Efficacy of aminocandin in the treatment of immunocompetent mice with haematogenously disseminated fluconazole-resistant candidiasis

M. A. Ghannoum*, H. G. Kim and L. Long

Center for Medical Mycology, University Hospitals of Cleveland/Case Western Reserve University, 11100 Euclid Avenue, Cleveland, OH 44106, USA

Received 26 July 2006; returned 2 October 2006; revised 28 November 2006; accepted 1 December 2006

Objectives: The objective of this study was to compare the activity of aminocandin, a new echinocandin with broad-spectrum activity against Candida spp., with that of amphotericin B, caspofungin and fluconazole, in an immunocompetent murine model of haematogenously disseminated candidiasis caused by a fluconazole-resistant Candida albicans.

Methods: Mice were infected with a fluconazole-resistant strain of C. albicans and treated with aminocandin 5 and 10 mg/kg intravenously (iv) once and twice weekly, amphotericin B 0.5 mg/kg iv every other day for 5 days, fluconazole 20 mg/kg orally (po) once a day for 5 days and caspofungin 0.5 mg/kg intraperitoneally (ip) once daily for 5 days.

Results: Treatment with aminocandin, given iv twice a week, resulted in 100% survival. Further, the tissue fungal burden of the aminocandin group was equivalent to that of amphotericin B (administered every other day) and caspofungin (administered daily).

Conclusions: Aminocandin may be an effective addition to the arsenal of antifungal compounds for the treatment of candidiasis caused by fluconazole-resistant C. albicans.

Keywords: echinocandins, Candida albicans, in vivo

Introduction

Candida albicans, an opportunistic pathogen, causes both mucosal and disseminated infections. Oropharyngeal candidiasis (OPC) is commonly treated with fluconazole. Recurring OPC caused by fluconazole-resistant C. albicans is the most common infection among persons infected with HIV. C. albicans has also been implicated in breakthrough fungaemias in immunocompromised cancer patients who received azole treatment during neutropenia. As an alternative to triazoles, the echinocandin class of antifungal, which inhibit the β-1,3-glucan synthase leading to cell wall inhibition, has shown broad-spectrum activity against Candida spp. in vitro, with an MIC₉₀ (the lowest concentration at which 90% of isolates were inhibited) of 0.25–0.50 mg/L.

Aminocandin is a new member of the echinocandin class of compounds that demonstrates favourable activity against Candida species and appears to be fungistatic for Aspergillus in vitro. Aminocandin does, however, exhibit some important differences compared with the other echinocandins. In a previous study, aminocandin was as efficacious in vivo as caspofungin against caspofungin-susceptible Candida glabrata, and at higher doses (40–100 mg/kg) it was more effective than caspofungin in reducing the tissue fungal burden in caspofungin-resistant strains. Moreover, aminocandin was recently shown to improve survival and reduce tissue fungal burden in a neutropenic murine model of fluconazole-resistant Candida tropicalis. This study was aimed at evaluating the antifungal efficacy of aminocandin in the treatment of haematogenously disseminated candidiasis caused by a fluconazole-resistant strain of C. albicans in an immunocompetent murine model. In this study, an immunocompetent model was chosen because: (1) such a model is routinely used to evaluate antifungal activity and is predictive of clinical outcomes; and (2) interactions between echinocandins and immunosuppressants have been shown to occur. Therefore, the use of an immunocompetent model avoided the possibility of interactions between antifungals and drugs used to render the animal immunocompromised.

Methods

Animal experiments were performed in accordance with the guidelines for animal health and welfare required by the Institutional Animal Care and Use Committee guidelines at Case
Aminocandin in the treatment of immunocompetent mice

Western Reserve University, School of Medicine, Cleveland, OH. Female BALB/c mice (6–8 weeks old and 20–25 g in weight; Charles River Laboratories, Wilmington, MA) were housed under standard laboratory conditions in microisolator cages (three or fewer per cage) and acclimatized for 5 days.

Fluconazole-resistant *C. albicans* strain MRL 648 was sub-cultured from frozen stock onto potato dextrose agar (Fisher Scientific) and incubated at 37°C for 24 h. MIC values of fluconazole, amphotericin, caspofungin and aminocandin were >64, 0.5, 1.0 and 0.25 mg/L, respectively. MFC values of aminocandin and amphotericin B were 16.0 and 1.0 mg/L, respectively. We selected a fluconazole-resistant isolate to investigate the efficacy of aminocandin because this may mirror the way this drug will be used clinically. *C. albicans* was grown overnight at 37°C in Sabouraud dextrose broth (Difco Laboratories). Cells were harvested by centrifugation and normal saline (0.85%) washes. The challenge inoculum, $5 \times 10^5$ blastospores in 0.1 mL of normal saline, was adjusted using a haemocytometer and injected via the lateral tail vein. Two identical experiments, each with survival study and tissue (kidney) fungal burden determination, were performed using five mice per group (selected randomly) for each component.

