

Efficacy of Amphotericin B or Itraconazole in a Murine Model of Central Nervous System *Aspergillus* Infection

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Given the greater than 90% lethality of clinical central nervous system (CNS) aspergillosis despite current therapies, there is a need for an animal model to study therapeutic strategies. We previously established a model of CNS aspergillosis by intracerebral infection and report here the results of treatment with the two therapies with the greatest clinical experience, i.e., treatments with amphotericin B (AMB) and itraconazole (ITZ). Mice were given cyclophosphamide to produce pancytopenia. AMB was given intraperitoneally (i.p.; 3 mg/kg of body weight) or intravenously (i.v.; 0.8 mg/kg) once daily. ITZ in cyclodextrin was given by gavage once daily at a dose of 100 mg/kg or twice daily at 50 mg/kg. Treatments were started at day 1 postinfection and given for 10 days. At day 15, survivors were euthanized. Ninety percent of the mice given no treatment died by day 6, and 100% died by day 10. Mice treated with AMB either i.p. or i.v. had 40% survival. Mice treated with ITZ either once or twice per day had a median survival time of 10 days, compared with 4 days for control animals, but a survival rate of only 10%. AMB and ITZ prolonged survival (P , <0.0001 to <0.05) compared with controls. Brains from surviving mice had CFU of *Aspergillus fumigatus*. This model can be used to compare newer antifungals and to study combination therapy or immunotherapy to find better therapeutic alternatives.

The central nervous system (CNS) is the most common site of hematogenous spread of *Aspergillus* species (2). Despite current antifungal therapy, *Aspergillus* infection in the CNS is almost always lethal, with mortality exceeding 90% (2). It is difficult to study treatment strategies against CNS aspergillosis in human trials given the limited numbers of patients with the disease and the associated mortality. However, there is a clear need to address this issue, as the number of susceptible hosts with *Aspergillus* infections is increasing (4). For these reasons, we developed a murine model of CNS aspergillosis to study the disease and treatment strategies (1).

In this study, we evaluated the efficacies of itraconazole (ITZ) and conventional deoxycholate-formulated amphotericin B (AMB) in a model of murine CNS aspergillosis.

Inocula. An isolate of *Aspergillus fumigatus*, AF-10, which was cultured from a patient with pulmonary aspergillosis and stored at the California Institute for Medical Research was used. The isolate was incubated on potato dextrose agar plates at 35°C for 48 h to form conidia. The conidia were extracted and filtered through sterile gauze. The conidia were then stored in a solution of 0.05% Tween 80 in normal saline at 4°C and were used within 1 month. Three days prior to infection, dilutions of the suspension were added to potato dextrose agar plates. The number of CFU of the conidial solution was determined 1 day prior to infection, and the solution was diluted

with 0.05% Tween 80 in normal saline to a concentration of 10^8 viable conidia/ml.

Mice. Five-week-old male CD-1 mice were purchased from Charles River Laboratories, Wilmington, Mass. Mice were used within 3 days of arrival and weighed an average of 25 g on the day of infection. Animals were housed five per cage and provided sterilized food and acidified water ad libitum. Treatment and control groups consisted of 10 mice each.

Immunosuppression. Mice were given cyclophosphamide intraperitoneally (i.p.) at a dose of 200 mg/kg of body weight beginning 2 days prior to infection and then every 5 days until euthanasia. Pancytopenia was documented by peripheral white blood cell counts beginning on the day of infection and throughout the experimental period (1).

Infection. Mice were anesthetized with methoxyflurane inhalation. A total of 5×10^6 conidia in a volume of 50 μ l was inoculated intracerebrally at a point midline on the cranium, 4 to 5 mm posterior to the eyes (1). A 27-gauge disposable needle was used to deliver the inoculum to a depth of 2 to 3 mm. Mice were fully recovered within 5 min of the procedure, and there were no deaths as a result of the inoculation procedure.

Drugs. AMB, a colloidal suspension in deoxycholate (Pharma-Tek, Huntington, N.Y.), was reconstituted per the manufacturer's instructions, diluted in 5% dextrose in water (D5W), and stored frozen, protected from light. Final concentrations were prepared in D5W on the first day of treatment. ITZ powder (Janssen, Beerse, Belgium, and Ortho Biotech, Raritan, N.J.) was prepared as an oral solution in hydroxypropyl- β -cyclodextrin (CDX) as previously described (3).

