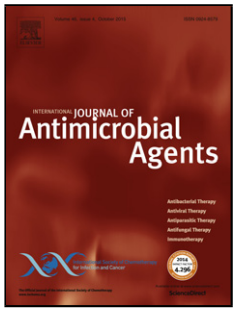

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Voriconazole minimum inhibitory concentrations are predictive of treatment outcome in experimental murine infections by *Candida glabrata*

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ABSTRACT

In this study, 27 clinical isolates of *Candida glabrata* with voriconazole (VRC) minimum inhibitory concentrations (MICs) ranging from ≤0.03 μg/mL to 8 μg/mL were tested to determine whether in vitro data are predictive of in vivo efficacy. The efficacy of VRC administered at 40 mg/kg was assayed in a neutropenic murine model of disseminated infection by *C. glabrata*. The reduction in fungal tissue burden in the kidneys was used as a marker of treatment efficacy. VRC reduced the fungal tissue burden in mice infected with strains that had MICs below the epidemiological cut-off value (ECV) of 0.25 μg/mL. Variable efficacy of VRC was obtained when the MIC equalled the ECV, and VRC was ineffective when the MIC exceeded the ECV. These results suggest that the use of in vitro data could be useful to predict the outcome for infections by this fungus.

1. Introduction

Candida glabrata is the second most frequent bloodstream pathogen affecting ca. 10–20% of patients with candidaemia in North America, especially in the USA [1]. The high incidence of infections caused by this species has been linked to the use of broad-spectrum antibiotics, the use of central venous catheters, stay in an intensive care unit, and renal failure in elderly patients [2]. Although it has been reported that *C. glabrata* is less virulent than *Candida albicans*, the mortality rate is higher due to the emergence of multidrug resistance in bloodstream isolates [3]. Current recommendations for invasive candidiasis (IC) in neutropenic patients include the use of echinocandins as primary therapy along with lipid formulations of amphotericin B [4]. However, recent studies have reported therapeutic failure or recurrence of *C. glabrata* infections following treatment with echinocandins linked to the emergence of strains with mutations in the *FKS* genes [5,6]. In the European and South American guidelines, fluconazole (FLC) is also recommended as prophylactic treatment [7,8]. Moreover, a rapid increase of *C. glabrata* strains showing resistance or low susceptibility to azoles, especially to FLC, has been recently reported, which could be associated with overexpression of the ABC transporter genes *CDR1* and *CDR2*, probably acquired due to its frequent use as prophylaxis [9–11]. Based on different clinical data, voriconazole (VRC) is considered an option for initial empirical treatment of IC in febrile neutropenic patients [7,8]. However, there are no experimental data on the efficacy of VRC in the treatment of disseminated *C. glabrata* infections, although it has been demonstrated in animal infections by other non-*albicans Candida* spp. [12,13]. Clinical breakpoints for VRC against *C. glabrata* have not been established, but recently the Clinical and Laboratory

Standards Institute (CLSI) has suggested an epidemiological cut-off value (ECV) of 0.25 μg/mL for VRC against *C. glabrata* [14]. To evaluate whether the ECV for VRC is predictive of in vivo efficacy, a murine model of disseminated *C. glabrata* infection using clinical isolates with different minimum inhibitory concentrations (MICs) was developed.

2. Materials and methods

For this study, 27 clinical isolates of *C. glabrata* representing a wide range of VRC MICs were selected. The MICs were determined previously in triplicate using the broth microdilution method according to the CLSI standard for yeasts [15] and, when discrepancies were found, the mode of the different values was taken. MICs were below the ECV in 9 isolates (33.3%), equal to the ECV in 3 isolates (11.1%) and above the ECV in 15 isolates (55.6%) (Table 1). The isolates were grown on potato dextrose agar (PDA) plates (Pronadisa, Madrid, Spain) at 35 °C for 48 h. Cultures were scraped off with a sterile loop, were suspended in sterile saline and were adjusted to the desired concentration by haemocytometer count. The viability of the inocula was confirmed by plating 10-fold dilutions onto PDA plates and counting the number of CFU.

