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IN VITRO ACTIVITIES OF TERBINAFINE, COMPARED WITH THOSE OF AMPHOTERICIN B AND AZOLES AGAINST CLINICAL CANDIDA ISOLATES

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SUMMARY

In vitro activity terbinafine, allylamine antifungal agent, was evaluated with 144 yeast isolates in comparison with amphotericin B, fluconazole, itraconazole and ketoconazole by broth microdilution method. The MICs of terbinafine at which 50% of isolates were inhibited were 1 µg/ml for *Candida albicans* (n=96), 32 µg/ml for *Candida glabrata* (n=32), 4 µg/ml for *Candida tropicalis* (n=12), and 0.125 µg/ml for *Candida parapsilosis* (n=4). These results suggest that terbinafine may be effective in vitro for *C.parapsilosis* but it has considerably less activity against other yeasts.

ÖZET

Klinik Candida izolatlarına karşı terbinafinin amfoterisin B ve azoller ile karşılaştırılmış in-vitro aktiviteleri.

Allilamin antifungal ajan olan terbinafinin, 144 maya suşuna karşı in-vitro aktivitesi buyyonda mikrodilüsyon yöntemi ile araştırılmış ve amfoterisin B, flukonazol, itrakonazol ve ketokonazol ile karşılaştırılmıştır. İzolatların % 50'sinin inhibe olduğu terbinafin MİK değerleri, *Candida albicans* (n=96) için 1 µg/ml, *Candida glabrata* (n=32) için 32 µg/ml, *Candida tropicalis* (n=12) için 4 µg/ml, *Candida parapsilosis* (n=4) için 0.125 µg/ml olarak belirlenmiştir. Terbinafinin aktivitesinin klinik göstergeleri henüz tam belirlenmemesine rağmen in-vitro *C.parapsilosis* suşları için etkili olabileceği fakat diğer mayalar için aktivitesinin daha az olduğu bu çalışmada gösterilmiştir.

INTRODUCTION

Terbinafine is an allylamine antifungal agent, which suppresses biosynthesis of ergosterol, an essential component of fungal cell membranes, via inhibition of the fungal enzyme squalene epoxidase (1). Terbinafine is an orally and topically active antifungal agent with a primarily fungicidal action in vitro. Its spectrum of in vitro activity includes a broad range of dermatophyte, filamentous, dimorphic and dematiaceous fungi, and some yeast species (3). Numerous earlier reports have documented the activity of terbinafine against a wide range of pathogenic fungi in vitro, as reviewed previously (1,3,8).

Terbinafine have been very well tolerated after oral or topical administration, with only minor adverse effects reported. In contrast to azole antifungal drugs, terbinafine is only weakly bound to cytochrome P450 and therefore does not interfere with steroid hormone pro-

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duction in the host such as ketoconazole, and it has little potential for interaction with other medications. In addition, mammalian squalene epoxidase is considerably less sensitive than the fungal enzyme to inhibition by terbinafine (1). This study was conducted to determine the activity of terbinafine against clinical yeast isolates in comparison with amphotericin B, fluconazole, itraconazole and ketoconazole.

MATERIALS AND METHODS

Test organisms: One hundred two yeast isolates were tested for their susceptibilities to amphotericin B, fluconazole, ketoconazole, and itraconazole by the broth microdilution method. The yeast isolate collection included *C.albicans* (n=96), *C.glabrata* (n=32), *C.tropicalis* (n=12), and *C.parapsilosis* (n=4). The strains were isolated from cutaneous lesion (n=59), mucosal (n=55), and body fluid (n=30). The isolates were identified with standard methods (11) and stored at -20°C in tryptic soy broth containing 10% glycerine until used in the study. Prior to use, yeasts were thawed, subcultured at least twice on Sabouraud dextrose agar plates.

Quality control was performed by testing the following strains according to the recommendations of NCCLS Document M27-A (6): *C.albicans* ATCC 90028, *C.parapsilosis* ATCC 22019.

