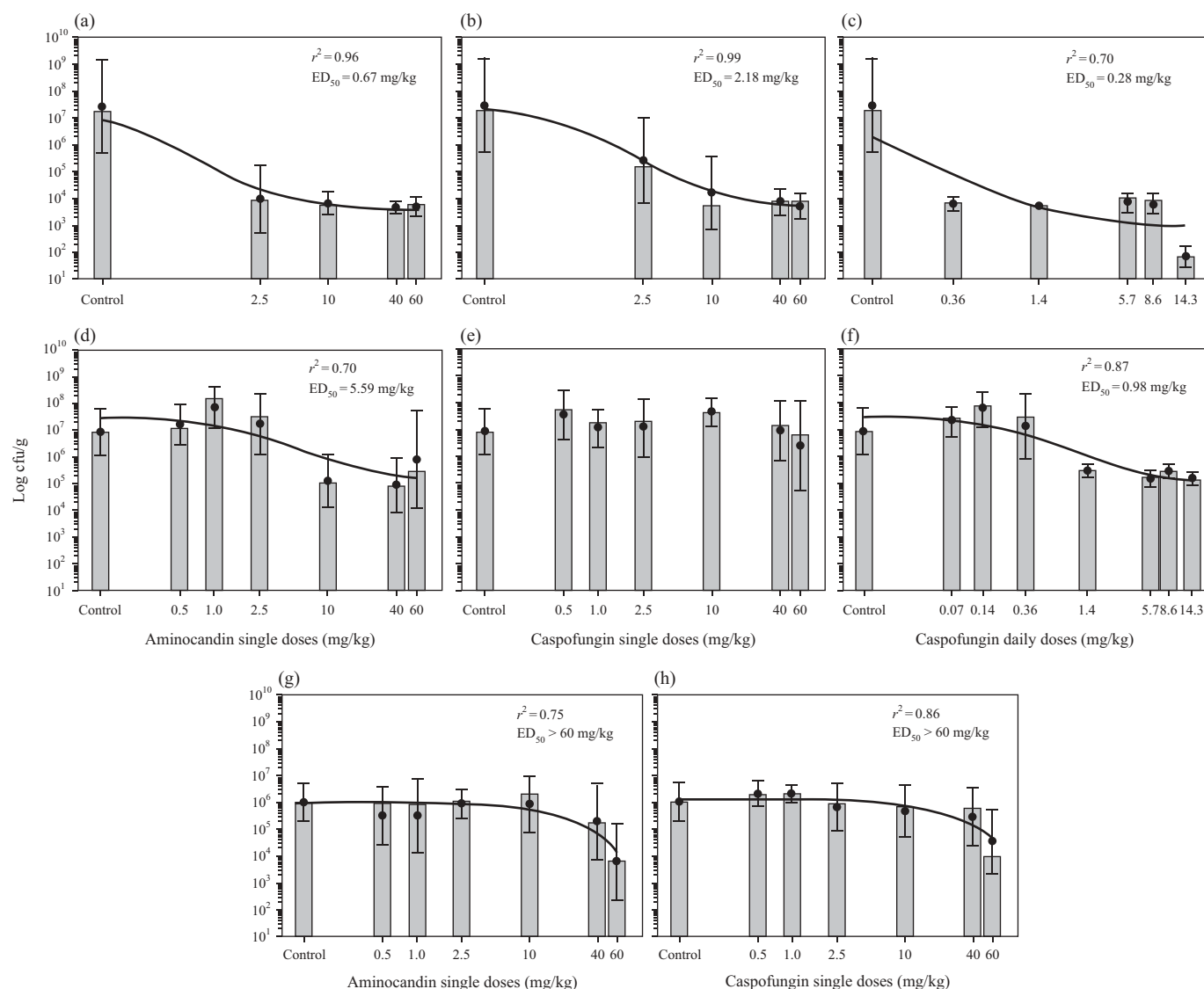
Figure 1. Sigmoidal dose–response curves for single dose against *C. glabrata* isolates A3 (CAS-S) (a, b and c), 05-62 (CAS-R1) (d, e and f) and 04-1748 (CAS-R2) (g and h) for single dose administration of aminocandin (a, d and g) and caspofungin (b, e and g), and daily doses of caspofungin (c and f). Symbols (closed circles) represent the means plus or minus the standard deviations of colony-forming units per gram of tissue, and the bars represent the corresponding median values. ED_{50}, dose resulting in 50% reduction in colony-forming units compared with controls.

animals treated with a single dose of aminocandin 100 mg/kg died immediately after the treatment (data not shown). Although the 100 mg/kg single doses of both aminocandin and caspofungin appeared to result in reductions in tissue fungal burden, these data were not included in the analysis due to the small number of animals that survived. However, daily doses of caspofungin 14.3 mg/kg (cumulative dose corresponding to the caspofungin single dose of 100 mg/kg) were well tolerated, suggesting that the deaths in the high-dose groups were potentially due to toxicity.

