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In Vitro Activities of 35 Double Combinations of Antifungal Agents against *Scedosporium apiospermum* and *Scedosporium prolificans*▽

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Activities of 35 combinations of antifungal agents against *Scedosporium* spp. were analyzed by a checkerboard microdilution design and the summation of fractional concentration index. An average indifferent effect was detected apart from combinations of azole agents and echinocandins against *Scedosporium apiospermum*. Antagonism was absent for all antifungal combinations against both species.

Scedosporium apiospermum (*Pseudallescheria boydii*) is considered to be susceptible to voriconazole, posaconazole, and miconazole and appears to have various susceptibilities to itraconazole, ketoconazole, and amphotericin B. *S. prolificans* seems to be more resistant than *S. apiospermum* to antifungals, tolerating virtually all systemically active antifungal agents, including the new triazoles and echinocandins (2, 4, 6–10, 16, 17, 22).

Combination therapy could be an alternative to monotherapy for patients with invasive infections that are difficult to treat, such as those due to multiresistant species, and for those who fail to respond to standard treatment (5, 13, 14).

We have analyzed the in vitro activities of 35 combinations of broad-spectrum antifungal agents against a panel of clinical isolates of *S. apiospermum* and *S. prolificans*.

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Fungi. Twelve *S. apiospermum* clinical strains and 12 *S. pro-*

TABLE 1. Susceptibility results of 24 clinical isolates of *Scedosporium* spp.

Antifungal agent	MIC or MEC (μg/ml)[a]									
	Scedosporium apiospermum					*Scedosporium prolificans*				
	Range	Mode[b]	Geometric mean	50%	90%	Range	Mode	Geometric mean	50%	90%
Amphotericin B	0.25–>16.0	4.0	4.87	4.0	16.0	2.0–>16.0	16.0	13.9	16.0	>16.0
Flucytosine	>64.0	>64.0	>64.0	>64.0	>64.0	>64.0	>64.0	>64.0	>64.0	>64.0
Itraconazole	0.25–>8.0	8.0	3.28	4.0	>8.0	2.0–>8.0	8.0	>8.0	8.0	>8.0
Voriconazole	0.12–>8.0	1.0	0.93	1.0	4.0	1.0–>8.0	8.0	>8.0	>8.0	>8.0
Ravuconazole	0.50–>8.0	4.0	3.92	4.0	>8.0	2.0–>8.0	>8.0	>8.0	>8.0	>8.0
Posaconazole	0.12–>8.0	1.0	1.18	1.0	>8.0	1.0–>8.0	>8.0	>8.0	>8.0	>8.0
Terbinafine	4.0–>16.0	16.0	>16.0	16.0	>16.0	8.0–>16.0	16.0	>16.0	16.0	>16.0
Caspofungin	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
Caspofungin[c]	0.03–>16.0	8.0	2.56	0.50	>16.0	1.0–>16.0	1.0	6.56	8.0	>16.0
Micafungin	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
Micafungin[c]	0.03–>16.0	0.03	0.39	0.12	>16.0	0.03–>16.0	>16.0	2.69	8.0	>16.0
Anidulafungin	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
Anidulafungin[c]	0.50–4.0	1.0	1.19	1.0	4.0	1.0–4.0	4.0	4.0	4.0	4.0

[a] The table displays average results of two repetitions performed on different days.
[b] Mode, most frequent MIC value.
[c] MEC values are shown in this row.

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TABLE 2. Average FICi by *Scedosporium* spp. per antifungal combination[a]

