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Oral glucan synthase inhibitor SCY-078 is effective in an experimental murine model of invasive candidiasis caused by WT and echinocandin-resistant *Candida glabrata*

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Background: Echinocandins are recommended as first-line therapy against *Candida glabrata* infections, although increased resistance to this class has been reported worldwide and they are currently only available for parenteral administration. SCY-078 is an investigational glucan synthase inhibitor that is orally available.

Objectives: To evaluate the *in vivo* efficacy of SCY-078 in an experimental model of invasive candidiasis due to WT and echinocandin-resistant *C. glabrata* isolates.

Methods: Neutropenic ICR mice were inoculated intravenously with a WT isolate (SCY-078 and caspofungin MICs 0.25 and 0.125 mg/L, respectively) or an echinocandin-resistant isolate (SCY-078 and caspofungin MICs 1 and 0.5 mg/L, respectively). Treatment with placebo, SCY-078 (8, 30 or 40 mg/kg orally every 12 h) or caspofungin (1 mg/kg by intraperitoneal injection once daily) began 24 h later. Kidney fungal burden was measured on day 8 post-inoculation.

Results: Significant reductions in kidney fungal burden were observed with 30 mg/kg SCY-078 against both isolates and with the 40 mg/kg dose against the echinocandin-resistant isolate. These results were supported by SCY-078 plasma concentration data at the higher doses, where levels above the MICs for both isolates were observed 12 h after the last oral dose. Reductions in fungal burden were also observed with caspofungin against the WT isolate, but not against the resistant isolate.

Conclusions: SCY-078 demonstrated *in vivo* efficacy against infections caused by both WT and echinocandin-resistant *C. glabrata* isolates in this experimental model. This orally available glucan synthase inhibitor has potential as a therapy against echinocandin-resistant *C. glabrata* infections.

Introduction

In the USA, the second most prevalent species associated with invasive infection is *Candida glabrata*.[1,2] Current treatment strategies for invasive infections caused by *C. glabrata* include the use of azoles, amphotericin B or echinocandins. Although effective, each of these classes has drawbacks that may limit clinical responses. For example, amphotericin B formulations are associated with nephrotoxicity and are only available intravenously. The echinocandins, although clinically safer than the polyenes, are also only available for intravenous administration, which may limit their use in outpatients. Fluconazole has been a safe and effective treatment option for many years for patients with invasive candidiasis and this antifungal is available in both oral and intravenous formulations. However, recent epidemiological studies have also demonstrated that fluconazole and echinocandin resistance in *C. glabrata* is increasing at many centres in the USA and worldwide.[3–6] Thus, the development of new therapeutic strategies for invasive candidiasis, including those infections caused by *C. glabrata*, is of paramount importance.

SCY-078 is an investigational glucan synthase inhibitor that has potent *in vitro* activity against *Candida* species, including isolates with mutations in *fks1* hot-spot regions that confer resistance to echinocandins.[7] This agent is also being developed for oral administration and thus may also overcome the limitation of the need for intravenous administration. The objective of this study was to evaluate the *in vivo* efficacy of SCY-078 administered orally against

invasive candidiasis caused by both WT and echinocandin-resistant *C. glabrata* isolates.

Materials and methods

Antifungals

SCY-078 (Scynexis, Inc., Jersey City, NJ, USA) was provided in a methylcellulose formulation for oral administration. The pharmaceutical formulation of caspofungin was used in the *in vivo* model. For *in vitro* susceptibility testing, powders of both agents were dissolved in DMSO and further dilutions were prepared in RPMI medium buffered with 0.165 M MOPS (pH 7.0).

Isolates

Both a susceptible WT *C. glabrata* isolate (05-761) and an echinocandin-resistant isolate (05-62; R1379S Fks2p amino acid substitution) were used. Antifungal susceptibility testing was performed per the CLSI M27-A3 methods with MICs read after 24 h of incubation at 50% inhibition of growth for both SCY-078 and caspofungin.[8] Prior to use in the *in vivo* model, isolates were subcultured at 37 °C for 48 h on Sabouraud dextrose agar three times before being washed in sterile saline with 0.1% Tween 20. Inoculum viability was determined by serially diluting an aliquot and plating it on Sabouraud dextrose agar to determine the number of cfu after incubation at 37 °C.

