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In vitro activity of caspofungin compared to amphotericin B, fluconazole, and itraconazole against *Candida* strains isolated in a Turkish University Hospital

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We investigated the *in vitro* activity of caspofungin compared to amphotericin B, fluconazole, and itraconazole against clinical strains of *Candida* spp. ($n = 239$). Antifungal susceptibility tests were done in accordance with NCCLS M27-A2 microdilution method and the results were read after 24 and 48 h. In general, 24 h MIC readings were similar to those at 48 h for most isolates and all antifungal agents. Caspofungin was active against all species tested. Caspofungin MICs of *Candida parapsilosis* were slightly higher than those for other *Candida* spp. Caspofungin MIC (μg/ml) ranges at 24 h for *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. kefyr*, *C. krusei*, *C. lusitaniae*, *C. norvegensis*, *C. guilliermondii* and *C. lipolytica* were 0.06–2, 0.125–2, 0.125–2, 1–4, 0.125–2, 1–2, 0.5–2, 0.5–1, 0.5–2 and 1–2, respectively. Eagle (paradoxical) effect was observed in 31 and 8% of the isolates at highest concentrations of caspofungin and itraconazole, respectively. The activity of caspofungin against fluconazole- and/or itraconazole-resistant isolates was similar to that detected for the susceptible ones. We conclude that caspofungin appears as a promising antifungal agent with enhanced activity against *Candida*, including the azole-resistant strains.

Keywords amphotericin B, *Candida*, caspofungin, fluconazole, itraconazole

Introduction

Treatment of invasive candidiasis in immunocompromized hosts has been troublesome so far. Unfavorable host factors, particularly the impaired immune status, and drawbacks of the antifungal drugs in current use, toxicity and resistance, are the major factors that complicate the issue. Caspofungin is a novel echinocandin that exerts antifungal activity via inhibition of (1-3)-ß-D-glucan synthesis [1–4]. It was licensed in the US to be used in cases of invasive aspergillosis who have been intolerant or refractory to currently used antifungal drugs [5].

Caspofungin is active *in vitro* against *Candida* [6] and *Aspergillus* [7–12]. It has also proved to be highly active against *Candida albicans* biofilms [13]. Similar to other echinocandins, one of the most significant advantages of caspofungin is its enhanced activity against azole-resistant *Candida* isolates as well as the susceptible ones [14–18]. This primarily originates from the distinctive mode of action of echinocandins compared to azoles. Caspofungin and other echinocandins also display selective toxicity against fungal cells due to the absence of the target molecule glucan in mammalian cells.

The activity of caspofungin against *Cryptococcus neoformans* [19], *Trichosporon* spp. [20] and *Fusarium* spp. remains limited. Caspofungin has proven to be efficacious *in vivo* in treatment of candidiasis [17,21–25], aspergillosis [12,21,26,27], and in experimental

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animal models of pneumocystosis [28] and coccidioidomycosis [29].

This study was undertaken (i) to investigate the *in vitro* activity of caspofungin against clinical *Candida* isolates compared to that of amphotericin B, fluconazole and itraconazole, and (ii) to determine its activity, particularly against fluconazole- and itraconazole-resistant isolates.

Materials and methods

Isolates

Clinical *Candida* isolates ($n=239$) of various species were included in the study. The test strains were isolated from blood ($n=44$; 18.4%), vaginal discharge ($n=47$; 19.7%), urine ($n=61$; 25.5%), oral sample ($n=43$; 18%), sputum/bronchoalveolar lavage fluid/tracheal aspirate ($n=19$; 7.9%), pus ($n=20$; 8.4%) and thoracentesis/paracentesis fluid ($n=4$; 1.7%) and consisted of *Candida albicans* ($n=107$), *Candida glabrata* ($n=29$), *Candida tropicalis* ($n=28$), *Candida parapsilosis* ($n=20$), *Candida kefyr* ($n=20$), *Candida krusei* ($n=19$), *Candida lusitaniae* ($n=8$), *Candida norvegensis* ($n=4$), *Candida guilliermondii* ($n=2$) and *Candida lipolytica* ($n=2$). The isolates were identified at species level according to their assimilation profiles as determined by ID 32C (BioMerieux, France) and morphological characteristics on cornmeal Tween 80 agar [30]. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included in each run of susceptibility tests for quality control.

