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In Vitro Interactions of Micafungin with Other Antifungal Drugs against Clinical Isolates of Four Species of *Cryptococcus*

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The combination of micafungin (MFG) with amphotericin B (AMB), fluconazole, itraconazole, voriconazole, or ravuconazole was evaluated against 37 strains of four species of *Cryptococcus* by the checkerboard method. Antagonism was never seen. Synergy was observed for some isolates for each combination and was most frequent with MFG-AMB.

Cryptococcosis is, despite aggressive antifungal therapy, an important cause of morbidity and mortality in immunocompromised patients, especially those with AIDS (4, 6, 12). Apart from *Cryptococcus neoformans*, other species of this genus are commonly involved in human infections, e.g., *C. gattii* and, less frequently, *C. albidus* and *C. laurentii* (13, 15, 16). The treatment of choice for cryptococcosis is amphotericin B (AMB), with or without flucytosine (5FC) and fluconazole (FLC) (24). The toxicity of AMB and 5FC and the increasing isolation of FLC-resistant strains (5, 25) underline the need for improved treatments and the use of new strategies. Combined therapies can be useful for this purpose (17). Several studies have evaluated the interactions of AMB or 5FC with other drugs or with each other against *Cryptococcus* (1, 2, 3, 10, 20, 21, 26), but little is known about the interactions between echinocandins and AMB or azoles (10, 23). We have evaluated the activity of micafungin (MFG) in combination with four other drugs against strains of the four species of *Cryptococcus* mentioned above.

We tested a total of 37 clinical isolates (Tables 1 and 2). Antifungal agents were obtained as pure powders. AMB, voriconazole (VRC), itraconazole (ITC), and ravuconazole (RVC) were diluted in dimethyl sulfoxide. MFG and FLC were diluted in sterile distilled water. For all drugs, the MIC was defined as the lowest drug concentration that produced 100% inhibition of visible fungal growth after 72 h of incubation. Antifungal agents were placed in rows or in columns of the trays to test all possible combinations; the highest concentrations were 4 μg/ml for AMB, 8 μg/ml for ITC, VRC and RVC, and 32 μg/ml for FLC and MFG. Drug interactions were assessed by a checkerboard microdilution method (8). The MIC of each drug alone was determined according to the NCCLS (19). The fractional inhibitory concentration index (FICI) was used to classify drug interactions (14). The procedure, conservation of the strains, and quality controls have all been detailed previously (22, 28). Approximately 80% of the tests were repeated, and interactions mainly showed the same tendencies (data not shown).

Table 1 shows the in vitro interactions between MFG and AMB or FLC against clinical isolates of *C. neoformans* and *C. gattii*. Due to the low MICs of ITC, VRC, and RVC for the strains of *C. neoformans* and *C. gattii* tested (<0.12 μg/ml in all cases), we could not evaluate the in vitro interactions of MFG with these azoles against these species. MFG-AMB showed the highest percentage of synergistic interactions (70% for *C. neoformans* and 80% for *C. gattii*). MFG-FLC showed a lower percentage of synergistic interactions (30% for *C. neoformans* and 20% for *C. gattii*).

Table 2 shows the in vitro interactions between MFG and AMB or azoles against clinical isolates of *C. albidus* and *C. laurentii*. MFG in combination with AMB showed synergy for five isolates of *C. albidus* (50%) and five of *C. laurentii* (71%). Synergistic interactions between MFG and FLC were observed for four isolates of *C. albidus* (40%) and three isolates of *C. laurentii* (15%). For the two species, MFG combined with ITC showed similar percentages of synergistic interactions, i.e., 30 and 29%, respectively. Interactions between MFG and VRC were highly dependent on the species tested. With six isolates of *C. albidus* (60%), this combination showed synergy, whereas for all isolates of *C. laurentii* tested, the results were indifferent. A high number of synergistic interactions were observed with MFG in combination with RVC for both species tested (60% for *C. albidus* and 71% for *C. laurentii*).

Antagonism was not detected for any of the antifungal combinations assayed, although in 14% of the cases MICs were higher than the highest concentrations used to detect any interaction.

