

Evaluation of the efficacy of rezafungin, a novel echinocandin, in the treatment of disseminated *Candida auris* infection using an immunocompromised mouse model

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Background: Multiple cases of *Candida auris* infection have been reported with high mortality rates owing to its MDR nature. Rezafungin (previously CD101) is a novel echinocandin with enhanced stability and pharmacokinetics that achieves high plasma drug exposure and allows for once weekly dose administration.

Objectives: Evaluate the efficacy of rezafungin in the treatment of disseminated *C. auris* infection using a mouse model of disseminated candidiasis.

Methods: Mice were immunosuppressed 3 days prior to infection and 1 day post-infection. On the day of infection, mice were inoculated with 3×10^7 *C. auris* blastospores via the tail vein. Mice were randomized into four groups ($n = 20$): rezafungin at 20 mg/kg, amphotericin B at 0.3 mg/kg, micafungin at 5 mg/kg and a vehicle control. Treatments were administered 2 h post-infection. Rezafungin was given additionally on days 3 and 6 for a total of three doses, while the remaining groups were treated every day for a total of seven doses. Five mice from each group were sacrificed on days 1, 4, 7 and 10 of the study. Kidneys were removed from each mouse to determine the number of cfu for each respective day.

Results: Rezafungin had significantly lower average \log_{10} cfu/g of tissue compared with amphotericin B- and vehicle-treated mice on all days when kidneys were harvested. Additionally, rezafungin-treated mice had significantly lower average \log_{10} cfu/g of tissue compared with micafungin-treated mice on day 10.

Conclusions: Our findings show that rezafungin possesses potent antifungal activity against *C. auris* in a disseminated model of candidiasis.

Introduction

The emergence of drug-resistant pathogens is an ever-growing concern. Originally reported in 2009,¹ *Candida auris* causes serious invasive infections with mortality rates approaching ~60%.² Reports of invasive infections caused by *C. auris* have emerged globally, including in Japan, South Korea, India, Kuwait, South Africa, Pakistan, the UK and, more recently, in Venezuela, Colombia and the USA.^{3–6} The majority of *C. auris* infections have been reported as secondary nosocomial infections.^{3,6,7} A high percentage of clinical strains of *C. auris* exhibit resistance to fluconazole and there are varying levels of resistance to the three major classes of currently available antifungals (azoles, polyenes and echinocandins), thus limiting treatment options.^{1,3–10}

Given the MDR nature of *C. auris*, development of new antifungal drugs that can combat this resistance issue is critical.

The echinocandin class of drugs is used to treat serious invasive fungal infections and they are recommended for the first-line treatment of suspected or confirmed invasive *Candida* infections.¹¹ Three echinocandins (caspofungin, micafungin and anidulafungin) are currently approved for the treatment of candidaemia, as well as other types of invasive candidiasis, by the US FDA. Although resistance to fluconazole and amphotericin B have been widely reported for different isolates of *C. auris*, only a limited number of strains of this emerging species have shown elevated MICs to currently available echinocandins. Rezafungin (previously CD101), a novel echinocandin with an extended $t_{1/2}$ and high plasma drug exposure that may help counter resistance,^{12,13} is in development as a once weekly intravenous formulation for the treatment and prevention of systemic fungal infections.¹⁴ Although its pharmacokinetic profile is a major distinguishing characteristic, rezafungin was designed to first be chemically and metabolically

stable to avoid hepatotoxicity while retaining the antifungal activity of an echinocandin.¹⁵ In our recent evaluation of antifungal susceptibility of a panel of *C. auris* isolates, in which 8 out of 16 isolates possessed susceptibility profiles that for other *Candida* spp. would be characterized as resistant to at least three of the antifungal agents tested, rezafungin demonstrated potent *in vitro* activity against both echinocandin-resistant and -susceptible *C. auris* isolates.^{16,17}

Herein, we evaluated the efficacy of rezafungin in the treatment of *C. auris* infection, utilizing a mouse model of disseminated candidiasis. Specifically, the temporal effect of rezafungin on kidney tissue fungal burden was evaluated.

Materials and methods

Ethics

All procedures in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

Test compounds

The following antifungals were evaluated: rezafungin, micafungin and amphotericin B. Rezafungin and micafungin were provided by Cidara Therapeutics, Inc. Amphotericin B was purchased from Sigma–Aldrich (St Louis, MO, USA).

Organism and inoculum preparation

A clinical isolate of *C. auris* (MRL 35368) was obtained from the Center for Medical Mycology Culture Collection and used as the infecting fungus. The MIC values (determined using the CLSI broth microdilution method, as described in the M27-A3 document) of rezafungin, amphotericin B and micafungin were 0.063, 4 and 1 mg/L, respectively. *C. auris* inoculum was prepared by plating onto potato dextrose agar (PDA) (Becton Dickinson, Sparks, MD, USA) and incubating at 35°C for 2 days. Next, *C. auris* blastospores were harvested by centrifugation followed by three washes with PBS. A challenge inoculum of 3×10^7 was prepared using a haemocytometer.

