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Therapeutic and prophylactic efficacy of aminocandin (IP960) against disseminated candidiasis in mice

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ABSTRACT

Extended interval dosing of the echinocandins has been suggested as a potential strategy to overcome the need for daily intravenous administration. This study evaluated the therapeutic and prophylactic efficacy of single doses of aminocandin, a new echinocandin in preclinical development, in a murine model of invasive candidiasis. For therapy, groups of mice were infected with *Candida albicans*, followed by a single dose of aminocandin (1–15 mg/kg) or placebo (mannitol 5% w/v) administered 1 day after inoculation. As prophylaxis, mice were given a single dose (5 or 30 mg/kg) of aminocandin, caspofungin, or placebo at increasing intervals between dose and inoculation. In both treatment and prophylaxis studies, survival was assessed at 21 days post-inoculation. The reduction in fungal burden was assessed in kidney tissue on day 8 post-inoculation. For treatment, single doses of aminocandin of ≥2.5 mg/kg prolonged survival significantly. In addition, the two doses evaluated for reductions in fungal burden (5 and 15 mg/kg) revealed fungicidal activity. As prophylaxis, both aminocandin and caspofungin 5 and 30 mg/kg prolonged survival when given 7 days before inoculation. Aminocandin and caspofungin 30 mg/kg were both able to prolong survival when the interval between dose and inoculation was increased to 10 days. When this interval was extended to 14 days, only aminocandin 30 mg/kg prolonged survival and reduced fungal burden. These results demonstrate that single doses of aminocandin are effective as treatment and prophylaxis, and suggest that extended interval dosing may be a useful strategy for treating invasive candidiasis.

Keywords Aminocandin, antifungal prophylaxis, *Candida albicans*, extended interval dosing, invasive candidiasis, mouse model

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INTRODUCTION

The echinocandin class of antifungal agents has been shown in both in-vivo and clinical studies to be effective in the treatment of invasive fungal infections. By inhibiting a fungal specific target, β-(1,3)-glucan synthase, members of this class avoid the drug interactions and toxicities associated with the azoles and amphotericin B [1]. Clinical studies have shown that the currently available echinocandins (anidulafungin, caspofungin and micafungin) are effective in the treatment of invasive infections caused by *Candida* spp. [2–4]. In addition, dose-escalation studies have demonstrated that members of this class are relatively well-tolerated at doses above those currently used clinically [5,6].

Aminocandin (IP960) is a new echinocandin currently undergoing preclinical development and phase I trials. Like other echinocandins, aminocandin has demonstrated in-vivo activity against various *Candida* spp., including fluconazole-resistant *Candida albicans* and *Candida tropicalis* [7–11]. Because of the long half-lives achieved clinically and the tolerability observed in dose-escalation

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trials, extended interval dosing of the echinocandins has been suggested as a means of overcoming the need for daily intravenous therapy. This strategy may allow antifungal drugs to be given at less frequent intervals for prophylaxis or treatment of invasive fungal infections; indeed, such an approach has been shown to be effective as treatment in murine models of invasive candidiasis [8,9].

The aim of the present study was to assess the utility of single doses of aminocandin as both treatment and prophylaxis against invasive candidiasis. An established murine model of disseminated candidiasis was used in which animals were infected with *C. albicans* and followed until 21 days post-inoculation. The primary endpoint was survival following single doses of aminocandin. Reductions in fungal burden within kidney tissue were also assessed as a secondary measure of efficacy.

MATERIALS AND METHODS

Antifungal agents

Stock solutions were prepared by dissolving aminocandin (Indevus Pharmaceuticals, Lexington, MA, USA) in mannitol 5% w/v, and by dissolving caspofungin (Merck, Whitehouse Station, NJ, USA) in water.

Test isolate

C. albicans clinical isolate 2823 was obtained from the Fungus Testing Laboratory at The University of Texas Health Science Center at San Antonio (San Antonio, TX, USA). Microdilution broth susceptibility tests were performed according to CLSI recommendations [12] before in-vivo experiments. The MICs of both aminocandin and caspofungin were 0.06 mg/L. For animal studies, isolate 2823 was grown overnight at 37°C in brain–heart infusion broth. Yeast cells were collected, washed in sterile saline, and verified with a haemocytometer. Inocula sizes were determined by plating serial dilutions and counting colonies.

