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NOTES

Activities of Amphotericin B and Antifungal Azoles Alone and in Combination against *Pseudallescheria boydii*

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In order to develop new approaches to treatment of infections due to *Pseudallescheria boydii*, the in vitro antifungal activity of amphotericin B alone and in combination with miconazole, itraconazole, and fluconazole was studied. Combinations of amphotericin B and antifungal azoles were synergistic, additive, or indifferent in their interaction against *P. boydii*. Antagonism was not observed.

Pseudallescheria boydii causes mycetoma, pneumonia, and disseminated infections, particularly involving the central nervous system, that are frequently refractory to antifungal chemotherapy (1–4, 6, 7, 10–16, 19–23, 25–28). Isolates of *P. boydii* are often considered to be resistant to amphotericin B; however, the frequency with which such resistance occurs is not well described. Antifungal azoles are often cited as the agents of choice for infections due to *P. boydii* (7, 13, 15), yet patients with pneumonia, cerebral abscesses, endophthalmitis, osteomyelitis, or disseminated infections due to *P. boydii* often fail to respond to single-agent azole therapy. New therapeutic approaches are clearly needed for the treatment of infections due to this organism. The combination of amphotericin B and antifungal azoles may provide an important therapeutic option.

Little is known, however, about the combination of amphotericin B and antifungal azoles for *P. boydii*. The combination of antifungal azoles and amphotericin B in treatment of *P. boydii* infections may not be considered feasible on the basis of theoretical concern about potential antagonism. We therefore determined the MICs and minimum lethal concentrations (MLCs) of amphotericin B and antifungal azoles in vitro against a large group of clinical isolates of *P. boydii* and further studied the potential synergistic interaction between these two classes of antifungal compounds against this frequently refractory pathogen.

The 22 isolates of *P. boydii* used in this study were identified at the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio. Cultures were maintained on the surface of potato dextrose agar slants (Remel, Lenaxa, Kans.) at −70°C.

MICs were determined by broth macrodilution methods, as previously described (8, 9, 18). Briefly, a suspension was adjusted with a spectrophotometer to 68 to 71% transmission at a wavelength of 530 nm and diluted 100-fold to yield an inoculum of 1×10^4 to 5×10^4 CFU/ml. Amphotericin B, fluconazole, itraconazole, and miconazole were tested in 0.165 M morpholinepropanesulfonic acid (MOPS)-buffered RPMI 1640 (Bio-Whittaker, Walkersville, Md.) at a pH of 7.0. A 100-μl quantity of concentrated antifungal compound was diluted 10-fold into 900 μl of inoculum suspension and incubated at 35°C for 24 and 48 h. The final concentration ranges for amphotericin B (Bristol-Myers Squibb, Princeton, N.J.) and itraconazole and miconazole (both from Janssen Pharmaceutica, Piscataway, N.J.) were 0.03 to 16.0 μg/ml, and that for fluconazole (Pfizer Central Research, Groton, Conn.) was 0.125 to 64.0 μg/ml. Growth was graded on a scale of 0 to 4+ as follows: 0, optically clear; 1+, slightly hazy; 2+, 50% reduction of growth; 3+, 25% reduction of growth; and 4+, growth equal to that of the growth control tube. The MIC was defined as the lowest concentration of antifungal compound which rendered no growth (0) for amphotericin B and 2+ growth for antifungal azoles. The MLC was determined by dispensing and streaking 100 μl of broth from the next four tubes with concentrations above the MIC, exhibiting no growth onto Sabouraud glucose agar (Media Department, National Institutes of Health, Bethesda, Md.), and incubating at 35°C. A growth of three or fewer colonies was considered to indicate fungicidal activity. The MLC was defined as the lowest concentration of antifungal compound causing growth of three or fewer colonies.

