In-vitro antifungal activity of sertaconazole, bifonazole, ketoconazole, and miconazole against yeasts of the Candida genus

A. J. Carrilo-Muñoz*, C. Tur, and J. Torres

Unitat de Micologia, Institut Municipal D'investigacio (IMIM), Universitat Autonoma de Barcelona, Avda. Dr. Aiguader 80, E-08003 Barcelona, Spain

The in-vitro antifungal activity of sertaconazole against 110 strains of Candida yeasts (50 Candida albicans, 15 Candida glabrata, 2 Candida guilliermodii, 8 Candida krusei, 1 Candida kefyr, 8 Candida parapsilosis and 26 Candida tropicalis) was assessed in comparison with bifonazole, ketoconazole, econazole and miconazole. The majority of the strains were clinical isolates; some reference strains were included. A commercial agar diffusion method (NeoSensitabs, Rosco, Taastrup, Denmark) in Shadomy's modified medium pH 7 was used. Using the manufacturer's criteria, 86.4% of the strains were classified as "sensitive" to sertaconazole. The only strain classified as "resistant" to sertaconazote was the control reference strain of C. albicans. The remaining strains were classified as "moderately sensitive". The sensitivity/resistance percentages for the other antifungals tested were 75.5/1.8 for ketoconazole, 71.8/2.7 for miconazole, 63.7/13.6 for econazole, and 59.1/5.5 for bifonazole. Sertaconazole showed a higher antifungal activity than that of the other antimycotics, tested in vitro which was statistically significant (P < 0.001), as well as a lower resistance rate than that of econazole, bifonazole and ketoconazole.

Introduction

Sertaconazole (7-chloro-3-[1-(2,4-dichlorophenyl-1-y1)ethoxy-methyl]benzo[b]thiophene) is a recently developed antifungal characterized by its broad spectrum of action against yeasts, dermatophytes and Gram-positive bacteria. (Torres-Rodriguez Alayeto & Palacin, 1986. Palacin Scristan, & Ortiz 1992a). Agut et al., (1992a,b) have shown that sertaconazole causes an inhibition of ergosterol synthesis and has a direct cell membrane effect producing irreparable damage. The good activity of this antifungal (Drohuet & Dupont, 1992; Carrilo-Muñoz & Torres-Rodriguez 1995), its low toxicity as a topical antifungal (Romero et al., 1992) and its efficacy in animal models of dermatophytosis and candidiasis (Palacin, Sacristan & Ortiz, 1990, 1992a) make it a promising candidate for the treatment of superficial mycosis.

This study was performed to compare the sensitivity of 110 strains of yeast of the genus Candida to sertaconazole, econazole, bifonazole, ketoconazole and miconazole. The antifungal activity of these substances was studied in vitro by means of a commercial agar diffusion method (NeoSensitabs) using Shadomy's modified medium (Yeast Nitrogen Base, asparagine, glucose, pH 7).

*Correspondence to: Dr A. J. Carrilo-Muñoz, Valle de Ordesa, Nª4,4ª,4ª. E-08031 Barcelona, Spain. Fax: + 34 3 429 71 20. E-mail: ajcm.acia@bcn.servicom.es.
Material and methods

Antifungals

The antifungals were used as 9-mm diameter standard tablets (NeoSensitabs Rosco, Tasstrup, Denmark) prepared and supplied by the manufacturer.

Organisms

One hundred and ten yeasts of Candida albicans, Candida glabrata, Candida guilliermondii, Candida krusei, Candida kefyr, Candida parapsilosis and Candida tropicalis, isolated from different patients, identified by standard biochemical and morphological methods (API 32C) and preserved in the Collection of IMIM, were used. Two reference strains (C. albicans, UPV 1096, C. albicans UPV 3153) were the gift of Dr G. Quindós and Dr J. Pontón (Department of Microbiology and Immunology, School of Medicine, Universidad del Pais Vasco UPV) and C. albicans ATCC 64550, C. glabrata ATCC 90030, C. glabrata ATCC 90028 and C. kefyr ATCC 28838 were supplied by the manufacturer of the NeoSensitabs.

Sensitivity testing in vitro.

