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Susceptibilities of Clinical and Laboratory Isolates of *Blastomyces dermatitidis* to Ketoconazole, Itraconazole, and Fluconazole

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Eighteen isolates of *Blastomyces dermatitidis* were evaluated for their in vitro susceptibilities to ketoconazole, itraconazole, and fluconazole. The MIC ranges were 0.1 to 0.4 μg/ml for ketoconazole, ≤0.018 to 0.07 μg/ml for itraconazole, and 2.5 to 4.0 μg/ml for fluconazole. The ranges for the minimal lethal concentrations were 0.2 to 0.8 μg/ml for ketoconazole, ≤0.018 to 0.07 μg/ml for itraconazole, and 10 to 40 μg/ml for fluconazole. Itraconazole was the most active agent against *B. dermatitidis* in vitro, while fluconazole was the least active. These results correlate with the clinical efficacies noted to date with doses of these agents used to treat blastomycosis.

Blastomyces dermatitidis is a thermally dimorphic fungus that grows in moist, rich soil, primarily in wooded areas such as those bordering the Great Lakes and the Mississippi, Ohio, and St. Lawrence rivers. Infection with *B. dermatitidis*, initiated by the inhalation of mycelial-phase conidia into the lungs, results in symptoms that are nonspecific and frequently mimic other respiratory infections. A disseminated disease, with involvement of the lungs, skin, central nervous system, and other organ systems, is common in individuals without preexisting immunological deficiency (1, 3). Although administration of amphotericin B is effective therapy for all forms of the disease, in recent years the azole class of antifungal agents has been used with increasing frequency in the treatment of blastomycosis. Itraconazole administration is now considered the initial therapy for patients with mild to moderate disease who have no central nervous system infection. Cure rates of 90% have been reported with itraconazole doses of 200 to 400 mg per day (4). Ketoconazole has also been effective, with cure rates of 79 and 100% having been reported for patients treated with 400 and 800 mg per day, respectively (12). Unfortunately, the utility of this therapy is limited by its side effect profile (2). Fluconazole at doses of 200 to 400 mg appears less efficacious, with cure rates of 62 and 70%, respectively, having been reported (13). However, a recent study reported successful treatment in 89 and 85% of patients who received 400 and 800 mg, respectively (14). We compared the in vitro activities of ketoconazole, itraconazole, and fluconazole against 18 isolates of *B. dermatitidis* using a previously described macrobroth dilution susceptibility test (5, 9, 15).

B. dermatitidis 10225 was obtained from the American Type Culture Collection (Rockville, Md.). The remaining 17 clinical isolates were obtained from patients with blastomycosis who were treated at the University of Mississippi Medical Center (Jackson, Miss.). Ten of the isolates were obtained prior to the use of azoles for antifungal therapy. No patient received an azole prior to culture. The isolates were maintained in our laboratory at the University of Mississippi Medical Center in yeast form on brain heart infusion agar at 37°C and in mycelial form on brain heart infusion agar at 25°C. The antifungal agents tested were ketoconazole and itraconazole (Janssen Pharmaceutica, Titusville, N.J.) and fluconazole (Pfizer Central Research, Groton, Conn.). Ketoconazole was solubilized in 0.2 N HCl, itraconazole was solubilized in polyethylene glycol, and fluconazole was solubilized in distilled water. *B. dermatitidis* isolates were grown in mycelial phase on potato flakes agar at 25°C for 5 to 7 days (16). Tubes were overlaid with sterile distilled water, and conidia were harvested by gentle scraping. Inocula were standardized spectrophotometrically by adjusting turbidity to 95% transmittance at 530 nm. The resulting suspension contained 1×10^5 to 5×10^5 conidia/ml. Inocula were diluted 1:10 in Synthetic Amino Acid Medium-Fungal to a final concentration of 1×10^4 to 5×10^4 conidia/ml, and 0.9 ml was added to each tube for each drug concentration tested (0.1 ml of drug per tube). Inoculated tubes with growth in the absence of drug and uninoculated tubes were included for each isolate as positive and negative growth controls, respectively. Tubes were then incubated at 25°C until the growth tube was positive, defined as the first visual evidence of turbidity by comparison to the simultaneously incubated negative control. The MIC was defined as the first concentration for which no growth or a marked reduction in growth was evident in an experimental tube as contrasted to the drug-free, positive control tube containing Synthetic Amino Acid Medium-Fungal. A marked reduction was considered to be as defined in standard M27-A of the National Committee for Clinical Laboratory Standards, that is, for azoles a less stringent end point of slight turbidity is allowed that is above the MIC (11). The amount of allowable turbidity was estimated by dilution of 0.2 ml of drug-free control growth with 0.8 ml of medium, producing an 80% inhibition standard (6). Aliquots