Immunosuppression

Female, 6–8-week-old, CD-1 mice received cyclophosphamide (Sigma–Aldrich), 200 mg/kg administered by intraperitoneal injection 3 days prior to challenge and 150 mg/kg 1 day after challenge. On the day of infection, blood was collected (20 µL) from one mouse from each group for a white blood cell count to verify immunosuppression.

Infection and evaluation of treatment efficacy

To evaluate the temporal effect of rezafungin on tissue fungal burden, immunocompromised mice ($n = 20$ mice per group) were infected with 3×10^7 *C. auris* blastospores in 100 µL of PBS via the lateral tail vein. Beginning 2 h post-infection, mice in the rezafungin group were given one dose (day 1) then subsequently treated on days 3 and 6 for a total of three doses. Additionally, mice in the remaining treatment groups (amphotericin B, micafungin and vehicle) were given one dose 2 h post-infection (day 1), then treated every day for a total of seven doses. Five mice from each group were sacrificed on days 1, 4, 7 and 10 of the study to determine the tissue fungal burden in the kidneys. Briefly, both kidneys from each animal were aseptically removed and weighed. Tissues were then homogenized in 1 mL of PBS and serially diluted. The dilutions were plated onto PDA and cultured at 35°C for 48 h to determine the number of cfu. Efficacy of rezafungin was evaluated as a reduction in \log_{10} cfu compared with all other groups.

Statistical analysis

Differences in the mean cfu in the kidneys were compared with the control and different treatment groups using a one-way ANOVA with a post-hoc Tukey test. A *P* value of <0.05 was considered statistically significant.

Results

Figure 1 shows the average \log_{10} cfu/g of tissue for mice by treatment group on the respective sacrifice day (days 1, 4, 7 and 10 post-infection). As shown in Figure 1, mice treated with rezafungin had significantly lower average \log_{10} cfu compared with amphotericin B- and vehicle-treated mice on all days when kidneys were harvested (Table 1). Additionally, rezafungin-treated mice had significantly lower average \log_{10} cfu compared with the micafungin-treated group on day 10 only.

Discussion

In this study, we examined the temporal effect of rezafungin compared with amphotericin B and micafungin dosed daily for 7 days, while rezafungin was administered three times (days 1, 3 and 6). Although dosed less frequently than amphotericin B and micafungin, rezafungin was significantly more efficacious in reducing fungal burden. The demonstrated superior activity of rezafungin compared with the other agents, even with less frequent dosing, reflects the clinical potential of rezafungin over current daily dosing regimens of approved echinocandins and can be attributed to its pharmacokinetic/pharmacodynamic profile. As seen with other antimicrobials that exhibit concentration-dependent killing and a prolonged $t_{1/2}$ (e.g. oritavancin), front-loaded dosing benefits rezafungin efficacy. Lakota *et al.*¹⁸ (2017) demonstrated that a single dose of rezafungin achieved efficacious drug exposures and greater efficacy than once daily and twice weekly regimens in a neutropenic mouse model of disseminated candidiasis. The time course of rezafungin concentrations (i.e. the shape of the AUC) was shown to be a determinant of its efficacy, which relates to the importance of achieving therapeutic drug exposure early in the course of therapy. The pharmacokinetics/pharmacodynamics of rezafungin may also play a role against resistance development, as suggested by the mutant prevention concentration (MPC) concept which calls for antifungal dosing above the MPC to inhibit growth of potentially resistant isolates.¹⁵

In our recent evaluation of the antifungal susceptibility of *C. auris*, 16 clinical isolates were tested against rezafungin and comparators, including currently available echinocandins, azole antifungals and amphotericin B. Eight of the 16 isolates possessed susceptibility profiles that for other *Candida* spp. would be characterized as resistant to at least three of the antifungal agents tested. These *in vitro* data showed that rezafungin possesses potent antifungal activity against a large panel of *C. auris* strains, including isolates that are resistant to other antifungal drugs, such as fluconazole and amphotericin B.¹⁷ Moreover, this study demonstrates that rezafungin possesses potent *in vivo* efficacy against *C. auris*. Further studies evaluating the efficacy of this novel antifungal against different strains of *C. auris* is recommended to rule out strain-specific findings.