Animal model

Immunocompetent outbred male ICR mice (Harlan Sprague Dawley) weighing *c.* 24 g were used in all experiments. Mice were housed five mice to a cage and had access to food and water *ad libitum*. On day 0, each mouse was infected intravenously with 0.2 mL of saline containing 10^6 CFU of *C. albicans*. All animal research procedures were approved by the Institutional Animal Care and Use Committee of The University of Texas Health Science Center at San Antonio.

Prophylaxis and treatment studies

For treatment, animals received aminocandin at doses of 1, 2.5, 5, 10, 12.5 or 15 mg/kg as a single intravenous dose 1 day after inoculation. In prophylaxis studies, aminocandin or caspofungin were administered as a single 5 or 30 mg/kg intravenous or intraperitoneal dose, respectively, at 7, 10 or 14 days before inoculation. Control animals received mannitol 5% w/v (placebo) by intravenous administration. To assess survival, mice were followed until day 21 post-inoculation. Any animal that appeared moribund (ruffled hair, hunched posture, cool to touch, >10% weight loss, or immobile) was killed humanely and death was recorded as occurring the next day. For tissue burden, separate groups of mice were followed until day 8 post-inoculation. The animals were then killed humanely and the kidneys were harvested and weighed. The kidneys of animals that died before day 8 were harvested on the day of death. Kidneys were then homogenised using a PT2100 tissue homogeniser (Kinematica, Cincinnati, OH, USA) in sterile saline supplemented with piperacillin and amikacin 60 mg/L. Serial dilutions were prepared and plated in duplicate on Sabouraud dextrose agar. After incubation for 24 h at 37°C, the number of CFU/g of tissue was calculated. Each experiment contained nine or ten animals/dose group.

Statistical analysis

Survival was assessed by Kaplan–Meier analysis, and differences in median survival time and survival among groups were analysed by the log-rank and chi-square test, respectively. The Mann–Whitney *U*-test was used for comparison of the tissue burden among groups. A p value of ≤0.05 was considered to be statistically significant. All analyses were performed using Prism v.4.0 (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Treatment

Aminocandin was effective at improving survival and reducing fungal burden following a single intravenous dose administered 1 day after inoculation with *C. albicans*. As shown in Table 1, single doses of ≥2.5 mg/kg were effective in prolonging the median survival to >21 days, which was

Table 1. Survival and tissue fungal burden following a single dose of aminocandin administered as therapy 1 day after intravenous inoculation of mice with *Candida albicans*

Treatment group	Percentage survival (p value vs. placebo)	Median survival (p value vs. placebo)
Placebo	15	11
AMN 1 mg/kg	0 (p 0.22)	15 (p 0.37)
AMN 2.5 mg/kg	55.6 (p <0.01)	>21 (p <0.01)
AMN 5 mg/kg	77.8 (p <0.01)	>21 (p <0.01)
AMN 7.5 mg/kg	66.7 (p <0.01)	>21 (p 0.02)
AMN 10 mg/kg	100 (p <0.01)	>21 (p <0.01)
AMN 12.5 mg/kg	90 (p <0.01)	>21 (p <0.01)
AMN 15 mg/kg	90 (p <0.01)	>21 (p <0.01)
Fungal burden	Median $\log_{10}$ CFU/g (range)	p value vs. placebo
Placebo	6.6 (5.8–7.0)	–
AMN 5 mg/kg	2.1 (1.3–2.6)	p <0.01
AMN 15 mg/kg	2.0 (1.1–3.5)	p <0.01

AMN, aminocandin.

significantly longer than the survival of controls (11 days; p ≤0.02). In addition, the same doses of aminocandin increased the percentage of animals surviving to the predetermined study endpoint of 21 days post-inoculation to ≥55%. These values were significantly greater than the survival rates for animals given placebo (15%; p <0.01) or aminocandin 1 mg/kg (0%; p 0.22 vs. placebo). Aminocandin also resulted in significant reductions in fungal burden when administered as single-dose therapy (Table 1). Both of the doses evaluated (5 and 15 mg/kg) reduced fungal burden within the kidney tissue by >4 $\log_{10}$ CFU/g of tissue (to 2.1 and 2.0 $\log_{10}$ CFU/g, respectively) compared to controls (6.6 $\log_{10}$ CFU/g; p <0.01) by day 8 post-inoculation.

