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ARTICLE

Paradoxical growth effect of caspofungin on *Candida* spp. sessile cells not only at high drug concentrations

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Abstract

According to the available literature, echinocandins display high anti-*Candida* spp. activity. Paradoxical growth (PG) of *Candida* spp. planktonic cells promoted by echinocandins is widely reported. Here we report on the ability of *Candida* spp. sessile cells to display PG when they are exposed to caspofungin in vitro, even at relatively low drug concentrations. Clinical significance of PG during echinocandin therapy of candidiasis remains uncertain. We assessed in vitro susceptibilities of *Candida* spp. sessile cells to caspofungin and analyzed the frequency of PG. The minimum inhibitory concentrations of caspofungin for sessile cells (SMICs) were determined for 70 clinical *Candida* spp. isolates (29 *Candida albicans*, 26 *Candida parapsilosis*, and 15 *Candida glabrata* isolates) and were defined as the lowest drug concentrations that resulted in at least 50% reduction in metabolic activity. PG was defined as a resurgence of growth (>50% of that in the drug-free growth control well) at drug concentrations above the MIC. The caspofungin SMICs ranged from ≤0.015 to >256 μg ml^{-1}. We observed PG in 26.9–93.1% of isolates tested, depending on the *Candida* species and age of sessile cells. Antibiofilm activity of caspofungin is species-specific, and strongly strain-depending among *C. albicans* and *C. parapsilosis* isolates. Interestingly, PG was present also at relatively low caspofungin concentrations.

Introduction

Echinocandins play a vital role in the treatment of invasive fungal infections [1, 2]. *Candida* spp. isolates are commonly susceptible to echinocandins in vitro, with the majority of isolates inhibited at MICs of 0.03–0.25 μg ml^{-1}. Intrinsically decreased susceptibility to the echinocandins is noted in *Candida orthopsilosis*, *C. parapsilosis*, *Candida lusitaniae*, and *Candida guilliermondii* [3]. Despite the growth-inhibitory activity of echinocandins at low drug concentrations, paradoxical growth (PG), defined as a re-growth at drug concentrations above the MIC, has been observed for *Candida* spp. isolates. To date no uniformly consistent incidence of PG phenomenon was observed. PG has been demonstrated in 14–90% of *Candida* spp. isolates, depending on echinocandin, *Candida* species, and group of isolates examined. The published data depicts that PG is more commonly observed with caspofungin than with other echinocandins [4–9].

To date, studies describing PG in *Candida* spp. isolates have focused mainly on planktonic cells and demonstrated the crucial role of high echinocandin concentrations in this phenomenon. There is few data on the PG incidence in *Candida* spp. biofilms [10–12]. We report here the ability of three *Candida* spp. growing as sessile cells to display PG, when they are exposed to caspofungin in vitro, even at relatively low drug concentrations. Clinical isolates from three *Candida* spp. were tested, *C. albicans*, *C. parapsilosis*, and *C. glabrata*. The in vitro susceptibilities of *Candida* spp. sessile cells to caspofungin and the frequency of PG were studied.

Materials and methods

Strains

A total of 70 *Candida* spp. isolates were examined: 29 of *C. albicans*, 26 of *C. parapsilosis*, and 15 of *C. glabrata*. All the strains were isolated in The Department of

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Table 1 Caspofungin SMICs and PG for *C. albicans* isolates ($n = 29$)

