In vitro Activity of Caspofungin against Planktonic and Sessile Candida sp. Cells

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This article is devoted to the memory of the late Prof. W.J.H. Kunicki-Goldfinger on the tenth anniversary of his passing away

Abstract

Candida sp. may be regarded as one of the leading etiologic agents of hospital-acquired infections, including those related with the indwelling medical devices, which become colonized by the yeasts, accompanied by biofilm formation. In this paper we assayed in vitro susceptibility to caspofungin of planktonic and sessile cells of nasopharyngeal isolates of Candida sp. Two types of biomaterials were used – silicone elastomer-coated latex urinary Foley catheter and PCV Thorax catheter. The minimal inhibitory concentrations (MIC) of caspofungin for planktonic Candida sp. cells ranged from 0.008 to 0.031 mg/l, while the minimal fungicidal concentrations (MFC) from 0.008 to 0.062 mg/l, with MFC/MIC ratios ≤2. The minimal concentration of caspofungin preventing adhesion process of Candida sp. on both biomaterials ranged from 0.004 to 0.031 mg/l, while preventing biofilm formation from 0.004 to 0.062 mg/l. In contrast, much higher minimal concentrations of caspofungin were needed to eradicate the mature biofilm (0.25 to >8 mg/l). In all cases, drug concentrations depended on the strain and the biomaterial used. Our preliminary data suggest that caspofungin, showing good anti-adherent activity in vitro against Candida sp., appears to be a potential agent rather for prophylaxis of the yeast infections associated with biomaterials but not for their treatment.

Key words: caspofungin, Candida sp., biomaterials, adhesion, biofilm

Introduction

Recently, the yeasts belonging to the genus Candida may be regarded as one of the leading etiologic agents of hospital-acquired infections, including those related with the indwelling medical devices such as catheters, prosthetic joints and heart valves, dentures etc. These devices become colonized by the yeasts, accompanied by biofilm formation (Hawser and Douglas, 1994; Douglas, 2002; Jabra-Rizk et al., 2004). Treatment of candidiasis often presents a challenge for clinicians, since the number of effective antifungal agents at their disposal is limited, due to increasing resistance of the pathogens. In addition, sessile cells of Candida sp. within a biofilm are even more insensitive to current antimicrobial therapy in comparison to their planktonic (free-floating) counterparts. Therefore, the pharmaceutical industry is still working on the discovery of novel drugs which might reinforce conventional antifungal armamentarium (Chandra et al., 2001; Bachmann et al., 2002; Kuhn et al., 2002; Jabra-Rizk et al., 2004).

Caspofungin is the first antifungal compound of a new echinocandin class with a unique mode of action. It inhibits the synthesis of 1,3-β-D-glucan, a key component of the fungal cell wall, that is essential for osmotic stability, cell growth and cell division (Letscher-Bru et al., 2003). This antibiotic should be effective in combating both planktonic and biofilm-associated Candida sp. populations. However, the literature data regarding the effect of caspofungin in vitro on Candida sp. biofilm formation and its eradication are controversial (Bachmann et al., 2002; Kuhn et al., 2002; Ramage et al., 2002; Soustre et al., 2004).

Here we presented data on the in vitro susceptibility to caspofungin of planktonic and sessile cells of nasopharyngeal isolates of Candida sp.
Experimental

Materials and Methods

Microorganisms. A total of 7 clinical isolates of Candida sp. possessing hydrophilic or hydrophobic cell structure were studied. The hydrophobicity of cell surface was assessed using salt aggregation test (Lindahl et al., 1981). The collection included the following isolates: C. albicans (1 hydrophilic and 2 hydrophobic isolates), C. famata (1 hydrophilic and 1 hydrophobic isolates), C. glabrata (1 hydrophobic isolate), C. krusei (1 hydrophilic isolate). The isolates were obtained from nasopharynx of patients with lung cancer undergoing pulmonary resection and were stored on Sabouraud dextrose agar and before each experiment they were subcultured on Sabouraud glucose broth.

Caspofungin. Standard antifungal powder of caspofungin (caspofungin acetate) was examined (Merck & Co., Inc., USA). Stock solution containing 16 mg/ml was prepared in distilled water and was stored frozen at –20°C.

