Effects of Caspofungin against Candida guilliermondii and Candida parapsilosis

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The in vitro activity of caspofungin (CAS) was investigated against 28 yeast isolates belonging to Candida albicans (n = 5), Candida guilliermondii (n = 10), and Candida parapsilosis (n = 13). CAS MICs obtained by broth dilution and Etest methods clearly showed a rank order of susceptibility to the echinocandin compound with C. albicans > C. parapsilosis > C. guilliermondii. Similarly, time-kill assays performed on selected isolates showed that CAS was fungistatic against C. albicans and C. parapsilosis, while it did not exert any activity against C. guilliermondii. In a murine model of systemic candidiasis, CAS given at doses as low as 1 mg/kg of body weight/day was effective at reducing the kidney burden of mice infected with either C. albicans or C. guilliermondii isolates. Depending on the isolate tested, mice infected with C. parapsilosis responded to CAS given at 5 and/or 5 mg/kg/day. However, the overall CFU reduction for C. guilliermondii and C. parapsilosis was approximately 100-fold less than that for C. albicans. Our study shows that CAS was active in experimental systemic candidiasis due to C. guilliermondii and C. parapsilosis, but this activity required relatively high drug dosages.

The frequency of invasive mycoses due to opportunistic fungal pathogens has increased dramatically over the past 2 decades, and now Candida spp. rank as the fourth most common cause of nosocomial bloodstream infections (17, 21). Although Candida albicans is the organism most associated with serious fungal infections, other Candida spp. have emerged as clinically important pathogens associated with opportunistic infections (17, 21). Candida parapsilosis is the second most common yeast species isolated from blood in Europe and South America (17, 20). It is particularly associated with bloodstream infections in neonates and with catheter-associated candidemia and intravenous hyperalimentation (17, 20).

Another emerging species of Candida is Candida guilliermondii (17). It has been shown to cause hematogenously disseminated candidiasis, and it is considered intrinsically less susceptible to amphotericin B (AMB) than other Candida spp. (17).

Although both C. parapsilosis and C. guilliermondii show a reduced innate virulence compared with C. albicans, there is a common trait regarding their susceptibility patterns to caspofungin (CAS), an echinocandin antifungal agent that has potent activity against many fungal species, including Candida spp. (1, 4, 8–12, 22, 23). Clinical studies have shown that CAS is at least as active as AMB and fluconazole in the treatment of invasive candidiasis (4, 12, 22, 23). However, in vitro susceptibility data on CAS indicate that C. guilliermondii and C. parapsilosis are the least susceptible species in the genus Candida (2, 7, 15, 18). In general, CAS MICs reported for these two species of Candida are from 8 to 32 times higher than those for C. albicans (7, 18). Whether this finding could be of clinical relevance is not still understood very well. Therefore, in this study, we analyzed the effects of CAS against these two species of Candida in either in vitro or in vivo experiments.

**MATERIALS AND METHODS**

**Isolates.** A total of 28 strains of Candida spp. were used in this study. Control organisms included Candida albicans ATCC 90029, C. albicans SC5314, and Candida parapsilosis ATCC 22019. An additional 3 clinical isolates of C. albicans, 10 isolates of Candida guilliermondii, and 12 isolates of C. parapsilosis were investigated. All clinical isolates were recovered from blood, and each represented a unique isolate from a patient. Yeasts were identified at the species level by conventional morphological and biochemical methods and stored at −70°C in 10% glycerol. Before the initiation of the study, yeast isolates were subcultured on antimicrobial agent-free medium to ensure viability and purity.

**TABLE 1. Caspofungin and amphotericin B MICs of 28 isolates of Candida spp. utilized in this study**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Candida species</th>
<th>Geometric mean MIC (range) (μg/ml) obtained by:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Broth dilution Etest</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>CA</td>
<td>0.03 (0.03)</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>0.85 (0.06–4.0)</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>2.0 (1.0–8.0)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>CA</td>
<td>1.15 (1.0–2.0)</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>0.69 (0.25–1.0)</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>0.66 (0.25–2.0)</td>
</tr>
</tbody>
</table>

*Five isolates of Candida albicans (CA), 10 isolates of C. guilliermondii (CG), and 13 isolates of C. parapsilosis (CP) were tested. Each isolate was tested from two to four times by each method. **MICs were significantly higher than those reported for CA (P values ranging from <0.001 to 0.023)**.