Tissue burden with CAS-R1

In the treatment of mice inoculated with the *C. glabrata* isolate with an elevated caspofungin MIC (4 mg/L) but a low aminocandin MIC (0.5 mg/L), isolate 05-62, single doses of aminocandin ≥10 mg/kg resulted in significant decreases in tissue burden compared with control ($P < 0.05$), while no single dose

administration of caspofungin resulted in reductions in fungal burden (Table 2 and Figure 1e). In contrast, caspofungin daily doses of ≥1.4 mg/kg (equal to a cumulative single dose of ≥10 mg/kg) were able to reduce cfu counts within the renal tissue compared with controls ($P < 0.05$). These results are consistent with the dose–response data that predicted ED_{50} values of 5.59 mg/kg for single dose aminocandin versus 0.98 mg/kg for daily dose caspofungin (Figure 1d and f). The results also suggest that higher doses of caspofungin may still be useful against an isolate with elevated MICs provided the drug is frequently dosed. Similar to that observed with the susceptible isolate, 7 of 10 mice treated with caspofungin single dose 100 mg/kg and 2 of 9 mice treated with aminocandin single dose 100 mg/kg died on the day of treatment, thus confirming that single doses of 100 mg/kg of each agent to be toxic. Based on these observations, the tissue fungal burden data were not included in the analysis, and single dose administration of

100 mg/kg of either agent was not studied against the remaining isolate. However, as before, daily doses of caspofungin 14.3 mg/kg (corresponding to the caspofungin total dose of 100 mg/kg) were well tolerated.

Tissue burden with CAS-R2 strain

Against clinical isolate 04–1748, which had increased MICs to both aminocandin (2 mg/L) and caspofungin (>16 mg/L), only single doses 60 mg/kg of each agent led to reductions in tissue fungal burden compared with control. These results are consistent with the ED_{50} values (>60 mg/kg) predicted by the dose–response curves for both agents (Figure 1g and h). Daily dosing of caspofungin was not evaluated for this organism.

Discussion

C. glabrata is a fungal species of increasing clinical importance. This opportunistic pathogen is responsible for 20% to 24% of *Candida* bloodstream infections in the USA and has been increasingly encountered in hospitals throughout the USA over the past decade as the second most commonly isolated yeast in patients with invasive candidiasis.[1,5,6,22] For many, the first choice of antifungal therapy for invasive candidiasis remains fluconazole due to its clinical effectiveness, lower cost and favourable adverse effect profile.[23,24] However, *C. glabrata* resistance to fluconazole and other azoles is becoming increasingly problematic, and breakthrough infections caused by this species in patients receiving fluconazole prophylaxis have been reported.[3,5,6,25] Although amphotericin B therapy has been successfully used for the treatment of such infections, this strategy may be associated with significant renal toxicity. Recently, the echinocandins have become increasingly used for the treatment of invasive fungal infections caused by *Candida* species. Clinical trials have demonstrated these agents to be efficacious and safe for the treatment of invasive candidiasis as well as for empirical therapy and antifungal prophylaxis.[7–10,26,27] Aminocandin is a new echinocandin undergoing preclinical development and Phase I trials with potent *in vitro* activity against many *Candida* spp. including *C. albicans, C. krusei, Candida lusitaniae, C. tropicalis* and *C. glabrata* with MICs ranging from 0.03 to 4 mg/L.[28] Phase I single dose escalation studies in healthy volunteers have also demonstrated this agent to be well tolerated.[29] Several *in vivo* studies with aminocandin reported efficacy in the treatment of infections caused by *C. albicans* and *C. tropicalis*, including isolates resistant to fluconazole.[11,12,30]

One of the major limitations of echinocandin therapy is the need for daily intravenous dosing, which may limit prolonged use in severely debilitated or immunocompromised patients due to increased risk of infection. To overcome this limitation, extended interval dosing has been suggested. This strategy is supported by the long half-lives of members of this antifungal class,[16,17,31] the excellent safety profiles observed in clinical trials and dosage escalation studies,[7–10,14,15] and the concentration-dependent activity reported in the animal models of invasive fungal infections.[18,32–35] Furthermore, this strategy has been shown to be effective for micafungin and aminocandin in the animal models of invasive candidiasis. Two studies have shown that aminocandin, administered as a single dose or twice weekly either as prophylaxis or treatment, reduces fungal burden and improves survival against infections caused by susceptible *C. albicans*.[12,36]

