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EXPERIMENTAL THERAPEUTICS

The Novel Arylamidine T-2307 Demonstrates *In Vitro* and *In Vivo* Activity against *Candida auris*

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ABSTRACT The *in vitro* and *in vivo* activity of the arylamidine T-2307 against *Candida auris* was evaluated. T-2307 demonstrated *in vitro* activity (MIC ranges ≤ 0.008 to 0.015 μg/ml at 50% inhibition; 0.125 to >4 μg/ml at 100% inhibition). Treatment with T-2307 (3 mg/kg subcutaneous [SC] once daily) also significantly improved survival (70% at 21 days postinfection) and reduced kidney fungal burden (5.06 $\log_{10}$ CFU/g) compared to control (0% survival and 7.09 $\log_{10}$ CFU/g) ($P < 0.01$).

KEYWORDS T-2307, *Candida auris*, invasive candidiasis, murine model, *in vitro* susceptibility, caspofungin, fluconazole

Candida auris is an emerging fungal pathogen that has now been detected in institutions on multiple continents (1, 2). Invasive infections caused by this species are associated with high mortality rates, up to 59% in one retrospective study (1). Unfortunately, treatment options are limited as *C. auris* isolates are often resistant to antifungals, including fluconazole and other azoles (1, 3). Up to one third of isolates may be resistant to amphotericin B, with echinocandin resistance also being reported (3–5). T-2307 is an investigational arylamidine that is similar in structure to pentamidine and causes the collapse of fungal mitochondrial membrane potential (6, 7). This agent has shown to have potent *in vitro* and *in vivo* activity against *Candida* species, including isolates that are resistant to the azoles and echinocandins (8, 9). *In vitro* and *in vivo* activity has also been demonstrated against *Cryptococcus*, *Aspergillus*, and *Fusarium* species (6, 10, 11). Our objective was to evaluate the *in vitro* and *in vivo* activity of T-2307 against *C. auris*.

In vitro susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) M27-A3 standard against 10 isolates available from the FDA CDC Antibiotic Resistance (AR) Bank and 13 clinical isolates that were received for testing by the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (UTHSCSA), representing isolates from the South Asian and South American clades (12). The MIC of T-2307 was measured as the lowest concentration that inhibited both 50% and 100% of growth compared to the drug-free control after 24 h of incubation at 35°C, while the MICs of fluconazole and caspofungin were measured at 50% growth inhibition. Male ICR mice were rendered neutropenic with a single dose of 5-fluorouracil (5 mg/mouse) administered 24 h prior to inoculation, and a clinical isolate of *C. auris* (DI 17-46) was used to infect mice via the lateral tail vein as previously described (13, 14). Treatment with vehicle control, T-2307 (0.75, 1.5, or 3 mg/kg SC once daily), fluconazole (20 mg/kg orally [p.o.] once daily), or caspofungin (10 mg/kg intraperitoneally [i.p.] once daily) began 1 day postinoculation and continued for 7 days. Ten mice were included in each group in each study arm. In the fungal burden arm, mice were humanely euthanized on day 8 postinoculation, and kidneys

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TABLE 1 *In vitro* activity of T-3207, fluconazole, and caspofungin against 23 *C. auris* isolates[a]

Activity level	T-2307 at 50% inhibition (μg/ml)	T-2307 at 100% inhibition (μg/ml)	Fluconazole at 50% inhibition (μg/ml)	Caspofungin at 50% inhibition (μg/ml)
MIC range	≤0.008–0.015	0.125–>4	0.5–>64	≤0.015–>8
MIC_{50}	0.015	>4	4	0.25
MIC_{90}	0.015	>4	>64	0.5
GM MIC	0.011	2.189	14.6	0.24

[a]MICs read after 24 h of incubation at 35°C. MIC_{50} and MIC_{90}, lowest concentrations that inhibited 50% and 90%, respectively, of the isolates tested; GM MIC, geometric mean MIC.

and brains were collected, weighed, and homogenized for analysis of CFU (CFU/g). In the survival arm, mice were followed off therapy for 14 days, until day 21 postinoculation. Fungal burden was also assessed in the survival arm on day 21 or on the day the mice succumbed to infection. Differences in survival were assessed by Kaplan-Meier analysis with the log-rank test. ANOVA with Tukey's post-hoc test for multiple comparisons were used to assess for differences in fungal burden and geometric mean (GM) MIC values.

