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In-vitro antifungal activity of sertaconazole, econazole, and bifonazole against *Candida* spp.

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The in-vitro activity of sertaconazole (7-chloro-3-[1-(2,4-dichlorophenyl-1-yl)ethoxy-methyl] benzo[b] thiophene) was compared with that of econazole and bifonazole against 150 strains of yeasts which includes six *Candida* species. Minimum inhibitory concentrations (MICs) were determined by a microdilution technique in Sabouraud's liquid medium, (pH 5.6). Sertaconazole (arithmetic mean MIC 0.77 mg/L) was more active than econazole (MIC 1.75 mg/L) and bifonazole (MIC 9.05 mg/L). MIC values for sertaconazole were generally and specifically lower for each tested species, *Candida parapsilosis* being the most susceptible (MIC 0.31 mg/L), in contrast to *Candida tropicalis*, which had the highest MIC (1.67 mg/L).

Introduction

Sertaconazole (7-chloro-3-[1-(2,4-dichlorophenyl-1-yl)ethoxy-methyl] benzo[b]thiophene) is a recently developed antifungal characterized by its broad spectrum activity against several yeasts and Gram-positive bacteria (Drouhet & Dupont, 1992; Palacín, Sacristán & Ortíz, 1992*a*). The fungicidal action of sertaconazole is due to inhibition of ergosterol synthesis and a direct cell membrane damaging effect. (Agut *et al.* 1992*a*,*b*). The good in-vitro activity of sertaconazole (Drouhet & Dupont, 1992) low toxicity after topical use (Romero *et al.*, 1992), and its efficacy in animal models of dermatophytoses (Palacín, Sacristán & Ortíz, 1990; Palacín *et al.*, 1992*a*,*b*) favour the use of this drug for the treatment of dermatophytoses.

In the present study we tested the susceptibility of 150 clinical strains of *Candida* spp. to sertaconazole, econazole and bifonazole. The in-vitro activity of sertaconazole was assessed by a microdilution method in Sabouraud's liquid medium (pH 5.6).

Materials and methods

Antifungal agents

Sertaconazole was kindly supplied by Ferrer Internacional, S.A. (Barcelona, Spain). Econazole and bifonazole were obtained from Sigma, S.A. (Sant Louis, USA). Stock solutions were prepared by dissolving each test substance in dimethyl sulphoxide at

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concentrations of 1 mg/mL. Then 1 mL of each stock solution was added to 11.5 mL of culture medium to prepare different concentrations of the antifungal agents.

Organisms

One hundred and fifty strains of *Candida albicans*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida parapsilosis* and *Candida tropicalis* isolated from different patients, identified by standard morphological and biochemical methods (API 32C) and preserved in our laboratory collection were used. Two reference strains (*C. albicans* NCPF 3153 and *C. krusei* NCPF 3100) from the National Collection of Pathogenic Fungi (UK) were used.

In-vitro susceptibility testing.

As in previous studies (Palacín, Sacristan & Ortíz, 1992*c*; Torres-Rodriguez JM, Carillo-Muñoz & Madrenys-Brunet, 1993) Sabouraud's liquid medium (Pronadisa, Spain) was used (pH 5.6). Susceptibility was assessed by a microdilution technique in liquid medium using 96 (8 × 12) sterile flat-bottom well microtitre plates (Corning, New York, USA). After inoculation, serial two-fold dilutions of each antifungal agent were prepared in rows of wells to give concentrations from 40 mg/L to 0.03 mg/L. In all cases, the first well, without antifungal, was used as growth control. Sterility of both the culture medium batches and the stock solutions of antifungals was monitored. 24 h cultures in Sabouraud's agar medium were used for the preparation of inocula. Suspensions of yeasts were prepared to an inoculum density of 1×10^4 cfu/mL. Seeding was performed by applying 100 μL of the inoculum suspensions on each well containing 100 μL of antifungal dilution. The plates were stirred for 5 min and then incubated at 35°C in moisture chambers for protection against desiccation.

Visual reading was performed 24 h after incubation using an inverted mirror (Dinatech, UK). Minimum inhibitory concentration, defined as the lowest concentration of antifungal that inhibited growth, was determined by comparing with the growth controls without antifungal. All assays were performed in duplicate the same day of the experiment.

