

The novel arylamidine T-2307 demonstrates *in vitro* and *in vivo* activity against echinocandin-resistant *Candida glabrata*

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Objectives: *Candida* species are major causes of invasive mycoses in immunocompetent and immunocompromised hosts. Treatment options are limited in the setting of antifungal resistance and increased rates of echinocandin-resistant *Candida glabrata* have been reported. The novel arylamidine T-2307 demonstrates potent *in vitro* antifungal activity against *Candida* species. Our objective was to evaluate the *in vitro* and *in vivo* activity of T-2307 against resistant *C. glabrata*.

Methods: *In vitro* activity was determined against 42 clinical *C. glabrata* isolates, including 17 echinocandin-resistant strains. Neutropenic ICR mice were inoculated intravenously with an echinocandin-resistant *C. glabrata* isolate (T-2307; caspofungin MICs \leq 0.008 and 0.5 mg/L, respectively). Therapy with vehicle control, T-2307 (0.75, 1.5, 3 or 6 mg/kg subcutaneously once daily) or caspofungin (1 or 10 mg/kg intraperitoneally once daily) began 1 day post-challenge. Kidneys were collected on day 8 and fungal burden was assessed by counting cfu.

Results: T-2307 demonstrated potent *in vitro* activity against *C. glabrata* (geometric mean MIC 0.0135 mg/L), which was maintained against echinocandin-resistant isolates (geometric mean MIC 0.0083 mg/L). T-2307 also demonstrated *in vivo* efficacy in mice infected with echinocandin-resistant *C. glabrata*. Significant reductions in fungal burden were observed at each dosage level of T-2307 compared with control. Reductions in fungal burden were also observed with high-dose caspofungin.

Conclusions: T-2307 demonstrated potent *in vitro* activity against *C. glabrata*, including echinocandin-resistant isolates, which translated into *in vivo* efficacy against invasive candidiasis caused by an echinocandin-resistant *C. glabrata* strain. These results demonstrate the potential for T-2307 as therapy against echinocandin-resistant *Candida*.

Introduction

Candida glabrata is a common species isolated from patients with invasive candidiasis.¹ The treatment of infections caused by this species may be limited, as many isolates may be resistant to fluconazole or require higher doses of this widely available azole.² Treatment guidelines recommend the use of the echinocandins for invasive infections caused by *C. glabrata* due to their *in vivo* efficacy and good tolerability.² However, echinocandin-resistant *C. glabrata* may be increasing at some institutions in the USA.^{3–5}

T-2307 is a novel arylamidine structurally similar to pentamidine and causes the collapse of mitochondrial membrane potential.^{6–8} This agent has potent activity against *Candida* species, including azole-resistant isolates,⁶ and was recently shown to be efficacious against echinocandin-resistant *Candida albicans*.⁹

Our objective was to assess the *in vitro* activity of T-2307 against *C. glabrata*, including echinocandin-resistant isolates, and determine whether this translated into *in vivo* efficacy in a murine model of invasive candidiasis caused by resistant *C. glabrata*.

Materials and methods

Antifungals

T-2307 (Toyama Chemical Company, Ltd, Toyama, Japan) and caspofungin powder (Merck & Company, Inc., Whitehouse Station, NJ, USA) powders were dissolved in DMSO to prepare a stock solution for susceptibility testing with further dilutions in RPMI buffered with 0.165 M MOPS (pH 7.0). For *in vivo* studies, a solution of T-2307 was prepared in sterile physiological saline and the commercially available formulation of caspofungin was used.

***Candida glabrata* isolates and in vitro susceptibility testing**

Forty-two *C. glabrata* clinical isolates from the University of Texas Health Science Center at San Antonio were used. This included 25 WT isolates and 17 that were echinocandin-resistant according to the CLSI M27-S4 clinical breakpoints.¹⁰ Antifungal susceptibility testing was performed according to the CLSI M27-A3 methods with MICs read after 24 h of incubation at both 50% and 100% inhibition of growth.¹¹ In the *in vivo* model an echinocandin-resistant *C. glabrata* clinical isolate (05-62) with a defined Fks2p amino acid substitution (R1379S) was used. This resistant isolate was chosen as it results in consistent infection in the murine model described below.^{12,13} The isolate was sub-cultured three times on Sabouraud dextrose agar, each for 48 h at 37°C. Colonies were taken from the third subculture by scraping the surface of the agar plates and washing three times in sterile saline with 0.1% Tween 20. The cells were then collected by centrifugation and resuspended in sterile saline with 0.1% Tween to the target inoculum and the number of viable cells/mL was determined by counting cfu.