A two-dimensional checkerboard macrodilution technique was used to characterize interactions between amphotericin B and the antifungal azoles. Serial twofold dilutions of amphotericin B and an antifungal azole, alone and in combination, were tested against a final inoculum concentration of 1×10^4 to 5×10^4 CFU/ml. Each of the two antifungal compounds in the tubes with two drugs was dispensed in 100-μl volumes to yield 200 μl. An 800-μl volume of the inoculum in broth was then added, thereby diluting each drug 10-fold. Each tube combination was again scored on a scale of 0 to 4+. The fractional inhibitory concentration index (FICI) was then calculated as (MIC of amphotericin B plus azole/MIC of amphotericin B) + (MIC of azole plus amphotericin B/MIC of azole). Drug interaction effects were defined on the basis of FICI values as follows: synergistic effect, ≤0.5; additive effect, >0.5 but ≤1; indifferent effect, >1 but ≤4; and antagonistic effect, >4.

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TABLE 1. MICs and MLCs of antifungal compounds against *P. boydii*[a]

Isolate no.	Amphotericin B		Miconazole		Itraconazole		Fluconazole	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
92-36	0.25	>16	0.5	8.0	0.03	>16	8	>16
91-753	2.0	2.0	0.125	0.5	0.25	2.0	16	16
91-895	2.0	2.0	0.125	0.125	≤0.03	>2.0	16	16
91-197	1.0	>16	0.5	4.0	0.5	>16	32	>64
91-725	2.0	4.0	0.5	1.0	0.25	>8.0	16	>64
91-1216	0.25	>8.0	0.125	1.0	0.25	>4.0	16	>64
91-1434	1.0	2.0	0.25	2.0	0.125	2.0	8	>16
92-194	0.5	1.0	0.125	0.125	0.25	>4.0	4	>64
285	0.5	2.0	0.5	4.0	0.25	>8.0	16	>32
1090	1.0	1.0	0.5	1.0	0.125	>8.0	32	64
1023	0.5	0.5	0.5	1.0	≤0.03	>4.0	16	32
90-9	1.0	1.0	0.25	>8.0	0.125	>4.0	8.0	32
91-635	0.5	>8.0	0.5	16.0	0.5	>16	8.0	>64
91-4	2.0	>16	0.5	8.0	0.25	>16	32	>64
92-116	1.0	>16	0.25	8.0	0.25	>16	32	>64
578	2.0	2.0	0.5	2.0	0.125	4.0	32	>64
370	0.5	>4.0	0.25	16	0.125	>2.0	10	32
90-423	2.0	>16	0.5	>16	2.0	>16	32	>64
92-49	0.5	0.5	0.5	16	≤0.03	>0.25	16	>64
1441	1.0	4.0	0.125	0.25	≤0.03	>0.25	16	32
91-514	2.0	>16	0.5	>4.0	4.0	>0.16	2.0	>64
Mean ± SEM	1.1 ± 0.15	12 ± 3.0	0.36 ± 0.04	6.9 ± 1.8	0.45 ± 0.20	15 ± 2.8	18 ± 2.2	87 ± 11

[a] All results are expressed as micrograms per milliliter.

Parallel testing of control isolates of *Candida parapsilosis* ATCC 90018 and *Paecilomyces variotii* ATCC 22319 was performed throughout the experiments for quality control. Results of in vitro susceptibility tests were similar ($P \geq 0.5$) for the two collaborating laboratories (National Cancer Institute and University of Texas Health Science Center at San Antonio).

Differences in means were determined by Student's paired *t* test. All *P* values were two-sided. A *P* value of ≤0.05 was considered to be significant. In determining the mean MIC and MLC for an antifungal compound, the next highest concentration was designated for single values exceeding the tested range of concentrations.