Antifungal drugs

Caspofungin (Merck, Research Laboratories, Rahway, NJ, USA), amphotericin B (Bristol–Myers Squibb Co., Princeton, NJ, USA), fluconazole (Pfizer Pharmaceuticals Group, New York, NY, USA) and itraconazole (a kind gift of Dr. John H. Rex, supplied by Janssen, Beerse Belgium) were provided by their respective manufacturers as standard powders to be used in susceptibility tests.

Susceptibility tests

NCCLS M27-A2 microdilution method [31] was used. Minimum inhibitory concentration (μg/ml) values were determined by using the MIC-0 (complete inhibition of growth, visually) endpoint for caspofungin and amphotericin B, and by MIC-2 ($\sim$50% reduction in turbidity, visually) endpoint for fluconazole and itraconazole. The results were read after 24 and 48 h incubation.

Analysis of the results

MIC_{50}, MIC_{90}, and MIC ranges were determined for each species–drug combination. Rates of resistance were determined for fluconazole and itraconazole according to the MIC breakpoints proposed by NCCLS [31] and the *in vitro* activity of caspofungin against fluconazole- and/or itraconazole-resistant isolates was evaluated. Isolates of *C. krusei* were accepted as fluconazole-resistant regardless of their fluconazole MICs. Given the current lack of definitive MIC breakpoint for amphotericin B and caspofungin, the results obtained for these drugs were analysed only by determining the MIC distributions.

Results

Caspofungin, amphotericin B, fluconazole and itraconazole MICs, and rates of resistance to fluconazole and itraconazole, are shown in Tables 1 and 2, respectively. In general, and for all antifungal agents, MICs obtained at 24 h remained the same or increased by one two-fold dilution for most of the isolates tested when the incubation period was extended to 48 h. Considering all species tested, the MIC range was widest for fluconazole, followed by itraconazole, caspofungin and amphotericin B in narrowing rank order. While caspofungin yielded low MICs for all species, caspofungin MICs were slightly higher for *C. parapsilosis*, compared to other species.

Amphotericin MICs were tightly clustered and did not display genus- or strain-based variations. Resistance to fluconazole was observed among isolates of *C. krusei* and *C. norvegensis* while itraconazole resistance was detected for *C. albicans*, *C. glabrata*, *C. kefyr* and *C. norvegensis*. Strain-based analysis of these results showed that caspofungin MICs of fluconazole- and/or itraconazole-resistant isolates were similar to those obtained for the susceptible ones at 24 (Table 3) and 48 h (data not shown).

Eagle (paradoxical) effect was observed in 73 (31%) and 20 (8%) of the isolates at highest concentrations of caspofungin and itraconazole, respectively. While caspofungin produced Eagle effect for various *Candida* species, itraconazole Eagle effect was observed for isolates of *C. albicans* and *C. tropicalis* only. Of note, Eagle effect was observed for both caspofungin and itraconazole for five *C. albicans* and two *C. tropicalis* strains.

Table 1 Amphotericin B, fluconazole, itraconazole and caspofungin MICs (μg/ml) after 24 and 48 h incubation ($n = 239$)

