ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2006, p. 113–120 Vol. 50, No. 1
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.1.113–120.2006


# Efficacy of Amphotericin B in Combination with Flucytosine against Flucytosine-Susceptible or Flucytosine-Resistant Isolates of *Cryptococcus neoformans* during Disseminated Murine Cryptococcosis

Patrick Schwarz,[1] Françoise Dromer,[1] Olivier Lortholary,[1,2] and Eric Dannaoui[1,3]*

*Centre National de Référence Mycologie et Antifongiques, Unité de Mycologie Moléculaire, CNRS FRE2849, Institut Pasteur, 75724 Paris Cedex 15, France[1]; Université Paris Descartes, Faculté de Médecine, AP-HP, Hôpital Necker-Enfants-Malades, Service des Maladies Infectieuses et Tropicales, 75743 Paris Cedex 15, France[2]; and Université Paris Descartes, Faculté de Médecine, AP-HP, Hôpital Européen Georges Pompidou, Unité de Parasitologie-Mycologie, 75015 Paris, France[3]*

Received 20 June 2005/Returned for modification 28 August 2005/Accepted 11 October 2005

**Whether or not flucytosine should be administered to patients infected with *Cryptococcus neoformans* isolates found to be resistant to flucytosine in vitro remains a controversial issue. Thus, the efficacy of amphotericin B and flucytosine in combination was investigated by mortality and fungal burden studies in a murine model of disseminated cryptococcosis using two clinical isolates of *Cryptococcus neoformans*, one susceptible and one resistant (i.e., 64 μg/ml) to flucytosine. Amphotericin B was given intraperitoneally at 0.25 or 0.5 mg/kg/day, while flucytosine was given at 100 or 250 mg/kg/day orally. Treatment was started 24 h or day 6 after inoculation and continued for 5 days in fungal burden and mortality studies, respectively. The combination of amphotericin B at 0.5 mg/kg/day and flucytosine at 250 mg/kg/day was significantly more effective than monotherapies for reducing fungal burden in brain, spleen, and lungs after infection by the flucytosine-susceptible isolate and in brain and spleen for the flucytosine-resistant isolate. For the flucytosine-resistant isolate, the combination of amphotericin B at 0.5 mg/kg/day with flucytosine at 100 mg/kg/day was significantly better than monotherapies for reducing the fungal burden in the brain. Survival obtained after the combination of amphotericin B at 0.5 mg/kg/day and flucytosine at 250 mg/kg/day increased compared to that obtained with monotherapies for both isolates, but the difference was statistically significant only for the flucytosine-susceptible isolate. Antagonism was never observed. This study demonstrates the beneficial effect of the addition of flucytosine to amphotericin B against experimental disseminated cryptococcal infection even when the *C. neoformans* isolate is resistant to flucytosine.**

Over the past decades, the frequency of systemic fungal infections has increased rapidly. *Cryptococcus neoformans* infections remain a major problem in immunocompromised patients with cellular immune deficiency, especially those with AIDS (22). Due to the introduction of highly active antiretroviral therapy in Western countries, the incidence of cryptococcosis has decreased in human immunodeficiency virus (HIV)-infected patients with AIDS (9, 21), but it is still associated with early high mortality (O. Lortholary, C. Droz, K. Sitbon, V. Zeller, S. Neuville, M. Alvarez, A. Boisbieux, F. Botterel, P. Dellamonica, F. Dromer, and G. Chêne, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1752, 2003). Recommended initial therapy for disseminated cryptococcal infections is amphotericin B (AMB), ideally with flucytosine (FC) (28). In 1979, Bennett et al. demonstrated the higher efficacy of AMB combined with FC in HIV-negative patients with cryptococcal meningitis than of AMB alone (2). In HIV-positive patients, the combination of AMB and FC therapy for 2 weeks has been shown to be independently associated with negative culture of cerebrospinal fluid at week 2 (32). Moreover, patients who received FC associated with AMB had a lower risk of relapse than those who received other primary treatment regimens (27). In addition, a recent study performed in Thailand demonstrated that this combination was the most fungicidal one for AIDS-associated cryptococcosis (4). These results underline the importance of antifungal combinations in cryptococcal infection. In patients infected with a FC-resistant *C. neoformans* isolate, the combination is generally not advocated, and FC is usually withdrawn. Indeed, it is assumed that the combination of AMB and FC is not beneficial in cases of infection with an FC-resistant isolate (14), since FC hematotoxicity may be increased by the subsequent renal insufficiency related to AMB. Nevertheless, there are no clinical data and rare experimental data (11) to support such opinions. The aim of the present study was to compare the efficacy of AMB in combination with FC against FC-susceptible or FC-resistant isolates of *C. neoformans* in a model of disseminated cryptococcosis in mice.