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TABLE 1. In vitro activities of ketoconazole, itraconazole, and fluconazole against *B. dermatitidis* isolates[a]

Isolate	Ketoconazole				Itraconazole				Fluconazole			
	MIC		MLC		MIC		MLC		MIC		MLC	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
1	0.4	0.8	0.8	1.6	≤0.018	0.07	0.07	0.15	20	40	40	80
2	0.4	0.4	0.4	1.6	≤0.018	≤0.018	0.035	0.30	5	20	20	40
3	0.2	0.2	0.4	0.8	0.035	0.035	0.035	0.035	10	20	20	20
4	0.1	0.4	0.4	0.8	≤0.018	0.035	0.035	0.035	2.5	20	20	40
5[b]	0.2	0.2	0.8	0.8	≤0.018	≤0.018	≤0.018	0.035	40	80	80	>80
6	0.1	0.4	0.4	1.6	≤0.018	0.035	0.035	0.035	2.5	20	20	20
7	0.4	0.4	0.4	3.2	≤0.018	0.035	0.035	0.035	10	40	40	40
8	0.2	0.2	0.2	3.2	≤0.018	≤0.018	≤0.018	≤0.018	10	20	20	80
9	0.2	0.2	0.2	6.4	≤0.018	0.035	0.035	0.15	40	40	40	>80
10	0.2	0.2	0.2	1.6	≤0.018	≤0.018	≤0.018	≤0.018	5	10	10	40
11	0.4	0.4	0.4	6.4	≤0.018	0.035	0.07	0.15	20	40	40	>80
12[b]	0.2	0.4	0.4	0.8	≤0.018	≤0.018	≤0.018	≤0.018	10	40	40	80
13	0.4	0.4	0.4	3.2	≤0.018	≤0.018	≤0.018	0.07	10	20	20	80
14[b]	0.4	0.4	0.8	0.8	0.07	0.07	0.07	0.15	10	20	20	80
15	0.2	0.4	0.4	0.4	≤0.018	≤0.018	≤0.018	0.15	2.5	10	10	20
16[b]	0.4	0.8	0.8	0.8	0.035	0.035	0.035	0.035	40	40	40	80
17[b]	0.2	0.4	0.4	0.4	≤0.018	0.035	0.035	0.035	40	40	40	80
18[b]	0.4	0.8	0.8	0.8	≤0.018	0.035	0.035	0.035	80	80	80	80
Geometric mean	0.25	0.37	0.43	1.37	≤0.02	≤0.03	≤0.03	≤0.06	12.0	28.2	28.2	≥54.4

[a] MICs and MLCs are expressed in micrograms per milliliter.

[b] Isolate required 72 h of incubation prior to the initial MIC and MLC determinations. All other isolates had sufficient growth to read the first MICs and MLCs at 48 h.

of 100 μl from tubes with no evidence of growth were plated on Sabouraud dextrose agar for determination of minimum lethal concentrations (MLCs). Plates with five or fewer colonies were considered negative, and the MLC was defined as the first concentration with a negative subculture. A reference strain of *Paecilomyces variotii* 36257 (American Type Culture Collection, Rockville, Md.) was tested simultaneously with clinical isolates.

