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Synergy between Pyrvinium Pamoate and Azoles against *Exophiala dermatitidis*

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KEYWORDS *Exophiala dermatitidis*, antifungal, azole, fungi, pyrvinium pamoate, synergy

The black yeast *Exophiala dermatitidis* causes phaeohyphomycosis in both immunocompetent individuals and immunosuppressed patients, resulting in localized cutaneous and subcutaneous infections and more-severe systemic forms, such as neurotropic infections (1–3). Additionally, this species frequently colonizes the pulmonary system of cystic fibrosis patients, which appears to be associated with more-advanced disease (4). Despite favorable *in vitro* susceptibilities to available antifungal agents, *E. dermatitidis* infection is clinically resistant to antifungal agents, with a success rate of only 40 to 70% (3–5). Due to the paucity of new antifungal drugs, combination therapy with drug repositioning has been considered a promising strategy.

Pyrvinium pamoate (PP), an anthelmintic drug approved by the FDA in 1955, is a quinoline-derived cyanine dye that has been used to treat pinworm (*Enterobius vermicularis*) infections (6) and strongyloidiasis (7) in humans. However, in recent years, PP has attracted considerable attention from several studies that demonstrated its potential antitumor properties (8–10). Interestingly, it has also been shown that PP strongly suppresses the growth of the *Candida albicans* isochromosome 5L strain [i(5L)], which contains two copies of the left arm of chromosome 5 and is known to confer resistance to fluconazole (11), and enhances the efficacy of fluconazole (12).

In the present study, the effects of PP alone and combined with azoles, including itraconazole (ITC), posaconazole (POS), and voriconazole (VRC), were investigated against a total of 18 strains of *E. dermatitidis*. *Candida parapsilosis* (ATCC 22019) was included to ensure quality control. All *E. dermatitidis* strains were clinical isolates and identified by microscopic morphology and by molecular sequencing of the internal transcribed spacer (ITS) ribosomal DNA (rDNA) (13). All tested agents were diluted in dimethyl sulfoxide (DMSO) as stock solutions (3,200 μg/ml). The working solution was made by dilution with sterile RPMI 1640 to achieve serial dilutions of PP (0.06 to 4 μg/ml; Selleck Chemicals, Houston, TX, USA) and azoles (0.03 to 4 μg/ml; Selleck Chemicals). The broth microdilution checkerboard technique adapted from Clinical and Laboratory Standards Institute broth microdilution method M38-A2 was performed (14). The MICs were determined as the lowest concentration resulting in complete inhibition (100%) of growth (14). The interactions between PP and azoles referred to the fractional inhibitory concentration index (FICI). The FICI was calculated by the formula FICI = (Ac/Aa) + (Bc/Ba), where Ac and Bc are the MICs of antifungal drugs in combination and Aa and Ba are the MICs of antifungal drugs A and B alone. An FICI of $\leq$0.5 is classified as synergy, an FICI of $>$0.5 to $\leq$4 indicates no interaction (indifference), and an FICI of $>$4 indicates antagonism (15). All experiments were conducted in triplicate.

Accepted manuscript posted online 5 February 2018

Citation Gao L, Sun Y, He C, Zeng T, Li M. 2018. Synergy between pyrvinium pamoate and azoles against *Exophiala dermatitidis*. Antimicrob Agents Chemother 62:e02361-17. https://doi.org/10.1128/AAC.02361-17.

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TABLE 1 MIC and FICI results with combinations of PP and azoles against *Exophiala dermatitidis*

