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In Vitro Susceptibilities of Clinical Yeast Isolates to the New Antifungal Eberconazole Compared with Their Susceptibilities to Clotrimazole and Ketoconazole

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The antifungal activity of eberconazole, a new imidazole derivative, against 124 clinical isolates of *Candida* comprising eight different species and to 34 isolates of *Cryptococcus neoformans* was compared to those of clotrimazole and ketoconazole. MICs of eberconazole, determined by the National Committee for Clinical Laboratory Standards standardized microbroth method, were equal to or lower than those of other azoles, especially for *Candida krusei* and *Candida glabrata*, which are usually resistant to triazoles.

Eberconazole (1-(2,4-dichloro-10,11-dihydro-5*H*-dibenzo-[*a*,*d*]cyclohepten-5-yl)-1H-imidazole) is a new antifungal imidazole derivative developed by Salvat Laboratories (Barcelona, Spain). In preliminary studies, eberconazole showed an excellent antifungal in vitro activity (3) against yeasts of the genus *Candida* and against dermatophytes and therefore has been developed as a topical antimycotic for the treatment of superficial fungal infections. It is currently in phase III clinical trials (1).

The first studies to determine its MICs were carried out between 1988 and 1989 using a nonstandardized micromethod with Sabouraud broth medium. In these studies, it was found that MICs for 116 yeast isolates were between 0.6 and 5.0 μg/ml, similar to or lower than that of clotrimazole, which was used as the reference (11).

The aim of the present study was to verify these earlier results and to compare the activity of eberconazole with those of clotrimazole and ketoconazole in clinical isolates of yeasts, using the micromethod standardized by the National Committee for Clinical Laboratory Standards (NCCLS).

A total of 124 isolates of *Candida* species were selected for the study: *C. albicans* (47 isolates, including 12 with MICs of fluconazole of >64 μg/ml), *C. tropicalis* (17 isolates), *C. parapsilosis* (14 isolates), *C. krusei* (13 isolates), *C. glabrata* (17 isolates), *C. guilliermondii* (7 isolates), *C. famata* (5 isolates), and *C. dubliniensis* (4 isolates). Thirty-four isolates of *Cryptococcus neoformans* were also included. All the strains were isolated from clinical samples, mainly from AIDS patients with oropharyngeal candidosis, and on a smaller scale from patients with candiduria, vaginal candidosis, and onyxis. *Cryptococcus neoformans* was isolated from AIDS patients with meningoencephalitis caused by this fungus.

Isolates were stored at −40°C in skim milk, when required, and they were subcultured on Sabouraud agar with chloramphenicol plus gentamicin. Eight reference strains from the American Type Culture Collection (ATCC) were included as quality controls (5).

Eberconazole was provided by Salvat S.A. Laboratories (Barcelona, Spain) as standard powder. Ketoconazole was from Roig-Farma (Barcelona, Spain) and clotrimazole was from Sigma (St. Louis, Mo.).

MICs were determined by the NCCLS microbroth dilution method M27-A (5) with the following modification: RPMI 1640 medium supplemented with glucose (18 g/liter) was used (10). Antifungal agents were distributed in the wells of microtiter plates, giving final drug concentrations of between 0.03 and 16 μg/ml.

Microdilution plates were incubated in a moist chamber at 35°C. The incubation times were 48 h for *Candida* species and 72 h for *Cryptococcus neoformans* isolates. Readings were made both visually and spectrophotometrically at 414 nm with an automatic reader (Multiscan MS; Labsystems, Barcelona, Spain). The MIC endpoints were defined as the lowest drug concentrations in the wells which resulted in an 80% reduction of fungal growth compared to the drug-free control (2).

The MIC range, the geometric mean (GM) MIC, and the MICs at which 90% and 50% of isolates are inhibited were determined (Table 1). Variance analysis and the Wilcoxon test were applied; a P value of <0.05 was considered to be statistically significant. The data were analyzed with SPSS software.

MICs of ketoconazole for the reference ATCC strains were within the acceptable quality control range described by the NCCLS (5). MICs ranged from 0.03 to 2 μg/ml for eberconazole and from 0.03 to 4 μg/ml for ketoconazole and clotrimazole.

