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Evaluation of the correlation of caspofungin MICs and treatment outcome in murine infections by wild type strains of *Candida parapsilosis*☆,☆☆

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ABSTRACT

We have evaluated the *in vitro* activity of caspofungin against 36 wild-type strains of *Candida parapsilosis sensu stricto* using 3 techniques: broth microdilution, disk diffusion, and the determination of minimal fungicidal concentration (MFC). The first 2 methods showed a good *in vitro* activity of caspofungin, but the MFCs were ≥2 dilutions above their corresponding MICs. In a murine model of disseminated infection, we evaluated the efficacy of caspofungin at 5 mg/kg against 8 strains of *C. parapsilosis* representing different degrees of *in vitro* susceptibility (0.12–1 μg/mL). All the isolates responded to treatment and (1→3)-β-D-glucan levels were reduced in all the cases; however, the study revealed differences among isolates, since caspofungin reduced the tissue burden of mice infected with isolates with MICs ≤0.5 μg/mL but was less effective against those with MICs of 1 μg/mL.

1. Introduction

Invasive candidiasis remains the most frequent opportunistic mycosis worldwide, mostly affecting immunocompromised patients. Even though the majority of infections are caused by *Candida albicans*, other species such as *Candida parapsilosis* have also become very clinically relevant (Pemán and Zaragoza, 2012; Trofa et al., 2008; van Asbeck et al., 2009). Current recommendations for invasive candidiasis in neutropenic patients include the use of echinocandins as the first therapeutic line and liposomal amphotericin B as the second line of treatment due its adverse effects observed in clinical trials (Pappas et al. 2009; Ullmann et al., 2012). Although *C. parapsilosis* shows higher MICs of echinocandins than other species of *Candida* (Garcia-Effron et al., 2008), more recent *in vitro* data from a multicentric study on bloodstream candidiasis have demonstrated that all the isolates of *C. parapsilosis* tested (n = 232) were susceptible to these drugs, which seems to indicate that echinocandins could be a good therapeutic choice for these infections (Pfaller et al., 2011a). Regarding that, the clinical efficacy of echinocandins against *C. parapsilosis* has recently been reported to be similar to that obtained against more susceptible species (Pfaller et al, 2011b). Clinical breakpoints of caspofungin/*C. parapsilosis* have been refined, and the values currently are: susceptible (S), ≤2 μg/mL; intermediate (I), 4 μg/mL; and resistant (R), ≥8 μg/mL (Pfaller and Diekema, 2012) and the epidemiological cut-off value (ECV) has been established at 1 μg/mL (Pfaller et al., 2010). However, data on the correlation of *in vitro* and clinical outcomes are inconclusive. Results of a clinical trial of 22 cases of invasive infection by *C. parapsilosis* showed an unfavourable outcome for 80% of patients infected with isolates with caspofungin MIC of 1 μg/mL, while for isolates with MIC of 2 μg/mL, it was only 13.3%, and in a case with an MIC of 4 μg/mL, the infection was resolved (Kartsonis et al., 2005). More recently, Pfaller et al. (2011b) reported a failure rate of approximately 25% for 72 clinical cases of infection with isolates of *C. parapsilosis* with MICs ≤1 μg/mL. Even though the data are based on a small number of isolates and accepting that the response of the patient to therapy can be influenced by numerous uncontrolled factors, the results are difficult to explain. Therefore, in an attempt to clarify whether there is a relationship between *in vitro* data and outcome, we have evaluated the *in vivo* efficacy of caspofungin in the treatment of an invasive murine infection by *C. parapsilosis*, testing a number of wild-type strains with different degrees of susceptibility to it.

2. Materials and methods

Thirty-six clinical isolates of *C. parapsilosis* were included in this study. The isolates were identified by sequencing the D1/D2 domains of the rDNA and comparing the sequences with those of the reference strain ATCC MYA-4646. The percentages of similarity were 98–100%. Their susceptibility to caspofungin was evaluated by 3 different methods. The broth microdilution method and the disk diffusion

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method were used to determinate the MICs and the inhibition zone diameters (IZDs), according to the respective Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2008a, 2008b).

The MIC values for caspofungin against *C. parapsilosis* were evaluated according to current criteria for susceptibility and resistance (Pfaller and Diekema, 2012). The IZD values were considered indicative of susceptibility and resistance for caspofungin when they were ≥11 mm and ≤10 mm, respectively (Pfaller et al., 2011b). Strain ATCC 22019 of *C. parapsilosis* was used as a quality control in the *in vitro* studies. The minimal fungicidal concentration (MFC) was determined by subculturing on Sabouraud dextrose agar (SDA) plates 20 μL of each well that showed complete inhibition or an optically clear well relative to the last positive well and the growth control. The plates were incubated at 35 °C until growth was seen in the control subculture. The MFC was considered to be the lowest drug concentration at which approximately 99.9% of the original inoculum was killed (Espinel-Ingroff, 1998). All *in vitro* determinations were evaluated in triplicate. In the cases in which the 3 MIC or MFC values did not coincide, the modal value was considered. When the 3 IZD values did not coincide, the mean was used.

Eight of the strains of *C. parapsilosis* were chosen for the *in vivo* study, representing the spectrum of MICs shown in the *in vitro* study. They were: 1 strain with MIC of 0.12 μg/mL; 2 strains with MICs of 0.25 μg/mL; 2 strains with MICs of 0.5 μg/mL; and 3 strains with MIC of 1 μg/mL.

