Anticandidal activity of SPA-S-843, a new polyenic drug

V. Strippoli\textsuperscript{a}, F. D. D’Auria\textsuperscript{b}, G. Simonetti\textsuperscript{b}, T. Bruzzese\textsuperscript{a} and N. Simonetti\textsuperscript{b*}

Institute of Microbiology, Faculties of \textsuperscript{a} Medicine and Surgery and \textsuperscript{b} Pharmacy, University of Rome ‘La Sapienza’, viale Regina Margherita, 255, 00198 Rome, Italy

The activity of a new, soluble and stable polyene (SPA-S-843) against \textit{Candida albicans} was assessed by contact and culture tests and by inhibition of germ-tube formation. The drug demonstrated a higher contact activity and lower MICs than amphotericin B. This antimicrobial activity was more evident under acid pH and low ionic strength. In addition, the ability of SPA-S-843 to inhibit \textit{Candida} sp. conversion from yeast to mycelial form was evident at low drug concentrations (0.25–0.62 mg/L).

**Introduction**

There are few available drugs for treatment of fungal infections in immunocompromised patients or in severe systemic pathology.\textsuperscript{1} Thus, the search for new compounds with low toxicity and stability is a priority in the field of anti-infectious therapy. Recently SPA-S-843, a new hydro-soluble partricine A polyene derivative, has been synthesized.\textsuperscript{2,3} This polyene possesses unusual stability and high activity against sensitive fungal isolates in comparison with amphotericin B. In this work we investigated the activity of SPA-S-843, compared with that of amphotericin B, against \textit{Candida albicans} in culture and in contact tests. Furthermore, we investigated the activity of the drug on yeast to mycelium transition of \textit{C. albicans}, an important step in the pathogenesis of candidosis.\textsuperscript{4}

**Materials and methods**

**Antifungal agents**

SPA-S-843 (N-dimethylaminoacetyl-partricin A 2-dimethylaminoethylamide diascorbate), potency 941 mg/g (Società Prodotti Antibiotici, Milan, Italy) and amphotericin B, potency 905 mg/g (Squibb, Rome, Italy) were used throughout. The polyenes were dissolved in dimethylsulphoxide (DMSO, Merck, Darmstadt, Germany) at a concentration of 4 g/L and then serially diluted with water to concentrations ranging from 100 mg/L to 0.002 mg/L. In the stability experiments, the polyene solutions (400 mg/L concentration) were protected from light and stored at 22°C.

**Microorganisms**

Ten clinical isolates and one stock isolate (ATCC 10261) of \textit{C. albicans} (from the Microbiology Institute’s collection) were tested. The isolates were identified by Microscan panels (Baxter, Milan, Italy) and by conventional methods.\textsuperscript{5} The cultures were grown in Sabouraud liquid medium overnight at 37°C with shaking, and the growth was estimated with a Thoma Zeiss Camera (Vetro Scientifica S.r.l., Rome, Italy) and controlled by determination of cfu. The final inoculum was \(5 \times 10^3\) cells/mL in the culture inhibition test, \(10^4\) cells/mL in the contact tests and \(10^6\) cells/mL in the \(K^+\) release tests.

**Electrical conductivity**

The media conductance was measured in \(\mu S/cm\) with an Hl 9032 conductivity meter (Hanna Instruments, S.p.A., Padova, Italy).

**Culture inhibition tests**

The experiments were performed in Sabouraud dextrose broth (SA B; Becton-Dickinson, Cockeysville, M D, USA) and diluted Sabouraud dextrose broth (1:3) plus 2% glucose. The inocula (\(5 \times 10^5\) cells/mL) were added to culture media containing serial dilutions of the drugs and to a control
V. Strippoli et al.

without drugs. The MIC was determined after 24 h incubation at 37°C.

Contact tests
C. albicans cells were incubated in phosphate buffer, at pH 5.6 and various molarities, in the presence of polyenic drugs for 1–15 min at 22°C. After contact, the cell suspensions were diluted 10³ times, then seeded in SA B agar. The cfu were determined after 48 h incubation at 37°C, and the results were reported as log cells/mL at that time point.

K⁺ release tests
The tests were performed with a K⁺ electrode connected to a Microion 2008 (CRISON Instruments S.A., Alella, Barcelona, Spain) with calibration curves adjusted to between 10⁻⁶ mol/L and 5 x 10⁻³ mol/L. The polyene concentrations ranged from 200 to 10 mg/L in sodium phosphate buffer 0.1 M and 0.001 M at pH 5.8. K⁺ ions released were measured after 1, 3, 5, 10 and 15 min and were expressed as percentage of K⁺ released in the test with respect to the K⁺ released from control cell suspensions treated at 100°C for 10 min.