Determination of minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of caspofungin. Determination of MIC of caspofungin for planktonic Candida sp. cells was performed by a broth microdilution method in accordance with the guidelines recommended by CLSI (Clinical Laboratory Standards Institute), using serial two-fold dilutions of caspofungin in Sabouraud glucose broth. Final concentrations of caspofungin ranged from 0.0002 to 16 mg/l. Stock inoculum suspensions of yeasts were prepared in Sabouraud medium and adjusted to optical density corresponding with 0.5 Mc Farland. Carbohydrate fermentation tests were performed according to the guidelines recommended by CLSI (2008), using phenol red broth (Difco). The yeasts, 10 µl from each tube that showed thorough growth inhibition, from the last positive one and from the growth control was streaked onto Sabouraud dextrose agar plates. After 48 h of incubation at 35°C, the MFCs were assessed visually as the lowest fungicidal concentration showing complete growth inhibition. In order to determine the MFC of caspofungin for the planktonic cells of the yeasts, 10 µl from each tube that showed thorough growth inhibition, from the last positive one and from the growth control was streaked onto Sabouraud dextrose agar plates. After 48 h of incubation at 35°C, the MFCs were assessed visually as the lowest drug concentration at which there was no growth. All experiments were done in triplicates. The representative data are presented.

Biomaterials. All assays were carried out on two types of catheters that differed in uneveness of surface from each other – silicone elastomer-coated latex urinary Foley catheter and PCV Thorax catheter.

The effect of caspofungin on adhesion of Candida sp. and biofilm formation on the biomaterials. The adhesion process and biofilm formation were determined by using the MTT (tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) reduction assay (Levitz et al., 1985). The catheters used were cut aseptically into 0.5 cm² fragments and placed into Petri dishes. The standardized yeast suspensions (optical density of 0.5 Mc Farland standard) were prepared. Various concentrations of caspofungin (0.004–0.062 mg/l) were used. (i) In order to assay the effect of caspofungin on adhesion process, the yeast suspensions in sterile PBS (phosphate-buffered saline) containing various concentrations of caspofungin were incubated with biomaterials for 1 h at 35°C. Nonadherent cells were removed by careful rinsing catheter discs with sterile PBS and then resuspended in fresh sterile PBS (phosphate-buffered saline) containing various concentrations of caspofungin were incubated with biomaterials for 24 h at 35°C. (ii) In order to assay the effect of caspofungin on biofilm formation, the yeast suspensions in Sabouraud glucose broth containing various concentrations of caspofungin were incubated with biomaterials for 24 h at 35°C. Nonadherent cells were removed by careful rinsing catheter discs with sterile PBS and then resuspended in fresh Sabouraud glucose broth. Medium changing and catheters washing procedures after overnight incubation at 35°C were repeated thrice (total incubation period lasted 72 h). (iii) In order to assay the effect of caspofungin on biofilm eradication, the mature biofilms were incubated in the presence of various concentrations of caspofungin for 24 h. In all assays a drop of 1% MTT solution was added to each dish. After incubation for 24 h at 35°C, in the presence of Candida sp. viable cells tetrazolium salt was reduced to the violet tetrazolium formazan product, accompanied by violet colour of the medium. In each experiment control free-drug assays were carried out. All experiments were done in triplicates. The representative data are presented.

Results

The MICs of caspofungin for planktonic Candida sp. cells ranged from 0.008 to 0.031 mg/l (Table I). Paradoxical turbidity at the highest drug concentrations, imitating fungal growth was observed for 3 isolates. However, this so-called “eagle” effect was ignored for the MIC determinations according to the literature data (Bartizal and Odds, 2003; Stevens et al., 2004). The MFCs of caspofungin for planktonic Candida sp. cells was 0.008–0.062 mg/l (Table I). For 6 strains tested MFC/MIC ratios were ≥2, while for one strain MFC/MIC = 1. It is worth notifying that the turbidity observed at the highest drug concentrations during the MIC readings was accompanied by the lack of fungicidal effect of caspofungin, which was revealed during MFC determinations.

All assayed Candida sp. isolates were able to adhere both to Thorax and Foley catheters, following by biofilm formation on both biomaterials. This was monitored by formation of violet tetrazolium formazan product inside and outside of the catheters and violet coloured medium after addition of MTT solution. The structure of the biofilm of hydrophobic strain of C. albicans on Thorax catheter was presented in Fig. 1.
Capsofungin activity against *Candida* sp.

The minimal concentration of capsofungin preventing adhesion process on both biomaterials ranged from 0.004 to 0.031 mg/l (Table II). The above data indicate that capsofungin significantly inhibited *in vitro* the adherence capacity of the yeasts to the biomaterials assayed at concentrations ranged from 0.5×MIC or 1×MIC.

The minimal concentration of capsofungin preventing biofilm formation ranged from 0.004 to 0.062 mg/l. In contrast, much higher minimal concentrations of capsofungin (0.25 to >8 mg/l) were needed to eradicate the mature biofilm of *Candida* sp., depending also on the strain and the biomaterial used (Table III). The total eradication of *Candida* sp. biofilms with the drug concentrations below 8 mg/l was obtained for 2 strains only (Table III). The above data indicate that capsofungin significantly inhibited *in vitro* the biofilm formation capacity of the yeasts to the biomaterials assayed at concentrations ranged from 0.5×MIC to 2×MIC, while eradication of the mature biofilm required concentrations from 4×MIC to >1032×MIC.