There was a broad range of antifungal activity, measured by high and low MICs, of amphotericin B, itraconazole, and fluconazole compared with that of miconazole, which had a consistently narrow range of low MICs (Table 1). The mean MICs ± standard errors of the mean were as follows: amphotericin B, 1.1 ± 0.15 μg/ml (range, 0.25 to 2.0 μg/ml); itraconazole, 0.45 ± 0.20 μg/ml (range, 0.03 to 4.0 μg/ml); fluconazole, 18 ± 2.2 μg/ml (range, 2.0 to 32 μg/ml); and miconazole, 0.36 ± 0.04 μg/ml (range, 0.125 to 0.50 μg/ml). As depicted in Table 1, the mean MLCs of amphotericin B, miconazole, itraconazole, and fluconazole against *P. boydii* were 5- to 33-fold higher than the corresponding mean MICs, as follows: amphotericin B, 12 ± 3.0 μg/ml; miconazole, 6.9 ± 1.8 μg/ml; itraconazole, 15 ± 2.8 μg/ml; and fluconazole, 87 ± 11 μg/ml.

Combinations of amphotericin B and antifungal azoles were synergistic, additive, or indifferent in their interaction against *P. boydii*. Antagonism was not observed. Sixteen (67%) of all amphotericin B-azole interactions studied were additive or synergistic. The mean FICIs ± standard errors of the mean for the combinations of amphotericin B and the azoles were as follows: with miconazole, 0.80 ± 0.09 (range, 0.49 to 1.07); with itraconazole, 1.03 ± 0.2 (range, 0.3 to 2.1); and with fluconazole, 0.61 ± 0.09 (range, 0.32 to 1.12) (Table 2). Figure 1 further illustrates the augmentation of antifungal activity by the combination of amphotericin with antifungal azoles. The mean MIC of amphotericin B against *P. boydii* was substantially reduced in the presence of miconazole ($P = 0.026$), itraconazole ($P = 0.042$), and fluconazole ($P = 0.11$).

TABLE 2. FICIs for combinations of amphotericin B and antifungal azoles against *P. boydii*

Isolate no.	FICI for amphotericin B plus:		
	Miconazole	Itraconazole	Fluconazole
92-36	1.07	1.24	1.12
91-197	0.49	0.75	0.57
91-895	0.75	1.12	0.32
92-194	0.60	1.25	0.32
91-753	0.62	0.37	0.75
91-1216	1.0	1.06	0.53
91-1434	0.62	0.31	0.75
91-725	1.01	2.12	0.50
Mean ± SEM (range)	0.77 ± 0.08 (0.49–1.07)	1.0 ± 0.2 (0.3–2.1)	0.61 ± 0.09 (0.32–1.125)