Species (n)	Drug	Incubation period (h)	MIC_{50}	MIC_{90}	MIC Range
Candida albicans (107)	AMB	24	1	1	0.25–2
		48	1	2	0.5–4
	FLU	24	0.125	0.5	<0.125–1
		48	0.125	0.5	<0.125–2
	ITRA	24	0.03	0.125	<0.015–0.5
		48	0.03	0.25	<0.015–1
	CASPO	24	0.5	2	0.06–2
		48	1	2	0.125–4
Candida glabrata (29)	AMB	24	1	2	0.5–2
		48	1	2	1–4
	FLU	24	2	4	<0.125–8
		48	4	8	0.25–16
	ITRA	24	0.25	0.5	<0.015–1
		48	0.25	1	0.03–1
	CASPO	24	0.5	2	0.125–2
		48	0.5	2	0.125–2
Candida tropicalis (28)	AMB	24	1	2	0.5–2
		48	2	2	0.5–4
	FLU	24	0.5	1	<0.125–4
		48	1	2	<0.125–4
	ITRA	24	0.06	0.125	<0.015–0.5
		48	0.125	0.5	<0.015–0.5
	CASPO	24	0.5	1	0.125–2
		48	1	2	0.25–4
Candida parapsilosis (20)	AMB	24	1	1	0.5–2
		48	1	2	1–2
	FLU	24	0.25	1	<0.125–1
		48	0.25	1	<0.125–2
	ITRA	24	0.06	0.125	<0.015–0.5
		48	0.06	0.25	<0.015–0.5
	CASPO	24	2	4	1–4
		48	4	4	1–8
Candida kefyr (20)	AMB	24	1	1	0.5–2
		48	1	2	0.5–2
	FLU	24	0.25	1	<0.125–2
		48	0.25	1	<0.125–4
	ITRA	24	0.06	0.25	<0.015–1
		48	0.06	0.25	<0.015–1
	CASPO	24	0.5	2	0.125–2
		48	1	2	0.125–2
Candida krusei (19)	AMB	24	1	2	0.5–2
		48	2	4	1–4
	FLU	24	16	32	4–32
		48	32	64	8– >64
	ITRA	24	0.25	0.5	<0.015–0.5
		48	0.5	0.5	<0.015–0.5
	CASPO	24	2	2	1–2
		48	2	2	1–4
Candida lusitaniae (8)	AMB	24	–	–	1–2
		48	–	–	1–2
	FLU	24	–	–	<0.125–1
		48	–	–	<0.125–1
	ITRA	24	–	–	0.03–0.125
		48	–	–	0.03–0.25
	CASPO	24	–	–	0.5–2
		48	–	–	1–4

Table 1 (*Continued*)

Species (*n*)	Drug	Incubation period (h)	MIC_{50}	MIC_{90}	MIC Range
Candida norvegensis (4)	AMB	24	–	–	0.5–2
		48	–	–	1–4
	FLU	24	–	–	0.25–32
		48	–	–	0.25–64
	ITRA	24	–	–	0.03–1
		48	–	–	0.03–1
	CASPO	24	–	–	0.5–1
		48	–	–	0.5–4
Candida guilliermondii (2)	AMB	24	–	–	1–2
		48	–	–	2
	FLU	24	–	–	1–2
		48	–	–	4–16
	ITRA	24	–	–	0.25–0.5
		48	–	–	0.25–0.5
	CASPO	24	–	–	0.5–2
		48	–	–	0.5–2
Candida lipolytica (2)	AMB	24	–	–	1
		48	–	–	2
	FLU	24	–	–	<0.125–0.25
		48	–	–	0.25–0.5
	ITRA	24	–	–	0.03–0.06
		48	–	–	0.125
	CASPO	24	–	–	1–2
		48	–	–	2–4

AMB, amphotericin B; CASPO, caspofungin; FLU, fluconazole; ITRA, itraconazole.

Discussion

In this study we investigated the *in vitro* activity of caspofungin against various *Candida* species and in comparison with amphotericin B, fluconazole and itraconazole. We used the NCCLS reference microdilution method and interpreted the results at 24 and 48 h. At both reading times, caspofungin MICs were low for all *Candida* species tested. Of note is that caspofungin MICs tended to be slightly higher for *C. parapsilosis*. As there is yet no established MIC breakpoint value for caspofungin, it is not possible to categorize the isolates according to their caspofungin susceptibility profile. Furthermore, the correlation of *in vitro* caspofungin data with clinical outcome is under question and demands further investigation [15,32].

In vitro activity of caspofungin has been explored in several studies. Although most of these studies employed the NCCLS microdilution method, the NCCLS macrodilution [33], disk diffusion assay [34] and E-test [35,36] have also been used by some investigators. In an effort to eliminate method-based MIC variations, we compared our results with the published data that used NCCLS microdilution method. Conclusively, the comparative evaluation of the MICs obtained in this study and those previously reported by other investigators suggest that genus- and strain-dependent MIC variations are possible for caspofungin. Our caspofungin MICs are similar to those reported by Espinel-Ingroff *et al*. [37] for most *Candida* species, except *C. guilliermondii*, for which our MICs are significantly lower (MIC range: 0.5–2.0 vs >16). However, the number of isolates of *C. guilliermondii* we have tested is very low and this might have attributed to the detected differences in MICs. On the other hand, our caspofungin MICs for *C. guilliermondii* were similar to those reported by Ostrosky-Zeichner *et al*. [38] (MIC range: 0.5–2.0 vs 0.5–2.0) despite the variation in MIC endpoint used in the two studies (MIC-0 in our study versus MIC-2 in the above-noted study). Caspofungin MIC ranges obtained by Lozano-Chiu *et al*. [34] for all *Candida* spp. tested at 24 and 48 h are also comparable to those obtained in our study.