Germ-tube inhibition test
The test was carried out in N-acetylglucosamine solution pH 6.6 with an inoculum of 3 x 10⁵ cells/mL. After 3 h incubation at 37°C the cultures were examined under a light microscope (Carl Zeiss, ×320) and the minimum concentration of the polyenes inhibiting >90% of germ-tube formation was microscopically estimated with respect to controls without drugs, where >95% of germ-tube formation usually occurs after 3 h incubation.

Statistical analysis
K (the velocity constant) was used as a measure of the efficiency of antimicrobial agents. K = (1/t) log(N₀/Nₜ), where N₀ is the initial number of cells, Nₜ is the final number of cells and t is the time for the viable count to fall from N₀ to Nₜ. Differences between mean MIC values were assessed by the Student’s t test. Regression analysis was performed using Excel 95.

Results and discussion
SPA-S-843 stock solution protected from light and stored at 22°C was highly stable for at least 10 days, whereas amphotericin B lost most of its activity after 72 h. The antifungal activity of SPA-S-843 in culture media was higher than that of amphotericin B, and was influenced by pH and medium electrical conductivity (Table).

In the contact experiments SPA-S-843 showed higher cytocidal activity than amphotericin B against C. albicans. The cell kill with SPA-S-843 was >99.99% in 3 min (Figure). This activity was also affected by pH and electrical conductivity.

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>pH</th>
<th>Electrical conductivity (µS/cm)</th>
<th>Mean MIC (mg/L) for amphotericin B</th>
<th>SPA-S-843</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabouraud</td>
<td>7.2</td>
<td>2500</td>
<td>0.182</td>
<td>0.0026</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sabouraud diluted 1:3</td>
<td>7.2</td>
<td>800</td>
<td>0.047</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nutrient broth</td>
<td>7.2</td>
<td>2500</td>
<td>0.158</td>
<td>0.0758</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nutrient broth diluted 1:3</td>
<td>7.2</td>
<td>800</td>
<td>0.208</td>
<td>0.0358</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sabouraud</td>
<td>5.4</td>
<td>2500</td>
<td>0.071</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sabouraud diluted 1:3</td>
<td>5.4</td>
<td>800</td>
<td>0.039</td>
<td>0.00067</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nutrient broth</td>
<td>5.4</td>
<td>2500</td>
<td>0.19</td>
<td>0.0206</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nutrient broth diluted 1:3</td>
<td>5.4</td>
<td>800</td>
<td>0.199</td>
<td>0.00378</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure. Cytocidal activity of SPA-S-843 and amphotericin B against C. albicans ATCC 10261 in different media. ●, SPA-S-843 0.31 mg/L in sodium phosphate buffer 0.001 M, pH 5.8; ○, SPA-S-843 0.31 mg/L in sodium phosphate buffer 0.1 M, pH 5.8; ▲, amphotericin B 100 mg/L in sodium phosphate buffer 0.001 M, pH 5.8; △, amphotericin B 100 mg/L in sodium phosphate buffer 0.1 M, pH 5.8.
conducitivity of the phosphate buffer solution used in the experiments. The remarkably rapid cytocidal activity of SPA-S-843 was better displayed in a medium with a low electrical conductivity (0.001 M buffer strength) and with acid pH (pH 5.8), with both factors favouring increase in cell permeability.

The SPA-S-843 activity was greater than 100 times that of amphotericin B, suggesting a higher candidicidal activity of the former compound. This activity seems to be related to the induction of a greater cellular damage, with accelerated leakage of K⁺ ions, as demonstrated by a significant difference between the K⁺ leakage induced by SPA-S-843 (80% ion leakage in 4 min) compared with that induced by amphotericin B (35% ion leakage in 4 min).

In the study of yeast to mycelium C. albicans cell transition the SPA-S-843 activity was remarkably higher (mean MIC 0.116 mg/L; range 0.045–0.31 mg/L) than that of amphotericin B (mean MIC 0.45 mg/L; range 0.25–0.62 mg/L). The differences between the mean values were statistically significant (P < 0.001).

Because there is evidence that the hyphal form may play a pathogenic role in the initial process of tissue invasion and germ tubes and hyphae adhere better than yeast cells to human buccal and vaginal epithelia,⁴ the better inhibition of the morphological transition by SPA-S-843 might improve the effectiveness of the drug.

References


Received 12 August 1998; returned 21 February 1999; revised 19 April 1999; accepted 19 October 1999