Only MICs for *C. glabrata* and *Cryptococcus neoformans* presented normally distributed values. Statistically significant differences were noted for *C. tropicalis* and *C. krusei* when all three antifungal agents were compared together. MICs of eberconazole for *C. krusei* and *C. glabrata* were lower than those of the other two azoles, and the differences between eberconazole and ketoconazole were statistically significant. For *C. tropicalis*, there was a significant difference between the MICs of ketoconazole and those of the other two agents. MICs for *Cryptococcus neoformans* were lower for ketoconazole (GM MIC, 0.035 μg/ml) than for clotrimazole (GM MIC, 0.042 μg/ml) and eberconazole (GM MIC, 0.162 μg/ml).

In a study performed before reference methods were published, we used a microdilution method with Sabouraud broth to compare the activity of eberconazole (WAS 2160) against yeasts and dermatophytes to those of clotrimazole and bifonazole (11). Bifonazole presented the lowest activity against

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TABLE 1. GM MICs, MIC ranges, MIC_{50}s, and MIC_{90}s[a] of eberconazole, ketoconazole, and clotrimazole for 124 isolates of *Candida* species

Isolate (*n*)	Result (μg/ml)											
	Eberconazole				Clotrimazole				Ketoconazole			
	GM MIC	Range	MIC_{50}	MIC_{90}	GM MIC	Range	MIC_{50}	MIC_{90}	GM MIC	Range	MIC_{50}	MIC_{90}
Candida albicans (35)	0.05	0.03–1	0.03	0.5	0.08	0.03–2	0.08	0.03	0.04	0.03–1	0.03	0.03
Candida albicans (12)[b]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Candida tropicalis (17)	0.27	0.03–2	0.27	2.0	0.16	0.03–2	0.125	1.0	0.06	0.03–1	0.03	0.25
Candida parapsilosis (14)	0.05	0.03–1	0.03	0.125	0.03	0.03–0.06	0.03	0.03	0.043	0.03–0.25	0.03	0.125
Candida krusei (13)	0.04	0.03–0.25	0.03	0.125	0.08	0.03–0.5	0.03	0.5	0.42	0.03–2	0.5	1.0
Candida glabrata (17)	0.1	0.03–2	0.06	0.05	0.4	0.5–4.0	0.5	2.0	0.4	0.03–4	1.0	2.0
Candida guilliermondii (7)	0.08	0.03–0.5	0.03	0.5	0.07	0.03–0.25	0.03	0.25	0.03	0.03–0.06	0.03	0.03
Candida famata (5)	0.046	0.03–0.125	0.03	0.06	0.061	0.03–0.125	0.03	0.03	0.03	0.03	0.03	0.03
Candida dubliniensis (4)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
All isolates (124)	0.064				0.079				0.068			

[a] MIC_{50} and MIC_{90}, the MICs at which 50% and 90% of the isolates, respectively, are inhibited.
[b] Isolates with MICs of fluconazole of >64 μg/ml.

Candida sp., and that of eberconazole was slightly greater than that of clotrimazole. This difference was especially evident with *C. glabrata*, which, as in this study, showed a high level of susceptibility to eberconazole. This antifungal agent showed great activity against *C. glabrata* and *C. krusei*, even higher than ketoconazole, which is especially relevant given that these two species have shown a high frequency of intrinsic resistance to fluconazole (8) and, on a smaller scale, to itraconazole (6, 9).

The in vitro activity of every new antifungal agent gives us an approximate idea of its therapeutic indications, which will be verified later in animal models and clinical trials. The MIC distributions of the studied yeasts showed that eberconazole's activity is comparable to that of ketoconazole but higher than that of clotrimazole against *C. albicans*, which still is the most common pathogenic clinical yeast isolate (7). Eberconazole also demonstrated a marked activity against *C. parapsilosis*, one of the most important species in skin and nail disorders (4).

In spite of the primary topical indication of eberconazole, it was considered relevant to include isolates of *Cryptococcus neoformans* because there is no information in the literature about the susceptibility of this species to the dichloroimidazoles and because other derivatives of them could be developed for systemic administration. This yeast was less sensitive to eberconazole than to ketoconazole and clotrimazole, but low MICs (GM MIC, 0.162 μg/ml) were observed.

The results demonstrate that this new azole exhibits good activity against a wide range of clinically relevant yeasts. Its profile is comparable to the most active topical antifungal azoles. Interestingly, some of the most triazole-resistant yeasts, such as *C. krusei* and *C. glabrata* and also fluconazole-resistant *C. albicans*, are sensitive to eberconazole.

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