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In Vitro Interactions of Approved and Novel Drugs against *Paecilomyces* spp.

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We have evaluated the in vitro activity of 15 combinations of antifungal drugs (amphotericin B, itraconazole, voriconazole, albaconazole, ravuconazole, terbinafine, and micafungin) against four isolates of *Paecilomyces variotii* and three of *P. lilacinus*. The interaction of terbinafine with the four azoles was synergistic for 53% of the combinations, while the interactions of both amphotericin B and micafungin with the rest of antifungal agents were mainly indifferent.

Paecilomyces species are saprophytic fungi usually recovered from soil and air which can cause the deterioration of grain, food, and paper. They can contaminate antiseptic creams and lotions of clinical use and colonize materials such as catheters and plastic implants, causing infections in immunocompetent and immunocompromised patients (4, 9). *Paecilomyces variotii* and *P. lilacinus* are the most ubiquitous species of the genus and also the most frequently involved in human infections (4, 14). Endophthalmitis and endocarditis are two of the most common infections produced by *P. lilacinus* and *P. variotii*, respectively, and have a very bad prognosis (4). Amphotericin B (AMB), alone or combined with flycytosine or azoles, is the standard treatment, but a failure rate of about 40% indicates that the proper treatment has not yet been found. Hence, new treatment regimens are needed, and the combination of antifungal agents constitutes an interesting new alternative to be tested. Allylamines and especially echinocandins are new classes of antifungal agents with novel targets, which make them very interesting for combination studies (6). In recent years, numerous studies have been performed to determine the in vitro activity of combinations of the available drugs against filamentous fungi, although the genus *Paecilomyces* was not included in any of them (2, 12).

Seven clinical isolates of *Paecilomyces* spp. (four strains of *P. variotii* and three strains of *P. lilacinus*) were tested. The isolates were grown on potato dextrose agar plates and incubated at 30°C for 7 to 10 days. Inocula were prepared by following the NCCLS guidelines (10) and adjusted to a final concentration of 1.1×10^4 to 3.4×10^4 conidia/ml. Antifungal agents were obtained as pure powders. AMB (USP, Rockville, Md.), itraconazole (ITZ) (Janssen Pharmaceutica, Beerse, Belgium), voriconazole (VCZ) (Pfizer Inc., Madrid, Spain), albaconazole (ABZ) (J. Uriach & Cia., Barcelona, Spain), ravuconazole (RVZ) (Bristol-Myers Squibb Company, New Brunswick, N.J.), and terbinafine (TBF) (Novartis, Basel, Switzerland) were dissolved in dimethyl sulfoxide. Micafungin (MFG) was obtained from Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan) and was dissolved in water.

The MICs of all drugs were defined as the lowest drug concentrations that produced a 100% inhibition of visible fungal growth after 48 to 72 h of incubation at 35°C. Drug interactions were assessed by a checkerboard microdilution method that also included the determination of the MIC of each drug alone in the same plate by using the parameters outlined in NCCLS document M38-A. Antifungal agents were placed in rows or in columns of the trays to test all possible combinations with the highest concentrations being 8 μg/ml for AMB, 32 μg/ml for TBF and MFG, and 16 μg/ml for the azoles. The fractional inhibitory concentration index (FICI) was used to classify drug interaction. FICI is the sum of the FIC of each of the drugs, which in turn is defined as the MIC of each drug when it is used in combination divided by the MIC of the drug when it is used alone. Interaction was synergistic if FICI was ≤ 0.5, indifferent if FICI was >0.5 and ≤ 4, and antagonistic if FICI was >4. Due to the multiple testing of single drugs (AMB, TBF, and MFG six times and azoles three times), MICs of single drugs were expressed as ranges when the values varied. Approximately 80% of the tests were repeated, and interactions showed mainly the same tendencies (data not shown).

All antifungal agents except TBF showed, in general, some activity against *P. variotii* when tested alone. For *P. lilacinus* only the novel azole derivatives, and especially RVZ, were active. These results generally confirmed our earlier studies (1, 3) and those of other authors (7).