Murine model

We utilized our established murine models of invasive candidiasis.[9–12] Outbred ICR male mice (Harlan) were housed five per cage and were rendered neutropenic with a single dose of 5-fluorouracil (150 mg/kg) administered intravenously 1 day prior to infection. Although this immunosuppression is temporary, mice are rendered neutropenic ($<2\times10^9$ neutrophils/L with a nadir of $<1\times10^9$/L) on days 1–8 post-inoculation, which corresponds to the duration of treatment and fungal burden analysis in this model. On the day of inoculation, animals were infected intravenously with 0.2 mL of *C. glabrata* with the number of *Candida* cells per animal adjusted to body weight (e.g. 4×10^6 *C. glabrata* cells/g = 1.0×10^8 cells per mouse for a 25 g mouse). Mice were monitored at least twice daily throughout the study to prevent and minimize unnecessary pain or distress post-inoculation. One day after inoculation, therapy was started with a placebo (methylcellulose), SCY-078 (8, 30 or 40 mg/kg by oral gavage every 12 h) or caspofungin (1 mg/kg by intraperitoneal injection once daily) and continued for 7 days. This dose of caspofungin was chosen based on our previous experience with this echinocandin against invasive candidiasis caused by resistant *Candida* species,[9,13,14] and the extensive tissue exposure that is achieved with repeated dosing, which exceeds the MIC_{90} for *C. glabrata*.[15,16] Mice were humanely euthanized on day 8, ∼12 h after the last dose of SCY-078. The kidneys were aseptically removed, weighed and homogenized, and serial dilutions of the homogenates were plated on Sabouraud dextrose agar. After 24 h of incubation, the number of cfu/g was calculated. Blood was also collected from the anaesthetized mice treated with SCY-078 via cardiac puncture. The plasma was separated and SCY-078 concentrations were measured. All animals were maintained in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care and this study was approved by the Institutional Animal Care and Use Committee at UTHSCSA (protocol no. 16038x).

SCY-078 plasma concentrations

Plasma samples were analysed by LC-MS/MS after protein precipitation as previously described.[17]

Data analysis

Differences in kidney fungal burden (cfu/g) and plasma concentrations among the groups were assessed for significance by ANOVA with Tukey's post-test for multiple comparisons. A P value of ≤ 0.05 was considered statistically significant for all comparisons.

Results

Fungal burden: WT isolate

SCY-078 was effective at reducing kidney fungal burden in this experimental model of invasive candidiasis caused by *C. glabrata*. Oral administration of SCY-078 at doses of 30 mg/kg significantly reduced the number of cfu (mean $\log_{10}$ cfu/g $\pm$ SD 3.57 ± 0.79) in the kidneys of mice infected with the WT isolate compared with both fungal burden measured prior to the start of therapy (24 h post-inoculation, 5.38 ± 0.85 $\log_{10}$ cfu/g) and on day 8 in mice administered a placebo (5.58 ± 1.11 $\log_{10}$ cfu/g; $P<0.01$ for both comparisons) (Figure 1a). Caspofungin also resulted in a significant reduction in the fungal burden compared with that observed prior to the start of therapy and in the placebo group on day 8 (2.74 ± 0.76 $\log_{10}$ cfu/g; $P<0.01$ for both comparisons). Neither SCY-078 at 8 mg/kg nor 40 mg/kg significantly reduced the fungal burden, although there was a trend with the higher dose compared with the placebo group (4.18 ± 0.96 $\log_{10}$ cfu/g; $P=0.074$).

