Susceptibility of Zygomycetes to Amphotericin B, Miconazole, and Ketoconazole

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Susceptibility to amphotericin B, miconazole, and ketoconazole was determined for 25 clinical isolates of zygomycetes in yeast nitrogen base broth and human serum. In yeast nitrogen base broth, 7 of 25 isolates were susceptible to an imidazole or amphotericin B. In serum, all were resistant to the imidazoles and inhibited by amphotericin B, the current drug of choice for infections caused by zygomycetes.

Human zygomycosis occurs as a sporadic infection in compromised hosts (5). Because of its rare and irregular occurrence and characteristically rapid progressive course, the effectiveness of antifungal chemotherapy in case studies is difficult to evaluate. Whether one antifungal agent is superior to another in the treatment of this infection is even more difficult to evaluate in humans. Mortality in the rhinocerebral form of zygomycosis remains high despite intensive amphotericin B therapy (1). However, in formation on the susceptibility of these organisms to amphotericin B is limited (8).

With the advent of the newer imidazoles, miconazole and ketoconazole (7), there is hope that these less toxic agents may offer an alternative to amphotericin B therapy for human zygomycosis (3). Hence, the susceptibility of clinical isolates of zygomycetes to amphotericin B, miconazole, and ketoconazole was defined, and the possibility of a beneficial interaction among these agents was explored.

All strains, including those in the genera Cunninghamella, Rhizopus, Absidia, and Mucor, were isolated from infected patients. Many were from the New York State Mycology Laboratory, the New York City Department of Health, and the U.S. Department of Agriculture. Isolates were grown and maintained on Sabouraud dextrose agar slants until tested.

Sabouraud dextrose agar slants were prepared by reconstitution in distilled water of powder purchased from Difco Laboratories, Detroit, Mich. Human serum (Microbiological Associates, Walkersville, Md.) containing no anticoagulants or preservatives was stored at −70°C to preserve complement activity until used. All tests were performed in sterile polyethylene tubes (12 by 75 mm; Falcon Plastics, Oxnard, Calif.).

Amphotericin B powder (867 μg/mg) was obtained from E. R. Squibb & Sons, Princeton, N.J. Miconazole and ketoconazole powders (>99% purity) were obtained from Janssen Pharmaceutical, New Brunswick, N.J. Amphotericin B was dissolved in N,N-dimethylformamide (Fisher Scientific Co., Springfield, N.J.) at 1,000 μg/ml, and the imidazoles were dissolved in dimethyl sulfoxide (Fisher Scientific) at 1,000 μg/ml and kept frozen at −70°C until used.

After 7 days of incubation on 10 ml of Sabouraud dextrose agar slants at 35°C, spores of each isolate were collected. Sterile distilled water (5 ml) was added to each slant, the fungal mat was teased with a wire probe, and the mycelia were then allowed to settle for 1 min. The spore suspension was collected by aspiration, washed three times by centrifugation, and resuspended in 10 ml of water. The final suspension used for inoculation was adjusted to 104 spores per ml.

The serum used was supplemented with a 0.1 volume of 10× YNB broth to ensure adequate nutrients to support fungal growth. The imidazole concentrations used in YNB broth and serum were 4, 2, 1, 0.2, and 0 μg/ml. The amphotericin B concentrations used were 2, 1, 0.5, 0.1, and 0 μg/ml. In addition, to test for polyene and
imidazole interactions, the same concentrations of imidazoles (4, 2, 1, 0.2, and 0 μg/ml) were prepared either in YNB broth or serum containing 0.1 μg of amphotericin B per ml, a concentration readily achievable in human serum.

Each dilution tube contained 10^6 test spores in 1 ml of test media. These dilution tubes were incubated at 35°C and examined visually after 48 and 72 h for the presence of fungal growth.

In YNB broth, most isolates were resistant to 4 μg of imidazole per ml. However, four of five isolates of Absidia were susceptible to 4 μg of ketoconazole per ml, and two of two isolates of Mucor were susceptible to both imidazoles (minimum inhibitory concentration, ≤ 4 μg/ml) (Table 1). One Cunninghamella bertholletiae isolate, one Rhizopus nigricans isolate, four Rhizopus arrhizus isolates, and one Rhizopus rhizopodiformis isolate were susceptible to human serum alone and did not exhibit growth up to 72 h in serum, whereas growth was easily detected in YNB broth at 48 h (Table 1). These data confirm the role of serum in host defenses against zygomycetes noted previously (4).

Our data collected for the drugs diluted in supplemented human serum differ from those for the drugs diluted in YNB broth. With no exceptions, all strains were resistant to the imidazoles (Table 1). Most strains were inhibited by 0.5 μg of amphotericin B per ml, a level readily achievable in human serum. No enhancement of imidazole activity was observed when 0.1 μg of amphotericin B per ml was added, suggesting that little if any benefit can be expected from the addition of an imidazole early during therapy with amphotericin B.

The application of in vitro susceptibility data for fungi to in vivo conditions of actual infection is difficult, as the minimum inhibitory concentration for the organism depends dramatically on the media used in the testing. The minimum inhibitory concentration of ketoconazole for Candida albicans can vary 1,000-fold, depending upon the concentration of serum in the culture media (2). Because of such a dependence of susceptibility results on the test media, in vitro data obtained under conditions which most resemble the in vivo conditions are desirable. Hence, susceptibility testing of drugs diluted in human serum with intact complement activity may be more appropriate than testing done with artificial culture media.

Our results indicate that all of the strains of zygomycetes tested were susceptible to amphotericin B in the presence of serum, whereas the imidazoles showed poor activity. Amphotericin B remains the drug of choice in the treatment of zygomycosis, and surgical intervention may remain instrumental in the recovery of patients.

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**LITERATURE CITED**