Statistics

SPSS/PC + para IBM 4.0 (USA) package was used for the statistical analysis of data.

Results

The statistical analysis (T-student and Wicoxon tests) of data, indicated that there was no significant differences ($P < 0.05$) in the replicate tests for each of the three antifungals. The results presented in the Table, show that sertaconazole (MIC 0.77 mg/L) was superior to econazole (MIC 1.75 mg/L) and bifonazole (MIC 9.01 mg/L) against all strains of *Candida* spp. *C. parapsilosis* was the most susceptible to sertaconazole, while *C. tropicalis* was the least susceptible. *C. tropicalis* was also the most resistant to econazole and bifonazole. MIC ranges of the three antifungals were lower for sertaconazole against all species tested. There were statistically significant differences (Student-Newman-Keuls)

Table. Minimum inhibitory concentrations of sertaconazole, bifonazole and econazole for 150 strains of *Candida* spp.

Organism	Compound	Range MIC values (mg/L)	MIC mean values (mg/L)
C. albicans	sertaconazole	0.03–15	1.02
($n = 73$)	econazole	0.03–15	2.24
	bifonazole	0.23–20	3.60
C. guilliermondii	sertaconazole	0.03–1.87	0.51
($n = 5$)	econazole	0.17–3.7	1.11
	bifonazole	1.87–5	3.87
C. krusei	sertaconazole	0.11–0.93	0.38
($n = 13$)	econazole	0.15–5	0.91
	bifonazole	0.93–5	2.20
C. parapsilosis	sertaconazole	0.03–2.5	0.31
($n = 22$)	econazole	0.03–3.75	0.75
	bifonazole	1.25–10	3.76
C. tropicalis	sertaconazole	0.23–5	1.67
($n = 21$)	econazole	0.31–10	3.14
	bifonazole	1.87–20	9.51
C. glabrata	sertaconazole	0.03–3.12	0.78
($n = 16$)	econazole	0.03–10	2.39
	bifonazole	0.03–20	4.09
Total	sertaconazole	0.03–5	0.77
($n = 150$)	econazole	0.03–15	1.75
	bifonazole	0.23–20	9.01

between the three antifungals in *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* ($P < 0.05$).

Discussion

The results obtained here are consistent with those reported by other authors (Drouhet & Dupont, 1992) who demonstrated that the in-vitro activity of sertaconazole was higher than that of miconazole against *Candida* spp. In other studies (Torres-Rodriguez, Alayeto & Palacín, 1986; Palacín *et al.*, 1992*c*), the fungistatic activity of sertaconazole was comparable with that of miconazole and clotrimazole, and superior to bifonazole and ketoconazole.

However, the results reported by Drouhet & Dupont (1992) for sertaconazole are lower than those obtained by us, perhaps because of the use of different culture media. However, Palacín *et al.* (1992*c*), who also used Sabouraud medium but tested a smaller number of strains of *C. albicans*, reported superior activity for sertaconazole (2 mg/L) and bifonazole (8 mg/L) against *C. albicans*, as compared with our values. The discrepancy between these values could be attributed to the use of different dilution procedures, since Palacín *et al.* (1992*c*) used an agar dilution technique and determined MIC_{90}s. Nevertheless, the MIC_{90}s reported using yeast nitrogen base medium (YNB) are consistent with ours for sertaconazole (1 mg/L) against *C. albicans*. To this effect, the values we achieved for sertaconazole (*C. albicans* MIC 6 mg/L) with Sabouraud's liquid medium were clearly lower than those previously published by us with YNB using an agar dilution method as well (Torres-Rodriguez *et al.*, 1986). Favourable results with

sertaconazole were also reported by us for *Scopulariopsis brevicaulis* in Sabouraud's medium (Torres-Rodriguez *et al.*, 1993).

The results of the present study of sertaconazole against pathogenic strains isolated from different clinical samples indicate good in-vitro activity, consistent with results obtained with several antifungals in other experimental conditions and predict better clinical efficacy than other topical azoles.

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