## MATERIALS AND METHODS

**Organisms.** From our collection of *C. neoformans* isolates maintained at the National Reference Center for Mycoses and Antifungals, two clinical strains isolated from the cerebrospinal fluid of HIV-positive patients with cryptococcal meningitis were selected for this study. These strains were chosen based on their susceptibility to FC and included one FC-susceptible isolate (isolate 2000/126; MIC = 4 μg/ml) and one FC-resistant isolate (isolate 1998/673; MIC = 64 μg/ml). The MIC of AMB was 1 μg/ml for both isolates. In vitro interactions between AMB and FC were found by checkerboard assay to be synergistic for both the FC-resistant and FC-susceptible isolates with fractional inhibitory con-

* Corresponding author. Mailing address: Centre National de Référence Mycologie et Antifongiques, Unité de Mycologie Moléculaire, Institut Pasteur, CNRS FRE2849, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France. Phone: 33 1 40 61 32 50. Fax: 33 1 45 68 84 20. E-mail: dannaoui@pasteur.fr.

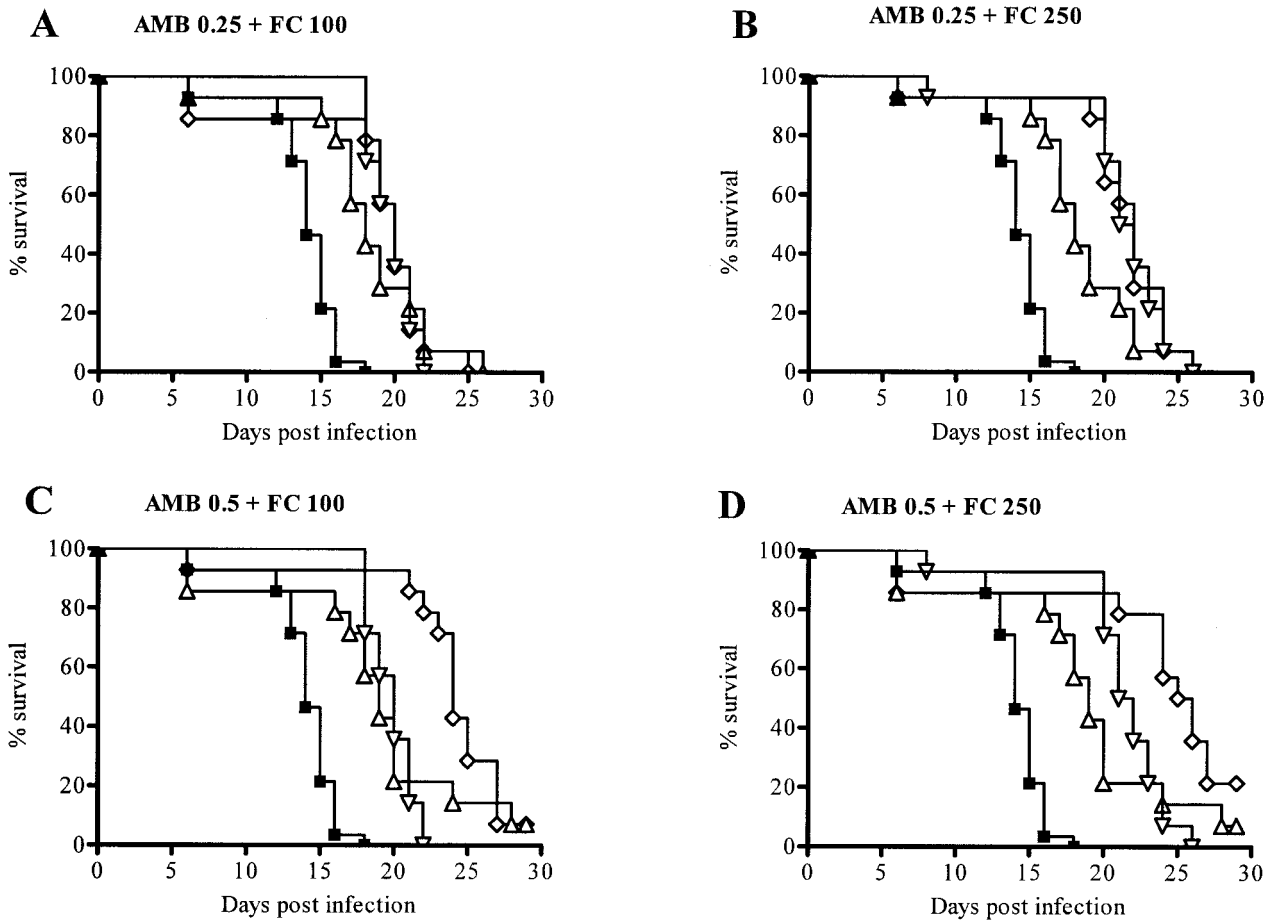


FIG. 1. Survival curves of mice infected with a flucytosine-susceptible isolate of *C. neoformans*. Mice were treated with AMB given either at 0.25 mg/kg/day (AMB 0.25) or at 0.5 mg/kg/day (AMB 0.5) or with FC given either at 100 mg/kg/day (FC 100) or at 250 mg/kg/day (FC 250) alone or in combination. ■, control; △, AMB alone at 0.25 mg/kg/day (A and B) or at 0.5 mg/kg/day (C and D); ▽, FC alone at 100 mg/kg/day (A and C) or at 250 mg/kg/day (B and D); ◇, AMB combined with FC at the indicated dosages.

centration indices of 0.5 and 0.08, respectively. In vitro susceptibility testing was performed according to CLSI (formerly NCCLS) guidelines (23). Strains were kept at −20°C in 50% glycerol and were subcultured once on Sabouraud agar slants before each experiment.

**Mice.** Outbred 7-week-old male OF1 mice (Charles River Laboratories, L'Arbresle, France) weighing 25 to 30 g were used for the experiments. Mice were housed in groups of seven and were given food and water ad libitum. Mice were maintained in a room at 21°C with a 12-h dark-light cycle. Animal studies were performed according to the recommendations of the European Community (directive 86/609/EEC, 24 November 1986) and were approved by the ethical committee of the Institut Pasteur.

**Infection.