The in vitro interactions of the seven antifungal drugs tested in this study are shown in Tables 1 to 3. Of the 105 combinations evaluated, 23 were synergistic and the rest were indifferent. We detected no antagonistic interactions in any case. TBF combined with the four azoles showed the highest percentage of synergistic interactions (53%). The combination TBF-VCZ was synergistic against six of the seven strains tested, and it was the only combination that was synergistic against all the strains of *P. lilacinus* tested. Highly favorable interactions obtained with TBF and azoles against other filamentous fungi have also been found by other authors and are probably due to their combined effects at different stages of the ergosterol biosyn-

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TABLE 1. MICs and FICI values of AMB combined with ITZ, VCZ, ABZ, RVZ, and TBF against clinical isolates of *Paecilomyces* spp.[a]

Strain	AMB-ITZ				AMB-VCZ			AMB-ABZ			AMB-RVZ			AMB-TBF		
	MIC (μg/ml)			FICI	MIC (μg/ml)		FICI	MIC (μg/ml)		FICI	MIC (μg/ml)		FICI	MIC (μg/ml)		FICI
	AMB	ITZ	AMB/ITZ		VCZ	AMB/VCZ		ABZ	AMB/ABZ		RVZ	AMB/RVZ		TBF	AMB/TBF	
P. variotii 4647	2	4	0.5/2	0.7	0.5	2/0.03	1.1	0.5	2/0.03	1.1	0.12	0.12/0.12	1.1	8	2/0.12	1
P. variotii 5516	0.12–0.25	4	0.12/1	0.7	16	ND	ND	2	0.12/0.5	0.7	4	0.12/2	1.5	8	ND	ND
P. variotii 5517	0.12–0.25	4	0.12/2	1	8	ND	ND	1	0.12/0.5	1	2	0.25/0.25	1.1	8	ND	ND
P. variotii 5518	0.12–0.25	1	0.25/0.03	1	2	0.12/0.03	0.5	0.5	0.12/0.06	0.6	0.25	0.12/0.25	2	8	ND	ND
P. lilacinus 5519	16	32	16/32	2	4	0.12/4	1	4	0.12/4	1	1	0.12/1	1	8	0.12/4	0.5
P. lilacinus 5522	16	32	16/32	2	2	0.12/2	1	4	8/0.12	0.5	0.25	0.12/0.5	2	4	0.12/4	1
P. lilacinus 5540	16	32	16/32	2	4	0.12/4	1	4	0.12/4	1	0.5	0.12/1	2	4	0.12/4	1

[a] ND, not determined.

thesis pathway (12). In contrast, the interactions of both AMB and MFG with any of the other antifungal agents and that between AMB and MFG were mainly indifferent; they were synergistic in only 10% of the tests. Concerning the combinations of MFG with azoles, we did not observe the favorable interactions against *Candida* spp. and *Aspergillus* spp. that other authors have reported (6). The lack of synergy of some AMB- or MFG-azole combinations against *P. variotii* may be related to the low MICs of AMB and MFG for this species. However, these in vitro findings do not exclude a positive interaction in vivo, which merits evaluation in animal models.

It is important to take into account the concentrations of each drug in the combination at which their effect is detected, especially if they are lower than those potentially achieved in serum. On this basis, for *P. variotii* the best results were obtained for TBF combined with ITZ and for *P. lilacinus* the best results were obtained for TBF combined with VCZ and RVZ.

Animal models can be important tools in evaluating the significance in vivo of the most promising combinations. We have recently developed a murine model of disseminated infection by the two above-mentioned *Paecilomyces* species (11), which could be useful for this purpose. However, the peculiar pharmacokinetics of TBF with high clearance from plasma and accumulation in skin and adipose tissues influences the choice of the adequate animal model to be used (8). In several in vivo studies using different animals, this drug was totally ineffective in spite of showing low in vitro MICs (13). However, there is some clinical evidence for the good activity of TBF combined

TABLE 2. MICs and FICI values of TBF combined with ITZ, VCZ, ABZ, RVZ, and MFG against clinical isolates of *Paecilomyces* spp.

