Caspofungin modulates in vitro adherence of Candida albicans to plastic coated with extracellular matrix proteins

Jérôme Soustre, Marie-Hélène Rodier, Sabrina Imbert-Bouyer, Gyslaine Daniault and Christine Imbert*

Laboratoire de Parasitologie et Mycologie Médicales, Centre Hospitalier Universitaire La Milétrie, BP 577, 86021 Poitiers Cedex, France

Received 12 September 2003; returned 22 October 2003; revised 19 November 2003; accepted 6 December 2003

Objectives: Some manifestations of candidiasis are associated with the formation of biofilms on inert or biological surfaces and the intrinsic resistance of Candida albicans biofilms to the most commonly used antifungal agents has been demonstrated. In this study, we report on the influence of the growth of C. albicans in medium containing a sub-inhibitory concentration (MIC/2) of caspofungin, on subsequent fungal adherence to plastic coated with extracellular matrix (ECM) proteins.

Methods: Eleven strains of C. albicans were studied: six strains were susceptible to fluconazole in vitro and five strains were resistant to this antifungal agent.

Results: Caspofungin induced a decrease in the adherence of all the tested strains that were susceptible to fluconazole but induced a decrease in the adherence of only 60% of the fluconazole-resistant strains.

Conclusions: This study demonstrated the anti-adherent activity of caspofungin but indicated a reduced effect in the case of in vitro fluconazole resistance. These results indicated a possible relationship between the efficiency of caspofungin to inhibit the first step of the development of C. albicans biofilm and the resistance of C. albicans to fluconazole in vitro.

Keywords: yeast, adhesion, antifungal agents, candidiasis

Introduction

Yeasts, mainly Candida albicans, are one of the most common causes of bloodstream infection in hospitalized patients.1 Adherence of Candida yeasts to implanted medical devices is a prerequisite for colonization and is, therefore, considered as a prerequisite in the process leading to infections. We know that medical devices introduced into the body are rapidly coated by adsorbed proteins.2 There is then an initial attachment of microorganisms, such as C. albicans blastospores, to the medical device coated by proteins and a biofilm layer rapidly forms on its surface. This biofilm is composed of yeasts embedded within extracellular matrix proteins.3

A small number of antifungal drugs can be used to treat candidiasis associated with implanted medical devices, and infected devices generally need to be removed.4 This type of infection can result in serious medical complications, expensive care and is noted as the most frequent factor limiting the prolonged use of central venous catheters.5

Echinocandins represent a new class of antifungal drug and act by inhibiting the synthesis of β-D-glucan in fungal cell walls.6 The cell wall is highly implicated in the adherence process of C. albicans, and so, in the first step of biofilm formation.3 Caspofungin is the first representative of echinocandins and shows in vitro antifungal activity against C. albicans.5 The aim of this study was to determine whether caspofungin, used in a concentration below its corresponding sub-inhibitory concentration, could prevent adherence of C. albicans to plastic coated with extracellular matrix proteins. This action could be effective before the emergence of infection by preventing the formation of C. albicans biofilm on implanted medical devices.

Materials and methods

Organisms and growth conditions

Eleven isolates of C. albicans were studied: five strains (92, 109, 163, 182 and 240) were isolated in our laboratory, from patients with candidaemia. The identification of these clinical isolates was carried out by using conventional physiological and morphological studies such as the germ-tube test in serum, agglutination (Bichro-Latex, Fumouze, Levallois Perret, France) and metabolic properties (API 20C, bioMérieux, Marcy-

*Corresponding author. Tel: +33-05-49-44-39-59; Fax: +33-05-49-44-39-08; E-mail: Christine.Imbert@univ-poitiers.fr
L’Étoile, France). The 1066 strain of *C. albicans*, originally isolated from a patient with septicemia, was kindly provided by Professor R. Robert (Laboratory of Immunology, Parasitology and Mycology, Angers, France). These six strains were susceptible to fluconazole (MIC < 8 mg/L, Etest method).

Five other strains were obtained from IHEM (Biomedical Fungi and Yeasts Collection, Brussels, Belgium) and were originally isolated from human mouth (IHEM-9581, IHEM-9582, IHEM-9584, IHEM-9586) or human blood (IHEM-10266); these strains were used in this study because they showed high MIC values of fluconazole by Etest method. These MICs have also been determined by the microdilution method (Table 1).