T-2307 demonstrated *in vitro* activity against *C. auris* (Table 1). The MIC range using the 50% inhibition endpoint was $\leq$ 0.008 to 0.015 μg/ml, but was markedly higher when measured using the 100% inhibition endpoint (0.25 to >4 μg/ml). Overall, the MICs for T-2307 using the 50% inhibition endpoint were lower than those for fluconazole (range 0.5 to >64 μg/ml) and caspofungin ($\leq$ 0.015 to >8 μg/ml), and the GM MIC for T-2307 (0.011 μg/ml) was significantly lower than that observed for both fluconazole and caspofungin (14.6 and 0.24 μg/ml, respectively; $P < 0.0001$). The MICs for T-2307, fluconazole, and caspofungin against the infecting isolate were ≤ 0.008, >64, and 0.25 μg/ml, respectively. Additional *in vitro* testing of T-2307 is warranted against a larger set of *C. auris* isolates, including those from other clades.

The *in vitro* activity of T-2307 did translate into *in vivo* efficacy, as the highest dose of T-2307 (3 mg/kg) resulted in significant improvements in median and percent survival (>21 days and 70%, respectively) compared to control (5 days and 0%, respectively) ($P < 0.01$) (Fig. 1). Similar improvements in survival were also observed in mice treated with high-dose caspofungin (>21 days and 100%, respectively) ($P < 0.001$). In contrast, neither the lower doses of T-2307 nor fluconazole improved survival.

In the fungal burden arm, significant reductions in kidney CFU were also observed in mice treated with T-2307 at 3 mg/kg (mean 5.06 $\log_{10}$ CFU/g) and caspofungin (3.21

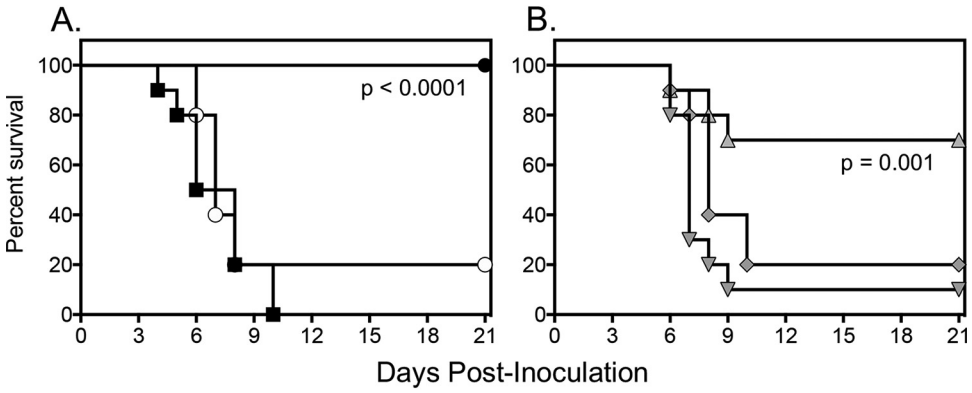

FIG 1 Survival curves in mice inoculated intravenously with *C. auris* and treated with vehicle control, fluconazole 20 mg/kg orally (p.o.) once a day (QD), or caspofungin 10 mg/kg intraperitoneally (i.p.) QD (A) or T-2307 at doses of 0.75 mg/kg, 1.5 mg/kg, or 3 mg/kg subcutenously QD (B). Treatment started 1 day postinoculation and continued for 7 days. Mice were then followed off therapy until day 21 postinoculation (14 days after therapy stopped). Black square, vehicle (untreated) control; white circle, fluconazole 20 mg/kg; black circle, caspofungin 10 mg/kg; inverted gray triangle, T-2307 0.75 mg/kg; gray rectangle, T-2307 1.5 mg/kg; gray triangle, T-2307 3 mg/kg. $n = 10$ mice per group.

