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

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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.1 Recurring OPC caused by fluconazole-resistant C. albicans is the most common infection among persons infected with HIV.2 C. albicans has also been implicated in breakthrough fungaemias in immunocompromised cancer patients who received azole treatment during neutropenia.3 As an alternative to triazoles, the echinocandin class of antifungals, 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 MIC50 (the lowest concentration at which 90% of isolates were inhibited) of 0.25–0.50 mg/L.4

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.5 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.5 Moreover, aminocandin was recently shown to improve survival and reduce tissue fungal burden in a neutropenic murine model of fluconazole-resistant Candida tropicalis.6 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 predicative 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.7

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...
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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 subcultured 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 × 10⁵ 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.

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

![Figure 1](https://academic.oup.com/jac/article-abstract/59/3/556/843637)
studies and the toxicity profile for amphotericin B. Therefore, it gals were not the same. These regimens were based on previous of administration used to treat animals with the different antifun-
treated mice. One caveat, however, is that the dosing and route for amphotericin B-treated mice and, as expected, fluconazole-
equally to caspofungin while surpassing the percentage survival and aminocandin 10 mg/kg once a week were 2.21 ± 0.73, 1.60 ± 0.97, 2.40 ± 0.87 and 1.92 ± 1.12 log₁₀ cfu, respect-
Thus, fluconazole-resistant clinical isolates of Candida spp., including 157 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 signifi-
cantly 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 effi-
cacy 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
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References

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