Fungal burden: echinocandin-resistant isolate

SCY-078 was also effective against infection caused by the echinocandin-resistant isolate (Figure 1b). Both the 30 and 40 mg/kg doses of SCY-078 significantly reduced the fungal burden (2.38 ± 0.66 and 2.34 ± 0.60 $\log_{10}$ cfu/g, respectively) compared with that measured prior to the start of therapy (3.66 ± 0.46 $\log_{10}$ cfu/g; $P\leq0.03$ for both comparisons) and in the placebo group on day 8 (3.99 ± 1.04 $\log_{10}$ cfu/g; $P<0.01$ for both comparisons). As expected, caspofungin was ineffective against infection caused by the resistant isolate, as the fungal burden remained unchanged (3.61 ± 1.22 $\log_{10}$ cfu/g). As observed against the WT isolate, 8 mg/kg SCY-078 was ineffective.

SCY-078 plasma concentrations

Bloodstream levels of SCY-078 were also elevated in mice treated with the 30 and 40 mg/kg doses. As shown in Figure 2, plasma concentrations at these two doses remained higher than the MICs for both the WT (SCY-078, 0.25 mg/L; caspofungin, 0.125 mg/L) and echinocandin-resistant (SCY-078, 1 mg/L; caspofungin, 0.5 mg/L) isolates 12 h after dosing had stopped (combined mean 3.225 ± 1.023 and 3.683 ± 0.761 mg/L, respectively). These concentrations were significantly higher than those achieved with the 8 mg/kg dose, which were similar to the MICs for the isolates used in this study (0.645 ± 0.221 mg/L; $P<0.01$). The plasma concentrations for SCY-078 were also consistent between the experiments involving the WT and resistant isolates.

Discussion

SCY-078 is an investigational semi-synthetic derivative of enfumafungin that inhibits glucan synthase, leading to a decrease in (1,3)-β-D-glucan polymers and a weakening of the fungal cell

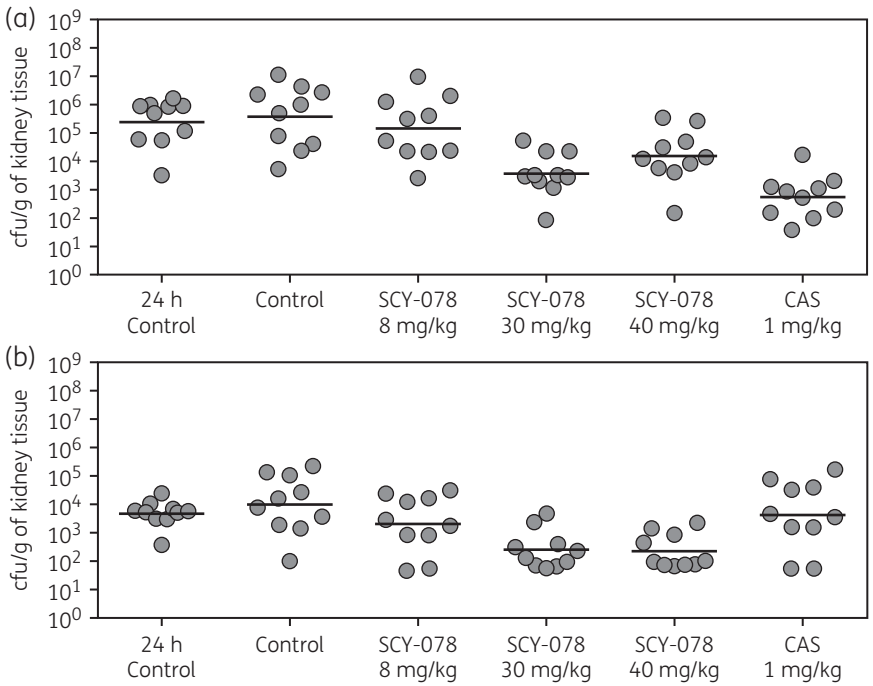

Figure 1. Kidney fungal burden in mice infected with (a) the WT *C. glabrata* isolate and (b) the echinocandin-resistant isolate at 24 h post-inoculation just prior to the start of therapy and on day 8 in mice treated with placebo, SCY-078 or caspofungin. CAS, caspofungin.