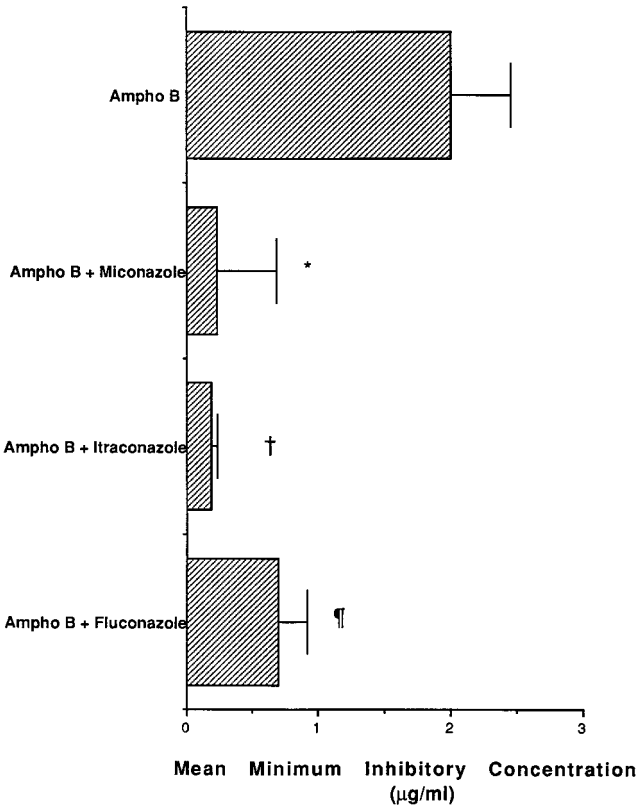

FIG. 1. Effect of antifungal azoles in reduction of the mean MIC of amphotericin B (Ampho B) against *P. boydii*, determined by checkerboard macrodilution. For each isolate ($n = 8$) of *P. boydii*, the effects of an antifungal azole on the MIC of amphotericin B were determined at the MIC of the azole for that isolate. The mean MIC of amphotericin B was reduced in the presence of miconazole ($P = 0.042$ [*]), itraconazole ($P = 0.026$ [†]), and fluconazole ($P = 0.11$ [¶]). Error bars indicate standard errors of the means.

P. boydii often has been described as being resistant to amphotericin B (4, 6, 13, 19, 22). While 7 (32%) of the 22 isolates of *P. boydii* used in this study were resistant to concentrations of amphotericin B of ≥2.0 μg/ml, for 8 isolates (36%), the MICs were ≤0.5 μg/ml. This strain-dependent in vitro response to amphotericin B suggests a potential role for in vitro susceptibility as a possible guide to selection of antifungal therapy and further suggests a potentially wider use of amphotericin B against *P. boydii* infections than has previously been recognized.

Immunocompromised patients with severe *Pseudallescheria* infections in the absence of host effector cells may depend upon the fungicidal activity of a compound for the successful eradication of organisms from tissue. This study, however, found a 5- to 33-fold disparity between mean MICs and mean MLCs for amphotericin B as well as for the antifungal azoles, suggesting that the potential fungicidal effects of these compounds are not attained at safely achievable concentrations in serum. Thus, strain-dependent antifungal activity and lack of fungicidal activity may limit the efficacy of single-agent therapy against *Pseudallescheria* infections in immunocompromised hosts.

The additional antifungal activity of combination antifungal therapy may be critical in profoundly neutropenic patients, in whom even moderate augmentation of antifungal activity may be important in preventing progression of infection until recovery from neutropenia. Perhaps the additive or synergistic activity of amphotericin B and antifungal azoles also may avert the need for disfiguring surgery in the management of *P. boydii* osteomyelitis. Combined topical administration of polyene and an antifungal azole also may have benefits in the management of *P. boydii* keratitis, which is refractory to single-agent systemic azole therapy (5, 17).

Combination therapy with amphotericin B and antifungal azoles has not been widely espoused as a therapeutic alternative because of the potential for antagonism. Schaffner and Frick demonstrated that when rats with experimental aspergillosis were exposed to ketoconazole, the in vivo activity of subsequently administered amphotericin B was reduced (24). *Aspergillus* isolates recovered from such animals had become resistant in vitro to amphotericin B. This antagonistic interaction may be related to the depletion of ergosterol by the initial nonlethal exposure to the antifungal azole, resulting in reduced efficacy of subsequent amphotericin B administration because of diminished binding of the polyene to the fungal cell membrane. This form of antagonism is predicated upon pretreatment with an antifungal azole and subsequent treatment with amphotericin B.

The augmented antifungal activity observed with amphotericin B-azole combinations against *P. boydii* in this study indicates that other mechanisms are likely to be operative. In a model of simultaneous exposure to amphotericin B plus an antifungal azole, amphotericin B binds to ergosterol at the fungal cell membrane, resulting in increased permeability to the antifungal azole. Increased amphotericin B-mediated permeability of fungal cell membranes may permit higher intracytoplasmic azole concentrations at the C-14-demethylase-cytochrome P-450 binding site, resulting in further inhibition of ergosterol synthesis in fungal cell membranes and augmentation of antifungal activity. This mechanism is consistent with findings of enhanced antifungal activity in this study.