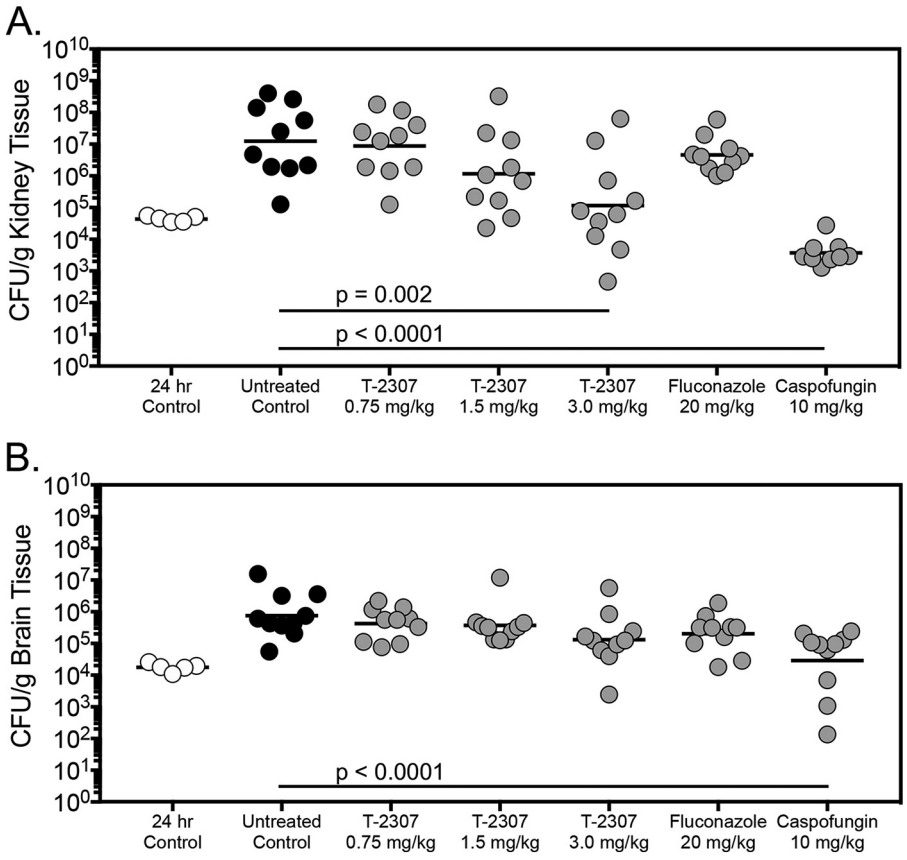

FIG 2 Kidney (A) and brain (B) fungal burden (CFU/g) in mice with invasive candidiasis secondary to *C. auris* in the fungal burden arm. CFU/g were measured on day 8 postinoculation after 7 days of therapy. $n = 10$ mice in the vehicle control and treatment groups; $n = 5$ mice in the 24 h control group.

$\log_{10}$ CFU/g) compared to untreated control (7.09 $\log_{10}$ CFU/g) ($P < 0.01$) (Fig. 2). The activity of T-2307 was static in nature, as the fungal burden in the 3 mg/kg group was similar to that observed in the 24 h group measured just prior to the start of therapy. Reductions in kidney fungal burden were not observed in mice treated with the lower doses of T-2307 or fluconazole. Brain fungal burden observed in the caspofungin group (4.45 $\log_{10}$ CFU/g) was significantly lower than in the untreated control (5.88 $\log_{10}$ CFU/g) ($P < 0.001$) on day 8 postinoculation, but not in mice treated with T-2307 or fluconazole.

In the survival arm, kidney fungal burden was significantly lower in the T-2307 3 mg/kg (6.28 $\log_{10}$ CFU/g) and caspofungin groups (3.11 $\log_{10}$ CFU/g) compared to untreated control (8.04 $\log_{10}$ CFU/g) ($P \leq 0.01$) (Fig. 3). Interestingly, brain fungal burden was significantly reduced in mice treated with T-2307 at 3 mg/kg (4.16 $\log_{10}$ CFU/g) and caspofungin (2.51 $\log_{10}$ CFU/g) versus untreated control (6.31 $\log_{10}$ CFU/g) ($P \leq 0.01$). Previous studies have demonstrated reductions in brain and ocular tissue fungal burden in mice infected with *Cryptococcus gattii* and *Candida albicans*, respectively, and treated with T-2307 (10, 11). In the current study, there was also a clear relationship between fungal burden and survival, as treated mice that survived to the day 21 endpoint had lower kidney and brain fungal burden compared to those that succumbed to infection. The survival and fungal burden results for the control, fluconazole, and caspofungin groups are consistent with those we have previously reported demonstrating the overall reproducibility of this model (13, 14).