Yeasts were first grown for 48 h at 28°C on Sabouraud agar slants (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France), to obtain a culture of synchronous stationary yeast-phase *C. albicans*. A loopful of this culture was transferred to 25 mL of Yeast Nitrogen Base medium (YNB, Difco, Detroit, MI, USA), supplemented with 50 mM glucose (Sigma, St Louis, MO, USA; YNB-glu), with or without a sub-MIC (YNB, Difco, Detroit, MI, USA), supplemented with 50 mM glucose (Sigma, St Louis, MO, USA; YNB-glu), with or without a sub-MIC of caspofungin and incubated for 18 h at 37°C, without shaking.

Before use in the adherence experiments, blastospores were harvested, washed twice in 0.1 M phosphate-buffered saline (PBS, pH 7.2; BioMérieux) and adjusted to 1.5 × 10⁷ blastospores/mL.

### MICs of caspofungin and fluconazole

Standard antifungal powder of caspofungin (caspofungin acetate, Merck, NJ, USA) and fluconazole (Pfizer, Orsay, France) was kindly provided by the manufacturers. Caspofungin was prepared as a stock solution of 10 mg/mL in DMSO and aliquots stored at −80°C. Fluconazole was prepared as a stock solution of 10 mg/mL in water, and aliquots stored at −80°C.

The MICs of antifungals were determined using YNB-glu medium, MICYPB-Glu (mg/L) MICYRPB (mg/L) MICYPB-Glu (mg/L) MICYRPB (mg/L)

<table>
<thead>
<tr>
<th>C. albicans strains</th>
<th>Caspofungin MICYPB-Glu (mg/L)</th>
<th>Caspofungin MICYRPB (mg/L)</th>
<th>Fluconazole MICYPB-Glu (mg/L)</th>
<th>Fluconazole MICYRPB (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>92</td>
<td>0.008</td>
<td>0.002</td>
<td>0.97</td>
<td>0.48</td>
</tr>
<tr>
<td>109</td>
<td>0.015</td>
<td>0.030</td>
<td>0.97</td>
<td>0.48</td>
</tr>
<tr>
<td>163</td>
<td>0.030</td>
<td>0.015</td>
<td>0.97</td>
<td>0.48</td>
</tr>
<tr>
<td>182</td>
<td>0.015</td>
<td>0.015</td>
<td>0.97</td>
<td>0.48</td>
</tr>
<tr>
<td>240</td>
<td>0.060</td>
<td>0.015</td>
<td>0.48</td>
<td>0.24</td>
</tr>
<tr>
<td>1066</td>
<td>0.015</td>
<td>0.015</td>
<td>0.48</td>
<td>0.24</td>
</tr>
<tr>
<td>9581</td>
<td>0.008</td>
<td>0.015</td>
<td>31.2</td>
<td>62.5</td>
</tr>
<tr>
<td>9582</td>
<td>0.001</td>
<td>0.002</td>
<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td>9584</td>
<td>0.001</td>
<td>0.015</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>9586</td>
<td>0.001</td>
<td>0.008</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>10266</td>
<td>0.015</td>
<td>0.060</td>
<td>31.2</td>
<td>31.2</td>
</tr>
</tbody>
</table>

The MICs were obtained with a broth microdilution method carried out in YNB-glu medium (MICYPB-Glu) or in RPMI medium (MICYRPB). Results are representative of two separate experiments.

### Immobilized extracellular matrix proteins

Extracellular matrix gel (ECM gel, Sigma) was coated onto wells of 96-well tissue culture plates (polystyrene, Evergreen Scientific, USA) according to the manufacturer’s instructions. This gel was composed primarily of laminin, collagen type IV, heparan sulphate proteoglycan and entactin. Briefly, the wells of microtitre plates were coated with 300 µL of ECM gel (10 µg/mL); after overnight incubation at 4°C, plates were washed twice with PBS.

### Adherence of *Candida albicans* to polystyrene coated with extracellular matrix proteins

Adherence experiments were carried out in untreated 96-well tissue culture plates as previously described (C. albicans plugs were washed every 2 h with PBS, pH 7.2). Tetrazolium salt XTT was used to assess the adherence of *C. albicans* blastospores to wells of tissue culture plates: the principle was based upon the reduction of XTT tetrazolium to tetrazolium formazan by mitochondrially active *C. albicans* blastospores in the presence of an electron-coupling agent, menadione. Briefly, *C. albicans* blastospores pre-incubated or not for 18 h with sub-MIC of caspofungin were added to 96-well tissue culture plates at an inoculum of 1.5 × 10⁷ cells/mL in 150 µL of PBS and were allowed to adhere to the polystyrene coated with extracellular matrix proteins for 2 h at 37°C; half of the cells were then washed twice with PBS to remove the non-adherent yeasts. Thereafter, 300 µg/mL XTT (Sigma) and 0.13 mM menadione (Sigma) were added to all wells. Plates were incubated for 3h at 37°C without shaking, then gently agitated and XTT formazan measured at A523 (micro-plate reader LPl400, Sanofi Diagnostics Pasteur) in washed and unwashed wells. The percentage adherence capacity of each isolate was calculated as a mean of absorbance units in washed wells/absorbance units in unwashed wells.
Background formazan values were determined with plates which contained PBS only or PBS, XTT and menadione; these values did not exceed 0.005 absorbance units and therefore were not significant. All experiments were carried out twice with six replicates.

