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In Vitro Synergistic Interaction between Amphotericin B and Micafungin against *Scedosporium* spp.

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The in vitro interaction between amphotericin B and micafungin against 36 isolates of *Scedosporium* spp. has been evaluated using checkerboard assays and the minimal effective concentration endpoint. Synergy was found for 82.4% of *Scedosporium prolificans* isolates and for 31.6% of *Scedosporium apiospermum* isolates. Antagonism was not observed.

The two species of *Scedosporium*, *Scedosporium prolificans* and *S. apiospermum*, have evolved into important agents of severe infections in immunocompromised patients (23). The outcome of these infections is very poor, and the most appropriate treatment is unknown. Although *Scedosporium* spp., particularly *S. apiospermum*, are more susceptible to azoles than polyenes, amphotericin B (AMB) is still the most-used drug (23). The high resistance of the two species, especially *S. prolificans*, to most of the conventional antifungal drugs forces testing of new possible therapeutic strategies (14). A promising approach might be to combine antifungal drugs with different active mechanisms. The advent of new echinocandins that can be combined with AMB or azoles has revived interest in finding antifungal combinations that are synergistic for refractory fungi (11, 12, 23).

Micafungin (MFG) is a novel echinocandin that exerts antifungal activity via inhibition of (1,3)-β-D-glucan synthase, interfering with fungal cell wall synthesis. This drug shows in vivo and in vitro activity against a variety of common pathogenic fungi (16). The in vitro activity of echinocandins against *Aspergillus* spp. is limited when measured using a conventional MIC endpoint. However, it has been suggested that the minimal effective concentration (MEC) endpoint correlates better with the in vivo activity of the echinocandins than the MIC does (2). Since the MICs of echinocandins at which no growth is visible are manyfold higher than the levels of the drugs achievable in blood and tissues, some authors have used the MEC as an alternative clinically relevant endpoint to evaluate the effectiveness of echinocandins (8). The MEC is the lowest concentration of the drug to cause abnormal hyphal growth, which can easily be detected by the presence of short abundant branches (2, 13), and appears to be a stable in vitro measurement for determining the activity of caspofungin against molds (2). Using MEC as an endpoint, Arikan et al. (3) showed in vitro synergy of AMB and caspofungin against *Aspergillus* and *Fusarium* spp. In the present study, we have evaluated the in vitro activity, using the MEC endpoint, of MFG combined with AMB against clinical strains of *Scedosporium*.

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Thirty-six clinical (80.5%) and environmental (19.4%) isolates of *Scedosporium* spp. (17 strains of *S. prolificans* and 19 of *S. apiospermum*) were tested. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used for quality control. Inocula were prepared by following the NCCLS guidelines (17). Isolation and identification of the isolates were carried out by using standard microbiological procedures. Antifungal drugs were obtained as pure powders. AMB was dissolved in dimethyl sulfoxide and MFG in water. The final concentrations of the drugs ranged from 0.12 to 8 μg/ml for AMB and from 0.06 to 32 μg/ml for MFG. Dilutions were made in RPMI 1640 medium buffered to pH 7.0 with 0.165 M MOPS (morpholinepropanesulfonic acid). Drug interactions were assessed by using checkerboard assays after incubation of the isolates for 48 h (for *S. prolificans*) or 72 h (for *S. apiospermum*) at 35°C in RPMI 1640 medium. Plates were scanned both visually and microscopically with a stereoscopic microscope at low magnification (×40). The MEC correlated with the visually assessed MIC that resulted in 50% reduction in turbidity compared to that of the growth control well, and the MEC was determined to be the lowest drug concentration to result in aberrant hyphal growth, characterized by an abundance of short branches (2, 13). The fractional inhibitory concentration index (FICI) was used to classify drug interactions (11). The FICI of the combination of AMB and MFG for each isolate was calculated using the MEC endpoint. Approximately 80% of the tests were repeated, and interactions showed mainly the same trends (data not shown).