*Five isolates of Candida albicans (CA), 10 isolates of C. guilliermondii (CG), and 13 isolates of C. parapsilosis (CP) were tested. Each isolate was tested from two to four times by each method. **MICs were significantly higher than those reported for CA (P = 0.03 [broth dilution] and P < 0.001 [E test])**.

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Drugs. CAS was used as commercial preparation (Cancidas; Merck Sharp & Dohme) for either in vitro or in vivo experiments. It was dissolved in sterile distilled water and in sterile saline solution for in vitro and in vivo studies, respectively. The pure powder (Sigma) of AMB was used for in vitro studies, and the commercial preparation (Fungizone; Bristol-Myers Squibb) was used for in vivo studies. It was dissolved in dimethyl sulfoxide and in sterile saline solution for in vitro and in vivo studies, respectively.

In vitro studies. (i) MICs. CAS and AMB MICs were determined either by the broth dilution method following the instructions established by the Clinical Laboratory Standards Institute (CLSI, formerly NCCLS) or by the Etest method (AB Biodisk, Skolne, Sweden) performed according to the manufacturer’s instructions. Both tests were performed in RPMI 1640 medium buffered with morpholinepropanesulfonic acid (MOPS) buffer. For broth dilution method, both drugs were used at concentrations ranging from 0.03 to 64 μg/ml. CAS MICs were read at 24 h and considered the lowest drug concentration causing a significant reduction of growth below control growth levels. Each isolate was tested from two to seven times by both methods (13, 14).

### TABLE 2. Caspofungin and amphotericin B susceptibilities of seven isolates utilized in killing experiments and in mice

<table>
<thead>
<tr>
<th>Strain or isolate</th>
<th>Median MIC (range) (μg/ml) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caspofungin</td>
</tr>
<tr>
<td>C. albicans SC5314</td>
<td>0.03 (0.03–1.0)</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td></td>
</tr>
<tr>
<td>Isolate 1</td>
<td>2.0 (2.0–4.0)</td>
</tr>
<tr>
<td>Isolate 2</td>
<td>2.0 (1.0–2.0)</td>
</tr>
<tr>
<td>Isolate 3</td>
<td>8.0 (2.0–8.0)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td></td>
</tr>
<tr>
<td>Isolate 1</td>
<td>1.0 (0.25–2.0)</td>
</tr>
<tr>
<td>Isolate 2</td>
<td>0.5 (0.5–1.0)</td>
</tr>
<tr>
<td>Isolate 3</td>
<td>4.0 (2.0–4.0)</td>
</tr>
</tbody>
</table>

* Each isolate was tested seven times.

FIG. 1. Time-kill studies conducted with *C. albicans* SC5314, *C. guilliermondii* isolates 1, 2, and 3, and *C. parapsilosis* isolates. (isolates 1, 2, and 3). Black diamonds, controls; closed squares, 1× AMB MIC; open squares, 8× AMB MIC; closed triangles, 1× CAS MIC; open triangles, 8× CAS MIC. The dotted lines represent a ≥99.9% growth reduction compared with the initial inoculum size (fungicidal effect). The limit of detection is 20 CFU/ml. Each data point represents the mean ± standard deviation (error bar) for three independent experiments.
(ii) Killing curves. *C. albicans* SC5314 and three isolates each of *C. guilliermondii* (isolates 1, 2, and 3) and *C. parapsilosis* (isolates 1, 2, and 3) were used in killing experiments (19). Briefly, three to five colonies of each strain from a 24-h growth plate were suspended in 10 ml of sterile distilled water, and the turbidity was adjusted using spectrophotometric methods to a 0.5 McFarland standard (approximately 1 x 10^6 to 5 x 10^6 CFU/ml). One milliliter of the adjusted fungal suspension was added to 9 ml of either RPMI 1640 medium buffered with MOPS buffer plus an appropriate amount of drug. Both CAS and AMB were used at 1 and 8 times the MIC obtained by the broth dilution method. CAS and AMB MICs for the selected isolates are reported below (see Table 2). Test solutions were placed on a shaker and incubated at 35°C. At time points 0, 2, 4, 6, and 24 h following the introduction of the test isolate into the system, 100-µl aliquots were removed from each test solution. After 10-fold serial dilutions, a 50-µl aliquot from each dilution was streaked on Sabouraud dextrose agar plates for colony count determination. The limit of detection was 20 CFU/ml. Fungicidal activity was considered to be achieved when the number of CFU per milliliter was reduced by ≥99.9% compared to the initial inoculum size (19). Experiments were performed in triplicate.