On the other hand, when our results are compared with some other previously published reports, variabilities are observed to a wider extend. Caspofungin MICs obtained by Bartizal *et al*. [39] at 24 h by NCCLS microdilution method are slightly lower than those generated in our hands for *C. albicans* (MIC_{90}: 0.5 vs 2.0), *C. parapsilosis* (MIC_{90}: 0.5 vs 4.0), *C. kefyr* (MIC_{90}: 0.5 vs 2.0), *C. lusitaniae* (MIC range 0.125–0.5 vs 0.5–2.0) and *C. guilliermondii* (MIC range: 0.25–2.0 vs 0.5–2.0) while the MICs obtained in the two studies are similar for *C. glabrata* (MIC_{90}: 1 vs 2),

Table 2 Rates of resistance of *Candida* spp. to fluconazole and itraconazole ($n = 239$)

Species (n)	No. (%) isolates in the denoted susceptibility category			
Incubation period (h)	Flu-S-DD	Flu-R	Itra-S-DD	Itra-R
Candida albicans (107)				
24	0	0	7 (6.5)	0
48	0	0	11 (10.3)	1 (0.9)
Candida glabrata (29)				
24	0	0	13 (44.8)	3 (10.3)
48	2 (6.9)	0	13 (44.8)	6 (20.7)
Candida tropicalis (28)				
24	0	0	3 (10.7)	0
48	0	0	7 (25)	0
Candida parapsilosis (20)				
24	0	0	2 (10)	0
48	0	0	4 (20)	0
Candida kefyr (20)				
24	0	0	2 (10)	1 (5)
48	0	0	5 (25)	1 (5)
*Candida krusei** (19)				
24	0	19 (100)	13 (68.4)	0
48	0	19 (100)	15 (78.9)	0
Candida lusitaniae (8)				
24	0	0	0	0
48	0	0	1 (ND)	0
Candida norvegensis (4)				
24	2 (ND)	0	1 (ND)	1 (ND)
48	1 (ND)	1 (ND)	0	2 (ND)
Candida guilliermondii (2)				
24	0	0	2 (ND)	0
48	1 (ND)	0	2 (ND)	0
Candida lipolytica (2)				
24	0	0	0	0
48	0	0	0	0

Flu-R, fluconazole-resistant; Flu-S-DD, dose-dependent susceptible to fluconazole; Itra-R, itraconazole-resistant; Itra-S-DD, dose-dependent susceptible to itraconazole; ND, the percentage was not determined because the total number of isolates was <10.
**Candida krusei* isolates were accepted to be fluconazole-resistant regardless of their fluconazole MICs.

C. tropicalis (MIC_{90}: 1 vs 1) and *C. krusei* (MIC_{90}: 2 vs 2). Compared to the data of Pfaller *et al*. [35] obtained by using NCCLS microdilution method and at 48 h, caspofungin MICs of our *C. albicans* (MIC_{90}: 2.0 vs 0.25), *C. glabrata* (MIC_{90}: 2.0 vs 0.25) and *C. tropicalis* (MIC_{90}: 2.0 vs 0.5) isolates are higher, while those of *C. parapsilosis* (MIC_{90}: 4 vs 2), *C. krusei* (MIC_{90}: 2 vs 1) and *C. lusitaniae* (MIC range: 1–4 vs 1–2) are similar, and of *C. guilliermondii* (MIC range: 0.5–2.0 vs >8) are lower.