Strain	TBF-ITZ			FICI	TBF-VCZ		FICI	TBF-ABZ		FICI	TBF-RVZ		FICI	TBF-MFG		FICI
	MIC (μg/ml)				MIC (μg/ml)			MIC (μg/ml)			MIC (μg/ml)			MIC (μg/ml)		
	TBF	ITZ	TBF/ITZ		VCZ	TBF/VCZ		ABZ	TBF/ABZ		RVZ	TBF/RVZ		MFG	TBF/MFG	
P. variotii 4647	4–16	4	2/0.12	0.5	0.5	4/0.03	0.6	0.5	2/0.03	0.6	0.12	−/−[a]	−	0.5	1/0.06	0.6
P. variotii 5516	2–16	1	2/0.12	0.6	16	8/0.03	0.5	2	0.5/2	1.2	4	16/0.03	1	0.12	1/0.06	0.7
P. variotii 5517	4–16	2	2/0.5	0.5	4	4/0.5	0.4	2	2/0.12	0.6	0.5	4/0.06	0.6	0.06	2/0.06	1.5
P. variotii 5518	2–16	1	2/0.06	0.6	1	4/0.03	0.5	0.5	1/0.12	0.5	0.12	2/0.03	0.5	0.12	1/0.06	1
P. lilacinus 5519	2–8	32	2/8	0.7	4	2/1	0.5	8	1/2	0.5	1	2/0.03	0.3	64	1/1	0.5
P. lilacinus 5522	1–8	32	1/4	0.4	4	2/0.5	0.4	4	1/0.5	0.6	0.5	1/0.06	0.4	64	1/0.06	1
P. lilacinus 5540	2–8	32	1/8	0.5	4	2/0.5	0.4	4	1/1	0.7	0.5	2/0.06	0.6	64	1/0.06	0.5

[a] Minus signs are as defined for Table 1.

TABLE 3. MICs and FICI values of MFG combined with ITZ, VCZ, ABZ, RVZ, and AMB against clinical isolates of *Paecilomyces* spp.

Strain	MFG-ITZ			FICI	MFG-VCZ		FICI	MFG-ABZ		FICI	MFG-RVZ		FICI	MFG-AMB		FICI
	MIC (μg/ml)				MIC (μg/ml)			MIC (μg/ml)			MIC (μg/ml)			MIC (μg/ml)		
	MFG	ITZ	MFG/ITZ		VCZ	MFG/VCZ		ABZ	MFG/ABZ		RVZ	MFG/RVZ		AMB	MFG/AMB	
P. variotii 4647	0.06–0.5	0.5	0.06/0.5	1.1	0.12	0.06/0.12	1.5	0.5	0.06/0.5	1.5	0.25	0.06/0.12	1.4	1	0.06/0.5	0.7
P. variotii 5516	0.12–2	0.25	0.06/0.25	1.5	4	0.25/0.12	1.0	4	0.06/4	1.5	4	0.06/0.5	0.6	0.12	0.06/0.12	1.0
P. variotii 5517	0.06–1	0.5	0.06/0.5	1.1	1	0.12/0.12	2.1	0.5	0.06/0.25	1.5	2	0.06/1	1	0.12	0.06/0.12	1.0
P. variotii 5518	0.06–0.25	0.12	0.06/0.12	2	1	0.06/0.5	0.7	0.25	0.06/0.25	2	0.12	0.06/0.12	2	0.12	0.06/0.12	2
P. lilacinus 5519	64	32	64/32	2	1	0.06/0.5	0.5	4	8/2	0.6	1	32/0.5	1	16	64/16	2
P. lilacinus 5522	64	32	8/4	0.2	0.25	0.06/0.25	1	0.5	8/0.25	0.6	0.5	0.6/0.5	1	16	64/16	2
P. lilacinus 5540	64	32	64/32	2	1	0.5/0.5	0.5	1	0.06/1	1	2	0.06/2	1	16	64/16	2

with several azoles in clinical practice, including one case of *P. lilacinus* infection (5).

Further studies are warranted to further elucidate the potential usefulness of these combination therapies.

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