In vivo studies. *C. albicans* SC5314, *C. guilliermondii* (isolates 1, 2, and 3) and *C. parapsilosis* (isolates 1, 2, and 3) were used for in vivo experiments. CD1 male mice (Charles River, Calco, Lecco, Italy) weighing 25 g were utilized in all experiments. In studies involving isolates of *C. guilliermondii* and *C. parapsilosis*, the mice were rendered neutropenic by intraperitoneal administration of cyclophosphamide (200 mg/kg of body weight/day) on days −4, −1, and +4 postinfection. Mice were infected intravenously with a yeast inoculum given in a 0.2-ml volume. Inoculum sizes were as follow: 2.2 x 10^5 CFU/mouse for *C. albicans* SC5314; 5.0 x 10^7 CFU/mouse, 8.5 x 10^8 CFU/mouse, and 1 x 10^9 CFU/mouse for *C. guilliermondii* isolates 1, 2, and 3, respectively; and 6.0 x 10^7 CFU/mouse, 3.4 x 10^6 CFU/mouse, and 3.5 x 10^8 CFU/mouse for *C. parapsilosis* isolates 1, 2, and 3, respectively. Both drugs were administered intraperitoneally for 4 consecutive days in a 0.2-ml volume starting 24 h postchallenge. CAS was given at 0.25, 1, and 5 mg/kg/day, while AMB was given at 1 mg/kg/day. Drug efficacy was assessed by determining the number of CFU per kidney pair. Briefly, the mice were sacrificed, the kidneys were homogenized, and diluted or undiluted aliquots, including the entire organ, were grown on Sabouraud dextrose agar for colony count determination. Tissue burden experiments were performed 24 h after the last dose (day 5 postinfection). There were seven or eight animals in
FIG. 1—Continued.
each control and treatment group. Animal experiments were conducted with the approval of the University of Ancona Ethics Committee.

Statistical analysis. The Mann-Whitney U test was used to compare either MICs or tissue burden counts. A P value of <0.05 was considered statistically significant.

RESULTS

CAS median MICs obtained by the broth dilution were 0.03 μg/ml for C. albicans ATCC 90029 and C. albicans SC5314 and 0.06 μg/ml for C. parapsilosis ATCC 22019. AMB median MICs obtained by the same method were 1.0 μg/ml for all three isolates.

CAS median MICs obtained by the Etest were 0.01 μg/ml, 0.06 μg/ml, and 0.125 μg/ml for C. albicans ATCC 90029, C. albicans SC5314, and C. parapsilosis ATCC 22019, respectively. AMB median MICs obtained by the Etest were 0.125 μg/ml for both ATCC isolates, while it was 0.06 μg/ml for C. albicans SC5314.

The overall susceptibilities of all 28 isolates tested are reported in Table 1. Isolates of C. albicans were shown to be significantly more susceptible to CAS than isolates of C. guilliermondii or C. parapsilosis. Furthermore, isolates of C. parapsilosis were more susceptible to CAS than isolates of C. guilliermondii were. Both the broth microdilution and Etest methods confirmed this trend of susceptibility. AMB MICs did not significantly differ among isolates belonging to the three species of Candida.
Then, we selected seven isolates for killing experiments (Table 2). Killing curves of the seven isolates tested are reported in Fig. 1.

AMB at the MIC was fungistatic against all seven isolates. AMB at eight times the MIC exerted a fungicidal activity against \textit{C. albicans} SC5314 and \textit{C. guilliermondii} isolate 2. This effect was reached upon approximately 6 and 22 h of incubation, respectively. The polyene, at the highest concentration, was fungistatic for the remaining five isolates.

CAS at the MIC exerted fungistatic activity only for 4 to 6 h of incubation against all isolates; afterwards, growth similar to that of the controls was often observed. The same phenomenon was seen in all isolates of \textit{C. guilliermondii}, even when CAS was utilized at eight times the MIC, while at this concentration, the drug maintained fungistatic activity upon 24 h of incubation against \textit{C. albicans} SC5314 and all isolates of \textit{C. parapsilosis}.