Aminocandin was provided by Indevus Pharmaceuticals, Inc. (Lexington, MA). This compound has a considerably longer half-life (48–58 h) than that of other drugs tested in this study (half-lives for caspofungin, amphotericin B and fluconazole are ~10 h, 24 h and 30 h, respectively). Due to the long half-life of aminocandin, we chose dosing schedules to include once- and twice-weekly dosing regimens. One group of animals was given a single dose per week, while the other group was treated with two doses of aminocandin, at 2 h and on day 3 post-inoculation. Treatment-control groups received amphotericin B 0.5 mg/kg intravenously (iv) every other day for 5 days, fluconazole 20 mg/kg orally (po) once a day for 5 days and caspofungin 0.5 mg/kg intraperitoneally (ip) once daily for 5 days. An untreated-control group received sterile water for injection ip. All treatments were given pre-emptive to infection, 2 h post-inoculation in 0.1 mL volumes. Route of administration (iv) and doses of aminocandin (5 and 10 mg/kg) used in this study were based on previous efficacy and toxicity data provided by the manufacturer, while doses and routes of administration of amphotericin B and caspofungin were selected based on a previous investigation where we showed that these agents were effective in the murine model used in the current study.5

Infected mice were monitored, and any signs of illness or mortality were recorded daily for up to 28 days. Moribund animals that failed to take food/drink were euthanized via CO2 gas asphyxiation. Kidney-tissue fungal burden was determined 6 days after infection. In cases where the animals died prior to 6 days, cfu were determined at the time of death. Kidneys were removed aseptically, weighed and homogenized in sterile saline. Diluted samples of homogenates were cultured on Sabouraud dextrose agar plates (Difco Laboratories) at 37°C for 48 h, and the number of cfu counted. The viable count detection limit was 4.5 log10 cfu/g of kidney.

The cfu values in kidneys were expressed as cfu/g of tissue, log-transformed and compared using the Mann–Whitney U test. Survival results were analysed by Kaplan–Meier. All statistical comparisons were performed using StatView version 4.5 for Windows 95. A P value <0.05 was considered significant.

**Results**

**Survival study**

Data from repeated experiments were combined. As expected, the untreated controls began dying on day 2 post-inoculation, with 100% mortality by day 13 (Figure 1). There was 100% survival in all aminocandin-treated groups irrespective of whether the drug was given once or twice a week, or the dose used. The caspofungin-treated group also had 100% survival. As expected, fluconazole-treated mice began dying on day 2 with 70% mortality by day 17. The amphotericin B-treated mice began dying 2 days post-inoculation, with 60% survival by day 17.

**Tissue fungal burden**

In the fluconazole group, three mice died prior to day 6 (on days 3, 4 and 5). In the untreated group, three animals died prior to day 6, with 40% mortality by day 17.

![Graph](https://academic.oup.com/jac/article-abstract/59/3/556/843637/f1) **Figure 1.** Survival rates for each treatment group.
day 6 (two on day 4 and one on day 5). The kidney tissue fungal burdens for aminocandin 5 mg/kg once a week, aminocandin 5 mg twice a week, aminocandin 10 mg/kg once a week and aminocandin 10 mg/kg twice a week were 2.21 ± 0.73, 1.60 ± 0.97, 2.40 ± 0.87 and 1.92 ± 1.12 log10 cfu, respectively (Figure 2). All aminocandin-treated groups had significantly lower fungal burdens compared with the untreated control (5.64 ± 1.11), P values <0.0001. Moreover, all aminocandin-treated groups also had significantly lower fungal burdens compared with the fluconazole-treated group (5.52 ± 1.56); P values for aminocandin 5 mg/kg once and twice a week were <0.0001, while P values for aminocandin 10 mg/kg once and twice a week were 0.002. There was no significant difference between the fungal burden for aminocandin treatment groups and amphotericin B or caspofungin treatment groups (3.41 ± 2.91 and 2.09 ± 0.40, respectively) and this was true even for treatment with aminocandin once a week.

Discussion

In this study, all doses of aminocandin prolonged survival equally to caspofungin while surpassing the percentage survival for amphotericin B-treated mice and, as expected, fluconazole-treated mice. One caveat, however, is that the dosing and route of administration used to treat animals with the different antifungals were not the same. These regimens were based on previous studies and the toxicity profile for amphotericin B. Therefore, it is possible that differences seen among these agents could be due to the differences in regimens used. There was no significant difference in the average tissue fungal burden between animals treated with aminocandin doses given once a week and caspofungin given daily. When given twice a week, aminocandin-treated animals had lower average tissue fungal burdens than those treated with caspofungin daily, although this difference was not statistically significant (P values of 0.2186 and 0.5942 for aminocandin 5 mg and 10 mg, respectively). When given once weekly, average tissue fungal burdens were only slightly higher than caspofungin daily. However, once-weekly dosing of aminocandin resulted in a lower average tissue fungal burden than that of mice treated with amphotericin B every other day and significantly lower than that of mice treated with fluconazole daily. Although there was a trend towards lower tissue fungal burdens, dosing of aminocandin twice a week did not significantly lower the average tissue fungal burden compared with the same doses of aminocandin given once a week. These data support previous findings indicating that large infrequent doses of aminocandin are the most effective.

Aminocandin is a promising novel antifungal agent. Its efficacy against fluconazole-resistant strains of Candida may mean that it is a valuable alternative to current therapies. The longer half-life of aminocandin could also have significant clinical and economic implications.

Transparency declarations

The Center for Medical Mycology would like to thank Indevus Pharmaceuticals, Inc. for their generous support of this work.

References

Aminocandin in the treatment of immunocompetent mice


