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Terbinafine susceptibility patterns for onychomycosis-causative dermatophytes and *Scopulariopsis brevicaulis*

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Abstract

The in vitro antifungal activity of terbinafine against 521 clinical isolates of seven species of dermatophytes, including four onychomycosis-causative species, as well as five *Scopulariopsis brevicaulis* isolates was determined by a modified Clinical and Laboratory Standards Institute microdilution method. Results showed a high antifungal activity of terbinafine against all dermatophyte isolates (geometric minimal inhibitory concentration (MIC) = 0.026 μg/mL; concentration inhibiting 50% of mycological growth (MIC_{50}) = 0.03 μg/mL; and concentration inhibiting 90% of mycological growth (MIC_{90}) = 0.06 μg/mL). The geometric mean MICs against onychomycosis-causative dermatophyte species was lower (0.024 μg/mL) than the global MIC. However, the in vitro activity of terbinafine against *S. brevicaulis* was considerably lower (geometric mean MIC = 1.38 μg/mL) in comparison with dermatophytes. The antifungal activity of itraconazole was lower than that of terbinafine against these fungi. These data confirm the high in vitro antifungal activity of terbinafine against dermatophytes, under standardised conditions.

Keywords: Terbinafine; Antifungal; Dermatophytes; Dermatophytosis; *Scopulariopsis*; *Trichophyton*; *Epidermophyton*; *Microsporum*

1. Introduction

The incidence of dermatophytoses has increased worldwide in recent years, especially in immunocompromised patients [1,2] with atypical manifestations and more severe lesions [3,4]. Dermatophytoses, among them onychomycosis, most commonly affect the elderly, children and some adults subgroups such as miners and sportsmen [5,6]. There is a wide geographical variation in the aetiological agents, with the most commonly isolated being the dermatophytes *Trichophyton rubrum*, *Trichophyton interdigitale*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum* [1–6].

Dermatophytes are susceptible to a great number of antifungal agents [7–15]. However, response to treatment of these infections is determined by the site and magnitude of the lesions rather than by the antifungal susceptibility of the isolated fungus. The safety profile, kinetics and clinical efficacy of the antifungal agent are important to decide on oral or topical antifungal administration. Although most dermatophytoses respond to topical therapy, systemic antifungal treatment is necessary for some tinea unguium, scalp ringworm, severe infections or skin lesions with folliculitis [7]. Oral drugs such as itraconazole, ketoconazole, fluconazole and terbinafine can be very active against dermatophytes

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[7,11,13,15–19], but the management of onychomycosis remains difficult. Determination of the in vitro susceptibility of dermatophytes may help to predict the efficacy of an antifungal drug. However, dermatophytes have not been included in the in vitro method proposed by the Clinical and Laboratory Standards Institute (CLSI) for testing filamentous fungi [20], but several authors have evaluated different testing conditions for determining minimal inhibitory concentrations (MICs) for dermatophytes [11,15–18]. The purpose of this study was to evaluate the in vitro antifungal activity of terbinafine against fresh clinical isolates of onychomycosis-causative dermatophytes, other dermatophytes and *Scopulariopsis brevicaulis* using a microdilution method based on CLSI document M38-A [11,15–18].

2. Materials and methods

2.1. Strains

In total, 521 clinical isolates of dermatophytes (*E. floccosum* ($n = 23$), *Microsporum canis* ($n = 99$), *Microsporum gypseum* ($n = 33$), *T. interdigitale* ($n = 24$), *T. mentagrophytes* ($n = 147$), *T. rubrum* ($n = 174$) and *Trichophyton tonsurans* ($n = 21$)) and 5 *S. brevicaulis* obtained from pathological specimens in different health centres in Spain and Argentina were tested.

2.2. Preparation of inocula

Inocula were prepared from 7-day-old cultures in potato dextrose agar (Biolife Italiana, Milan, Italy) by covering plates with 1 mL of sterile saline solution containing 1% Tween 80 (Difco, St. Louis, MO). Conidia were collected by probing the colonies with the tip of a sterile Pasteur pipette. The obtained mixture of non-germinated conidia was adjusted spectrophotometrically to 80–82% transmittance. Suspensions were later diluted 1:50 in sterile saline solution and final inoculum densities were $0.4–5 \times 10^4$ colony-forming units/mL [15–18].

2.3. Antifungal agents

Terbinafine and itraconazole (Sigma Aldrich Química, Madrid, Spain) were obtained as standard powder and the stock solution was prepared in RPMI 1640 with glutamine and without bicarbonate, buffered to pH 7 with morpholinepropanesulphonic acid (MOPS) (0.165 mol/L) (Sigma Aldrich Química) to yield twice the final concentration required for the test.

2.4. Microdilution method

The microdilution method was modified from CLSI document M38-A [15–17,19,20]. All tests were performed in round-bottomed 96-well microplates. Aliquots of 100 μL of the double drug dilutions were inoculated into the wells with a multichannel pipette followed by 100 μL of the diluted inocula suspensions to bring the drug dilutions to the final test concentrations (range 0.001–64 μg/mL). MIC readings were made after 48, 72 and 96 h of incubation at 28 °C. Incubation was prolonged when no growth was observed in the control wells after these incubation times. MICs for the quality control strains *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were determined. MIC endpoints were determined as the lowest concentrations that showed 50% growth inhibition. Geometric means and MIC ranges were calculated for each species. MIC_{50} and MIC_{90} values were defined as the concentrations of terbinafine that were able to inhibit 50% and 90% of the mycological growth, respectively. The significance of differences in mean values was determined using Student's *t*-test. *P*-values <0.05 were considered statistically significant.

3. Results

Table 1 shows the greater antifungal activity (i.e. lower MICs) of terbinafine against the studied dermatophyte species. Geometric mean MIC, MIC_{50} and MIC_{90} values

Table 1
In vitro antifungal activity of terbinafine against dermatophytes and *Scopulariopsis brevicaulis*

Species	*N*	MIC (μg/mL)			
		GM	Range	MIC_{50}	MIC_{90}
Onychomycosis-causative dermatophytes					
Trichophyton rubrum	174	0.015	0.003–16	0.01	0.06
Trichophyton interdigitale	24	0.0171	0.007–2	0.007	0.06
Trichophyton mentagrophytes	147	0.038	0.007–0.5	0.06	0.06
Epidermophyton floccosum	23	0.024	0.01–1	0.03	0.06
Other dermatophytes					
Microsporum canis	99	0.036	0.007–32	0.06	0.06
Microsporum gypseum	33	0.0416	0.007–0.06	0.06	0.06
Trichophyton tonsurans	21	0.009	0.03–0.06	0.003	0.03
Scopulariopsis sp.					
S. brevicaulis	5	1.38	0.01–16	N.C.	N.C.

MIC, minimal inhibitory concentration; GM, geometric mean; $MIC_{50/90}$, concentration of terbinafine able to inhibit 50% and 90% of mycological growth, respectively; N.C., values not calculated for species with less than 10 isolates.