Strain	MIC (μg/ml)				MICs of drug A/drug B (μg/ml), or FICI (susceptibility[a])		
	PP	ITC	VRC	POS	ITC-PP	VRC-PP	POS-PP
109140	2	0.5	0.5	1	0.03/0.125 (S)	0.125/0.5 (S)	0.03/0.25 (S)
109144	2	1	0.5	1	0.03/0.25 (S)	0.125/0.25 (S)	0.03/0.25 (S)
109145	2	0.5	0.5	1	0.125/0.25 (S)	0.125/0.25 (S)	0.03/0.25 (S)
109148	2	1	0.5	0.5	0.03/0.25 (S)	0.25/0.25 (I)	0.06/0.25 (S)
109149	2	1	0.5	0.5	0.03/0.25 (S)	0.125/0.25 (S)	0.03/0.25 (S)
109152	2	0.5	0.5	0.5	0.25/0.25 (I)	0.25/0.125 (I)	0.03/0.25 (S)
BMU00028	2	1	0.5	0.5	0.03/0.25 (S)	0.25/0.25 (I)	0.03/0.25 (S)
BMU00029	2	1	0.5	0.5	0.03/0.25 (S)	0.125/0.25 (S)	0.03/0.25 (S)
BMU00030	2	0.5	0.5	0.5	0.03/0.25 (S)	0.125/0.25 (S)	0.03/0.25 (S)
BMU00031	2	1	0.5	0.5	0.06/0.25 (S)	0.25/0.25 (I)	0.03/0.25 (S)
BMU00034	2	0.5	0.5	0.5	0.03/0.25 (S)	0.125/0.5 (S)	0.03/0.25 (S)
BMU00035	2	1	0.5	0.5	0.03/0.25 (S)	0.25/0.25 (I)	0.06/0.25 (S)
BMU00036	2	1	0.5	0.5	0.25/0.125 (S)	0.25/0.25 (I)	0.03/0.25 (S)
BMU00037	2	1	0.5	1	0.03/0.25 (S)	0.125/0.125 (S)	0.03/0.25 (S)
BMU00038	2	1	0.5	0.5	0.25/0.125 (S)	0.25/0.25 (I)	0.03/0.125 (S)
BMU00039	2	1	0.5	0.5	0.06/0.25 (S)	0.25/0.25 (I)	0.06/0.25 (S)
BMU00040	2	1	0.5	1	0.5/0.25 (I)	0.125/0.25 (S)	0.03/0.25 (S)
BMU00041	2	1	0.5	0.5	0.03/0.25 (S)	0.25/0.25 (I)	0.03/0.25 (S)

[a]S, susceptible; I, intermediate resistance.

The MIC ranges of individual tested agents against *E. dermatitidis* isolates were 2 μg/ml for PP, 0.5 to 1 μg/ml for ITC, 0.5 μg/ml for VRC, and 0.5 to 1 μg/ml for POS (Table 1). The combination of PP with POS, ITC, or VRC showed synergistic antifungal effects against 18 (100%), 16 (88.9%), or 9 (50%) strains of *E. dermatitidis*, respectively. The effective MIC ranges of PP were mostly within the range of 0.125 to 0.5 μg/ml (0.1 to 0.4 μM) (Table 1).

PP exerts potential anticancer effects via CK1α activation and mitochondrial respiration inhibition, which affects multiple critical signaling pathways and biological processes, such as energy, autophagy, and Akt- and Wnt-β-catenin-dependent pathways (8). In the pathogenic fungus *C. albicans*, PP potentiates the activity of fluconazole by targeting aneuploid chromosomes (12). Excitingly, in the present study, pyrvinium showed antifungal activity alone and favorable synergistic effects with all azoles against *E. dermatitidis*. Previous studies have demonstrated that PP is well tolerated in humans at doses as high as 35 mg/kg of body weight without any toxic side effects (7). However, the facts that PP is not completely soluble in aqueous solutions and there is no measurable absorption of pyrvinium from the gastrointestinal tract (16) have to some extent limited the possible application of the drug to bloodstream infections. Nevertheless, with the advances in pharmaceutical manufacturing technology, innovative formulations of PP that enable a wider application can be anticipated. More insights into the mechanism of the antifungal properties of PP might help establish novel antifungal strategies.

ACKNOWLEDGMENTS

We thank Ruoyu Li and Wei Liu from the Research Center for Medical Mycology, Peking University First Hospital, Peking University, Beijing, China, and G. Sybren de Hoog from the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, for kindly providing us with the *E. dermatitidis* isolates studied.

This work was supported by grants 31400131 (Lujuan Gao) and 81401677 (Yi Sun) from the National Natural Science Foundation of China and grant WJ2015MB281 from the Hubei Province Health and Family Planning Scientific Research Project (Yi Sun).

The funders had no role in study design, data analysis, decision to publish, or preparation of the manuscript.

We declare that we have no conflicts of interest.

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