Generally, using the Thorax catheter higher concentrations of capsofungin were needed in order to achieve the same effects on adhesion process of *Candida* sp. and the yeast biofilm formation as were obtained with the Foley catheter (Table II and III).

**Discussion**

*Candida* sp. infections can be regarded as an important medical problem, especially in the immunocompromised patients, e.g. in lung cancer patients. Additionally, according to our unpublished data, *Candida* sp. isolates have shown to possess a potential ability to colonize pleural drains in patients undergoing pulmonary resection. Although *C. albicans* remains a predominant etiologic agent of candidiasis, other species that tend to be less susceptible to the commonly used antifungal agents such as *C. krusei*, *C. glabrata* or *C. famata* have emerged as opportunistic pathogens (Chandra *et al.*, 2001; Jabra-Rizk *et al.*, 2004).

The results presented in this paper confirmed the good inhibitory activity of capsofungin at low concentrations against planktonic cells of *Candida* sp., observed also by other authors (Bachmann *et al.*, 2002;
Ramage et al., 2002; Bartizal and Odds, 2003; Stevens et al., 2004). The MICs and MFCs determined for the nasopharyngeal Candida sp. isolates were lower than those reported in the literature (Bartizal et al., 2003; Stevens et al., 2004). However, such discrepancies are possible since the type of medium used in antifungal susceptibility testing is of the utmost importance. The standard medium for testing drug susceptibility of Candida sp. was RPMI 1640 medium, whereas Sabouraud glucose broth was used for studies on Candida sp. biofilm formation (Chryssanthou and Cuenca-Estrella, 2002; Bartizal et al., 2003). The MFC/MIC ratio, not higher than 2 for the tested strains, confirmed the excellent in vitro fungicidal activity of caspofungin against planktonic cells of Candida sp.

Our data demonstrating in vitro anti-adherent activity of caspofungin are in accordance with those from literature (Soustre et al., 2004). The drug concentrations as low as the 0.5×MIC and 1×MIC were sufficient to deprive biofilm-precursor planktonic cells of Candida sp. of adherence capacity and ability to form a mature biofilm. It’s worth mentioning that these values are within the range of mean through levels of caspofungin in serum in humans after standard dosing – 1–2 mg/l (Letscher-Bru and Herbrecht, 2003). As shown by other authors (Bachmann et al., 2002; Kuhn et al., 2002; Ramage et al., 2002; Soustre et al., 2004), exposure of planktonic Candida sp. cells even to subinhibitory concentrations of caspofungin inhibited adhesion process and subsequent biofilm formation. On the other hand, all examined strains of Candida sp. became much more resistant to caspofungin when adhered to the biomaterials and embedded in the mature biofilm. Eradication of sessile Candida sp. populations required much higher concentrations of the antibiotic in comparison with their free-floating counterparts. Analogous investigations also have shown that the complete sterility of the mature Candida sp. biofilms as well as the thorough eradication of adherent cells was hard to attain (Bachmann et al., 2002; Ramage et al., 2002). Nevertheless, a significant reduction in metabolic activity in caspofungin-treated sessile yeast cells was detected by other authors (Bachmann et al., 2002; Ramage et al., 2002; Soustre et al., 2004). In addition, caspofungin-treated yeast biofilms appeared to be less hyphal and showed minor defects in the overall biofilm architecture (Bachmann et al., 2002; Ramage et al., 2002; Soustre et al., 2004). However, Ramage et al. (2002) found that caspofungin killed >99% of sessile cells of some strains of C. albicans within the mature biofilm at therapeutically attainable concentrations (0.125 mg/l and 1 mg/l). The highest concentration of caspofungin (8 mg/l) was less efficacious due to paradoxical lack of fungicidal effect of caspofungin at this concentration. Therefore, in studies on the effect of caspofungin on removal of Candida sp. cells adhered to the biomaterials or embedded in the biofilm, the “eagle” effect observed in case of some yeast strains should be taken into account (Bartizal and Odds, 2003; Stevens et al., 2004).

The observed in this paper differences in antifungal activity of caspofungin against catheter-associated cells of Candida sp. depended on the type of biomaterial used. Using the Thorax catheter higher concentrations of the drug were needed in order to achieve the same outcomes as were obtained with the Foley catheter.

To conclude, the controversial literature data (Bachmann et al., 2002; Kuhn et al., 2002; Ramage et al., 2002; Soustre et al., 2004) and our studies concerning in vitro effect of caspofungin against sessile Candida sp. cells suggest that this antibiotic, showing good anti-adherent activity in vitro, appears to be a potentially effective agent rather for prophylaxis of the yeast infections associated with biomaterials but not for their treatment.

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Literature


Capsofungin activity against Candida sp.