Drugs: Amphotericin B (Sigma Co. St. Louis, USA), fluconazole (Fako Co. Istanbul, Turkey), ketoconazole (Bilim Co. Istanbul, Turkey), itraconazole (Nobel Co. Istanbul, Turkey), and terbinafine (Sandoz Co, Istanbul, Turkey) were supplied as powders, and 1280 µg/ml stock solution of amphotericin B, ketoconazole, itraconazole, and terbinafine were prepared by dissolving in dimethyl sulfoxide and fluconazole in sterile water. The stock solutions were stored at -70°C until used. The final drug concentrations ranged from 16 to 0.03 µg/ml for amphotericin B, ketoconazole, itraconazole, and terbinafine and from 64 to 0.125 µg/ml for fluconazole and were obtained by 10 twofold serial dilutions.

Antifungal susceptibility testing methods: Reference broth microdilution testing was performed according to NCCLS guidelines by using the spectrophotometric method of inoculum preparation. An inoculum concentration of $0.5X10^3$ to $2.5X10^3$ cells per ml, and RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropane sulphonic acid (MOPS) buffer (Sigma Co. St. Louis, USA) was used. The trays were incubated in air at 35°C and were observed for the presence or absence of growth at 24 h. The MIC endpoint was determined according to NCCLS recommendations (complete absence of growth for amphotericin B and 80% reduction in turbidity for azoles) (6,7).

RESULTS

The range of MICs of all antifungal agents for yeasts as well as the MICs at which 50% (MIC_{50}) and 90% (MIC_{90}) of isolates inhibited are summarized in the table.

Table. In vitro susceptibilities of 144 yeast isolates to amphotericin B, fluconazole, ketoconazole, itraconazole, and terbinafine.[a]

Organism (no. tested)	Antifungal agent	MIC_{50}	MIC_{90}	range
C.albcians (n: 96)	Amphotericin B	0.125	0.5	0.003-0.5
	Fluconazole	0.25	8	0.06-16
	Ketoconazole	0.03	1	0.03-8
	Itraconazole	0.06	0.5	0.03-1
	Terbinafine	1	8	0.03-32
C.glabrata (n: 32)	Amphotericin B	0.125	0.25	0.03-0.25
	Fluconazole	16	64	0.06-64
	Ketoconazole	0.25	0.5	0.06-2
	Itraconazole	0.125	0.5	0.03-1
	Terbinafine	32	32	0.25-32
C.tropicalis (n: 12)	Amphotericin B	0.125	0.5	0.06-0.5
	Fluconazole	0.5	0.5	0.125-8
	Ketoconazole	0.25	0.25	0.06-2
	Itraconazole	0.06	0.125	0.06-1
	Terbinafine	4	8	1-32
C.parapsilosis (n: 4)	Amphotericin B	0.06		0.03-0.125
	Fluconazole	0.125		0.125-0.5
	Ketoconazole	0.06		0.03-0.125
	Itraconazole	0.06		0.03-0.125
	Terbinafine	0.125		0.06-0.5

[a]MICs (μg/ml) were determined by the RPMI broth method.

The control organisms were tested three times versus all four antifungal agents. Fluconazole, ketoconazole, amphotericin B, itraconazole, and terbinafine MICs for *C.albicans* ATCC 90028 were found as 0.5-1, 0.06-0.125, 0.25-1, 0.06-0.125, and 1 μg/ml, respectively, in different tests. Fluconazole, ketoconazole, amphotericin B, itraconazole, and terbinafine MICs for *C.parapsilosis* ATCC 22019 were found as 1-2, 0.06-0.125, 0.25-0.5, 0.06-0.125, and 0.03-0.06 μg/ml, respectively.

The terbinafine MICs for *C.albicans, C.glabrata, C.tropicalis,* and *C.parapsilosis* ranged from 0.03 to 32, 0.25 to 32, 1 to 32, 0.06 to 0.5 μg/ml, respectively. The terbinafine was active against *C.parapsilosis* strains at 0.5 μg/ml or lower concentrations. The terbinafine had higher MIC values for other yeasts. MICs of amphotericin B were near for the four species of *Candida* tested. Fluconazole, ketoconazole, and itraconazole were most active against *C.parapsilosis,* least to *C.glabrata.*

The MICs of terbinafine for all *C.albicans* strains were higher in comparison with those of amphotericin B, ketoconazole, and itraconazole. For *C.glabrata* and *C.albicans,* the MICs of terbinafine and fluconazole were near. For *C.tropicalis,* the MICs of terbinafine were higher than those of amphotericin B, fluconazole, ketoconazole, and itraconazole. For *C.parapsilosis,* the MICs of terbinafine were lower than other yeasts.