The in vitro activities of ketoconazole, itraconazole, and fluconazole against all 18 isolates are presented in Table 1. The majority of isolates (12 of 18) had sufficient in vitro growth to allow initial MIC and MLC determinations at 48 h of incubation. Six isolates had slower growth, and the initial susceptibility readings for these isolates were performed at 72 h (Table 1). After the initial reading (T1), all cultures were incubated for an additional 24 h, and MIC and MLC determinations were repeated (T2).

The MLCs of ketoconazole for two isolates (Table 1, isolates 9 and 11) were >5 μg/ml after extended incubation. Changes in the MICs of ketoconazole were minimal (≤fourfold rise) between T1 and T2. When the MLCs of ketoconazole were analyzed at T2 relative to these at T1, there was an increase of ≤4-fold for 12 isolates, an increase of 8-fold for 3 isolates, an increase of 16-fold for 2 isolates, and an increase of 32-fold for 1 isolate. The MICs at T2 were essentially the same as the MLCs at T1.

The highest MIC and MLC of itraconazole were only 0.07 μg/ml and 0.15 μg/ml, respectively. Little or no change in MIC (<fourfold increase for all isolates) was noted when the incubation time was extended. The maximum increase in MLC with the longer incubation period was only eightfold.

The MICs and MLCs were highest for fluconazole. The MICs at T2 were essentially the same as those at the first MLC reading. No isolates were considered susceptible by MLCs at T2.

Previous studies have reported serum ketoconazole and itraconazole concentrations that were above the MICs and MLCs shown here (7, 17, 20). In contrast, reported serum fluconazole concentrations above the MICs and the initial MLCs have not been consistently achieved, even at doses of 400 mg/day (19). In AIDS patients with cryptococcal meningitis, fluconazole doses of 800 to 1,000 mg/day achieved concentrations in serum of 42.47 ± 26.31 μg/ml (10). This result suggests that even doses of up to 1,000 mg/day do not consistently result in concentrations in serum that exceed the MICs and MLCs reported in our study.

While these in vitro results correlate with the clinical efficacies noted to date with these agents in the treatment of blastomycosis, in vitro susceptibilities do not necessarily reflect in vivo responses to therapy. Specifically, fluconazole at doses of ≤200 mg/day has been less effective than either ketoconazole (400 mg/day) or itraconazole (200 mg/day) (3, 4, 13). Our study demonstrates that itraconazole and ketoconazole have comparable in vitro activities, and both have superior in vitro activities compared to that of fluconazole. Itraconazole and ketoconazole at currently used doses consistently achieve concentrations in serum above the MICs and MLCs for *B. dermatitidis*, while fluconazole, even at doses exceeding 400 mg, does not.

Susceptibility studies were conducted employing the saprobic (mold) form of the fungus rather than the parasitic (yeast) form. This was done for two reasons: (1) the ease of preparation of conidial inocula and (2) previous testing accomplished in the Fungus Testing Laboratory had shown no differences between results obtained with either morphological form of the fungus (18). Other authors have noted that there are differences in susceptibility results for the parasitic form versus the saprobic form of *B. dermatitidis* (8). It is recognized that there is currently no standardization of the in vitro susceptibility testing of filamentous or dimorphic fungi. Investigations are currently under way via the auspices of the National Committee for Clinical Laboratory Testing Subcommittee on Antifungal Susceptibility Testing to develop consensus standards for molds; however, no present examination of dimorphic fungi

is being conducted. All such test results reflect the methods of testing and the known variabilities which may occur as test conditions differ between investigators. Hence, as with all antimicrobial testing (including standardized methods), in vitro test results do not always correlate with or reflect therapeutic outcomes in vivo.

In vitro test data may offer clinicians additional, potentially valuable, information upon which to base decisions regarding therapy. Clearly, prospective studies employing standardized methods of laboratory testing are needed to address these issues. It is hoped that data such as those presented here will assist in the development of such standardization for this particular dimorphic mycotic pathogen.

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