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In Vitro Activities of the New Antifungal Drug Eberconazole and Three Other Topical Agents against 200 Strains of Dermatophytes

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We have compared the in vitro activity of the new antifungal drug eberconazole with those of three other topical antifungal agents, clotrimazole, ketoconazole, and miconazole, against 200 strains of dermatophytes. MICs were determined by a microdilution method with optimal conditions determined in a previous study (an inoculum of 10^4 CFU/ml, an incubation temperature of 28°C, an incubation period of 7 days, and a MIC endpoint of 100% inhibition of growth). In general, the four drugs tested showed low MICs. However, eberconazole was more active ($P < 0.05$) than the other three drugs against the majority of the species tested. Eberconazole represents an advantageous alternative for dermatophytoses where a topical therapy is required.

Dermatophytoses constitute an important public health problem as yet unresolved. These infections are frequent not only in underdeveloped countries but also in elderly and immunocompromised patients worldwide (20). Systemic drugs, such as terbinafine and itraconazole, are currently used for the treatment of severe and chronic dermatophytoses (13). However, in less-severe infections, topical antifungal therapy based on the use of imidazoles, such as clotrimazole (CTZ), miconazole (MCZ), and ketoconazole (KTZ), is most commonly used (17). Comparative studies on the in vitro activity of these drugs against a representative number of species are scarce. Eberconazole (EBZ) {1-(2,4-dichloro-10,11-dihydro-5*H*-dibenzo-[*a*,*d*]cyclohepten-5-yl)1H-imidazole} ($C_{18}H_{15}Cl_2N_3O_3$) is a novel topical imidazole with a mode of action similar to that of other azole antifungals, namely inhibition of fungal lanosterol 14α-demethylase (19). This antifungal agent has shown excellent in vivo and in vitro activity against dermatophytes in the studies performed so far (5; J. M. Torres-Rodriguez, A. Carrillo, C. Gallach, N. Madrenys, and J. Julve, abstract from the 10th Congr. Int. Soc. Hum. Anim. Mycol., Rev. Iberoam. Micol. **5**(Suppl. 1)**:**84, 1988). However, its in vitro activity against an important number of strains representing the wide spectrum of dermatophytes, and following a standardized and reproducible method, has not yet been investigated. Even though a reference method for testing dermatophytes has still not been developed, some experience has been accumulated by different authors. Several studies have been performed, generally consisting of modifications of the NCCLS methods for yeasts and molds (8) but also using very different techniques (11, 14). In a recent multicenter study, we determined the optimal conditions for testing of dermatophytes using a microdilution method (7). Therefore, the aim of this study has been to evaluate the in vitro activity of EBZ in comparison with that of the conventional topical drugs CTZ, MCZ, and KTZ against 200 strains of dermatophytes using our own previously standardized method (7).

A total of 200 strains of dermatophytes belonging to 19 species were tested. They included 8 *Epidermophyton floccosum* strains, 5 *Microsporum audouinii* strains, 23 *Microsporum canis* strains, 3 *Microsporum ferrugineum* strains, 2 *Microsporum fulvum* strains, 1 *Microsporum gallinae* strain, 10 *Microsporum gypseum* strains, 1 *Microsporum praecox* strains, 1 *Microsporum racemosum* strain, 2 *Trichophyton ajelloi* strains, 2 *Trichophyton balcaneum* strains, 7 *Trichophyton erinacei* strains, 13 *Trichophyton interdigitale* strains, 31 *Trichophyton mentagrophytes* strains, 1 *Trichophyton phaseoliforme* strain, 65 *Trichophyton rubrum* strains, 2 *Trichophyton simii* strains, 15 *Trichophyton tonsurans* strains, and 8 *Trichophyton violaceum* strains. The isolates were stored as suspensions in water at room temperature until used in the study. Prior to testing, each isolate was subcultured onto potato dextrose agar (PDA) to ensure purity and optimal growth. *Aspergillus fumigatus* NCPF 7099 was included as a reference strain and was tested each time a set of isolates was evaluated. CTZ was provided by Química Farmaceútica Bayer (Barcelona, Spain), EBZ by Laboratorios Salvat, S. A. (Barcelona, Spain), and MCZ and KTZ by Janssen Research Foundation (Beerse, Belgium). Drug dilutions were prepared in 100% dimethyl sulfoxide at 100 times the final concentration, followed by further dilutions (1:50) in RPMI 1640 medium to yield twice the final strength required for the test. Aliquots (0.1 ml) of each antifungal agent were dispensed into the wells of microdilution trays, which were stored at −70°C until they were used. The final concentrations of all drugs ranged from 0.01 to 16 μg/ml. Stock inoculum suspensions were prepared from 7- to 10-day-old cultures grown on PDA at 28°C as described previously. The suspensions were adjusted to an optical density that ranged from 0.13 to 0.17 (65 to 70% transmittance). The final concentrations of the stock inoculum suspensions ranged from 0.3×10^5 to 6.4×10^6 CFU/ml, as demonstrated by quantitative colony counts on PDA. Each suspension was diluted 1:50 in RPMI 1640 to obtain the final test inoculum twice. Each microdilution well was inoculated with 100 μl of the diluted suspensions. Growth