Animal model and fungal burden

We utilized our established murine model of invasive candidiasis for this study.^{12,13} Outbred male ICR mice were used and had access to food and water *ad libitum*. Mice were rendered neutropenic by a single dose of 150 mg/kg 5-fluorouracil 1 day prior to inoculation. On day 0, mice were infected intravenously with *C. glabrata* at a target inoculum of 1×10^8 cfu/mouse. Twenty-four hours after inoculation, therapy was started with control (sterile physiological saline), T-2307 (0.75, 1.5, 3 or 6 mg/kg once daily by subcutaneous injection) or caspofungin (1 or 10 mg/kg once daily by intraperitoneal injection), and continued for 7 days. These doses were chosen based on our previous experience with both T-2307 and caspofungin against invasive candidiasis caused by echinocandin-susceptible and -resistant isolates of *C. glabrata* and *C. albicans*.^{9,13} On day 8, mice were humanely euthanized and the kidneys were removed, weighed and homogenized in sterile saline. Serial dilutions of the homogenates were prepared and plated on Sabouraud dextrose agar. Following incubation, colonies were counted and the number of cfu/g within the kidney tissue was calculated. This study was approved by the Institutional Animal Care and Use Committee at UTHSCSA (protocol number 10129x) and all animals were maintained in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care.

Data analysis

For the *in vitro* analysis, MIC ranges, MIC₅₀ values, MIC₉₀ values and geometric mean (GM) MIC values were determined. Differences in GM MICs were assessed for significance using a paired *t*-test. Differences in fungal burden were assessed for significance using ANOVA with Tukey's test for multiple comparisons. A *P* value of ≤ 0.05 was considered statistically significant.

Results

***In vitro* susceptibility**

T-2307 demonstrated potent *in vitro* activity against *C. glabrata*, including echinocandin-resistant isolates. The MICs for all isolates ranged from ≤ 0.008 to >4 mg/L for a single isolate using the 50% inhibition endpoint, with the T-2307 MIC ≤ 0.008 mg/L for the majority of isolates (Table 1). Caspofungin MICs ranged from 0.06 to 4 mg/L and the GM MIC of caspofungin was significantly higher than that observed with T-2307 (0.3773 versus

Table 1. MICs (mg/L) of T-2307 (0.008–4 mg/L) and caspofungin (0.015–8 mg/L) for 42 *C. glabrata* isolates, including caspofungin-susceptible ($n=25$) and caspofungin-resistant ($n=17$) strains

Parameter	T-2307	Caspofungin
All isolates ($n=42$)		
MIC range	≤ 0.008 to >4	0.06–4
MIC ₅₀	≤ 0.008	0.25
MIC ₉₀	≤ 0.008	2
GM MIC	0.0135	0.3773
Echinocandin-resistant isolates ($n=17$)		
MIC range	≤ 0.008 –0.015	0.5–4
MIC ₅₀	≤ 0.008	0.5
MIC ₉₀	≤ 0.008	2
GM MIC	0.0083	0.8849

MIC₅₀ and MIC₉₀, MICs at which 50% and 90% of the isolates were inhibited, respectively.

The 50% inhibition endpoint is shown for both T-2307 and caspofungin.

0.0135 mg/L, respectively; $P < 0.0001$). The potency of T-2307 was also maintained against the echinocandin-resistant isolates (MIC range ≤ 0.008 –0.015 mg/L; GM MIC 0.0083). In contrast, caspofungin MICs ranged from 0.5 to 4 mg/L for these 17 isolates (GM MIC 0.8849). The MICs of T-2307 and caspofungin for the strain used to infect mice (05-62) were ≤ 0.008 and 0.5 mg/L, respectively. As previously reported, T-2307 did not result in complete inhibition of growth, as reflected by the MICs of >4 mg/L when the 100% inhibition endpoint was used, which is consistent with the trailing effect observed with this agent.^{9,14} Thus, it is unknown whether the isolate with the elevated T-2307 MIC (>4 mg/L) was truly resistant to this agent or whether this was due to the trailing effect. However, no visual reductions in growth were observed against this isolate on two separate occasions and its growth rate was not reduced compared with the others.

Fungal burden

The *in vitro* potency of T-2307 translated into *in vivo* efficacy in our neutropenic murine model of invasive candidiasis. Significant reductions in fungal burden were observed with each T-2307 dose (range of mean \log_{10} cfu/g 4.78–5.01) compared with control (6.60 \log_{10} cfu/g; $P \leq 0.001$ for all comparisons) (Figure 1). These results were reproducible for the T-2307 dose levels of 1.5, 3 and 6 mg/kg, which were evaluated in two independent experiments. Fungal burden with T-2307 was also significantly lower than that observed with 1 mg/kg caspofungin (6.75 \log_{10} cfu/g; $P \leq 0.001$ for all comparisons). In contrast, high-dose 10 mg/kg caspofungin also resulted in significant reductions in fungal burden compared with both control and the lower dose level of this echinocandin (5.00 \log_{10} cfu/g; $P < 0.001$). When two low fungal burden outliers were removed from the 6 mg/kg T-2307 and 10 mg/kg caspofungin groups, the reductions in fungal burden compared with control and 1 mg/kg caspofungin were still significant (5.02 and 5.19 \log_{10} cfu/g for 6 mg/kg T-2307 and 10 mg/kg caspofungin, respectively; $P < 0.001$ for both comparisons).