The MECs and FICI values obtained for each isolate of *S. apiospermum* and *S. prolificans* tested are shown in Table 1. The high off-scale MEC of AMB, >8 μg/ml, was converted into the next highest concentration, 16 μg/ml, for calculation of the FICI, and the high off-scale MEC of MFG, >32 μg/ml, was converted into 64 μg/ml. The geometric mean of the MECs of AMB for all the *S. apiospermum* isolates was 4.62 μg/ml (range, 0.51 to >8 μg/ml), and that of MFG was 5.76 μg/ml (range, 1 to >32 μg/ml). These drugs were clearly less active against *S. prolificans*. For this species, the mean MECs of AMB and MFG were 11.56 and 64 μg/ml, respectively. Synergistic interaction was found for 14 (82.4%) of the 17 *S. prolificans* isolates and for 6 (31.6%) of the 19 *S. apiospermum* isolates. Antagonism was not detected.

This study has shown that the combination of AMB and

TABLE 1. In vitro results of the AMB-MFG combination against isolates of *Scedosporium* spp.

Isolate	MEC(s) (μg/ml) of:			FICI	Result[b]
	AMB	MFG	AMB/MFG[a]		
S. apiospermum isolates[c]					
6922	>8	4	4/1	0.5	S
6694	>8	>32	2/4	0.19	S
4167	2	2	0.12/1	0.56	I
7885	>8	>32	4/4	0.32	S
4072	2	4	0.12/2	0.56	I
3743	>8	>32	4/4	0.31	S
6697	8	32	2/8	0.5	S
6921	8	>32	2/2	0.28	S
6918	1	2	0.5/1	1	I
6920	1	2	0.5/0.12	0.62	I
8346	4	2	2/1	1	I
8348	8	2	2/2	1	I
8349	8	2	2/1	0.75	I
8350	2	2	1/0.5	0.75	I
8352	0.5	2	0.12/1	0.74	I
8353	2	1	1/0.5	1	I
8358	4	1	1/0.5	0.75	I
8359	>8	>32	8/8	0.62	I
8361	8	2	1/1	0.62	I
S. prolificans isolates[d]					
3569	>8	>32	4/16	0.5	S
6642	4	>32	0.5/16	0.37	S
6719	>8	>32	2/16	0.37	S
6721	>8	>32	4/16	0.5	S
6802	1	>32	0.5/0.06	0.51	I
7252	>8	>32	4/8	0.37	S
7257	>8	>32	4/8	0.37	S
7258	>8	>32	2/16	0.5	S
7294	8	>32	1/16	0.37	S
7297	>8	>32	2/8	0.25	S
6645	8	>32	2/16	0.5	S
6646	>8	>32	4/16	0.5	S
6650	>8	>32	8/2	0.51	I
6651	>8	>32	4/16	0.5	S
6652	>8	>32	8/0.06	0.51	I
6653	>8	>32	4/8	0.38	S
6655	>8	>32	4/16	0.5	S

[a] MECs of AMB and MFG when used in combination.
[b] S, synergistic; I, indifferent.
[c] Incubated for 72 h.
[d] Incubated for 48 h.

MFG has potential for the treatment of scedosporiosis. In the case of *S. prolificans*, it is especially important because so far practically all the treatments tested have failed. Although recent studies have demonstrated that high doses of liposomal AMB (19, 20) and the new triazole albaconazole (6) have been effective in the treatment of animal-disseminated infections, the combinations AMB-MFG and azoles-terbinafine (15) also merit investigation. For *S. apiospermum* infections, more optimism exists because numerous clinical reports (9, 18, 24) and experimental data (4, 5) have demonstrated that voriconazole is effective in the treatment of severe infections. However, in some cases this drug has also failed (21), and it is in these cases where other alternatives, such as the combination AMB-MFG, could perhaps play some role. Peak levels of MFG in human serum have been reported to be up to 11 mg/liter (1, 7, 10). This level is within the range of concentrations of this drug which, when the drug is combined with AMB, produce synergistic effects.

This combination has also shown beneficial effects in animal models of other fungal infections (22). The mechanism to explain why this combination works is not clear. It can be speculated that MFG causes some structural alteration at the cell wall level which facilitates the action of AMB on the cell membrane at a lower concentration. However, the inverse effect, i.e., how echinocandin MICs are lowered by AMB, is more difficult to explain (3, 11).

In conclusion, our results indicate that the combination AMB-MFG shows an in vitro synergistic effect mainly against *S. apiospermum*. If this high activity is confirmed in appropriate animal studies, we would have a potential treatment for invasive scedosporiosis.

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