Our findings are in agreement with recent studies that showed rezafungin possesses potent *in vivo* activity against *Candida*

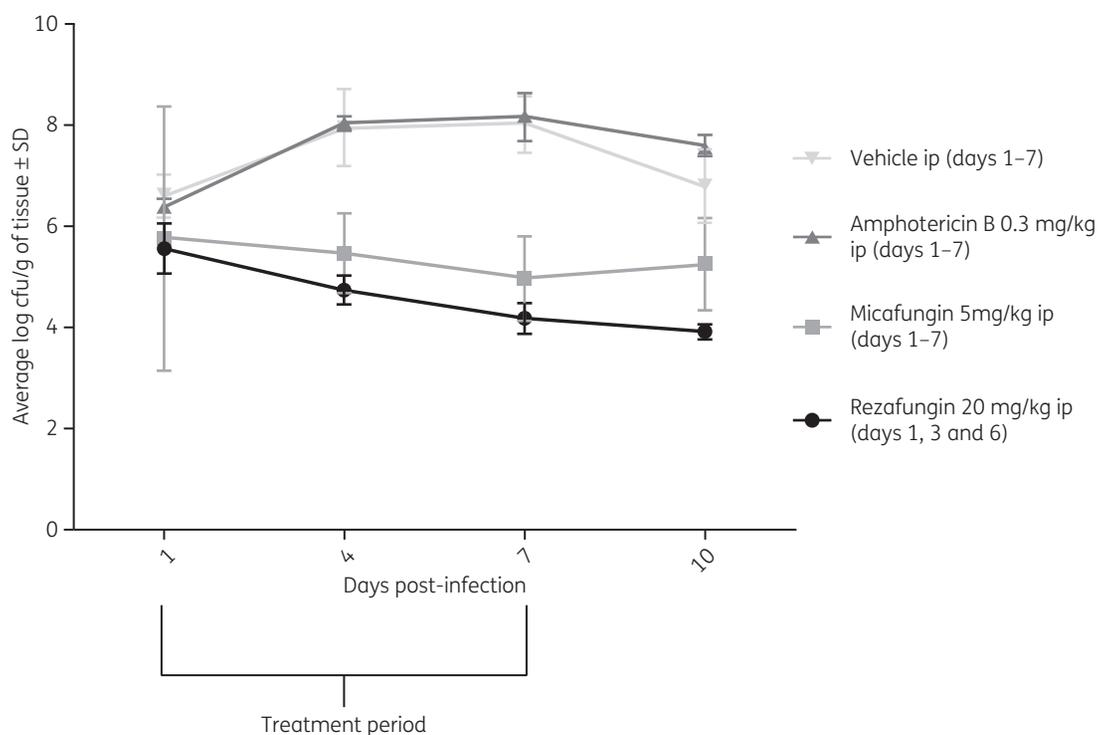


Figure 1. Average log cfu/g (\pm SD) in kidney tissue on days 1, 4, 7 and 10. ip, intraperitoneally.

Table 1. Statistical summary of between-group comparisons^a of average log cfu/g of tissue

Comparison		Mean difference	95% CI	Adjusted P value
group A	group B			
1 day post-infection				
rezafungin 20 mg/kg ip	micafungin 5 mg/kg ip	-0.20	-0.99 to 0.59	0.8801
rezafungin 20 mg/kg ip	amphotericin B 0.3 mg/kg ip	-0.85	-1.59 to -0.10	0.0231 ^b
rezafungin 20 mg/kg ip	vehicle ip	-1.04	-1.79 to -0.30	0.0052 ^b
4 days post-infection				
rezafungin 20 mg/kg ip	micafungin 5 mg/kg ip	-0.72	-1.75 to 0.32	0.2339
rezafungin 20 mg/kg ip	amphotericin B 0.3 mg/kg ip	-3.30	-4.34 to -2.27	<0.0001 ^b
rezafungin 20 mg/kg ip	vehicle ip	-3.21	-4.24 to -2.17	<0.0001 ^b
7 days post-infection				
rezafungin 20 mg/kg ip	micafungin 5 mg/kg ip	-0.78	-1.83 to 0.27	0.1836
rezafungin 20 mg/kg ip	amphotericin B 0.3 mg/kg ip	-3.99	-5.04 to -2.94	<0.0001 ^b
rezafungin 20 mg/kg ip	vehicle ip	-3.85	-4.90 to -2.80	<0.0001 ^b
10 days post-infection				
rezafungin 20 mg/kg ip	micafungin 5 mg/kg ip	-1.34	-2.41 to -0.26	0.0128 ^b
rezafungin 20 mg/kg ip	amphotericin B 0.3 mg/kg ip	-3.69	-4.76 to -2.61	<0.0001 ^b
rezafungin 20 mg/kg ip	vehicle ip	-2.89	-3.96 to -1.81	<0.0001 ^b

ip, intraperitoneally.

^aTukey's multiple comparisons test.

^bStatistically significant results.

albicans and *Aspergillus fumigatus* in neutropenic mouse models of disseminated infection.^{15,19,20} These results collectively indicate the broad-spectrum *in vivo* activity of rezafungin against multiple fungal species and support further development of rezafungin for the treatment of *C. auris* infections.

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Transparency declarations

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