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In Vitro Activities of 10 Combinations of Antifungal Agents against the Multiresistant Pathogen *Scopulariopsis brevicaulis*

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The activities of 10 combinations of antifungal agents against 25 clinical isolates of *Scopulariopsis brevicaulis* were tested by the checkerboard technique. An average indifferent effect was detected for all combinations. Synergy was observed for some isolates and combinations, particularly with posaconazole-terbinafine (68% of strains), amphotericin B-caspofungin (60%), and posaconazole-caspofungin (48%).

Scopulariopsis brevicaulis is a rare and emerging pathogen that has been increasingly reported in the past 2 decades as a cause of deep mycosis in hosts presenting factors that predispose them to infection. This dermatomycotic species and other *Scopulariopsis* spp. have mainly been associated with onychomycosis (32, 37), but their spectrum of human infections includes posttraumatic keratitis and endophthalmitis (15, 20, 21), disseminated skin lesions and meningitis in AIDS patients (12, 29), endocarditis related to valvuloplasty or prosthetic valves (16, 22, 25), subcutaneous hyalohyphomycosis in immunocompromised hosts (4, 30, 33, 34), fungus ball and pneumonia (13, 23, 39), and disseminated infections in stem cell transplant patients or hosts with leukemia (24, 27, 28, 36, 38).

S. brevicaulis has been reported to be resistant in vitro to amphotericin B, flucytosine, terbinafine, and azole compounds (1, 5, 8, 9, 14, 18). Invasive infections due to *S. brevicaulis* are unlikely to respond to particular antifungal treatment (24, 27, 36, 38), and several therapeutic approaches have been considered, such as debridement or excision of necrotic tissue plus chemotherapy (22, 30), prolonged monotherapy with azole agents or terbinafine (27, 28), and combinations of antifungal agents (36).

To date, combined activity in vitro of antifungal agents against *S. brevicaulis* has not been assessed. This study describes the activities of 10 combinations of antifungal compounds against clinical isolates of this species.

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Fungi. A collection of 25 clinical isolates was used. All strains were recovered over a 5-year period (2000 to 2005) from 15 Spanish hospitals. Each clinical isolate represented a unique isolate from a patient. Strains were isolated from nails (20/25 [80%]), skin scrapings (2/25 [12%]), sputum (1/25 [4%]), and blood (1/25 [4%]). Isolates were identified by macroscopic and microscopic examination (10).

Antifungal agents. Antifungal agents utilized were amphotericin B (Sigma-Aldrich Química, Madrid, Spain), terbinafine (Novartis Pharma AG, Basel, Switzerland), itraconazole (Janssen Pharmaceutica, Madrid, Spain), voriconazole (Pfizer Ltd., Sandwich, United Kingdom), posaconazole (Schering-Plough, Kenilworth, NJ), and caspofungin (Merck & Co., Inc., Rahway, NJ).

Antifungal susceptibility testing. The individual MICs were determined by following the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) reference method (26) with the following minor modifications: the medium was RPMI 1640 with L-glutamine (buffered to pH 7 with 0.165 M morpholinepropanesulfonic acid [MOPS] and 10 M NaOH) supplemented with 2% glucose (Oxoid, Madrid, Spain); the inoculum size was 1×10^5 to 5×10^5 CFU/ml; and the inoculum was prepared by microscopic enumeration with a cell-counting hemocytometer (Neubauer chamber; Merck, S.A., Madrid, Spain). Several reports have demonstrated that these modifications generate reproducible, reliable, and accurate in vitro susceptibility data (9, 11, 14, 17, 31). For caspofungin, two different visual determinations of the end point were performed: (i) complete inhibition of growth (MIC) and (ii) the lowest drug concentration resulting in aberrant hyphal growth as determined by examination with an inverted microscope, the minimum effective concentration (MEC) (2, 35).

Interaction of drugs in vitro. Drug interaction was evaluated in a checkerboard microdilution design. Combinations tested were amphotericin B plus itraconazole, amphotericin B plus voriconazole, amphotericin B plus posaconazole, amphotericin B plus caspofungin, itraconazole plus caspofungin, voriconazole plus caspofungin, posaconazole plus caspofungin, itraconazole plus terbinafine, voriconazole plus terbinafine, and posaconazole plus terbinafine. The combined effects were analyzed by the summation of the fractional inhibitory concentration index (FICi) (2, 35). For combinations including caspofungin, FICi was calculated by taking into account both the MIC and the MEC of the echinocandin. The interactions were defined as synergistic when FICi was ≤ 0.5, as antagonistic when FICi was >4, and as indifferent, or no interaction, when FICi was >0.5 but ≤ 4. Triplicate testing on three separate days was performed.