Isolate no. /origin	2-hours old sessile cells		6-hours old sessile cells		24-hours old sessile cells	
	SMIC (μg ml^{-1})	Caspofungin concentration range producing PG (μg ml^{-1})	SMIC (μg ml^{-1})	Caspofungin concentration range producing PG (μg ml^{-1})	SMIC (μg ml^{-1})	Caspofungin concentration range producing PG (μg ml^{-1})
1/catheter	0.06	8–16	0.125	4–32	0.125	8–16
2/urine	0.125	16	0.125	4–64	0.125	4–32
3/urine	0.125	8–16	0.125	2-64	0.125	8–32
4/urine	0.125	4–16	64	ND	64	ND
5/urine	0.06	816	0.06	4–32	0.06	8–16
6/ear	0.25	8–16	2	4–16	32	ND
7/urine	0.06	16	0.125	8–32	0.125	8–16
8/ear	0.125	8	0.125	4–32	0.125	2–32
9/urine	0.125	8–32	0.125	2–64	0.125	2–32
10/SM	0.125	4–16	0.125	2–16	0.125	4–16
11/urine	0.125	8–16	0.125	1–64	0.125	1–64
12/ear	0.125	8	0.25	0.5–32	128	ND
13/urine	0.125	ND	0.25	ND	0.25	1–128
14/urine	0.03	8–16	0.25	16–64	0.25	1–64
15/ pharynx	0.03	8–16	0.25	4–32	0.25	1–32
16/urine	0.125	8–16	0.125	4–32	0.125	8–32
17/RTS	0.125	16	0.125	2–64	0.125	4–64
18/ pharynx	0.125	16	0.125	0.25–32	0.125	2–32
19/urine	0.125	8–16	0.125	4–32	0.125	1–64
20/BAL	0.25	16–32	0.25	16–64	0.25	8–64
21/BAL	0.125	16–32	64	ND	128	ND
22/PF	0.125	16–32	0.125	2–32	64	ND
23/urine	0.25	16–32	64	ND	128	ND
24/ pharynx	0.06	4–8	0.25	4–64	0.5	2–64
25/urine	0.25	4	0.25	2–16	0.25	2–16
26/urine	0.125	16	0.25	16	0.25	16
27/urine	0.125	16	0.25	16–32	0.25	16–32
28/urine	0.25	16–32	0.25	2–32	0.25	8–32
29/urine	0.125	ND	0.25	32	0.25	16–32

SMIC sessile minimal inhibitory concentration, *PG* paradoxical growth, *ND* not detected, *SM* surgical mesh, *RTS* respiratory tract swab, *BAL* bronchoalveolar lavage, *PF* peritoneal fluid

Microbiology of Dr. A. Jurasz University Hospital No.1 in Bydgoszcz, Nicolaus Copernicus University in Torun, in 2007–2010. One isolate per patient was studied. Isolates were derived from various specimens: urine ($n = 23$), blood cultures ($n = 9$), bronchoalveolar lavage fluid ($n = 4$), middle ear discharge ($n = 7$), pharyngeal swab ($n = 6$), wound swab ($n = 4$), peritoneal fluid ($n = 5$), tip of vascular catheter ($n = 3$), gastrostomy swab ($n = 2$), and one strain per tip of drain, piece of surgical mesh, respiratory tract swab, insertion-site skin swab, peritoneal cavity swab, tracheotomy swab, and groin swab. Biofilm-forming strain *C. albicans* GDH 2346 served as a positive control in the whole experiment. All examined strains were stored at −70 °C. Species identification was confirmed before research by means of germ-tube tests [13], and MALDI-TOF mass spectrometry carried out according to the manufacturer's protocol (Bruker).

Antifungal susceptibility testing

Caspofungin acetate (Merck & Co., Inc.) concentrations ranging from 0.015 to 256 μg ml^{-1} were tested. MICs for

Table 2 Caspofungin SMICs and PG for *C. parapsilosis* isolates ($n = 26$)

Isolate no./origin	MIC (μg ml^{-1})	2- hours old sessile cells		6- hours old sessile cells		24- hours old sessile cells	
		SMIC (μg ml^{-1})	Caspofungin concentration range producing PG (μg ml^{-1})	SMIC (μg ml^{-1})	Caspofungin concentration range producing PG (μg ml^{-1})	SMIC (μg ml^{-1})	Caspofungin concentration range producing PG (μg ml^{-1})
1/blood	2	4	16–128	128	ND	>256	NA
2/blood	0.25	2	4–32	2	4–32	2	16–32
3/blood	1	2	4–32	64	ND	>256	NA
4/GS	0.125	4	16–32	64	ND	>256	NA
5/catheter	0.5	16	ND	32	ND	128	ND
6/PF	0.5	32	ND	32	ND	128	ND
7/blood	2	2	8–64	128	ND	>256	NA
8/ear	0.5	0.5	2–16	32	ND	128	ND
9/wound	≤0.015	0.25	ND	0.25	ND	0.5	ND
10/blood	0.25	0.5	1–8	1	2–16	1	2–64
11/ear	0.25	0.5	4–16	8	16	>256	NA
12/blood	0.5	16	ND	32	ND	128	ND
13/urine	0.5	2	4–32	32	ND	128	ND
14/PF	0.25	0.25	8	0.25	8–16	1	4–128
15/wound	1	1	ND	2	ND	4	64–128
16/blood	0.25	0.25	4–32	0.25	4–32	0.25	0.5–32
17/TS	0.25	0.25	0.5–8	32	ND	128	ND
18/GS	0.5	0.25	0.5–16	32	ND	128	ND
19/ear	1	1	4–32	2	8–32	8	16–64
20/wound	0.25	0.5	16–32	2	8–128	128	ND
21/groin	0.25	0.5	ND	0.5	2–16	4	8–32
22/ear	0.5	32	ND	64	ND	128	ND
23/wound	0.5	0.5	2–16	32	ND	128	ND
24/ catheter	0.5	1	ND	1	ND	128	ND
25/blood	0.5	0.5	16–32	64	ND	128	ND
26/ pharynx	0.5	0.5	8–16	1	2–128	128	ND

