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Combined In Vitro Effect of Amphotericin B and Rifampin on *Cryptococcus neoformans*†

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The combination of amphotericin B and rifampin was synergistic in vitro in both inhibiting and killing seven strains of *Cryptococcus neoformans* by the checkerboard microtitration technique.

The current anticryptococcal drug regimen of choice is the combination of amphotericin B and 5-fluorocytosine (3, 14). The combination of amphotericin B and rifampin has proven to be synergistic in vitro against a number of fungi, including *Candida* species (2, 5), *Histoplasma capsulatum* (9), *Coccidioides immitis* (12), and *Saccharomyces cerevisiae* (10). As yet, no studies on the combined effects of amphotericin B and rifampin have been reported for *Cryptococcus neoformans*. Because of the potential clinical use of this combination, an in vitro study was performed, utilizing a range of amphotericin B and rifampin concentrations which are clinically achievable.

Seven strains of *C. neoformans* were studied; six were clinical isolates from patients at Harbor-UCLA Medical Center and one was from the American Type Culture Collection, Rockville, Md. (strain ATCC 2526). All strains were vitalized from the lyophilized state and maintained on Sabouraud dextrose agar slants before testing. A small loopful of each strain was inoculated into yeast-nitrogen base broth supplemented with 0.15% L-asparagine and 1% glucose (adjusted to pH 7.0 with 8 N sodium hydroxide) and incubated at 37°C for 24 h. After incubation, each strain was standardized with a hemacytometer to a concentration of 4×10^5 organisms per ml of the yeast-nitrogen base broth, so that after the final inoculation into the test well, a concentration of 10^5 organisms per ml was achieved. The quantitative cultures of each strain revealed a range of 2.5×10^4 to 9.5×10^4 colony-forming units/ml. The final volume in each well was 0.2 ml.

The susceptibility testing with amphotericin B and rifampin was performed by a modified checkerboard microtitration technique (6). Serial twofold dilutions of amphotericin B (0.015 to 1.0 μg/ml) were tested with serial twofold dilutions of rifampin (0.06 to 64 μg/ml). Stock solutions of amphotericin B (1.10 mg/ml) were prepared from USP reference standard amphotericin B 0672-G (U.S. Pharmacopeial Convention, Bethesda, Md.) in dimethyl sulfoxide (J. T. Baker Chemical Co., Phillipsburg, N.J.); rifampin (32 mg/ml) (lot 601426; Calbiochem, San Diego, Calif.) was similarly dissolved in dimethyl sulfoxide. Both were stored at −20°C in the dark. The tests were performed in duplicate with each strain.

The minimum inhibitory concentration (MIC) was designated as the concentration in the first well having no visible turbidity (read with a microtiter reading device, Dynatech Laboratories, Inc., Alexandria, Va.) after incubation for 48 h at 37°C. The minimum cidal concentration (MCC) was designated as the concentration in the first well from which <3 colony-forming units were observed from a subculture of 50 μl of the test suspension placed with a microdiluter device (Dynatech Laboratories, Inc.) onto Sabouraud dextrose agar. If the MIC and the MCC differed between the duplicate plates, the higher concentration was utilized for analysis. The maximum discrepancy for the MIC and the MCC of amphotericin B in the presence or absence of rifampin was a twofold concentration difference; the maximum discrepancy for rifampin in the presence of amphotericin B was a fourfold concentration difference, which occurred in 5 of 14 experiments. Synergism was examined in relation to both the inhibitory and the bactericidal effects and was defined as the reduction of the respective MIC or MCC by at least one fourfold concentration of one antibiotic in the presence of the other.

The MICs and MCCs of amphotericin B for all seven strains of *C. neoformans* were between 0.25 and 0.5 μg/ml. None of the strains was

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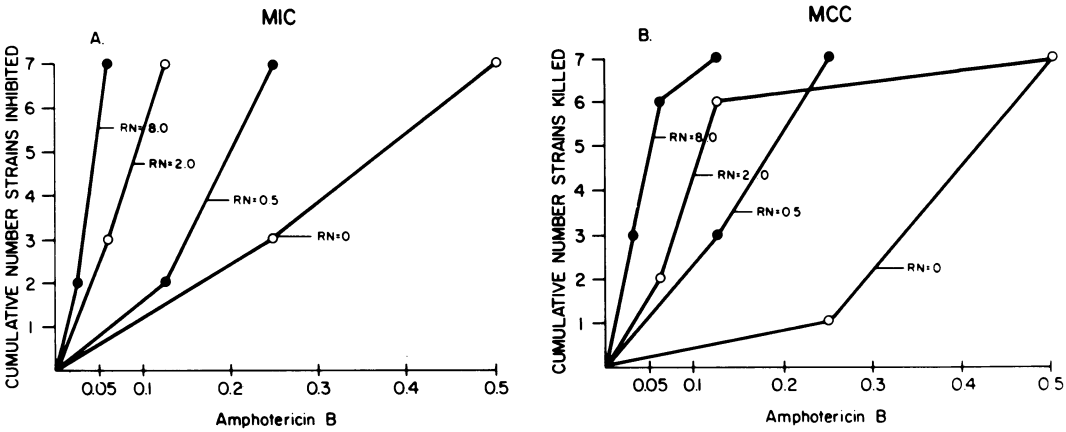

FIG. 1. *Cumulative number of strains inhibited (A) and killed (B) by combinations of amphotericin B and rifampin (RN). Concentrations of both drugs are given in micrograms per milliliter. Points represent actual concentrations tested.*

inhibited or killed by rifampin alone up to a concentration of 64 μg/ml. The combination of amphotericin B and rifampin was synergistic for all seven strains. The MIC and MCC of amphotericin B for all strains were progressively lowered by increasing concentrations of rifampin (Fig. 1A and B). All strains were inhibited by only 0.06 μg of amphotericin B per ml in the presence of 8 μg of rifampin per ml. All strains were killed by 0.125 μg of amphotericin B per ml in the presence of 8 μg of rifampin per ml. Similar but not as pronounced decreases in MIC and MCC of amphotericin B were observed with the addition of 2 μg of rifampin per ml. The concentrations of dimethyl sulfoxide present in the test wells were not inhibitory to any of the strains of *C. neoformans*.

The combination of amphotericin B and rifampin was synergistic at achievable serum levels of both drugs against all seven strains of *C. neoformans* tested (usual average peak serum concentrations for rifampin and amphotericin B, 7 μg/ml [15] and 1 to 2 μg/ml [11], respectively).

The concentration of rifampin achieved in cerebrospinal fluid has ranged from 0.2 to 1.06 μg/ml in patients with tuberculous meningitis (4, 13). No data are available for cerebrospinal fluid levels of rifampin in patients with cryptococcal or other fungal meningitides. However, rifampin has been found to attain high tissue levels, especially in the lungs (4). Studies on the efficacy of the combination of amphotericin B and rifampin in various forms of cryptococcosis are needed. This combination has been shown to be somewhat more efficacious than amphotericin B alone in the treatment of murine aspergillosis (1), histoplasmosis (8), and blastomycosis (8), but not in murine coccidioidomycosis (7).

Although only seven strains were tested, the reductions of the inhibitory and cidal concentrations of the two drugs were consistent and striking.

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