These results demonstrate that T-2307 may be effective against invasive infections caused by *C. auris*, as both *in vitro* and *in vivo* activity were observed against

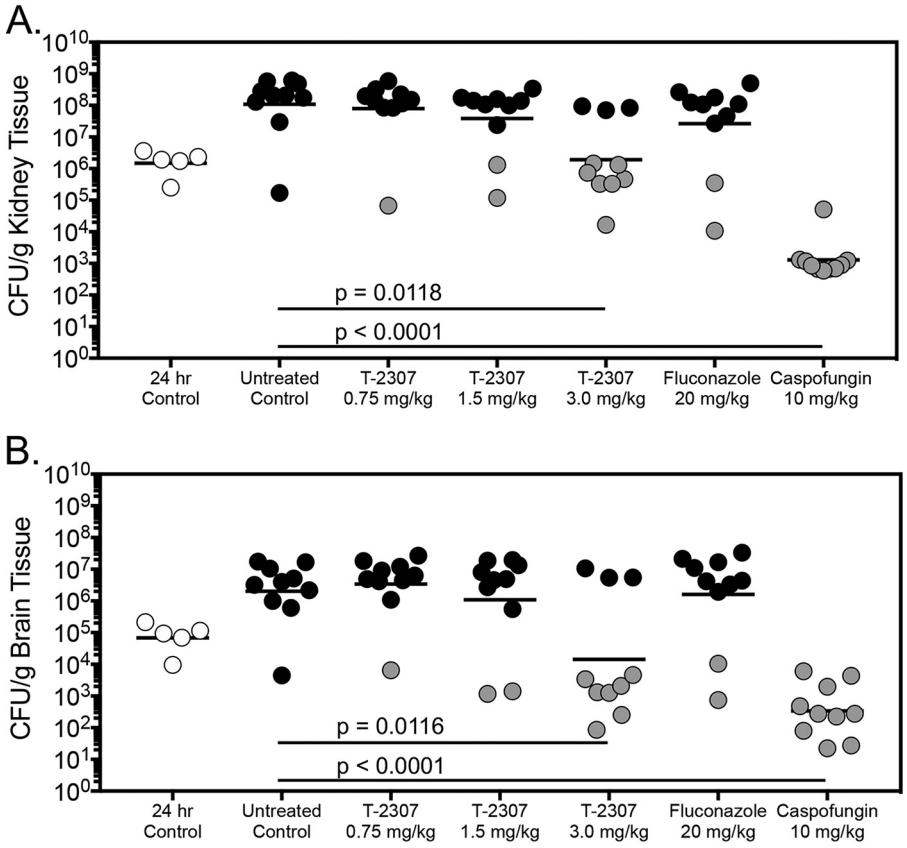

FIG 3 Kidney (A) and brain (B) fungal burden (CFU/g) in mice with invasive candidiasis secondary to *C. auris* in the survival arm. CFU/g were measured on day 8 postinoculation after 7 days of therapy. $n = 10$ mice in the vehicle control and treatment groups; $n = 5$ mice in the 24 h control group. Black circles represent mice that succumbed to infection prior to day 21; gray circles represent mice that survived to the survival endpoint.

this emerging pathogen. The reductions in fungal burden observed in this study were less than those previously observed by our group against echinocandin-resistant *C. albicans* in immunocompetent mice (9), but were similar to those observed against echinocandin-resistant *C. glabrata* infections in neutropenic mice (8). This suggests that the *in vivo* efficacy of T-2307 may be influenced by host immune status, similar to what has been reported for other antifungals in murine models of infection (15, 16). It is unknown if efficacy could be improved with higher doses or more frequent administration. Additional studies are warranted, including pharmacokinetic/pharmacodynamic assessments.

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