SMIC sessile minimal inhibitory concentration, *PG* paradoxical growth, *NA* not applicable, *ND* not detected, *GS* gastrostomy swab, *PF* peritoneal fluid, *GS* gastrostomy swab, *TS* tracheotomy swab

planktonic cells were determined by EUCAST (European Committee on Antimicrobial Susceptibility Testing) method for antifungal susceptibility testing of yeasts [14]. *C. parapsilosis* ATCC 22019 served as quality control strain. Sessile MICs (SMICs) were tested by means of methods published by Ramage et al.[15], and Krom et al. [16]. These methods had been previously used in our study on micafungin and described in detail [17]. The colorimetric assay based on MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H* tetrazolium bromide) (AlfaAesar) reduction was engaged to assess cell viability and by this means drug susceptibility of *Candida* spp. sessile cells [16, 17]. The metabolic activity of *Candida* spp. sessile cells were determined quantitatively by the absorbance of obtained solutions measurement in a microtiter plate reader (spectrophotometer using KC4™ v3.4 and KC4 ™ Signature programs) (BIO-TEK) at 550 nm, after transferring the solutions to a new 96-well plates. SMICs were established at ≥50% biofilm inhibition comparing to untreated biofilms.

Paradoxical growth

The PG frequency in *Candida* spp. isolates exposed to caspofungin was studied. PG was considered as a resurgence of growth, >50% of that in the drug-free growth control well, at drug concentrations above the MIC.

Table 3 Caspofungin SMICs and PG for *C. glabrata* isolates ($n = 15$)

Isolate no. /origin	2- hours old sessile cells		6- hours old sessile cells		24- hours old sessile cells	
	SMIC (μg ml^{-1})	Caspofungin concentration range producing PGE (μg ml^{-1})	SMIC (μg ml^{-1})	Caspofungin concentration range producing PGE (μg ml^{-1})	SMIC (μg ml^{-1})	Caspofungin concentration range producing PGE (μg ml^{-1})
1/PF	0.03	64	0.06	ND	0.06	ND
2/drain	0.06	4–16	0.06	8–16	0.125	4
3/BAL	0.015	2–128	0.06	64	0.06	ND
4/urine	0.125	0.5–128	0.125	8–32	0.5	ND
5/urine	0.03	32–64	0.125	64	0.125	ND
6/urine	0.06	ND	0.06	ND	0.25	4–32
7/urine	0.015	8	0.06	8–16	0.06	4–16
8/PS	0.015	0.5–32	0.125	ND	0.25	ND
9/BAL	0.015	ND	0.015	32–128	0.06	16–128
10/blood	0.015	1–32	0.03	16–32	0.06	ND
11/ pharynx	0.015	ND	0.015	ND	0.03	32–128
12/ pharynx	0.015	128	0.015	128	0.015	128
13/PF	0.015	ND	0.125	ND	0.125	32–64
14/IS	0.015	ND	0.25	ND	0.25	ND
15/urine	0.125	4–128	0.125	16–128	0.125	ND

SMIC sessile minimal inhibitory concentration, *PG* paradoxical growth, *ND* not detected,
PF peritoneal fluid, *BAL* bronchoalveolar lavage, *PS* peritoneal swab, *IS* insertion site

Results

MICs of caspofungin

A total of 29*C. albicans* (MICs ≤ 0.015 μg ml^{-1}) and 15*C. glabrata* (MICs ranged from ≤ 0.015 to 0.06 μg ml^{-1}, $MIC_{90} = 0.06$ μg ml^{-1}) isolates were susceptible, and 26*C. parapsilosis* isolates (MICs ranged from ≤ 0.015 to 2 μg ml^{-1}, $MIC_{90} = 1$ μg ml^{-1}) were intermediate to caspofungin according to EUCAST interpretative criteria [18].

