In Vitro Inhibition of *Coccidioides Immitis* Strains with Amphotericin B Plus Rifampin

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Rifampin was shown in vitro to decrease the amphotericin B minimal inhibitory concentrations of all of 10 strains of *Coccidioides immitis* tested. These decreases ranged from two- to fourfold and occurred with rifampin concentrations of 10 to 40 µg/ml. Rifampin alone was without effect.

At the present time, amphotericin B is the only therapeutic agent available for the treatment of coccidioidomycosis. In the localized pulmonary form of the disease no specific therapy is used, because the toxicity associated with the antibiotic administration seems unwarranted in this self-limited process. In the chronic pulmonary infection with cavitation, amphotericin B and surgical resection are frequently necessary (5, 11). In the disseminated disease, a mortality rate of about one-half still remains despite antifungal therapy and further, when the central nervous system is involved, cures are extremely rare (8).

Recently, Medoff and co-workers (6, 7) have shown an interaction of amphotericin B with rifampin in the inhibition of the yeast-phase of *Histoplasma capsulatum*, and with 5-fluorcytosine in the inhibition of candida, cryptococcus, and saccharomyces strains.

The present study explores the interaction of amphotericin B with rifampin in the in vitro inhibition of *Coccidioides immitis*.

MATERIALS AND METHODS

Coccidioides immitis strains. Ten strains of *C. immitis* were tested for susceptibility of antibiotics. Three strains (Kr, Co, and Si) had been maintained in the laboratory for varying periods of time on Sabouraud agar. The remaining seven strains were recent clinical isolates.

Arthrospore-mycelial suspensions. Strains of *C. immitis* were cultured in screw-top tube slants of Sabouraud agar for 6 to 12 weeks at room temperature. The growth was harvested in 0.85% saline and plate counts were made on Sabouraud agar plates incubated at 37 C. For susceptibility testing, the suspensions were adjusted to approximately 50 to 200 colony-forming units per 0.1 ml in 0.85% saline on the basis of the plate counts. The suspensions were stored at 4 C prior to use, usually for a period of 1 to 2 weeks.

Antibiotic preparations. Amphotericin B was pre-pared from the material packaged for clinical intravenous use (Fungizone Intravenous; Squibb) by reconstitution and dilution in water. Preliminary studies using amphotericin B standard dissolved initially in ME_2SO and then in phosphate-buffered saline (pH 8.0) showed no significant differences in susceptibility test results.

Rifampin was prepared by dissolving the contents of a 300-mg capsule of the drug (Rimactane; CIBA-Geigy) in 10 ml of absolute alcohol. Further dilutions were then made in water.

Susceptibility testing. Antibiotic-susceptibility testing was done in 1-ounce screw-capped glass prescription bottles. Sterilized melted Sabouraud agar (4.5 ml) and 0.5 ml of antibiotic or antibiotic combinations were dispensed and the bottles were placed on their sides to allow the agar to solidify. The arthrospore-mycelial suspension (0.1 ml) described above was added and the bottles were incubated at 37 C for 3 to 5 days. Counts were made of the colonies when they were just visable to the unaided eye. Each concentration of antibiotic was tested in duplicate bottles. The minimal inhibitory concentration (MIC) was determined on the basis of complete absence of growth.

RESULTS

The MICs of amphotericin B for the 10 strains of *C. immitis* varied from 0.8 to 3.2 µg/ml (Table 1).

Rifampin alone was tested against the 10 strains in concentrations up to 40 µg/ml and in no case was there any inhibition of growth (not shown in Table 1).

The MICs of amphotericin B in the presence of varying concentrations of rifampin were then assayed (Table 1). In all 10 strains, rifmapin decreased the MIC of amphotericin B. These decreases were twofold in five strains (Kr, Ma, Mc, Sy, Ro) and fourfold in the remaining five strains. An effect was produced by a rifampin concentration of as little as 10 µg/ml in five of

TABLE 1. *Effect of rifampin on the amphotericin B MICs of 10 strains of Coccidioides immitis* .

Strain	Rifampin concn (µg/ml)[a]				
	0	5	10	20	40
Kr	0.8	0.8	0.8	0.8	0.4
Da	1.6	1.6	1.6	0.8	0.4
Ma	1.6	1.6	1.6	0.8	0.8
Mc	1.6	1.6	0.8	0.8	0.8
Co	1.6	1.6	1.6	0.8	0.4
Si	1.6	1.6	0.8	0.8	0.4
In	3.2	3.2	0.8	0.8	0.8
La	3.2	3.2	1.6	1.6	0.8
Sy	3.2	3.2	1.6	1.6	1.6
Ro	3.2	1.6	3.2	1.6	1.6

[a] Data in table are the amphotericin B MICs (µg/ml) in the presence of the indicated concentrations of rifampin.

the ten strains (Mc, Si, In, La, Sy), whereas the other five strains required 20 to 40 µg of rifampin per ml to influence the amphotericin B MICs.

DISCUSSION

The amphotericin B MICs for all of the 10 strains of *C. immitis* tested were decreased two- to fourfold by rifampin. Rifampin, tested in concentrations up to 40 µg, had no inhibitory effect when tested alone against any of the strains.

In this study, the combined effect of rifampin and amphotericin B was shown directly by inhibition of colonial growth. In the study by Kobayashi et al. (6), the combined effect of these two drugs against the yeast-phase of *H. capsulatum* was assayed on the basis of decreases in tritiated guanine uptake into ribonucleic acid. In addition, however, they state that similar results were obtained by the colony count method.

It is of course unknown whether the combined effect of amphotericin B and rifampin would be useful in the treatment of patients with coccidioidomycosis. This question awaits appropriate animal and then controlled clinical trials. Two notes of caution should be considered in evaluating such a program. First, it was demonstrated by both in vitro and in vivo methods that rifampin inhibits manifestations of the cell-mediated immune reaction (4). As this immune response is central to the defense against fungal infections its suppression would adversely affect recovery. However, in disseminated coccidioidomycosis, specific cell-mediated immunity is defective and so the rifampin effect might not be further detrimen-

tal. Second, the development of resistance to rifampin has proved a problem in the treatment of certain bacterial infections (3) and such might also arise during combined therapy of coccidioidomycosis with rifampin and amphotericin B.

The concentrations of rifampin (10 to 40 µg/ml) shown here to decrease the amphotericin B MICs are similar to those obtained in patients given the usual therapeutic doses. Following a single 600-mg oral rifampin dose, peak serum levels range from 5.5 to 32 µg/ml in one study (9) and 3.4 to 16.9 µg/ml in another (10). Thus, serum concentrations of rifampin shown in vitro to be effective would be achievable in patients.

In contrast, cerebrospinal fluid levels of rifampin are low after oral therapy, even in the presence of inflammation, and accordingly, central nervous system infection would pose an unique problem (2). It should be noted that such is also the case with amphotericin B (1), and administration of this antifungal agent directly into the cerebrospinal fluid is essential in the treatment of coccidioidal meningitis. Possibly rifampin could be admixed with amphotericin B for intrathecal administration.

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