A summary of MICs of individual antifungal agents against isolates is given in Table 1. All organisms were highly resistant

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TABLE 1. Susceptibility results[a] for 25 clinical isolates of *Scopulariopsis brevicaulis*

Antifungal agent	Mode		Geometric mean		50%[b]		90%[b]		Range	
	MIC	MEC	MIC	MEC	MIC	MEC	MIC	MEC	MIC	MEC
Amphotericin B	16.0		13.0		16.0		>16.0		8.0–>16.0	
Itraconazole	>8.0		>8.0		>8.0		>8.0		>8.0	
Voriconazole	>8.0		>8.0		>8.0		>8.0		>8.0	
Posaconazole	>8.0		>8.0		>8.0		>8.0		>8.0	
Terbinafine	>16.0		14.4		>16.0		>16.0		4.0–>16.0	
Caspofungin	>16.0	8.0	>16.0	12.0	>16.0	8.0	>16.0	16.0	>16.0	4.0–>16.0

[a] Results are given in micrograms per milliliter. MECs were calculated for caspofungin only.
[b] 50% and 90%, MICs or MECs at which 50% and 90% of isolates, respectively, were inhibited.

in vitro to amphotericin B, azole agents, caspofungin, and terbinafine.

Regarding interactions in vitro, an average indifferent effect was detected for all combinations. Synergy was observed for some isolates with some combinations. Posaconazole plus terbinafine exhibited synergy for 17/25 (68%) strains; this combination showed the highest rate of positive effect in vitro. The average FICi for this combination was 0.64. Voriconazole plus terbinafine and itraconazole plus terbinafine also showed synergy for some strains, with rates of 40% and 28%, respectively. Amphotericin B in combination with azole agents did not exhibit synergy for a significant number of isolates. Combinations including caspofungin were largely indifferent when MICs were used for FICi calculation. However, synergy was observed for some organisms if MECs were included. FICi values under 0.5 were found in 15/25 (60%) strains for amphotericin B plus caspofungin, in 12/25 (48%) strains for posaconazole plus caspofungin, and in 5/25 (20%) strains for the voriconazole-caspofungin combination. Average FICi values were 0.56 for posaconazole plus caspofungin and 0.51 for amphotericin B plus caspofungin.

Notably, antagonism was absent for all antifungal combinations. Table 2 displays average FICi values and numbers and percentages of strains for which synergy was detected per combination analyzed.

TABLE 2. FICi values and numbers and percentages of strains for which combinations showed synergy

Antifungal combination	FICi[a]		No. (%) of strains for which the indicated combination showed synergy	
	With MICs	With MECs[b]	With MICs	With MECs[b]
Amphotericin B + itraconazole	1.94		0 (0)	
Amphotericin B + voriconazole	1.81		1 (4)	
Amphotericin B + posaconazole	1.96		0 (0)	
Amphotericin B + caspofungin	0.90	0.51	3 (12)	15 (60)
Itraconazole + caspofungin	1.78	1.49	0 (0)	2 (8)
Voriconazole + caspofungin	1.86	1.25	0 (0)	5 (20)
Posaconazole + caspofungin	1.44	0.56	1 (4)	12 (48)
Itraconazole + terbinafine	1.02		7 (28)	
Voriconazole + terbinafine	0.88		10 (40)	
Posaconazole + terbinafine	0.64		17 (68)	

[a] FICi values are arithmetic means from three repetitions with 25 clinical strains.
[b] MECs were calculated for caspofungin only.

S. brevicaulis seems to be a multiresistant species that can usually cause fatal invasive infections in immunocompromised hosts (27, 36, 38). Taking into account the resistance of *S. brevicaulis*, other therapeutic approaches should be considered and combined therapy could be useful, particularly for immunosuppressed patients with disseminated infections (6, 7).

Until now, the combined activity of antifungal agents against *S. brevicaulis* had not been evaluated. Here we present the in vitro activities of 10 combinations of antifungal compounds against 25 clinical isolates of *S. brevicaulis*. An average indifferent effect was observed for all combinations, but synergistic interaction was detected for a significant percentage of strains with some combinations. The rate was particularly high with posaconazole plus terbinafine, where synergy was observed for 68% of strains analyzed. Other combinations, such as amphotericin B plus caspofungin, posaconazole plus caspofungin, and voriconazole plus caspofungin, also showed synergistic effects against a number of organisms.

Data on the clinical efficacy of combination therapy in cases of *Scopulariopsis* infection are too scarce to draw any firm conclusions. Steinbach et al. (36) reported a case of disseminated infection due to *S. brevicaulis* in a child with graft-versus-host disease after stem cell transplantation who failed lipid amphotericin B therapy as well as combination therapy with voriconazole plus caspofungin. For other *Scopulariopsis* spp., successful therapy has been reported (3, 19). Combination therapy could be an alternative for treating deep infections due to *Scopulariopsis*, but the effect of combinations is not predictable and depends on the strain tested. Therefore, studies of interaction in vitro would be needed before therapeutic recommendations could be made.

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