In a murine model of invasive candidiasis caused by a susceptible *C. glabrata* isolate, Gumbo *et al.*[18] demonstrated that a single high dose of micafungin 100 mg/kg was effective in reducing fungal burden. The results of the current study are consistent with these data as well as with other studies that have reported concentration-dependent activity of caspofungin and aminocandin with both the C_{max}/MIC and AUC/MIC associated with efficacy.[30,33] In the current study, single doses of aminocandin and caspofungin ≥2.5 mg/kg were effective in reducing tissue fungal burden after inoculation with a *C. glabrata* isolate with low MIC values to both agents. Interestingly, single doses of aminocandin and caspofungin 100 mg/kg were associated with rapid deaths in animals in the current study, but such an effect has not been reported with micafungin at this same dose.[18]

A second question addressed by our study was whether differences in the *in vitro* potency favouring aminocandin could be reflected by greater *in vivo* efficacy. Against the 11 isolates of *C. glabrata* that had elevated caspofungin MIC values, aminocandin appeared to maintain *in vitro* potency. However, when animals were inoculated with an isolate with an elevated caspofungin MIC, higher single doses of aminocandin (≥10 mg/kg) were required to reduce the fungal burden within the renal tissue, while single doses of caspofungin were ineffective. These data suggest that a *C. glabrata* isolate with decreased *in vitro* susceptibility to caspofungin may not respond to single doses of this drug, but single doses of aminocandin could still be effective. Interestingly, daily doses of caspofungin did reduce tissue fungal burden against this isolate, suggesting that more frequent administration may be an alternative to extended interval dosing against isolates with reduced caspofungin susceptibility. The *in vivo* efficacy of aminocandin was markedly reduced against the isolate with severely reduced *in vitro* susceptibility to caspofungin, as only the single doses of 60 mg/kg of each agent were able to lead to reductions in fungal burden. This observation, coupled with the toxicity observed with 100 mg/kg single doses of aminocandin and caspofungin, suggests that the strategy of extended interval dosing of aminocandin and caspofungin may be limited against *C. glabrata* isolates with reduced *in vitro* susceptibility.

One limitation of this study is that we did not assess the pharmacokinetics of either aminocandin or caspofungin in our model. Other investigators have reported long half-lives in mice (20.2–23.2 h) for both agents when serum concentration data alone are assessed.[30,33] When caspofungin serum concentration data were co-modelled with kidney tissue concentrations, the terminal half-life increased significantly (59.2 h).[33] This long terminal half-life in mice is similar to that reported in humans for aminocandin (53 h) and caspofungin (40–50 h).[16,31] Another potential limitation is that these agents were administered by different routes (iv for aminocandin and ip for caspofungin). However, previous animal studies have demonstrated rapid absorption of caspofungin from the peritoneal space into the bloodstream following ip administration.[33,34] Thus, differences in the time to peak concentrations between these two agents that occur between iv and ip administration are negligible. Furthermore, similar pharmacokinetic parameters for caspofungin (e.g. plasma clearance, total exposure as measured by the area under the concentration curve) are expected when equivalent doses up to 5 mg/kg are administered ip or iv in mice.[33,37] However, it is unknown if this would be consistent at the higher doses used in this study. Finally, we did not evaluate the efficacy of daily dosing of aminocandin to determine if this strategy

would be effective against isolates with reduced susceptibility. As suggested by the daily dose caspofungin data, this strategy may be effective against isolates to which this agent has reduced *in vitro* potency. However, further studies would need to be conducted to test this hypothesis.

The results of our study as well as those by others demonstrate that single doses of aminocandin, caspofungin and micafungin are effective as treatment against susceptible *C. glabrata* isolates in murine models of invasive candidiasis. This suggests that extended interval dosing may be a potentially useful strategy against such infections. However, as demonstrated by the data from this study, this strategy may be limited against infections caused by isolates with reduced susceptibilities to these agents as well as potential toxicity associated with high doses (e.g. 100 mg/kg). Thus, although aminocandin has been shown to be well tolerated in single dose escalation studies in healthy volunteers, it is unknown if the maximum tolerated doses will be high enough to allow for extended interval dosing of this echinocandin as empirical therapy. Further investigations are warranted to assess the feasibility of this approach.

Transparency declarations

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