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Comparison of Fluconazole and Amphotericin B in Treating Histoplasmosis in Immunosuppressed Mice

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Fluconazole (UK-49,858) was compared with amphotericin B in treating histoplasmosis in female AKR mice immunosuppressed with either cyclophosphamide or cortisone. Both drugs protected animals from a lethal challenge with *Histoplasma capsulatum*, but neither regimen resulted in cures since viable organisms were cultured from spleens of survivors.

We have previously shown that the triazole fluconazole (UK-49,858) given orally was as effective as amphotericin B given by intraperitoneal (i.p.) injection in treating normal AKR and C57BL/6 mice infected with *Histoplasma capsulatum* (7). Others have reported that fluconazole was more effective than the imidazole ketoconazole in treating normal mice (12, 14) and rats (12, 13) infected systemically with *Candida albicans*. Similar results were obtained with mice intracerebrally infected with *Cryptococcus neoformans* (10). Concern with the increasing morbidity and mortality of systemic mycoses in the immunocompromised host (2, 4, 8, 9) has prompted investigators to test newer drug regimens in *C. albicans*-infected mice immunosuppressed by treatment with cyclophosphamide (12) or dexamethasone (14). Richardson et al. (12) found fluconazole to be at least 50 times more potent than ketoconazole in mice made neutropenic with cyclophosphamide and infected with *C. albicans*. In another study, Troke et al. (14) reported that fluconazole was more than 20 times as effective as ketoconazole in *C. albicans*-infected mice that were immunocompromised by daily administration of dexamethasone. Fluconazole is an attractive antifungal agent in that (i) it is less toxic than amphotericin B, (ii) it is renally excreted, (iii) it readily penetrates cerebral spinal fluid, and (iv) it has a high serum half-life that can be maintained by daily oral administration (5). In the present study, we evaluated the efficacy of fluconazole and amphotericin B in the treatment of histoplasmosis in mice immunosuppressed with either cyclophosphamide or cortisone.

For these studies, 6- to 8-week-old female AKR mice (average weight before beginning the experiment was 22 g) were purchased from Charles River Mouse Farms, Wilmington, Mass. All mice were housed and held for 1 week before experimentation. They were fed Rodent Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) and given water ad libitum. Groups of animals were rendered leukopenic by treatment with cyclophosphamide (Cytoxan; Meade Johnson and Co., Evansville, Ind.). Leukopenia was induced by a modification of the procedure of Cryz et al. (3). Briefly, four successive i.p. injections of 100 mg of cyclophosphamide per kg in 0.2 ml of phosphate-buffered saline (pH 7.4) were given 6, 4, 2, and 0 days before intravenous infection with *H. capsulatum*. At various intervals, leukopenia was quantitated by collecting a sample of blood directly from the tail vein into a leukocyte pipette and immediately mixing it with 9 volumes of leukocyte diluent (Unopette; Becton Dickinson and Co., Rutherford, N.J.) and counting according to the Unopette technique (1).

After the final injection of cyclophosphamide, there were fewer than 250 leukocytes per mm^3, and the leukocyte count remained at this level for at least 4 additional days. The mice were infected intravenously with 5×10^5 cells of *H. capsulatum* G217B (ATCC 26032) within 1 h after the last cyclophosphamide injection according to procedures previously described (6, 7). At this infecting dose, the cyclophosphamide-induced leukopenic control mice that were sham treated had a median survival time of 7 days and were all dead by day 9. The 50% lethal dose (LD_{50}) of *H. capsulatum*, calculated by the method of Reed and Muench (11), for the cyclophosphamide-treated mice was 5×10^4 organisms per mouse, compared with a value of 4×10^5 organisms per mouse in normal mice (7). The drug dosages for fluconazole were tested in twofold increments between 1.42 and 90.91 mg/kg per mouse, given twice a day in 0.5-ml volumes orally for a total of 6 consecutive days. For amphotericin B, the range of dosages tested was in twofold increments between 0.85 and 2.73 mg/kg per mouse, given every other day for a total of six injections by the i.p. route. Each dosage of drug was tested in groups of 10 animals, and the experiments were repeated at least three times with essentially the same results. The doses were calculated on the basis of milligrams per kilogram of average body weight of the animals at the start of therapy. According to procedures previously described (7), both fluconazole, given by gavage twice daily in 0.5-ml volumes for a total of 6 consecutive days, and amphotericin B, administered by i.p. injections on alternate days for a total of six injections begun 24 h after infection, protected the cyclophosphamide-induced leukopenic mice from fatal histoplasmosis (LD_{100} dose of 5×10^5 organisms per mouse). The dosage of drugs that protected 50% of the infected mice (PD_{50}) was 9.6 ± 2.9 mg/kg per day of fluconazole and 0.54 ± 0.1 mg/kg per day of amphotericin B (Table 1). With this drug regimen, the PD_{90} for fluconazole was 14.4 mg/kg per day and the PD_{10} was 6.4 mg/kg per day; for amphotericin B, the PD_{90} was 0.78 mg/kg per day and the PD_{20} was 0.31 mg/kg per day.