As with all findings of in vitro antifungal activity, correlation with appropriate animal models and patient outcome is important. Given the difficulty in performing a large clinical trial with sufficient predictive power for assessing antifungal therapy against *Pseudallescheria* infections, animal studies assume even greater importance in studying the treatment of uncommon but emerging opportunistic fungi, such as *P. boydii*. We currently are investigating the mechanisms of host response and strain-dependent virulence in rabbit models of pulmonary and disseminated *P. boydii* infection. Following characterization of these models, the in vivo interaction of antifungal azoles and amphotericin B can be appropriately assessed.

REFERENCES

1. **Alsip, S. G., and C. G. Cobbs.** 1986. *Pseudallescheria boydii* infection of the central system in a cardiac transplant recipient. South. Med. J. **79:**383–384.
2. **Anaissie, E., G. P. Bodey, H. Kantarjian, J. Ro, S. E. Vartivarian, R. Hopfer, J. Hoy, and K. Rolston.** 1989. New spectrum of fungal infections in patients with cancer. Rev. Infect. Dis. **11:**369–378.
3. **Armin, A. R., V. B. Reddy, and E. Orfei.** 1987. Fungal endocarditis caused by *Pseudallescheria* (*Petriellidium*) *boydii* in an intravenous drug user. Texas Heart Inst. J. **14:**321–324.
4. **Berenguer, J., J. Diaz-Mediavilla, D. Urra, and P. Munoz.** 1990. Central nervous system infection caused by *Pseudallescheria boydii*: case report and review. Rev. Infect. Dis. **11:**890–896.
5. **Bloom, P. A., D. A. Laidlaw, D. L. Easty, and D. W. Warnock.** 1992. Treatment failure in a case of fungal keratitis caused by *Pseudallescheria boydii*. Br. J. Ophthalmol. **76:**367–368.
6. **Cooper, C. R., Jr., and I. Salkin.** 1993. Pseudallescheriasis, p. 335–358. *In* J. Murphy (ed.), Fungal infections and immune responses. Plenum Press, New York.
7. **Dworzack, D. L., R. B. Clark, W. J. Borkowski, Jr., D. L. Smith, M. Dykstra, M. P. Pugsley, E. A. Horowitz, T. L. Connolly, D. L. McKinney, M. K. Hostetler, J. F. Fitzgibbons, and M. Galant.** 1989. *Pseudallescheria boydii* brain abscess: association with near drowning and efficacy of high-dose,