Prophylaxis

Based on the prolonged survival and reduced tissue burden observed in the treatment studies with aminocandin, single-dose prophylaxis studies were initiated, using increasing intervals between dose administration and inoculation. As a positive comparator, caspofungin prophylaxis was administered at the same doses and intervals between administration and inoculation. Both doses of aminocandin and caspofungin tested (5 and 30 mg/kg) were effective at prolonging survival compared to controls receiving placebo when administered 7 days before inoculation (Fig. 1a). While caspofungin 5 mg/kg increased median survival (15.5 days) compared to controls (2 days; p <0.01), only 30% of animals survived to day 21 post-inoculation. This difference was not significant compared to controls (0%; p 0.06). Furthermore, this dose of caspofungin did not result in a decrease in fungal burden (5.8 vs. 6.2 $\log_{10}$ CFU/g for controls; p 0.63) (Fig. 1d). This may reflect the higher inoculum achieved in this experiment compared to the other prophylaxis studies (Fig. 1). However, this higher inoculum did not affect the potency of either aminocandin 5 and 30 mg/kg or the high dose of caspofungin. These regimens increased the median survival to >21 days and the percentage of animals in each group surviving to day 21 compared to controls (90%, 100% and 60%, respectively; p <0.01). Each of these doses also reduced tissue fungal burden significantly compared to controls (4.3, 1.3 and 5.1 $\log_{10}$ CFU/g, respectively).