To see whether the strains grown at the highest drug concentrations upon 24 h of incubation represent CAS- or AMB-resistant mutants, two single colonies from each strain/drug combination were randomly selected and tested by the broth dilution method. All strains tested maintained a susceptibility pattern similar (within a double dilution) to that of their respective parent isolates for both drugs (data no shown).

The results of in vivo studies are reported in Fig. 2.

In mice infected with \textit{C. albicans} SC5314, all treatments, with the exception of CAS at 0.25 mg/kg/day, were effective at reducing the counts against the controls (\( P < 0.001 \)). Similarly, in mice infected with \textit{C. guilliermondii} isolates 1 and 3, all treatments, with the exception of the lowest dose of CAS, were effective (\( P \) ranging from \(<0.001\) to 0.04). Only CAS at 1 mg/kg/day (\( P = 0.008 \)) and 5 mg/kg/day (\( P = 0.001 \)) significantly reduced the counts with respect to the counts of controls in mice infected with \textit{C. guilliermondii} isolate 2.

In mice infected with \textit{C. parapsilosis} isolates 1 and 2, only AMB at 1 mg/kg/day and CAS at 5 mg/kg/day were effective at reducing fungal burden with respect to the control (\( P \) ranging from \(<0.001\) to 0.006). Finally, in mice infected with \textit{C. parapsilosis} isolate 3, not only AMB at 5 mg/kg/day and CAS at 5 mg/kg/day but also CAS at 1 mg/kg/day reduced the counts in
FIG. 2—Continued.

*Candida parapsilosis* #1
CAS MIC 1.0 μg/ml
AMB MIC 0.5 μg/ml
6.0 x 10⁴ CFU/mouse

C AMB 1* CAS 0.25 CAS 1 CAS 5*

*Candida parapsilosis* #2
CAS MIC 0.5 μg/ml
AMB MIC 1.0 μg/ml
3.4 x 10⁵ CFU/mouse

C AMB 1* CAS 0.25 CAS 1 CAS 5*

*Candida parapsilosis* #3
CAS MIC 4.0 μg/ml
AMB MIC 1.0 μg/ml
3.5 x 10⁵ CFU/mouse

C AMB 1* CAS 0.25 CAS 1* CAS 5*
the kidney compared with the controls ($P = 0.004$ for AMB and $P < 0.001$ for both CAS doses).

DISCUSSION

In this study, we analyzed the in vitro and in vivo activities of CAS against *C. guilliermondii* and *C. parapsilosis*. Although we utilized only 5 to 13 strains of each species, we confirmed that *C. guilliermondii* and *C. parapsilosis* isolates are less susceptible in vitro to CAS than *C. albicans* is. This was demonstrated by the broth dilution method and confirmed by the Etest. Our MIC data, which are in agreement with those previously reported by others (7, 18), clearly showed a precise rank order of susceptibility to the echinocandin compound with $C. albicans > C. parapsilosis > C. guilliermondii$. These findings partially mirrored those obtained by killing experiments. Although CAS did not exert a fungicidal activity against any of the strains tested, at the highest concentration, after 24 h it reduced the CFU of *C. albicans* SC5314 and *C. parapsilosis* isolates from 0.2 to 1.5 mg/kg/dose twice a day was effective at reducing the kidney burden of DBA/2N mice challenged with *C. parapsilosis* (1).

The reason why *C. parapsilosis* and *C. guilliermondii* isolates with higher CAS MICs responded in vivo to this drug as well as isolates with lower MICs did can be explained with data reported by Louie et al. (11). They found that CAS levels persisted in kidney tissue well after serum concentrations fall below the MIC and thus exerted a greater effect than might be expected.

Since echinocandins show a concentration-dependent activity in experimental models of infections due to *Candida* species other than *C. guilliermondii* and *C. parapsilosis* (19), further studies employing additional doses are needed to clarify this issue in these two species as well.

In conclusion, we demonstrated that CAS was active in experimental systemic candidiasis due to *C. guilliermondii* and *C. parapsilosis*, but this activity required relatively high drug dosages and the overall CFU reduction was approximately 100-fold less than that for *C. albicans*.

REFERENCES