SMICs of caspofungin

Caspofungin appeared to have excellent activity against *C. glabrata* sessile cells; SMICs ranged from 0.015 to 0.5 μg ml^{-1}, and the median SMIC was 0.125 μg ml^{-1}, and $SMIC_{90}$ was 0.25 μg ml^{-1}, for 24-hours old sessile cells. The median SMIC for *C. albicans* 24-hours old sessile cells was 0.25 μg ml^{-1}, however, SMICs > 2 μg ml^{-1} were observed for 6 of 29 (20.7%) isolates (SMICs ranged from 32 to 128 μg ml^{-1}).

The SMICs ≤ 2 μg ml^{-1} (0.25–2 μg ml^{-1}) were observed for 5 of 26 (19.2%) *C. parapsilosis* 24-hours old sessile cells; the rest SMICs ranged from 4 to >256 μg ml^{-1}. Detailed data are presented in Tables 1–3.

Paradoxical growth

PG was observed for three tested *Candida* species, at each sessile cells age tested. The frequency of PG differed by species, as well as by sessile cells age.

PG was the most frequent among *C. albicans* isolates, and among 2-hours old sessile cells in case of each *Candida* species tested. PG was noted as follows: for 2-hours old sessile cells: in 93.1% of *C. albicans* (27 of 29 isolates), in 69.2% of *C. parapsilosis* (18 of 26 isolates), in 66.7% of *C. glabrata* (10 of 15 isolates); for 6-hours old sessile cells: in 86.2% of *C. albicans* (25 of 29 isolates), in 60.0% of *C. glabrata* (9 of 15 isolates), in 34.6% of *C. parapsilosis* (9 of 26 isolates); for 24-hours old sessile cells: in 79.3% of *C. albicans* (23 of 29 isolates), in 46.7% of *C. glabrata* (7 of 15 isolates), in 26.9% of *C. parapsilosis* (7 of 26 isolates).

Interestingly, PG effect of caspofungin on *Candida* spp. sessile cells was observed not only at high caspofungin concentrations (>2 μg ml^{-1}), but also at concentrations ≤2 μg ml^{-1} (0.25–2 μg ml^{-1}). PG at ≤2 μg ml^{-1} was noted in 34.5% of *C. albicans* for 6- and 24-hours sessile cells, in 26.7% of *C. glabrata* 2-hours old sessile cells, and in 19.2%, 11.5%, and 7.7% of *C. parapsilosis*, growing as 2-, 6-, and 24-hours old sessile cells, respectively. PG of *Candida* spp. was observed over one to nine dilutions

(Tables 1–3). The range of caspofungin concentrations promoting PG differed by the stage of biofilm maturation. The widest range of caspofungin concentrations triggering PG of *C. albicans* was noted for 6-hours old sessile cells (0.25–64 μg ml^{-1}), of *C. glabrata* was noted for 2-hours old sessile cells (0.5–128 μg ml^{-1}).

Discussion

Echinocandins are antifungals, that inhibit the glucan synthesis in the fungal cell wall, by 1,3-β glucan synthase inhibition.We have described the effect of caspofungin on sessile *Candida* spp. cells. We focused on their susceptibility to caspofungin and the PG effect of the drug.

Our studies revealed the highest antibiofilm activity against *C. glabrata* isolates. All the examined strains of this species displayed SMICs ≤ 0.5 μg ml^{-1}, regardless of sessile cells age, with $SMIC_{90}$ value of 0.25 μg ml^{-1} for 24-hours old sessile cells. Similarly, Choi et al. [19] described caspofungin $SMIC_{90}$ of 0.5 μg ml^{-1} for mature biofilms of 9 *C. glabrata* strains. Besides, Choi et al. [19] revealed also very high antibiofilm activity of caspofungin against 12 *C. albicans* strains, with SMICs ≤ 0.5 μg ml^{-1}. In contrast, in our investigation, 23.3% of the *C. albicans* strains displayed SMICs > 2 μg ml^{-1} for 24-hours old sessile cells, associated with $SMIC_{90}$ 128 μg ml^{-1}. Melo et al. [10] noted caspofungin SMIC of 2 μg ml^{-1} for 3 out of 4 *C. albicans* biofilms, and 4 μg ml^{-1} for one tested biofilm. These inter-study variations highlight the differences in susceptibilities of *C. albicans* biofilms, which can be due to differences in the biofilm-forming abilities and biofilm properties of the particular tested isolates.