prolonged miconazole therapy in patients with multiple abscesses. Medicine **68:**218–224.
8. **Espinel-Ingroff, A., K. Dawson, M. Pfaller, E. Anaissie, E. Breslin, D. Dixon, A. Fothergill, V. Paetznik, J. Peter, M. Rinaldi, and T. Walsh.** 1995. Comparative and collaborative evaluation of standardization of antifungal susceptibility testing for filamentous fungi. Antimicrob. Agents Chemother. **39:**314–319.
9. **Espinel-Ingroff, A., and T. M. Kerkering.** 1991. Spectrophotometric method of inoculum preparation for in vitro susceptibility testing of filamentous fungi. J. Clin. Microbiol. **29:**393–394.
10. **Fessler, R., and F. D. Brown.** 1989. Superior sagittal sinus infection with *Petriellidium boydii*: case report. Neurosurgery **24:**604–607.
11. **Fisher, J. F., S. Shadomy, J. R. Teabaut, J. Woodward, G. E. Michaels, M. A. Newman, E. White, P. Cook, A. Seagraves, F. Yagmai, and P. Rissing.** 1982. Near drowning complicated by brain abscess due to *Petriellidium boydii*. Arch. Neurol. **39:**511–513.
12. **Freeman, D., U. DeGirolami, D. Tarsy, S. Hoffman, G. Eliopoulos, and A. W. Karchmer.** 1990. *Pseudallescheria boydii* infection of the central nervous system. Arch. Neurol. **47:**468–472.
13. **Galgiani, J. N., D. A. Stevens, J. R. Graybill, D. L. Stevens, A. J. Tillinghast, and H. B. Levine.** 1984. *Pseudallescheria boydii* infections treated with ketoconazole. Clinical evaluations of seven patients and *in vitro* susceptibility results. Chest **86:**219–224.
14. **Gaya, J. G., S. G. Farmer, G. A. Williams, T. R. Franson, R. A. Komorowski, and J. C. Kies.** 1988. Bilateral *Pseudallescheria boydii* endophthalmitis in an immunocompromised patient. Wis. Med. J. **87:**11–14.
15. **Goldberg, S. L., D. J. Geha, W. F. Marshall, D. J. Inwards, and H. C. Hoagland.** 1993. Successful treatment of simultaneous pulmonary *Pseudallescheria boydii* and *Aspergillus terreus* infection with oral itraconazole. Clin. Infect. Dis. **16:**803–805.
16. **Lutwick, L. I., J. N. Galgiani, R. H. Johnson, and D. A. Stevens.** 1976. Visceral fungal infection due to *Petriellidium boydii* (*Allescheria boydii*). *In vitro* drug sensitivities studies. Am. J. Med. **61:**632–640.
17. **Mills, R., and G. Garrett.** 1992. *Pseudallescheria boydii* keratitis. Aust. N. Zealand J. Ophthalmol. **20:**253–256.
18. **National Committee for Clinical Laboratory Standards.** 1992. Reference method for broth dilution antifungal susceptibility testing of yeasts. Proposed standard. NCCLS document M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
19. **Patterson, T. F., V. T. Andriole, M. J. Zervos, D. Therasse, and C. A. Kauffman.** 1990. The epidemiology of pseudallescheriasis complicating transplantation: nosocomial and community-acquired infection. Mycoses **33:** 297–302.
20. **Peréz, R. E., M. Smith, M. McClendon, J. Kim, and N. Eugenio.** 1988. *Pseudallescheria boydii* brain abscess. Complication of an intravenous catheter. Am. J. Med. **84:**359–362.
21. **Pfiefer, J. D., M. G. Grand, M. A. Thomas, A. R. Berger, M. J. Lucarielli, and M. E. Smith.** 1992. Endogenous *Pseudallescheria boydii* endophthalmitis. Clinicopathologic findings in two cases. Arch. Ophthalmol. **109:**1714–1717.
22. **Rippon, J. W.** 1988. Medical mycology. The pathogenic fungi and the pathogenic actinomycetes, p. 651–680. W. B. Saunders Co., Philadelphia.
23. **Rippon, J. W., and J. W. Carmichael.** 1976. Petriellidiosis (allescheriasis): four unusual cases and review of literature. Mycopathologia **58:**117–124.
24. **Schaffner, A., and P. G. Frick.** 1985. The effect of ketoconazole on amphotericin B in a model of disseminated aspergillosis. J. Infect. Dis. **151:**902–910.
25. **Scherr, G. R., S. G. Evans, M. T. Kiyabu, and E. C. Klatt.** 1992. *Pseudallescheria boydii* infection in the acquired immunodeficiency syndrome. Arch. Pathol. Lab. Med. **116:**535–536.
26. **Travis, L. B., G. D. Roberts, and W. R. Wilson.** 1985. Clinical significance of *Pseudallescheria boydii*: a review of 10 years' experience. Mayo Clin. Proc. **60:**531–537.
27. **Welty, F. K., G. X. McLeod, C. Ezratty, R. W. Healy, and A. W. Karchmer.** 1992. *Pseudallescheria boydii* endocarditis of the pulmonic valve in a liver transplant recipient. Clin. Infect. Dis. **15:**858–860.
28. **Yoo, D., W. H. S. Lee, and K. J. Kwon-Chung.** 1985. Brain abscess due to *Pseudallescheria boydii* associated with primary non-Hodgkin's lymphoma of the central nervous system: a case report and literature review. Rev. Infect. Dis. **7:**272–277.