Combination[b]	*Scedosporium apiospermum*				*Scedosporium prolificans*			
	MIC		MEC		MIC		MEC	
	FICi	No. (%) of strains showing synergy	FICi	No. (%) of strains showing synergy	FICi	No. (%) of strains showing synergy	FICi	No. (%) of strains showing synergy
AMB plus 5-FC	1.80	0/12 (0)	NC[c]	NC	2.0	0/12 (0)	NC	NC
AMB plus TBF	2.0	0/12 (0)	NC	NC	2.0	0/12 (0)	NC	NC
AMB plus ITC	0.93	1/12 (8.3)	NC	NC	2.0	0/12 (0)	NC	NC
AMB plus VRC	0.68	2/12 (16.6)	NC	NC	1.14	2/12 (16.6)	NC	NC
AMB plus POS	0.79	2/12 (16.6)	NC	NC	1.89	0/12 (0)	NC	NC
AMB plus RVC	0.82	3/12 (25)	NC	NC	1.51	0/12 (0)	NC	NC
AMB plus CPF	1.26	2/12 (16.6)	0.68	1/12 (8.3)	2.0	0/12 (0)	0.89	2/12 (16.6)
AMB plus MCF	1.87	0/12 (0)	0.85	4/12 (33.3)	2.0	0/12 (0)	1.55	0/12 (0)
AMB plus ADF	1.52	0/12 (0)	1.30	0/12 (0)	2.0	0/12 (0)	1.02	0/12 (0)
5-FC plus ITC	1.84	1/12 (8.3)	NC	NC	2.0	0/12 (0)	NC	NC
5-FC plus VRC	1.83	1/12 (8.3)	NC	NC	2.0	0/12 (0)	NC	NC
5-FC plus POS	1.86	0/12 (0)	NC	NC	2.0	0/12 (0)	NC	NC
5-FC plus RVC	1.84	1/12 (8.3)	NC	NC	2.0	0/12 (0)	NC	NC
5-FC plus CPF	2.0	0/12 (0)	2.0	0/12 (0)	2.0	0/12 (0)	2.0	0/12 (0)
5-FC plus MCF	2.0	0/12 (0)	2.0	0/12 (0)	2.0	0/12 (0)	2.0	0/12 (0)
5-FC plus ADF	2.0	0/12 (0)	1.87	0/12 (0)	2.0	0/12 (0)	1.88	0/12 (0)
TBF plus ITC	0.66	4/12 (33.3)	NC	NC	2.0	0/12 (0)	NC	NC
TBF plus VRC	1.44	0/12 (0)	NC	NC	1.13	3/12 (25)	NC	NC
TBF plus POS	1.09	0/12 (0)	NC	NC	1.91	0/12 (0)	NC	NC
TBF plus RVC	0.89	1/12 (8.3)	NC	NC	1.01	3/12 (25)	NC	NC
TBF plus CPF	1.29	1/12 (8.3)	1.57	1/12 (8.3)	1.38	0/12 (0)	0.65	1/12 (8.3)
TBF plus MCF	1.89	0/12 (0)	0.99	5/12 (41.7)	2.0	0/12 (0)	1.85	1/12 (8.3)
TBF plus ADF	1.81	0/12 (0)	1.91	0/12 (0)	2.0	0/12 (0)	1.77	1/12 (8.3)
ITC plus CPF	0.24	12/12 (100)	0.29	12/12 (100)	2.0	0/12 (0)	1.32	0/12 (0)
ITC plus MCF	0.54	6/12 (50)	0.24	8/12 (66.6)	2.0	0/12 (0)	2.0	0/12 (0)
ITC plus ADF	1.44	0/12 (0)	0.54	4/12 (33.3)	1.91	0/12 (0)	2.0	0/12 (0)
VRC plus CPF	0.62	3/12 (25)	0.41	8/12 (66.6)	2.0	0/12 (0)	1.41	2/12 (16.6)
VRC plus MCF	0.87	1/12 (8.3)	0.36	7/12 (58.3)	2.0	0/12 (0)	1.56	0/12 (0)
VRC plus ADF	1.73	0/12 (0)	0.66	0/12 (0)	2.0	0/12 (0)	1.14	1/12 (8.3)
POS plus CPF	0.39	8/12 (66.6)	0.39	9/12 (75)	2.0	0/12 (0)	1.70	0/12 (0)
POS plus MCF	0.92	5/12 (41.7)	0.39	6/12 (50)	2.0	0/12 (0)	1.89	0/12 (0)
POS plus ADF	1.73	0/12 (0)	0.66	2/12 (16.6)	2.0	0/12 (0)	1.79	0/12 (0)
RVC plus CPF	0.35	11/12 (91.7)	0.34	10/12 (83.4)	2.0	0/12 (0)	0.75	5/12 (41.7)
RVC plus MCF	0.38	7/12 (58.3)	0.28	11/12 (91.7)	2.0	0/12 (0)	2.0	0/12 (0)
RVC plus ADF	0.47	7/12 (58.3)	0.44	6/12 (50)	2.0	0/12 (0)	1.63	0/12 (0)

[a] The table shows results after two repetitions performed on different days.
[b] AMB, amphotericin B; 5-FC, flucytosine; TBF, terbinafine; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; RVC, ravuconazole; CPF, caspofungin; MCF, micafungin; ADF, anidulafungin.
[c] NC, not calculated.

lificans strains were tested. The majority of isolates ($n = 16$) were obtained from blood cultures and the remainder ($n = 8$) from specimens of deep sites. *Aspergillus fumigatus* ATCC 204305 and *Aspergillus flavus* ATCC 204304 were included as quality control organisms (9, 20).

Antifungal agents. Antifungal agents used were amphotericin B (range, 16.0 to 0.03 μg/ml; Sigma-Aldrich Quimica S.A., Madrid, Spain), flucytosine (range, 64.0 to 0.12 μg/ml; Sigma-Aldrich), itraconazole (range, 8.0 to 0.015 μg/ml; Janssen S.A., Madrid, Spain), voriconazole (range, 8.0 to 0.015 μg/ml; Pfizer S.A., Madrid, Spain), ravuconazole (range, 8.0 to 0.015 μg/ml; Bristol-Myers Squibb, Princeton, NJ), posaconazole (range, 8.0 to 0.015 μg/ml; Schering-Plough, Kenilworth, NJ), terbinafine (range, 16.0 to 0.03 μg/ml; Novartis, Basel, Switzerland), caspofungin (range, 16.0 to 0.03 μg/ml; Merck & Co., Inc., Rahway, NJ), micafungin (range, 16.0 to 0.03 μg/ml; Astellas Pharma, Inc., Tokyo, Japan), and anidulafungin (range, 16.0 to 0.03 μg/ml; Pfizer S.A.).