Our results indicate the lowest antibiofilm activity of caspofungin against *C. parapsilosis* isolates. Caspofungin SMICs ≤ 2 μg ml^{-1} for 24-hours old sessile cells were noted for 21.4% of the tested isolates. The $SMIC_{50}$ value for 24-hours old sessile cells was established at 128 μg ml^{-1}. Our results are consistent with those published by Choi et al. [19], in which caspofungin SMICs ≤ 2 μg ml^{-1} for mature biofilms were noticed for 3 out of 12 (25%) tested strains, and $SMIC_{50}$ and $SMIC_{90}$ values were 8 and >16 μg ml^{-1}, respectively. Melo et al. [10] noted caspofungin SMICs of 2 μg ml^{-1} for 37.5%, 4 μg ml^{-1} for 25%, and 256 μg ml^{-1} for 37.5% of 8 *C. parapsilosis* isolates tested. Similarly, Simitsopoulou et al. [20] noted SMIC range from 2 to 128 μg ml^{-1}, with mean value 64 μg ml^{-1}, for 6 *C. parapsilosis* biofilms examined.

Our studies, in comparison with studies published before, revealed that antibiofilm activity of caspofungin is species-specific, and strongly strain-depending among *C. albicans* and *C. parapsilosis* isolates.

We observed PG in 26.9–93.1% of tested isolates, depending on the *Candida* species and age of sessile cells. The lower frequency of PG among *C. parapsilosis* than *C. albicans* and *C. glabrata* isolates seems to be mainly due to the fact that 6- and 24-hours old sessile cells of *C. parapsilosis* were less susceptible to caspofungin than the biofilms of the rest two *Candida* species tested. Similarly, the lower frequency of PG among *C. parapsilosis* and *C. albicans* in 6- and 24-hours old sessile cells than 2-hours old sessile cells was probably due to the lower susceptibility of these forms of living to caspofungin. In contrast, among *C. glabrata* sessile cells the frequency of PG was lower in older sessile cells, as follows: 2-hours old sessile cells (66.7%) > 6-hours old sessile cells (60.0%) > 24-hours old sessile cells (46.7%), despite the fact that the SMICs of caspofungin persisted low, ranged from 0.015 to 0.5 μg ml^{-1}.

The correlation between origins of the isolates and their susceptibility to caspofungin, as well as PG were analyzed. Among *C. parapsilosis* strains there were six isolates (23.1%) without PG effect. Their origins were different, including: wound swab, blood, peritoneal fluid, middle ear discharge, and the tip of vascular catheters – two isolates. Otherwise, *C. albicans* originating from the catheter displayed PG effect. The *C. parapsilosis* strain without PG effect, isolated from the wound expressed the lowest MIC, i.e. ≤0.015 μg ml^{-1}, and also the lowest SMICs –0.25 μg ml^{-1}. However, MICs of the rest five *C. parapsilosis* isolates without PG effect, were 0.5 μg ml^{-1}, and their SMICs ranged 1–128 μg ml^{-1}, thus they did not outstand.

All *C. albicans* isolates displayed PG in at least one biofilm-maturation step. High SMICs of caspofungin for 24-hour *C. albicans* biofilms, ranging 32–128 μg ml^{-1}, were noted for six isolates, originating from different specimens: urine samples—two isolates, middle ear discharge—two isolates, BAL, and peritoneal fluid. When analyzing the most numerous *C. albicans* strains isolated from urine samples, it was noticed that SMICs of caspofungin widely ranged 0.06–128 μg ml^{-1}, $SMIC_{50}$ was 0.25 μg ml^{-1}, and $SMIC_{90}$ –64 μg ml^{-1}. Likewise, no correlation between the origins of *C. glabrata* isolates and PG effect of caspofungin was observed. Only one isolate originating from the insertion-site skin swab did not display PG effect at all.

In our previous study PG effect of other echinocandin, i.e. micafungin was less frequent. PG effect of micafungin was detected in 3.3–31% *C. albicans*, and 7.1–14.2% *C. parapsilosis*. None *C. glabrata* displayed PG although SMICs values were low, ranged 0.015-0.25 μg ml^{-1}. For six *C. parapsilosis* isolates without PG effect of caspofungin, the lack of PG effect of micafungin was also noted [17].

MICs and SMICs of caspofungin seem to be unrelated to the origins of the *Candida* spp. isolates. Although the

highest MICs of caspofungin, i.e. 2 μg ml^{-1} were observed only in two *C. parapsilosis* strains isolated from blood, MICs for the rest isolates from blood ranged 0.25–1 μg ml^{-1}.