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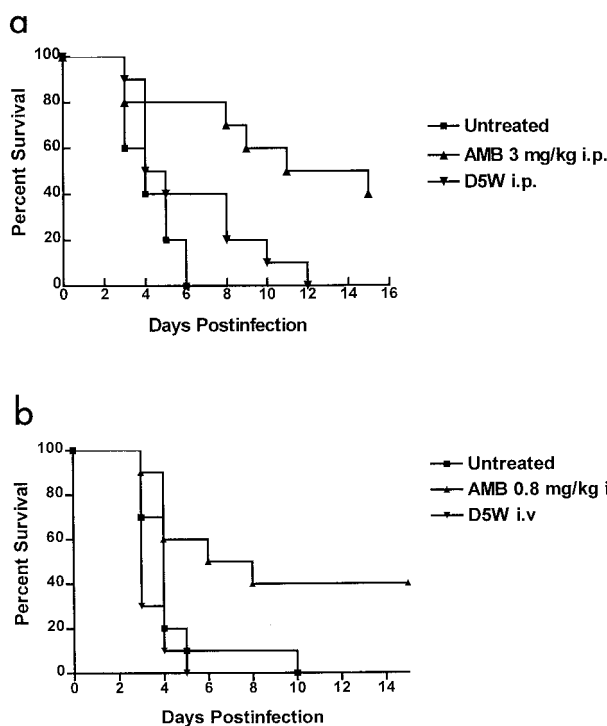


FIG. 1. Survival of groups of 10 male 5-week-old immunosuppressed CD-1 mice infected intracerebrally with *A. fumigatus* and left untreated, given D5W, or treated with AMB at a dose of 3 mg/kg i.p. (a) or 0.8 mg/kg i.v. (b). Treatments were started on day 1 postinfection and continued for 10 days.

On repeated in vitro testing (5), the MIC and minimum fungicidal concentration of AMB for AF-10 were 1 and 2 $\mu\text{g/ml}$ and those of ITZ were 3.13 and 6.25 $\mu\text{g/ml}$, respectively.

Treatment. All treatments began 24 h after infection and continued daily for 10 days. Mice treated with AMB i.p. received a dose of 3 mg/kg of body weight/day in 0.2 ml of diluent (D5W), and mice treated with AMB i.v. received 0.8 mg/kg/day in 0.2 ml of diluent. Controls consisted of untreated mice and those given 0.2 ml of diluent either i.p. or i.v. to correspond to the treatment route. ITZ at either 100 mg/kg once a day (QD) or 50 mg/kg twice a day (BID) was given by gavage in 0.1 ml of CDX solution prepared the day prior to treatment. Control animals were left untreated or given 0.1 ml of CDX solution by gavage. Earlier studies in our laboratories have suggested some toxicities with higher doses of these drugs administered by these routes.

Survival studies. Cages were inspected twice daily. All animals were observed for 4 days after the end of treatment, for a total period postinfection of 15 days. Mice surviving to day 15 were euthanatized using CO_2 anoxia. The brains were removed, homogenized in sterile saline with a Tissuizer (Tekmar, Cincinnati, Ohio), and further diluted in saline. Homogenates were cultured on plates containing Sabouraud's dextrose agar and 50 mg of chloramphenicol/liter and incubated at 35°C for 48 h. Colonies were counted to determine the fungal burdens remaining in the entire organ. Plates containing no colonies were kept for 5 days and reexamined before being discarded.