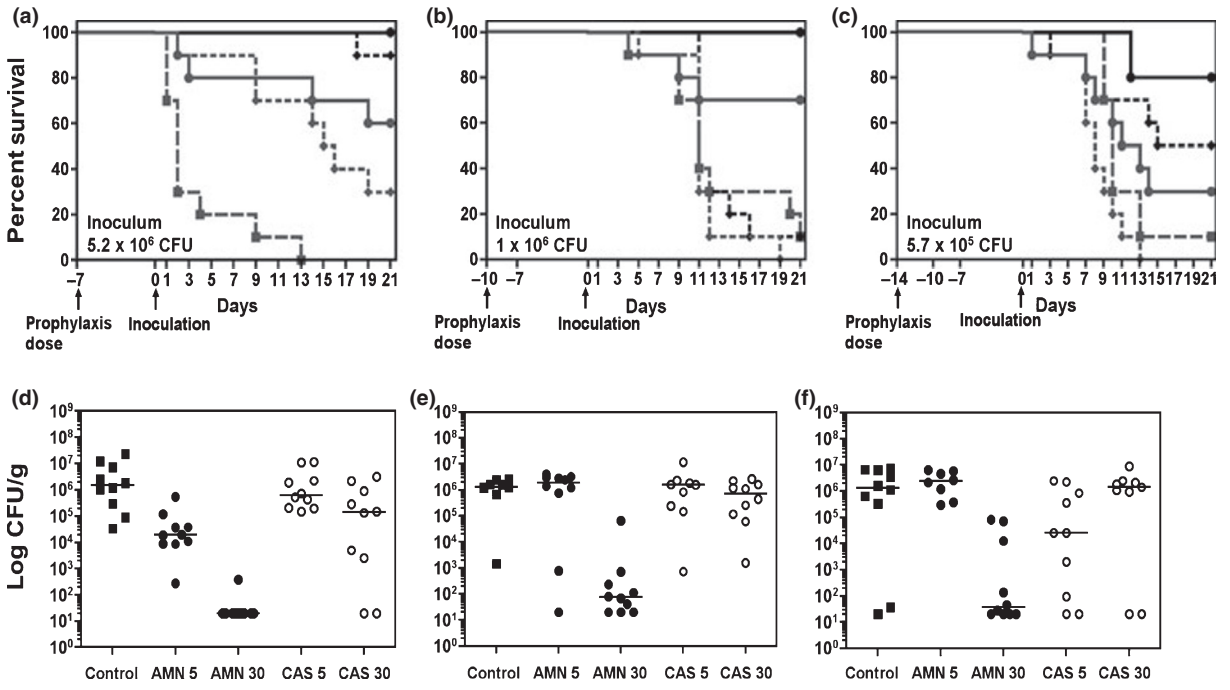

Fig. 1. Survival (a, b and c) and tissue fungal burden (d, e and f) of mice given placebo (■), aminocandin 5 mg/kg (◆), aminocandin 30 mg/kg (●), caspofungin 5 mg/kg (◆) or caspofungin 30 mg/kg (●) as a single dose at 7 days (a and d), 10 days (b and e) and 14 days (c and f) before inoculation with *Candida albicans*.

When the interval between the single prophylactic dose and inoculation was increased to 10 days, only aminocandin and caspofungin 30 mg/kg remained effective at prolonging survival (>21 days for both drugs) and improving the survival rate (100% and 70%) compared to controls (median survival 11 days, p ≤0.02; 10% survival, p ≤0.02) (Fig. 1b). In addition, aminocandin 30 mg/kg reduced tissue fungal burden by >4 $\log_{10}$ CFU/g compared to controls (1.9 vs. 6.1 $\log_{10}$ CFU/g, respectively; p <0.01) (Fig. 1e). Despite improvements in survival, caspofungin 30 mg/kg did not reduce the fungal burden significantly (5.8 $\log_{10}$ CFU/g; p 0.28 vs. controls).

As the interval between the single prophylactic dose and inoculation was further extended to 14 days, only aminocandin 30 mg/kg remained effective at prolonging survival (median >21 days) and the percentage of animals surviving to the predetermined endpoint (80%) compared to controls (median survival 10 days, p <0.01; 10% survival, p <0.01) (Fig. 1c). This dose of aminocandin also suppressed the fungal burden effectively following a single dose administered 2 weeks before inoculation (1.5 vs. 6.1 $\log_{10}$ CFU/g for controls, p <0.01) (Fig. 1f). Although aminocandin 5 mg/kg showed a trend toward improved survival (median survival 18 days, p 0.093; 50% survival, p 0.051), these values did not reach statistical significance. In contrast, caspofungin 30 mg/kg was not effective at improving survival (median survival 12 days, p 0.40) or reducing fungal burden (6.1 $\log_{10}$ CFU/g, p 0.84) when administered as a single dose 14 days before inoculation.

DISCUSSION

Members of the echinocandin class of antifungal agents have improved tolerability and similar efficacy, compared to azole antifungals and amphotericin B, for the treatment of invasive candidiasis. In randomised controlled trials, each of the echinocandins currently available for clinical use (anidulafungin, caspofungin and micafungin) has been shown to yield a favourable response in *c.* 70% of patients [2–4]. In addition, trials that have compared caspofungin and micafungin to amphotericin B deoxycholate and liposomal amphotericin B, respectively, have revealed that the echinocandins were better tolerated, with fewer infusion-related reactions and less nephrotoxicity [2,3].

While no clinical efficacy data are currently available for aminocandin, several in-vivo studies have evaluated this echinocandin for the treatment of infections caused by *Candida* spp. Using an immunocompromised murine model of invasive candidiasis caused by a fluconazole-resistant strain of *C. tropicalis*, Warn *et al.* [7] demonstrated that daily doses of aminocandin of ≥1 mg/kg were as effective as amphotericin B deoxycholate 5 mg/kg/day in prolonging survival and reducing tissue fungal burden. In a murine model of invasive candidiasis caused by *C. albicans*, Andes *et al.* [13] demonstrated that aminocandin has concentration-dependent pharmacodynamic activity *in vivo*, with large infrequent drug administration being most effective at reducing the fungal burden [14]. Similarly, other in-vivo studies have revealed concentration-dependent activity for anidulafungin, caspofungin and micafungin, thus raising the possibility of extended interval dosing for the echinocandins [9–11].