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TABLE 1. In vitro activity of four topical antifungal agents against 19 species of dermatophytes determined by the microdilution broth method

Species (n^a)	Antifungal drug	Range	Geometric mean MIC	$MIC_{50}{}^b$	MIC_{90}
E. floccosum (8)	CTZ	0.125–1	0.32	0.25	
	EBZ	0.03–0.25	0.06	0.06	
	KTZ	0.03–0.5	0.12	0.125	
	MCZ	0.03–0.5	0.10	0.06	
M. audouinii (5)	CTZ	0.06–0.25	0.09	0.06	
	EBZ	0.03–0.125	0.06	0.06	
	KTZ	1–2	1.52	1	
	MCZ	0.06–2	0.22	0.125	
M. canis (23)	CTZ	0.01–4	0.23	0.25	1
	EBZ	0.03–2	0.11	0.06	0.25
	KTZ	0.06–4	0.58	0.5	2
	MCZ	0.03–2	0.38	0.5	1
M. ferrugineum (3)	CTZ	0.03–0.5	0.08	0.03	
	EBZ	0.03	0.03	0.03	
	KTZ	0.125–0.5	0.31	0.5	
	MCZ	0.06	0.06	0.06	
M. fulvum (2)	CTZ	0.125	0.13	0.125	
	EBZ	0.03–2	0.24	0.03	
	KTZ	1–2	1.41	1	
	MCZ	1–2	1.41	1	
M. gallinae (1)	CTZ	0.03			
	EBZ	0.06			
	KTZ	1			
	MCZ	1			
M. gypseum (10)	CTZ	0.125–2	0.5	0.5	2
	EBZ	0.03–0.125	0.07	0.06	0.125
	KTZ	0.06–8	0.65	0.5	2
	MCZ	0.06–4	0.93	1	2
M. praecox (1)	CTZ	0.06			
	EBZ	0.03			
	KTZ	8			
	MCZ	8			
M. racemosum (1)	CTZ	>16			
	EBZ	1			
	KTZ	16			
	MCZ	0.5			
T. ajelloi (2)	CTZ	1	1	1	
	EBZ	0.06	0.06	0.06	
	KTZ	2	2	2	
	MCZ	1	1	1	
T. balcaneum (2)	CTZ	0.03–0.25	0.09	0.03	
	EBZ	0.03–0.06	0.04	0.03	
	KTZ	0.25–2	0.71	0.25	
	MCZ	0.25–1	0.5	0.25	
T. erinacei (7)	CTZ	0.125–1	0.37	0.25	
	EBZ	0.03–0.5	0.15	0.25	
	KTZ	0.06–8	1.99	2	
	MCZ	0.06–2	0.50	0.5	
T. interdigitale (13)	CTZ	0.06–1	0.27	0.25	1
	EBZ	0.125–1	0.26	0.125	0.5
	KTZ	0.25–4	1.56	2	2
	MCZ	0.125–4	0.74	0.5	4
T. mentagrophytes (31)	CTZ	0.03–2	0.27	0.25	1
	EBZ	0.03–0.5	0.14	0.125	0.5
	KTZ	0.06–8	0.64	0.5	2
	MCZ	0.125–4	0.62	1	2
T. phaseoliforme (1)	CTZ	0.25			
	EBZ	0.25			
	KTZ	8			
	MCZ	0.125			
T. rubrum (65)	CTZ	0.03–2	0.21	0.125	0.25
	EBZ	0.03–0.5	0.12	0.125	0.125
	KTZ	0.125–4	0.65	0.5	1
	MCZ	0.03–2	0.37	0.25	0.5
T. simii (2)	CTZ	0.25–0.5	0.35	1	
	EBZ	0.125–0.5	0.24	0.125	
	KTZ	8	8	8	
	MCZ	2–>16	2	>16	
T. tonsurans (15)	CTZ	0.03–1	0.16	0.25	0.5
	EBZ	0.03–0.125	0.09	0.125	0.125
	KTZ	0.25–4	0.72	0.5	2
	MCZ	0.06–2	0.55	0.5	1
T. violaceum (8)	CTZ	0.06–1	0.09	0.06	
	EBZ	0.03–0.25	0.07	0.06	
	KTZ	0.06–1	0.35	0.5	
	MCZ	0.06–0.5	0.23	0.25	
All organisms (200)	CTZ	0.01–>16	0.22	0.25	1
	EBZ	0.03–2	0.11	0.125	0.5
	KTZ	0.03–16	0.72	1	2
	MCZ	0.03–>16	0.43	0.5	2