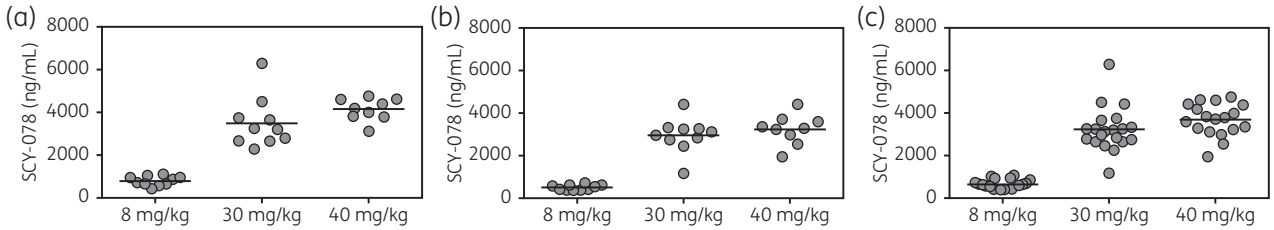

Figure 2. SCY-078 plasma concentrations at ∼12 h after the last dose in mice infected with (a) the WT *C. glabrata* isolate and (b) the echinocandin-resistant isolate. (c) Combined results.

wall.[18] This agent is structurally different from the echinocandins, which act in the same fashion. Unlike the echinocandins, SCY-078 is also absorbed from the gastrointestinal track following oral administration. Potent *in vitro* activity for SCY-078 has been reported against *Candida* species, including some isolates that harbour *fks1* and *fks2* point mutations that cause echinocandin resistance.[18] Efficacy has also been reported in an established murine model of infection caused by *Candida albicans*, *C. glabrata* and *Candida tropicalis*.[19] In this previous study, the SCY-078 MICs for the *C. glabrata* isolates ranged from 0.03 to 0.25 mg/L and it is unknown whether any of these were echinocandin-resistant or harboured *fks* mutations.

The results of our study support the *in vitro* findings that SCY-078 maintains activity against echinocandin-resistant *C. glabrata* isolates. This agent was effective at reducing the kidney fungal burden against both WT and resistant isolates in our established neutropenic murine model. At the most effective oral dose used, SCY-078 resulted in a 1.28–1.81 $\log_{10}$ cfu/g reduction in fungal burden compared with that measured prior to the start of therapy and a 1.61–2.01 $\log_{10}$ cfu/g reduction compared with that of mice administered placebo. These results were supported by the plasma concentrations that were achieved 12 h after the last oral dose of SCY-078 and result in exposures that are achievable in humans.[17,20] Interestingly, the highest dose of SCY-078 did not result in a statistically significant reduction in fungal burden against the WT isolate. Although paradoxical reductions in activity have been reported for the echinocandins at higher concentrations against *Candida* species, which also target the production of (1,3)-β-D-glucan polymers, this has not been observed against *C. glabrata*,[21,22] and was not observed when SCY-078 was tested *in vitro* against the WT isolate in this study. Thus, the lack of a statistically significant reduction in fungal burden at the highest dose used against this isolate may be due to model variability, as there was a trend toward a reduction in fungal burden ($P = 0.074$ as reported above).

Overall, these results suggest that SCY-078 may be potentially useful for the treatment of invasive *C. glabrata* infections, including those caused by echinocandin-resistant isolates. One limitation of this study is the use of a resistant isolate with a relatively rare *fks* mutation compared with other more frequent mutations that lead to higher echinocandin MICs (e.g. Fks1p S629P, Fks2p S663P, F659S

and F659del).[4,5,23,24] Thus, it cannot be extrapolated that SCY-078 would maintain *in vivo* efficacy against all *fks* mutations. However, in another experiment conducted by our group, SCY-078 did maintain *in vitro* activity compared with caspofungin against two resistant *C. glabrata* isolates, including one harbouring a S629P amino acid change in Fks1p and the other a F659del in Fks2p (SCY-078 MICs 1 and 0.5 mg/L versus caspofungin MICs >8 and 8 mg/L, respectively). Thus, further *in vivo* studies with other isolates harbouring different *fks* mutations are warranted.

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Transparency declarations

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