Antifungal susceptibility testing. The individual MICs were determined by following the recommendations of the European Subcommittee for Antifungal Susceptibility Testing of the European Committee for Antimicrobial Susceptibility Testing (AFST-EUCAST) (1, 11, 15, 21, 23).

For amphotericin B, flucytosine, and azole compounds, the MIC was defined as the lowest concentration of the antifungal agent that completely inhibited fungal growth. For echinocandins, two different visual determinations of the endpoint were performed: (i) complete inhibition of growth (MIC) and (ii)

the lowest drug concentration resulting in aberrant hyphal growth by examination with an inverted microscope, or the minimum effective concentration (MEC) (3, 24).

Interaction of drugs in vitro. Drug interaction was evaluated in a checkerboard microdilution design. The combined effects were analyzed by summation of the fractional concentration index (FICi). For combinations including echinocandin, the FICi was also calculated taking into account both the MIC and the MEC of the echinocandin. The interactions were defined as synergistic if the FICi was ≤0.5, as antagonistic if the FICi was >4, and indifferent (or no interaction) if the FICi was >0.5 but ≤4. Duplicate testing was performed on two separate days.

Analysis of data. Descriptive statistical analysis of MIC, MEC, and FICi values was done with the Statistical Package for the Social Sciences (SPSS, version 15.0; SPSS S.L., Madrid, Spain).

Results and discussion. Table 1 shows the susceptibility testing results of *Scedosporium* clinical strains.

Regarding interactions of antifungal agents, a summary of the combined effects in vitro is displayed in Table 2. For *S. apiospermum*, an average indifferent effect was detected for combinations including amphotericin B, flucytosine, and terbinafine. However, average synergy was detected for some isolates and combinations of azole agents and echinocandins. In addition, some combinations showed synergy against a percentage of strains of *S. apiospermum*. Amphotericin B exhibited a positive effect against 15 to 25% of isolates when combined with azole agents. The combination of terbinafine plus itraconazole was synergistic against one-third of the isolates. The highest rates of synergy were obtained for azole agents plus echinocandins, particularly with itraconazole plus caspofungin, which exhibited synergy against 100% of strains. Other azole and echinocandin combinations also showed synergy against significant percentages of *S. apiospermum* isolates.

As for *Scedosporium prolificans*, all combinations were indifferent but synergy was detected for 15 to 25% of isolates for some combinations. The most active combination was ravuconazole plus caspofungin, which was synergistic against 41.7% of *S. prolificans* isolates when MEC values were used as endpoints.

Notably, antagonism was absent for all antifungal combinations against both *Scedosporium* spp.

The combined activity of antifungal agents against *Scedosporium* has rarely been evaluated previously. A report on the combined effect in vitro of amphotericin B and azole agents (miconazole, itraconazole, and fluconazole) against *S. apiospermum* was published in 1995 (26). Authors used the checkerboard technique and described an average indifferent effect, but synergy was found for some isolates, particularly for the amphotericin B and miconazole combination. Antagonism was not reported.

Meletiadis et al. reported that the combination of terbinafine with miconazole, voriconazole, or itraconazole showed synergy in vitro against *S. prolificans* (18, 19). The synergistic effects were more potent after 72 h of incubation. Authors used the checkerboard technique with three different reading methods, one spectrophotometrical and two colorimetric techniques. An alternative response surface approach method was used for assessing drug interaction as well (18).

Our results concur with those found years ago for *S. apiospermum*. Amphotericin B and azole agents showed synergy against a number of isolates, although an indifferent effect was the most common interaction. However, our data are somehow different from results reported by Meletiadis et al. We did not find a synergistic effect between terbinafine and azole agents in all cases. We observed synergy for 25% of *S. prolificans* isolates and only between voriconazole plus terbinafine and ravuconazole plus terbinafine.

We have obtained some novel results as well. Combinations including an azole agent plus an echinocandin exhibited synergy against most *S. apiospermum* isolates. The positive effect was particularly common for combinations including an azole compound plus caspofungin. Differences in combined activity of distinct echinocandins may be explained by different molecular interactions and limitations of the analysis by the FICi method. Further experiments are warranted in order to assess these interactions.

Data on the clinical efficacy of combination therapy in cases of *Scedosporium* infection are too scarce. Combinations of voriconazole plus terbinafine with or without aggressive surgical debridement have resulted in the cure or control of deep infections due to *S. prolificans* (12). In addition, a case of *S. prolificans* osteomyelitis was treated successfully with systemic administration of voriconazole and caspofungin (25).

There is insufficient evidence to make any recommendations for combination therapy, but azole agents plus echinocandins against *S. apiospermum* and terbinafine plus voriconazole against *S. prolificans* could have clinical efficacy.

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