Similarly, caspofungin MICs obtained by Vazquez *et al*. [40] for *Candida* are in general lower than our MICs. The difference is most pronounced for *C. parapsilosis* (MIC_{50} at 48 h: 0.2 vs 4.0). Caspofungin MICs of *C. albicans*, *C. glabrata* and *C. tropicalis* reported by Laverdiere *et al*. [36] are two- to three-fold lower than our MICs for the corresponding species, while the MICs obtained for *C. parapsilosis* are similar in the two studies. Caspofungin MICs reported by Ostrosky-Zeichner *et al*. [38] for various *Candida* species are also one to two two-fold lower than our MICs (MIC_{90}: 0.5–2.0 vs 2–4). However, Ostrosky-Zeichner *et al*. used MIC-2 as the reading endpoint while we used MIC-0. This variation in the MIC reading endpoint may possibly have resulted in higher MICs obtained in our study.

One of the major aims of our study was to investigate the activity of caspofungin against fluconazole- and itraconazole-resistant *Candida* isolates. Strain-based detailed analysis of the results (Table 3) show that there is no correlation between the MICs of caspofungin and fluconazole or itraconazole. Caspofungin appeared to be similarly active against fluconazole- and/or

Table 3 Caspofungin MICs in reference to (a) fluconazole and (b) itraconazole MICs for the clinical isolates included in the study ($n = 239$)

(a) Caspofungin MIC (μg/ml)											
16											
8											
4	1	2	2	2							
2	9	13	6	5	2	4	2	5	5		
1	31	18	10	3	3	2	2	1	2		
0.5	31	10	12	3	4	2	2	1			
0.25	10	3	3	4	1	3	1				
0.125	10	3	2	2	1						
0.06	1										
0.03											
	0.125	0.25	0.5	1	2	4	8	16	32	64	Fluconazole MIC (μg/ml)
(b) Caspofungin MIC (μg/ml)											
16											
8											
4	4	2		1							
2	7	8	9	10	7	9	1				
1	27	10	12	14	4	4	1				
0.5	26	3	13	9	6	7	1				
0.25	4	3	8	5	3	1	1				
0.125	2	1	8	4	2		1				
0.06	1										
0.03											
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	Itraconazole MIC (μg/ml)

The MICs were determined after 24 h incubation. The numbers denote the number of isolates with the corresponding MICs for caspofungin and fluconazole (a) or itraconazole (b). (a) MIC <8: fluconazole-susceptible; MIC $=16-32$: dose-dependent susceptible to fluconazole; MIC >64: fluconazole-resistant [31]. (b) MIC <0.125: itraconazole-susceptible; MIC $=0.25-0.5$: dose-dependent susceptible to itraconazole; MIC >1: itraconazole-resistant [31].

itraconazole-resistant and itraconazole-susceptible isolates. These findings are in accordance with the data published previously by several investigators [14,18,39,40]. Pfaller *et al*. [18] tested caspofungin against 351 fluconazole-resistant *Candida* isolates. In this study, 90% of the isolates were inhibited at an MIC of 1 μg/ml and 99% were inhibited at an MIC ≤ 2 μg/ml. No caspofungin MICs greater than 4 μg/ml were observed for any of the isolates. Favourable activity of caspofungin against azole-resistant isolates mainly originates from the distinctive mode of action of caspofungin compared to azole compounds and appears promising for potential use of caspofungin in treatment of infections due to azole-resistant *Candida* isolates. Validation of these *in vitro* data by *in vivo* experiments is required. We also attempted to comparatively evaluate the amphotericin B MICs with those of caspofungin. However, given the existence of a remarkably narrow MIC range and the lack of an established MIC breakpoint value, amphotericin B MICs were not discriminatory to detect any possibly existing putatively-resistant isolates and the comparison of the *in vitro* activity of amphotericin B and caspofungin remained limited.

We observed Eagle effect at highest concentrations of caspofungin and itraconazole in some isolates. This phenomenon has previously been reported for caspofungin against *Candida* spp. [38] as well as for the other novel echinocandins, anidulafungin and micafungin against *Aspergillus* and *Fusarium* [41], and for itraconazole against *C. albicans* [42,43]. Clinical significance of Eagle effect remains unclear and demands further investigation.

In conclusion, caspofungin appears active *in vitro* against *Candida* strains. Its activity is slightly less pronounced against *C. parapsilosis* compared to other species. The favourable activity of caspofungin against fluconazole- and/or itraconazole-resistant isolates is noteworthy and appears promising for potential use of caspofungin in treatment of infections due to azole-resistant isolates. Further investigations are required for clarification of the clinical implications of these results.

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