In our study, AMB generally showed low MICs against all the isolates tested. AMB alone or in combination with 5FC is commonly used in the treatment of *C. neoformans* and *C. gattii* infections (24), but its toxicity limits its usefulness. FLC is an alternative regimen for colonization and mild to moderate pulmonary disease in the immunocompetent host and constitutes a consolidation therapy for severe and progressive pulmonary and central nervous system disease (24). However, in our study we observed a wide range of FLC MICs, with a

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TABLE 1. Interactions of MFG with AMB and FLC against isolates of *C. neoformans* and *C. gattii*

Species (*n*) and isolate[a] tested	MIC (μg/ml)			FICI[b] for AMB-MFG	MIC (μg/ml)		FICI[b] for FLC-MFG
	AMB	MFG	AMB-MFG		FLC	FLC-MFG	
C. neoformans (10)							
FMR 8393	1	64	0.25/16	0.5	16	16/64	2
FMR 8398	1	64	0.25/16	0.5	32	32/64	2
FMR 8400	0.5	64	0.12/8	0.4	16	16/64	2
FMR 8401	0.5	64	0.12/8	0.4	8	2/2	0.3
FMR 8408	0.25	64	0.12/4	0.6	2	2/64	2
FMR 8409	0.5	64	0.12/16	0.5	1	1/64	2
FMR 8411	1	64	0.25/2	0.3	32	4/0.25	0.1
FMR 8415	0.5	64	0.25/4	0.6	8	8/64	2
FMR 8416	0.5	64	0.12/16	0.5	8	8/64	2
FMR 8420	0.25	64	0.12/8	0.6	4	1/16	0.5
C. gattii (10)							
FMR 8402	0.5	64	0.12/16	0.5	64	64/64	2
FMR 8403	0.06	64	0.06/0.06	1	32	32/64	2
FMR 8404	0.5	64	0.12/0.25	0.2	64	64/64	2
FMR 8405	0.25	64	0.12/2	0.6	64	64/64	2
FMR 8406	0.5	64	0.12/0.5	0.2	32	32/64	2
FMR 8407	0.25	64	0.06/16	0.5	64	64/64	2
FMR 8410	0.25	64	0.06/16	0.5	32	8/16	0.5
FMR 8412	0.25	64	0.06/16	0.5	8	2/16	0.5
FMR 8413	0.25	64	0.06/16	0.5	32	32/64	2
FMR 8414	0.5	64	0.12/0.25	0.2	32	32/64	2

[a] FMR, Faculty of Medicine, University of Rovira i Virgili, Reus, Tarragona, Spain.
[b] FICI scores: ≤0.5, synergistic; >0.5 and ≤4, indifferent; >4, antagonistic (14).

predominance of MICs of 64 μg/ml. ITC can be an alternative to FLC in *C. neoformans* infections (24). In our study this drug showed low MICs for *C. neoformans*, *C. gattii*, and *C. laurentii* strains, but MICs as high as 16 μg/ml were obtained for 3 of the 10 isolates of *C. albidus*, suggesting a lack of activity. No clinical reports exist on the use of ITC for the treatment of *C. laurentii* and *C. albidus* infections. The high toxicity of AMB, the variable activity of FLC, and the poor experience in the management of *C. laurentii* and *C. albidus* infections have led to the testing of new therapeutic approaches. Combined treatments seem to be good candidates for this purpose. In a preliminary study (data not shown), we observed a higher percentage of synergistic interactions with MFG-azole or MFG-AMB than with AMB-azole combinations. Echinocandins are inactive against *C. neoformans* (7), and data showing their in vitro activities against other *Cryptococcus* spp. are scarce (9, 28, 29). In this study, MFG alone was also inactive against all of the 37 isolates tested. However, when MFG was combined with other

TABLE 2. Interactions of MFG with AMB and various azoles against isolates of *C. albidus* and *C. laurentii*