Four-week-old male OF1 mice (Charles River Laboratories; Criffa SA, Barcelona, Spain) weighing 30 g were housed under standard conditions. Animals were immunosuppressed 2 days prior to infection by intraperitoneal injection of 200 mg/kg body weight of cyclophosphamide and once every 5 days thereafter (Genoxal®; Laboratories Funk S.A., Barcelona, Spain) [16]. Groups of 16 mice were challenged intravenously via the lateral tail vein with a yeast suspension consisting of 2×10^8

CFU/animal of each isolate in 0.2 mL of sterile saline. In a previous study using different strains, it was demonstrated that this inoculum resulted in a high fungal load in the kidneys [17]. Groups of 8 infected mice received VRC (VFEND®; Pfizer S.A., Madrid, Spain) at 40 mg/kg once daily by oral gavage [18,19]. Previous pharmacokinetic studies in an experimental murine model had demonstrated that this dose provided drug plasma levels above all MIC for all strains tested [17]. From 3 days before infection, mice receiving VRC were given diluted (1:2) grapefruit juice instead of water in order to increase the drug concentration in murine serum [20]. Treatments began 24 h after challenge and lasted 7 days. Controls received no treatment. In addition, to prevent bacterial infections all animals received 5 mg/kg day ceftazidime subcutaneously. The efficacy of VRC was evaluated by reduction of tissue burden in the kidneys. Animals were euthanised by CO_2 anoxia 7 days post-infection, 4 h after the last drug administration, in order to compare the results with untreated controls. Kidneys were then aseptically removed, were homogenised in 1 mL of sterile saline and serial 10-fold dilutions of the homogenates were plated on PDA and were incubated for 48 h at 35 °C to determine tissue burden (CFU/g of tissue). All animal care procedures were supervised and approved by the Universitat Rovira i Virgili Animal Welfare and Ethics Committee (Reus, Spain). Colony counts from tissue burden studies were analysed using the Mann–Whitney *U*-test. Differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

In this study, the in vitro activity of VRC was determined against 27 clinical isolates of *C. glabrata* with MICs ranging from ≤0.03 μg/mL to 8 μg/mL. Table 1 shows the VRC MICs and the fungal load in the kidneys 7 days after infection with all strains. To our knowledge, this is the first study evaluating the correlation between the in vitro activity and in vivo efficacy of VRC against experimental murine IC by a wide number of *C. glabrata* strains. The in vitro study was performed in triplicate and no significant variations in the VRC MICs between repeat measurements were found, with only a difference of one two-fold dilution in nine strains (33.3%). The in vivo study showed that VRC was able to significantly reduce the fungal tissue burden in the kidneys in mice infected with those strains showing MICs below the ECV. Efficacy was only obtained against one strain (UTHSC DI15-89) among the three strains showing MICs equal to the ECV (P = 0.0317), whilst no response was found against isolates with MICs higher than the ECV. These results demonstrated that the ECV of VRC is a good predictor of response to IC due to *C. glabrata*. Previously, VRC has demonstrated improvement in experimental and clinical infections by other *Candida* spp. such as *Candida krusei*, *Candida tropicalis* and *Candida guilliermondii*, although the correlation between ECV and outcome has not been established [12,13,21]. Although there are limited clinical data on the usefulness of VRC in the treatment of invasive infections by *C. glabrata*, this drug has shown efficacy in the treatment of myocarditis [22] and as salvage therapy treatment of IC by *C. glabrata* in immunosuppressed and critically ill patients, even in those previously exposed to azoles [23]. In addition, two recent cases of vulvovaginal candidiasis caused by FLC-resistant isolates were successfully treated with VRC [24],

suggesting that VRC can constitute a therapeutic option [7]. The present study has some limitations, such as the use of a single efficacy marker (fungal load in the kidneys) and the relatively low number of *C. glabrata* isolates with VRC MICs equal to the ECV. To determine more accurately these findings it would be interesting in future studies to expand the number of isolates as well as the markers used for efficacy evaluation.

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Competing interests: None declared.

Ethical approval: Procedures were supervised and approved by L. Loriente Sanz (ID 39671243) of the Veterinary and Animal Welfare Advisory of the Universitat Rovira i Virgili Animal Welfare and Ethics Committee (Reus, Spain).