A commercial agar diffusion method (NeoSensitabs, Rosco, Taastrup, Denmark), which has been described previously (Casals, 1979), was used. Shadomy's modified medium (Yeast Nitrogen Base, asparagine and glucose) was buffered to pH 7 with Na₂HPO₄ and 0.2 M K₂HPO₄. Standard Petri dishes (9 cm diameter) containing medium to a depth of 0.5 cm were used and stabilised by warming at 35°C for 30 min before use. The sterility of the lots was controlled before use. Inocula were prepared by suspending 1 or 2 colonies from 24 h cultures in Sabouraud medium into tubes containing 10 mL of sterile saline until turbidity was equivalent to 0.5 on the McFarland scale. The tubes were diluted 1/2 with saline. The concentration of the final inoculum was 5 × 10⁵ cfu/mL. For C. krusei strains, a final dilution of 1/10 was used. The inoculum (0.5 mL) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the antifungal tablets on the surface. After incubation in reverse-position at 35°C for 24 h, the halos of inhibition around the tablets were measured. Strains were classified as sensitive, moderately sensitive or resistant according to the manufacturer's criteria. For sertaconazole, econazole, miconazole, and ketoconazole sensitive strains produced inhibition diameters of > 20 mm, moderately sensitive strains diameters of 12–19 mm and resistant strains diameters of < 11 mm. For bifonazole the criteria were sensitive for halos with diameters greater than 15 mm, moderately sensitive if their halos measured between 10 and 14 mm, and resistant if there was no inhibition halo. Experiments were performed in duplicate on the same day.

Statistics

The analysis of data was performed using SPSS/PC + for IBM 4.0(USA) package.
Results
Sertaconazole had the greatest activity overall with the highest number of sensitive strains and the least number of resistant strains (Table I). The only resistant strain found was that used as resistance control (C. albicans ATCC 64550). The statistical assessment revealed a similarity between the halos produced by sertaconazole and ketoconazole which were the ones with the largest size, as well as a statistically significant coefficient of correlation (0.6518) despite the difference between the sensitivity rates for both drugs. Similarly, sertaconazole was the antifungal with best-activity against C. albicans and C. glabrata (Table II). None of the strains of C. krusei, C. guilliermondii, C. parapsilosis and C. tropicalis were resistant to sertaconazole. The number of strains (C. albicans, C. glabrata and C. guilliermondii) with moderate sensitivity to sertaconazole was lower than that of the other antifungals. The activity of sertaconazole was statistically higher than that of bifonazole, ketoconazole and econazole (P < 0.01).

Discussion
The results obtained are consistent with those reported by several authors who demonstrated that the activity of sertaconazole in vitro against yeasts of the Candida genus was higher than other antifungals (Drouhet & Dupont, 1992). In other studies (Torres-Rodriguez, et al., 1986; Palacin et al., 1992b), the fungistatic activity of sertaconazole was found to be comparable to that of miconazole and higher than that of bifonazole and ketoconazole. Nevertheless, there is a variety of results in the literature due to the diversity of experimental variables. However, Drouhet & Dupont (1992) and Palacin et al., (1992b), who used a medium other than Shadomy’s modified medium, demonstrated that the activity of sertaconazole was higher than that of bifonazole, or bifonazole and econazole (Carrillo-Munoz & Torres-Rodriguez, 1995) against strains of Candida as well. Although there may also be discrepancies between the values resulting from different methods (Torres-Rodriguez et al., 1986; Palacin et al., 1992b), sertaconazole was always found to be more active than other antifungals.

The values from the present study with sertaconazole against pathogenic yeast strains from different clinical isolates show good activity in vitro, which is in agreement with the results reported by other authors under different experimental conditions and has already described by us using a microdilution method in liquid medium for assessment.

Table I. Sensitivity overall values (%) to five antifungals determined by means of the agar diffusion method (NeoSensitab, Rosco, Taastrup, Denmark)

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>Sensitive</th>
<th>Moderately sensitive</th>
<th>Resistant</th>
<th>Mean halo (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sertaconazole</td>
<td>86.4</td>
<td>12.7</td>
<td>0.9</td>
<td>24.95</td>
</tr>
<tr>
<td>Bifonazole</td>
<td>59.1</td>
<td>35.4</td>
<td>5.5</td>
<td>16.80</td>
</tr>
<tr>
<td>Econazole</td>
<td>63.7</td>
<td>22.7</td>
<td>13.6</td>
<td>19.73</td>
</tr>
<tr>
<td>Miconazole</td>
<td>71.8</td>
<td>25.5</td>
<td>2.7</td>
<td>21.50</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>75.5</td>
<td>22.7</td>
<td>1.8</td>
<td>26.13</td>
</tr>
</tbody>
</table>
of sensitivity in vitro. The present study uses the diffusion method in agar, as currently this is the only commercial method for assessing sensitivity to sertaconazole.

**References**


*(Received 2 August 1995; accepted 13 December 1995)*