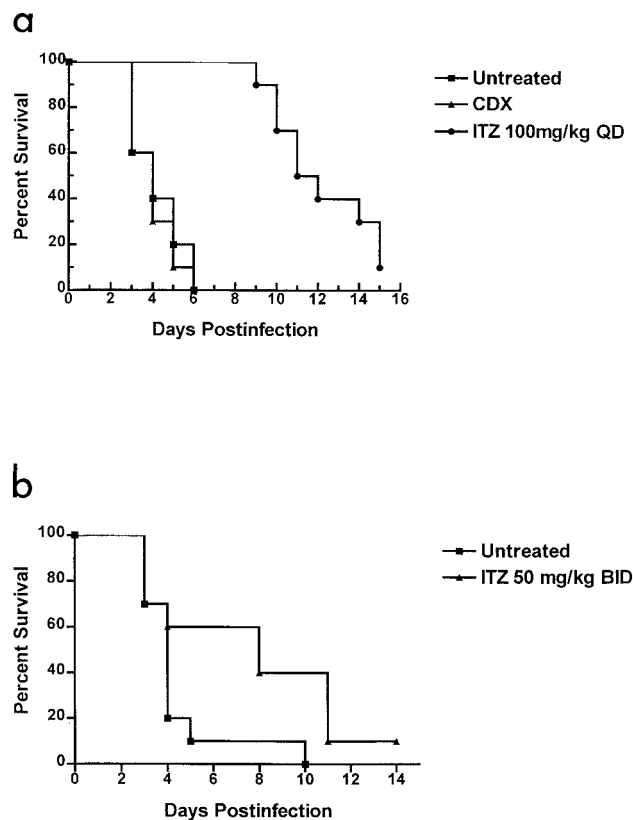


FIG. 2. Survival of groups of 10 male 5-week-old immunosuppressed CD-1 mice infected intracerebrally with *A. fumigatus* and left untreated, given CDX, or treated with ITZ orally at a dose of 100 mg/kg QD (a) or 50 mg/kg BID (b). Treatments were started on day 1 postinfection and continued for 10 days.

Histopathology. Histopathologic examination of survivors from some replicate experiments was performed as previously described (1).

Statistics. Survival was analyzed with the Mantel-Haenszel log rank test using Prism software (version 3.02; GraphPad Software, San Diego, Calif.). Organ burdens were converted to \log_{10} for comparison using a Mann-Whitney U test.

AMB therapy. Figure 1a illustrates the survival curves for mice treated with AMB i.p. versus those for the control animals. Survival of the AMB-treated group was greater than that of the untreated group as well as that of the group treated with D5W ($P = 0.0007$ and 0.014 , respectively). Four of 10 mice treated with AMB survived until day 15, compared with none in the control groups. Similar results were obtained in two additional experiments (data not shown). Treatment with AMB i.v. and that with AMB i.p. gave similar results (Fig. 1b). Mice treated with AMB i.v. survived longer than those given no treatment or those given D5W i.v. ($P = 0.018$ and 0.0011 , respectively). No control animals survived through day 15, compared with 4 of 10 mice treated with AMB.

All euthanatized mice from treatment groups, except one mouse treated with AMB i.p., had CFU in brain tissue. Mice treated with AMB i.p. ($n = 4$) had a mean \log_{10} CFU of 1.44, and those treated i.v. ($n = 4$) had a mean \log_{10} CFU of 2.66. Histopathological examination, in replicate experiments, of

surviving mice treated with AMB revealed brains with inflammation and meningitis, which were milder than what was previously described in untreated animals (1) and without the focal abscesses or cerebritis previously described (1); kidneys showed no evidence of infection, unlike the dissemination described previously (1).

ITZ therapy. Figure 2a shows the survival of mice treated once daily with ITZ in comparison with that of control animals. ITZ-treated mice survived longer than untreated or CDX-treated mice ($P < 0.0001$ for both comparisons). At day 15, only one mouse in the ITZ-treated group was alive. Figure 2b shows that mice treated with ITZ BID survived significantly longer than untreated control mice ($P = 0.041$). ITZ QD and ITZ BID gave similar results (median survival, 12 versus 8 days, respectively). Both mice that survived until day 15 in the ITZ treatment groups had CFU in brain tissue. Indistinguishable results were seen in two additional experiments, in which the results with ITZ administered QD were compared directly with the results with AMB administered i.p. (data not shown). Histopathology of brain and kidneys from one surviving ITZ-treated mouse demonstrated the presence of scattered lymphocytes in both organs.

The treatment studies described here represent the first evaluation of therapies against *Aspergillus* infections in a new murine model of CNS aspergillosis. The two drugs gave similar results. Although these two antifungals did prolong survival, they did not successfully eradicate the infection, as demonstrated by survival rates of only 10 to 40% at day 15 and the

presence of *Aspergillus* in brain tissue. These results are consistent with current experience in patients, in whom therapy with AMB or ITZ is often not successful at stopping progression of the disease or eradicating the infection (2). The results with this model would not suggest that one of these therapeutic agents is preferred for *Aspergillus* infection at this site.

This study demonstrates the potential use of this murine model (1) for evaluating therapies against CNS aspergillosis. This model offers the potential to evaluate both prolongation of survival and effect on residual infection by examination of fungal burden and histopathology in brain tissue. This could provide an opportunity to study new agents and new approaches to the treatment of *Aspergillus* infections in the CNS, including combination therapy and immunotherapy.

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