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Table 1

In vitro activity and in vivo efficacy of voriconazole (VRC) in reducing the fungal load from the kidneys of mice challenged with 27 strains of *Candida glabrata* [a]

Strain	VRC MIC (μg/mL)	Mean (± S.D.) log_{10} CFU/g of kidney tissue		
		Controls	Treated group	*P*-value
UTHSC DI15-86	≤0.03	5.480± 0.03916	2.372 ± 0.1406	0.0159 *
FMR 11377	0.03	7.258 ± 0.2215	4.672 ± 0.2414	0.0079 *
UTHSC DI15-87	0.03	7.386 ± 0.3025	5.048 ± 0.4077	0.0079 *
FMR 11382	0.03	7.972 ± 0.6472	4.984 ± 0.7027	0.0079 *
UTHSC DI15-88	0.06	7.162 ± 0.2114	4.394 ± 0.2000	0.0159 *
FMR 11379	0.06	7.436 ± 0.3133	5.538 ± 0.4659	0.0079 *
FMR 11378	0.06	7.508 ± 0.3930	5.360 ± 0.5240	0.0159 *
FMR 8502	0.12	7.474 ± 0.8935	5.406 ± 0.7624	0.0079 *
FMR 11383	0.12	7.648 ± 0.6871	5.850 ± 0.1838	0.0079 *
JMI 002-729	0.25	7.305 ± 0.5510	6.305 ± 0.5889	0.0571
JMI 002-297	0.25	6.093 ± 0.1617	5.230 ± 0.4062	0.0571
UTHSC DI15-89	0.25	7.143 ± 0.3067	6.330 ± 0.3603	0.0317 *
UTHSC DI15-90	0.5	6.424 ± 0.1324	6.096 ± 0.4043	0.2222
JMI 002-206	0.5	7.022 ± 0.4034	6.520 ± 0.5070	0.0952
JMI 801705	1	7.612 ± 0.4183	6.650 ± 0.7040	0.0566
JMI 787195	1	6.473 ± 0.2943	5.834 ± 0.3247	0.0635
UTHSC DI15-91	1	7.550 ± 0.6399	6.840 ± 0.3621	0.0952
UTHSC DI15-92	2	7.412 ± 0.5903	7.038 ± 0.3819	0.2857
JMI 780445	2	6.822 ± 0.6613	6.112 ± 0.3312	0.0556
JMI 766341	2	6.566 ± 0.3439	6.088 ± 0.5572	0.3095
JMI 787587	4	6.392 ± 0.2647	6.070 ± 0.1478	0.1111
JMI 815914	4	6.330 ± 0.2736	5.876 ± 0.3819	0.1508
JMI 766356	4	6.312 ± 0.3337	5.760 ± 0.7036	0.3095
JMI 815905	4	7.740 ± 0.4123	7.144 ± 0.2493	0.0556
JMI 663371	8	6.400 ± 0.1177	6.072 ± 0.2009	0.3095

JMI 030-10956	8	5.312 ± 0.2860	4.946 ± 0.3156	0.0873
JMI 698837	8	5.718 ± 0.1574	5.616 ± 0.2053	0.4127

MIC, minimum inhibitory concentration; S.D., standard deviation.

[a] The treatment group received 40 mg/kg/day for 7 days, whilst controls received no treatment.

* Differences were considered statistically significant at a *P*-value of ≤0.05.

Highlights

- We evaluated the efficacy of voriconazole (VRC) against 27 *Candida glabrata* clinical isolates with varying MICs.
- We assessed whether VRC in vitro activity is predictive of in vivo efficacy using an immunosuppressed murine model of disseminated candidiasis by *C. glabrata*.
- VRC showed efficacy against strains with MICs < 0.25 μg/mL, variable efficacy when MIC = 0.25 μg/mL and no efficacy at MIC ≥ 0.5 μg/mL.
- The current epidemiological cut-off value for voriconazole against *C. glabrata* is 0.25 μg/mL; our results showed efficacy at MIC < 0.25 μg/mL.

[25]