[a] *n*, number of isolates tested.
[b] MIC for 50% of the isolates tested.

and sterility control wells were included for each isolate tested. The microplates were incubated at 28°C and read at 7 days of incubation. For all drugs, the MIC was defined as the lowest concentration showing 100% growth inhibition. Geometric mean MICs were determined to facilitate comparisons of the activities of the drugs. MIC ranges and the MICs at which 50% and 90% (MIC_{90}) of the isolates are inhibited were also calculated for all species tested. Comparisons of the geometric mean MICs were calculated by Student's *t* test. *P* values of <0.05 were considered statistically significant.

Table 1 summarizes the in vitro susceptibility of the four drugs tested. The MIC ranges of CTZ, MCZ, and KTZ were broader in all cases (0.01 to ≥16 μg/ml) than those of EBZ (0.03 to 2 μg/ml). In general, all drugs showed good activity, although EBZ displayed the lowest geometric mean MIC (0.11 μg/ml) of the four drugs tested ($P < 0.05$). Those of CTZ, MCZ, and KTZ were 0.22, 0.43, and 0.72 μg/ml, respectively. KTZ and MCZ showed the highest MIC_{90} (2 μg/ml). EBZ (considering only the species with eight or more strains included in the study) was the most active against *E. floccosum* (mean MIC, 0.06 μg/ml), *M. canis* (mean MIC, 0.11 μg/ml), *M. gypseum* (mean MIC, 0.07 μg/ml), *T. mentagrophytes* (mean MIC, 0.14 μg/ml), *T. rubrum* (mean MIC, 0.12 μg/ml), and *T. tonsurans* (mean MIC, 0.09 μg/ml) ($P < 0.05$). The differences between the MICs of CTZ and EBZ were not statistically significant for *T. interdigitale* (0.27 and 0.26 μg/ml, respectively) and *T. violaceum* (0.09 and 0.07 μg/ml, respectively).

Our results agree with those of Torres-Rodriguez et al. (10th Congr. Int. Soc. Hum. Anim. Mycol.), who tested EBZ against 44 strains of dermatophytes by a microdilution method, although they used Sabouraud dextrose broth as the culture medium. The MIC range obtained by those authors (0.078 μg/ml to 1.2 μg/ml) was very similar to that obtained by us.

As mentioned above, KTZ, CTZ, and MCZ are currently the most used in the topical treatment of dermatomycoses (2, 17). In recent years, there has been an important increase in

the use of topical agents, probably due to the fact that they produce fewer adverse effects than systemic therapy. They are also commonly used in the prevention of recurrence of onychomycosis and tinea pedis (1, 10). In our country, KTZ is clearly the topical antifungal agent most used (27%), followed by CTZ (17%) and MCZ (17%) (2). EBZ is a novel antifungal agent which has potential against human mucosal and cutaneous mycoses (15). Another interesting feature of this drug is its anti-inflammatory activity (9). Preliminary in vitro data have indicated that this drug is also active against some triazole-resistant yeasts, such as *Candida glabrata* and *Candida krusei* and even gram-positive bacteria (4, 19).

In our study, EBZ and CTZ showed the lowest MICs against all of the strains tested. In vivo efficacy of both drugs has also been investigated in clinical trials with patients with tinea. In a double-blind phase III study of dermatophytoses that included 133 patients, del Palacio et al. (4) compared the efficacy of EBZ 1% cream with that of CTZ 1% cream. Both antifungals showed similar results. EBZ was effective in 72% of treated sites, whereas CTZ effective was in 61% of them. The relapse rate in both groups was 1% for the EBZ group and 4% for the CTZ group.

In our study the other two antifungals tested, KTZ and MCZ, also showed low MICs. Other authors have also obtained low MICs testing these drugs, although these results are difficult to compare because they have used different techniques (11, 14).

Although terbinafine has been shown to be at least as effective as MCZ and CTZ in tinea pedis (12, 16) or KTZ in tinea corporis (3) when it is administered topically, it was not included in our study because it is used mainly systematically to treat onychomycoses. In addition, in a previous article (6) members of our group had already reported the in vitro activity of this compound against an important number of dermatophyte strains.

In summary, ECZ was more active in vitro against a broad range of species of dermatophytes than the other topical drugs tested. Therefore, this antifungal agent may be a good alternative for the treatment of dermatophytoses that require a topical therapy.

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