DISCUSSION

The results of our study show that terbinafine was highly active against *C.parapsilosis* (MIC ≥0.5 μg/ml). Terbinafine had higher MICs (MIC_{90} = 8 μg/ml) for *C.albicans* and

C.tropicalis. Terbinafine possesses essentially the same potent in vitro activity showed as fluconazole against *C.albicans* and *C.glabrata* strains. However, terbinafine was the least active against *C.tropicalis* strains in comparison with the other three antifungal agents in this study.

In comparative in vitro tests, terbinafine was generally found less active against yeast species than ketoconazole, itraconazole, and amphotericin B (1,3). The activity of terbinafine against yeasts is more variable; the list of susceptible organisms includes *C.parapsilosis, Cryptococcus neoformans* and *Malessezia furfur. C. albicans* (yeast form), *C.tropicalis*, and *C.glabrata* are relatively resistant, with MICs of 6.25 to > 128 μg/ml, 10 to 128 μg/ml and 100 to > 128 μg/ml, respectively (3). Shadomy et al (9) compared the activities of terbinafine, ketoconazole and itraconazole against various yeast species, and found terbinafine less active than the azole drugs. Terbinafine shows primarily fungicidal activity against dermatophytes and *Aspergillus* species, *Scopulariopsis brevicaulis, Blastomyces dermatitis, Histoplasma capsulatum* and *C.parapsilosis* at concentrations near to MICs, but only fungistatic against *C.albicans* (9). Ryder et al (8) reported that terbinafine was highly active against *C.parapsilosis* (MIC_{90} = 0.125 μg/ml). Our in vitro data indicates that MICs of terbinafine for *C.parapsilosis* was lower than those for other yeasts. These data suggest that identification of these species may be necessary for appropriate therapy.

A number of studies have estimated the incidence of clinical fluconazole resistance to be from 6 to 36%, depending on the patient group studied and the case definition used (2). The risk factors for the development of fluconazole-resistant *Candida* infections included duration of exposure to fluconazole and degree of immunosuppression (4). None of our *C.albicans* isolates were resistant to fluconazole. However, terbinafine showed higher MICs against azole-resistant *Candida* strains such as *C.glabrata* for which fluconazole has higher MICs than for other non-*C.albicans* strains. No difference was demonstrated when terbinafine and fluconazole were compared against *C.glabrata*. Terbinafine was 128 times less active than amphotericin B on the basis of MIC_{90}, although the MIC_{50} of terbinafine was 256 times lower than of amphotericin B against *C.glabrata*. The higher MICs of terbinafine are encouraging and indicate that this antifungal agent has not alternative for use against this fungus. Six of 12 *C.tropicalis* strains showed high MICs to terbinafine. These six strains of *C.tropicalis* were also susceptible to amphotericin B, itraconazole and fluconazole (except one strain for itraconazole and ketoconazole). As similarly, Ryder et al (8) reported that it was not active against the *C.tropicalis* and *C.glabrata*. This observation is in agreement with our finding.

Oral terbinafine (250 mg/day) was not found to be effective in a pilot study about AIDS-associated oral candidiasis (5), but it is not yet known whether this lack of efficacy is due to pharmacokinetic factors or to low susceptibility of the pathogens. On the other hand, a systemic *Candida* infection was reported to respond to the treatment with higher doses of terbinafine (10).

In conclusion, these studies demonstrate that terbinafine was not superior to amphotericin B, fluconazole, ketoconazole, and itraconazole against clinical yeasts except for *C.parapsilosis*. However, further study is needed to confirm the efficacy of terbinafine in an experimental fungal infection model.

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