Species (*n*) and isolate[a] tested	MIC (μg/ml)			FICI[b] for AMB-MFG	MIC (μg/ml)		FICI for FLC-MFG	MIC (μg/ml)		FICI for ITC-MFG	MIC (μg/ml)		FICI for VRC-MFG	MIC (μg/ml)		FICI for RVC-MFG
	AMB	MFG	AMB-MFG		FLC	FLC-MFG		ITC	ITC-MFG		VRC	VRC-MFG		RVC	RVC-MFG	
C. albidus (10)																
IHEM 4786	0.5	64	0.25/8	0.6	64	64/64	2	16	16/64	2	16	16/64	2	16	8/32	1
IHEM 2740	0.25	64	0.06/16	0.5	64	64/64	2	1	1/64	2	2	2/64	2	1	0.25/16	0.5
IHEM 3267	0.5	64	0.12/16	0.5	64	64/64	2	0.25	0.25/64	2	8	2/8	0.4	8	1/8	0.2
IHEM 5516	0.5	64	0.25/8	0.6	64	16/16	0.5	0.5	0.12/16	0.5	0.5	0.12/0.25	0.2	1	0.25/16	0.5
IHEM 6283	0.25	64	0.12/8	0.6	32	16/0.06	0.6	4	1/8	0.3	16	4/0.06	0.2	16	8/8	0.6
IHEM 6286	0.5	64	0.25/4	0.6	64	64/64	2	16	16/64	2	16	16/64	2	16	16/64	2
IHEM 6699	0.25	64	0.06/0.25	0.4	64	16/4	0.3	0.5	0.5/64	2	2	0.5/16	0.5	0.25	0.06/0.06	0.2
IHEM 6723	1	64	0.25/16	0.5	64	64/64	2	16	16/64	2	16	16/64	2	16	16/64	2
IHEM 6895	0.5	64	0.12/16	0.5	64	16/0.5	0.2	1	0.25/16	0.5	1	0.25/16	0.5	2	0.5/16	0.5
IHEM 10432	0.25	64	0.12/1	0.6	16	4/0.25	0.2	0.25	0.12/2	0.6	0.25	0.06/16	0.5	0.25	0.06/16	0.5
C. laurentii (7)																
IHEM 8060	0.5	64	0.12/16	0.5	16	16/64	2	1	0.25/16	0.5	0.25	0.25/64	2	0.25	0.06/16	0.5
IHEM 8061	0.5	64	0.12/8	0.3	4	4/64	2	4	4/64	2	2	2/64	2	4	1/16	0.5
IHEM 8062	0.5	64	0.12/16	0.5	32	16/0.06	0.6	1	0.25/16	0.5	2	2/64	2	0.25	0.12/8	0.6
IHEM 8063	0.5	64	0.12/16	0.5	2	1/0.06	0.6	0.25	0.25/64	2	1	1/64	2	0.25	0.06/16	0.5
IHEM 8064	0.25	64	0.12/64	0.7	64	64/64	2	0.12	0.12/64	2	0.12	0.12/64	2	0.5	0.5/64	2
FMR 8123	0.25	64	0.06/0.06	0.2	32	32/64	2	0.5	0.5/64	2	0.25	0.25/64	2	0.12	0.12/64	2
FMR 8515	0.5	64	0.12/16	0.5	64	16/0.06	0.2	0.5	0.12/16	0.5	0.5	0.25/16	0.7	0.5	0.5/64	2

[a] FMR, Faculty of Medicine, University of Rovira i Virgili, Reus, Tarragona, Spain; IHEM, Scientific Institute of Public Health, Louis Pasteur Institute, Brussels, Belgium.
[b] FICI scores: ≤0.5, synergistic; >0.5 and ≤4, indifferent; >4, antagonistic (14).

antifungals, especially AMB, MFG MICs often went down by as much as 10 dilutions, and in many cases they went below peak plasma levels achieved by this drug in humans (7) and experimental infection (11). Although combinations of MFG with azoles have also frequently resulted in synergistic interactions, MICs were generally over the therapeutic values.

MFG combined with AMB or azoles has also demonstrated synergy and efficacy in animal model infections of *Aspergillus fumigatus* (18) and *Trichosporon asahii* (27). The good results obtained in this study encourage us to perform further studies with animal models to confirm the potential of these combinations for the treatment of cryptococcosis.

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