Large single doses of micafungin and aminocandin have been shown to be effective as therapy against invasive candidiasis. Gumbo *et al.* [9] demonstrated that a single dose of micafungin 100 mg/kg resulted in fungicidal activity in mice infected with *Candida glabrata*. Similarly, aminocandin doses of 5 and 10 mg/kg, administered either once- or twice-weekly, reduced fungal burden significantly and resulted in 100% survival when begun 2 h after inoculation with a fluconazole-resistant strain of *C. albicans* [8]. The results of the current study are consistent with these data and support the strategy of extended interval dosing. In the present study, single intravenous doses of aminocandin ≥2.5 mg/kg, begun 24 h after inoculation with *C. albicans*, prolonged survival significantly compared with controls. In addition, both of the single doses evaluated (5 and 15 mg/kg) resulted in a significant reduction in the fungal burden (>3 $\log_{10}$ CFU/g reduction).

The excellent safety and drug interaction profile of the echinocandins, as well as the significant morbidity and financial burden associated with invasive candidiasis, mean that there is considerable interest in the use of members of this class as prophylaxis against invasive fungal infections in high-risk patients [14,15]. In a prospective double-blind randomised trial involving autologous and

allogeneic stem-cell transplant recipients in the pre-engraftment period, micafungin was shown to be of similar effectiveness to fluconazole in preventing breakthrough candidiasis [16]. One of the limitations of echinocandin prophylaxis is the requirement for daily intravenous administration for the period during which patients are at risk for fungal infections. In the animal model used in the present study, single doses of aminocandin and caspofungin at 5 and 30 mg/kg were effective at prolonging survival and reducing fungal burden when administered as a single prophylactic dose 7 days before inoculation. Aminocandin 30 mg/kg maintained efficacy when the interval between prophylaxis and inoculation was extended to 10 and 14 days, while caspofungin 30 mg/kg remained protective at an interval of 10 days. Thus, extended interval dosing could potentially overcome the limitation of daily administration of an echinocandin. However, further study is required to clarify this point, as daily administration of aminocandin or caspofungin was not evaluated.

One potential limitation of the present study was the difference in the route of administration between aminocandin (intravenous) and caspofungin (intraperitoneal). Previous animal studies have demonstrated rapid absorption of caspofungin from the peritoneal space into the bloodstream following intraperitoneal administration [11,17]. Thus, differences in the time required to attain peak concentrations for these two agents that occur between the two routes of administration may be negligible, and would probably have no effect on the results of the current study, since the minimal interval between the prophylactic dose and inoculation was 7 days. In addition, the pharmacokinetics of the two agents in the mouse model were not assessed as a potential explanation for the effectiveness of single-dose prophylaxis at extended intervals between administration and inoculation. Other investigators have reported long half-lives in mice (20.2–23.2 h) for both agents when serum concentration data alone are assessed [11,13]. When caspofungin serum concentration data were co-modelled with kidney tissue concentrations, the terminal half-life increased significantly to 59.2 h [11]. However, this strategy needs to be evaluated in an immunocompromised model of invasive candidiasis, as the immune status may affect treatment outcome [18].

While aminocandin has been shown to be well-tolerated in single-dose-escalation studies in healthy volunteers, no efficacy data currently exist. The results of the present study, as well as those from other investigators, demonstrate that single doses of aminocandin, caspofungin and micafungin are effective, either as treatment or prophylaxis, in murine models of invasive candidiasis. These data suggest that extended interval dosing may be a useful strategy for combating and preventing infections caused by *Candida* spp. However, it remains to be determined whether the maximum tolerated doses will be sufficiently high to allow clinical trials of once-weekly administration of echinocandins for treatment or prophylaxis of invasive candidiasis. Further investigations are warranted to determine the clinical feasibility of this approach.

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