We also studied the efficacy of fluconazole and amphotericin B in a cortisone-induced immunosuppressed model of murine histoplasmosis. For these studies, groups of 10 AKR mice, weighing an average of 22 g before the experiment was begun, were injected subcutaneously in the nape of the neck with 5-mg doses of hydrocortisone (Elkins-Sinn, Inc.,

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TABLE 1. Efficacy of fluconazole and amphotericin B in treating histoplasmosis in the immunosuppressed AKR mouse model[a]

Immunosuppression treatment	*H. capsulatum* infecting dose (organisms/mouse)		PD_{50}[b] (mg/kg per day)	
	LD_{50}	LD_{100}	Fluconazole[c]	Amphotericin B[d]
None	4×10^5	5×10^6	6.2 ± 1.0[e]	1.8 ± 0.6[e]
Cyclophosphamide (100 mg/kg × 4)	5×10^4	5×10^5	9.6 ± 2.9	0.54 ± 0.1
Hydrocortisone (4 mg subcutaneously × 2)	5×10^4	1.3×10^5	7.2 ± 3.1	0.42 ± 0.2

[a] All experiments were done at least three times.
[b] PD_{50}, Dose of drug preventing 50% of deaths of mice infected with an LD_{100} inoculum of *H. capsulatum* (± 95% confidence interval).
[c] Administered twice daily for 6 consecutive days beginning 24 h after infection.
[d] Administered by i.p. injection once daily on alternate days beginning 24 h after infection for a total of six injections.
[e] Data taken from reference 7.

Cherry Hill, N.J.) on 4 and 0 days before intravenous infection with *H. capsulatum* G217B (see above for details of infections). The control mice receiving cortisone and sham treatment were all dead by day 10 after infection. The LD_{50} of *H. capsulatum* in the cortisone-treated mice was 5×10^4 organisms per mouse. Both fluconazole and amphotericin B given by the same dosage schedule described in the cyclophosphamide study to groups of 10 animals prolonged the survival of cortisone-immunosuppressed animals given a lethal challenge of *H. capsulatum* (LD_{100} was 1.3×10^5 organisms per mouse; Table 1). The PD_{50} values for both drugs were in the same range of those observed when these drugs were used to treat histoplasmosis in cyclophosphamide-induced leukopenic mice (7.2 ± 3.1 mg/kg per day for fluconazole and 0.42 ± 0.2 mg/kg per day for amphotericin B; Table 1). For mice immunosuppressed with cortisone, the PD_{90} for fluconazole was 13.2 mg/kg per day and the PD_{10} was 3.8 mg/kg per day; for amphotericin B, the PD_{90} was 0.86 mg/kg per day and the PD_{10} was 0.36 mg/kg per day.

Both the cyclophosphamide and hydrocortisone led to immunosuppression as evidenced by the approximately 10-fold decrease in the LD_{50} levels after both treatments (Table 1). However, despite the immunosuppression, the PD_{50} of fluconazole was unchanged and the PD_{50} of amphotericin B actually decreased compared with the levels previously found in the treatment of murine histoplasmosis of nonimmunosuppressed mice (7). In the latter study (7) and the present report, neither drug was curative, since viable *H. capsulatum* organisms were cultured from spleens removed from surviving mice sacrificed 4 weeks after infection.

Recently, it was reported (J. R. Graybill, J. Ahrens, and E. Palou, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 920, 1985) that amphotericin B and fluconazole but not ketoconazole significantly increased survival of ICR mice infected with *H. capsulatum*. Those authors further reported that all three drugs prolonged survival and lowered colony counts and antigen concentrations in *H. capsulatum*-infected athymic (*nu/nu*) mice. Our study extends these reports. We found that fluconazole given orally was effective in the treatment of murine histoplasmosis in animals that have been immunosuppressed with either cortisone or cyclophosphamide, but not as effective as amphotericin B given parenterally.

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