Further analyzes showed that higher MICs of caspofungin in *C. parapsilosis* isolates did not result in higher SMICs of the drug. Susceptibility of the *C. parapsilosis* biofilm to caspofungin seems to depend on other mechanisms than the susceptibility of planktonic cells.

Although PG was more frequent at high caspofungin concentratrions, this was noted also at realtively low caspofungin concentrations, i.e. ≤2 μg ml^{-1}. Interestigly, PG was noted in 7.7–34.5% of *Candida* spp. isolates at the drug concentrations of 0.25–2 μg ml^{-1}, depending on *Candida* species, and sessile cells age.

PG of *Candida* spp. biofilms exposed to caspofungin was described before by Melo et al. [10]. The SMICs of caspofungin were determined for 30 clinical *Candida* spp. isolates (4 *C. albicans*, 6 *Candida tropicalis*, 7 *C. parapsilosis*, 8 *C. orthopsilosis*, and 5 *Candida metapsilosis* isolates), when they were grown as biofilms. The SMICs ranged from 2 to 512 μg ml^{-1}. PG was observed for 80.0% of *Candida* spp. isolates and the range of caspofungin concentrations promoting PG ranged from 4 to 512 μg ml^{-1}. Melo et al.[10] did not observe PG at caspofungin concentrations lower than 4 μg ml^{-1}, as well as they did not note SMICs lower than 2 μg ml^{-1}.

Next, Walraven et al. [12] analyzed biofilms formed by 12 *C. albicans* clinical strains, resistant to caspofungin due to *FKS1* hot-spot mutations. The sessile antifungal activities of anidulafungin, caspofungin, and micafungin were compared, and PG effects were assessed. The majority of strains displayed PG to particular echinocandins, in either planktonic or sessile forms. Caspofungin at the concentrations of 64 to ≥128 μg ml^{-1} promoted PG in *C. albicans* biofilms in 58.3% of isolates tested, but did not in planktonic forms.

The clinical significance of PG effect during echinocandin therapy of candidiasis remains unclear. Clinical data on paradoxical effect of echinocandins are limited. An international, multicenter, randomized, double-blind clinical trial included 204 patients with proven invasive candidiasis; 104 patients receiving a standard (70 mg followed by 50 mg/day), and 100 patients receiving high-dose (150 mg/day) caspofungin treatment regimen [21]. Favorable responses were similar between the two groups, 71.6% of patients who received the standard regimen, and 77.9% of patients who received the high-dose regimen. Obtained results suggest no paradoxical effect associated with high caspofungin concentrations in regards to efficacy. In another study, Safdar et al. [22] demonstrated that high-dose caspofungin regimen may have favorably influenced outcomes. In the retrospective analysis 34 patients receiving high-dose caspofungin (150 mg/day) were compared with 63 patients receiving standard-dose caspofungin (70 mg followed by 50 mg/day). After 12 weeks of treatment 44% of patients revealed complete or partial response compared with 29% of patients receiving standard-dose caspofungin regimen. Further clinical studies are needed to evaluate efficacy of high-dose echinocandins in patients with invasive fungal infections.

Clemons et al. [23] observed paradoxical effect of caspofungin in animal model, in only one instance of murine candidiasis, however, the effect was not reproducible in a subsequent experiment. Published clinical, and animal data indicate that the PG is rather an in vitro phenomenon, or is somehow balanced by in vivo conditions. Shields et al. [6] demonstrated that PG is eliminated in human serum in vitro. Furthermore, brief exposures to caspofungin resulted in killing of *C. albicans* cells in vitro, but did not lead to PG, suggesting compensatory mechanisms occur with prolonged exposure to drug [6]. There are some potential mechanisms responsible for PG hypothesized; [5, 7, 9] they include (i) an increased cell wall chitin content, (ii) upregulation of the protein kinase C cell wall integrity pathway, and (iii) involvement of the calcineurin pathway.

In conclusion, caspofungin presents high anti-*Candida* spp. biofilm activity, however, this activity is species-specific, and strongly strain-depending among *C. albicans* and *C. parapsilosis* isolates. *Candida* spp. sessile cells can display PG in the presence of high, but also relatively low concentrations of caspofungin. This finding makes the significance of the PG in vivo even more complex and difficult to examine. In fact, the PG phenomenon might be one of the reasons for the lack of success in therapy, even when standard-dose echinocandin treatment is applied.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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