Statistical analyses
An analysis of variance (ANOVA, \( P < 0.05 \)) and a Scheffé’s test were conducted to determine differences among the test groups.

Results and discussion
Our results comparing MICYNB-glu to MICRPMI values were similar and showed some differences only for two strains (IHEM-9584 and IHEM-9586, Table 1). This is consistent with previous studies showing that the composition and the pH of the culture medium can influence MIC values.8 The MICYNB-glu values of caspofungin obtained for the different strains of \( C. \) albicans were similar and ranged between 0.001 and 0.015 mg/L. This result is in accordance with other studies which suggested that caspofungin showed broad-spectrum antifungal activity against \( C. \) albicans in vitro, without cross-resistance to existing agents.10

It is important to understand the mechanisms involved in biofilm formation on implanted medical devices, which could improve the prevention and the treatment of systemic candidiasis. Previous studies have demonstrated the intrinsic resistance of \( C. \) albicans biofilms to the most commonly used antifungal agents, fluconazole and amphotericin B.10 These authors recently suggested that caspofungin could affect the cellular morphology and the metabolic status of \( C. \) albicans cells within the biofilm. Results obtained in a previous study demonstrated that the activity of some antifungal agents on adherence and on metabolic activity could be different and even opposite, depending on the tested molecule.8 Caspofungin is the first representative of a new antifungal class, and its influence on fungal colonization is not yet well characterized. This paper deals with the effect of caspofungin on the adherence capacity of \( C. \) albicans blastospores to plastic coated with ECM proteins, which is considered as the first step in the development of \( C. \) albicans biofilm.3

Our results showed that the growth of \( C. \) albicans blastospores in medium containing a sub-inhibitory concentration (MIC/2) of caspofungin significantly inhibited (Scheffé’s test, \( P \leq 0.001 \)) the adherence capacity of yeasts to plastic coated with ECM proteins (Figure 1): nine of the 11 strains used (81.8%) were less adherent \( (P \leq 0.001) \) than without contact with caspofungin during growth. These results showed that caspofungin was able to significantly inhibit \( (P \leq 0.001) \) the adherence capacity of all tested strains that were susceptible to fluconazole in vitro. So the anti-adherent activity of caspofungin is obvious and could be explained by the original mechanism of action of caspofungin on the yeast’s cell wall which contains some adhesins implicated in the contact of \( C. \) albicans with the different components of ECM.3 So a disruption of the parietal surface could easily perturb this cellular adherence process.

In the experimental conditions of this work, the anti-adherent activity of caspofungin was less obvious when \( C. \) albicans strains were resistant to fluconazole in vitro: only three of the five fluconazole-resistant strains tested (60%) were significantly less adherent \( (P \leq 0.001) \) in this case. So, this result suggested that the anti-adherent activity of caspofungin could be reduced in the case of in vitro fluconazole resistance and suggested a possible relationship between in vitro fluconazole resistance and the activity of caspofungin. Our results also indicated that under these experimental conditions, caspofungin (MIC/2) did not modify the metabolic activity of the yeasts independently of their resistance to fluconazole (data not shown).

In conclusion, this study demonstrated the efficiency of caspofungin in preventing adherence of \( C. \) albicans to plastic coated with proteins. This indicates that this antifungal drug could be a good candidate in the prevention of the early stage of \( C. \) albicans biofilm development and so in the prevention of candidiasis related to medical devices. Our results indicated a reduced anti-adherent activity of caspofungin when the \( C. \) albicans strains were resistant to fluconazole in vitro; further studies will be conducted to confirm this relationship.
Caspofungin and Candida albicans adherence

Acknowledgements

We thank IHEM (Biomedical Fungi and yeasts Collection, Brussels, Belgium) for providing C. albicans strains, Dr Françoise Symoens for advice and critical review of the manuscript and Mrs Hays-Cloutour for correction of the English.

References