Male OF1 mice (Charles River, Criffa, Barcelona, Spain) weighing 30 g were used in this study. Animals were housed under standard conditions. All animal care procedures were supervised and approved by the Universitat Rovira i Virgili Animal Welfare and Ethics Committee. Animals were immunosuppressed 1 day prior to infection by administering a single intraperitoneal (i.p.) injection of 200 mg/kg of cyclophosphamide (Genoxal; Laboratories Funk, Barcelona, Spain), plus a single intravenous (i.v.) injection of 150 mg/kg of body weight of 5-fluorouracil (Fluorouracilo; Ferrer Farma, Barcelona, Spain). This immunosuppressive regimen provokes severe neutropenia with peripheral blood polymorphonuclear leukocyte counts <100/μL from day 3 to day 9 or later (Ortoneda et al., 2004). Mice were challenged with 4×10^6 CFU in 0.2 mL of sterile saline injected into the lateral tail vein. For each isolate, the inoculum was adjusted to the desired concentration based on haemocytometer counts, and the viability of the inocula was checked by serial plating onto potato dextrose agar (PDA) plates. In a previous study, we had demonstrated that this inoculum provoked a significant fungal load in organs but did not cause the animals to die. Thus, the mice were able to receive the entire treatment regimen and be compared with controls.

We tested caspofungin (Cancidas, Merck and Co., Whitehouse Station, NJ, USA) administered at 5 mg/kg of body weight i.p. once a day (Barchiesi et al., 2006). To prevent bacterial infection, all animals received ceftazidime at 5 mg/kg day subcutaneously. All treatments began 24 h after challenge and lasted for 7 days. The efficacy of caspofungin was evaluated through tissue burden reduction and by the determination of mannan antigenemia and (1→3)-β-D-glucan serum levels. For the tissue burden studies, groups of 10 mice were established. The animals were sacrificed on day 8 postinfection, 4 hours after the last dose, in order to compare the results with untreated controls. Kidneys, liver, and spleen were aseptically removed and homogenized in 1 mL of sterile saline. Serial 10-fold dilutions of the homogenates were plated on SDA and incubated for 24 h at 35 °C, the CFUs/g of tissue being calculated. Before being sacrificed, approximately 1 mL of blood from each mouse belonging to the tissue burden groups was extracted by cardiac puncture for determining antigenemia. Pooled serum samples from mice of each group were taken to determine the mannan and (1,3)-β-D-glucan levels, using Platelia *Candida* Ag (BioRad, Marnes la Coquette, France) and the Fungitell kit (Associates of Cape Cod, E. Falmouth, MA, USA), respectively.

Colony counts from tissue burden studies were analysed using the Mann-Whitney *U* test. The Kolmogorov-Smirnov test was carried out to determine the normal distribution of (1→3)-β-D-glucan and mannan serum levels so that they could be analysed using the *t* test.

3. Results

The results for antifungal *in vitro* susceptibility are shown in Table 1. Caspofungin showed MICs and IZD values that are within the suggested current ranges of susceptibility. MICs ranged from 0.12 to 1 μg/mL with a geometric mean MIC of 1 μg/mL and MIC50 and MIC90 values of 0.5 and 1 μg/mL, respectively. The IZDs ranged from 11 to 23 mm with a geometric mean of 15.80 mm and a SD of 3.3 mm. A good correlation was obtained between the microdilution and the disk diffusion methods results. The MFCs were in most cases ≥2 dilutions above their corresponding MICs.

In general, all the isolates tested responded to some extent to treatment, but caspofungin was able to reduce the fungal load with respect to the controls in all 3 organs tested only for the strains with MICs ≤0.5 μg/mL. For the 3 isolates that showed an MIC of 1 μg/mL, caspofungin only reduced the fungal burden in 1 or 2 of the organs assayed. With strains from our collection at the Facultat de Medicina de Reus (FMR) 11554 and FMR 10292 (MIC 0.12 and 0.5 μg/mL, respectively), the fungal burden in the kidneys of untreated mice was from 1 to 4 log higher than that of the other isolates, which could suggest a higher virulence of these isolates (Fig.1). The drug was also able to reduce the (1→3)-β-D-glucan serum concentrations in comparison with the untreated group, but in all

Table 1
In vitro activity of caspofungin against 36 isolates of *C. parapsilosis*.

Strains	MIC (μg/mL)	IZD (mm)	MFC (μg/mL)
FMR 9544	0.5	15	>16
FMR 9613	0.25	16	4
FMR 9609	0.25	11	>16
FMR 9612	0.5	11	>16
FMR 9611	0.25	19	>16
FMR 9608	0.5	19	>16
FMR 9614	0.5	16	>16
FMR 9615	0.5	14	2
FMR 9610	0.5	15	2
FMR 9601	0.25	18	>16
FMR 10291	0.5	16	4
FMR 10292*	0.5	21	2
FMR 11566	0.25	15	2
FMR 11573*	0.5	14	>16
FMR 11571	1	17	4
FMR 10288	1	11	8
FMR 10289	1	11	>16
FMR 10296*	0.25	20	4
FMR 11563*	1	15	8
FMR 11769	0.5	16	2
FMR 10304	0.25	23	>16
FMR 11572	0.12	15	>16
FMR 10301*	0.25	19	4
FMR 10293	0.5	13	0.5
FMR 10297	0.5	11	>16
FMR 10290	0.5	16	8
FMR 11565	0.5	19	4
FMR 11554*	0.12	16	2
FMR 11562*	1	11	4
FMR 11564	1	18	>16
FMR 10302	0.5	16	>16
UTHSC 03-3108*	1	17	8
UTHSC 07-3845	1	19	16
UTHSC 06-3476	1	15	4
UTHSC 07-2985	0.25	23	4
UTHSC 09-13	0.5	20	8
C. parapsilosis (ATCC 22019)	1	15	2

* Strains that have been used in the *in vivo* studies.