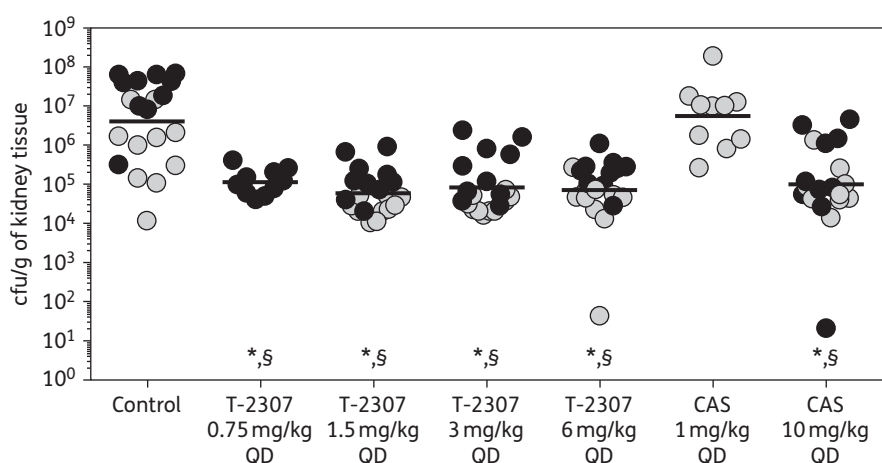


Figure 1. Kidney fungal burden (cfu/g of kidney tissue) on day 8 in mice infected with *C. glabrata* 05-62 and treated with placebo by subcutaneous injection once daily as follows: control (physiological saline); T-2307 at doses of 0.75, 1.5, 3 or 6 mg/kg by subcutaneous injection once daily; or caspofungin at doses of 1 or 10 mg/kg by intraperitoneal injection once daily. Treatment began 1 day post-inoculation and continued for 7 days. Grey circles represent data from the first experiment and black circles represent data from the repeat experiment. Lines represent mean values. * $P < 0.001$ versus vehicle control; \$ $P < 0.001$ versus 1 mg/kg caspofungin. CAS, caspofungin; QD, once daily.

Discussion

T-2307 is an investigational antifungal that causes collapse of fungal mitochondrial membrane potential following uptake.^{7,8} Previous studies have demonstrated potent *in vitro* activity against *Candida* species, *Cryptococcus neoformans* and *Aspergillus fumigatus*, which has translated into *in vivo* efficacy in murine models of invasive disease caused by these species.⁶ We have recently demonstrated that T-2307 also maintains potent *in vitro* activity against echinocandin-resistant *C. albicans*, which translated into *in vivo* efficacy in a murine model of invasive candidiasis.⁹ This activity against resistant *Candida* is encouraging, because treatment options against such infections are limited. Recently, several institutions in the USA have reported increases in the incidence of echinocandin-resistant *C. glabrata*.³⁻⁵ Unfortunately, it has also been reported that many of these isolates may also be resistant to fluconazole.^{5,15}

In the current study, T-2307 demonstrated potent *in vitro* and *in vivo* activity against echinocandin-resistant *C. glabrata*. The *in vitro* potency of this agent was similar between WT and echinocandin-resistant strains and each T-2307 dose resulted in significant reductions in tissue fungal burden compared with control and 1 mg/kg caspofungin. In fact, there did not appear to be a dose-response relationship, as the reductions in fungal burden observed with each dose of T-2307 were similar. The reductions in fungal burden that were observed in this study were not as great as those we recently reported when T-2307 was evaluated against invasive candidiasis caused by an echinocandin-resistant *C. albicans* isolate.⁹ This difference may be due to the use of a neutropenic model in the current study for mice infected with *C. glabrata* versus an immunocompetent model for the *C. albicans* study. We have previously demonstrated that the immune status (i.e. neutropenic versus non-neutropenic) may influence treatment outcomes in mice infected with *C. albicans*.¹³ Another explanation may be that the doses used in the current study were not low enough to observe

a dose-response relationship. Previous studies with T-2307 against invasive candidiasis caused by echinocandin-susceptible *C. glabrata* and *C. albicans* isolates did demonstrate a dose-response relationship, but with lower doses.^{6,14} In addition, the reduction in fungal burden observed against echinocandin-susceptible *C. glabrata* was similar to what we observed with higher doses of T-2307 against the resistant isolate.¹³

Overall, these results suggest that T-2307 may have potential for the treatment of invasive infections caused by echinocandin-resistant *C. glabrata*. Further *in vivo* studies with other isolates and pharmacokinetic/pharmacodynamics evaluations are warranted.

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Transparency declarations

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