** Each inoculum was prepared from a 24-h-old culture grown in 10 ml minimum medium (20 g glucose, 6.7 g yeast nitrogen base without amino acids [Difco], 1 liter distilled water) at 30°C with agitation at 150 rpm. Yeasts were washed three times with 0.9% NaCl, cells were counted in a hemacytometer, and the inoculum was adjusted to the desired concentration. Viability was determined by plating dilutions of the yeast suspension onto Sabouraud agar. CFU determination was done after incubation at 30°C for 48 h. Infections were performed by injecting 100 μl of the yeast suspension into a lateral tail vein, leading to a disseminated infection closely mimicking that observed in AIDS patients (18). After infection, cages were randomized in the different treatment groups. Preliminary studies were performed to determine the 90% lethal dose at 14 days for both isolates by testing three different inocula. The 90% lethal dose for both isolates was $5 \times 10^5$ CFU/mouse.

**Drugs and therapy.** AMB deoxycholate (Fungizone; Bristol-Meyers Squibb, Puteaux, France) diluted in 5% glucose was given intraperitoneally (i.p.) once a day. Due to the short half-life in mice and the known time-dependent effect of the drug (1, 31), FC (Ancotil; ICN Pharmaceuticals, Orsay, France) was given in the drinking water (orally [p.o.]) to ensure a more stable concentration of the drug over time. FC dosages were based on the consumption of 4 ml/mouse/day of drinking water as determined in preliminary experiments. Extensive preliminary experiments were performed to assess the efficacy of both drugs as monotherapies in this murine model. For mortality studies, four dosages of AMB ranging from 0.06 to 0.5 mg/kg/day and three dosages of FC ranging from 250 to 1,000 mg/kg/day were tested. Similarly for CFU studies, the efficacies of AMB alone given at 0.03 to 0.25 mg/kg/day (four dosages) and of FC alone given at 50 to 250 mg/kg/day (three dosages) were evaluated. Based on the results of these preliminary experiments, drug dosages that showed a limited efficacy were used in the subsequent combination studies in order to detect a potential synergistic activity. Each combination experiment was performed once.

**Mortality studies.** For mortality studies, treatment was begun 6 days postinfection and was continued for 5 days. This infection model is reproducible. In untreated mice, data from at least two independent experiments carried out for both isolates showed an identical mortality rate by the end of the experiment and a variation of the median survival time of no more than 2 days. For both isolates, 10 groups of 14 mice were used (two cages/group), and the groups were as follows: water p.o. (control 1), 5% glucose i.p. (control 2), AMB i.p. at 0.25 mg/kg/day (AMB 0.25) or 0.5 mg/kg/day (AMB 0.5), FC p.o. at 100 mg/kg/day (FC 100) or 250 mg/kg/day (FC 250), AMB at 0.25 mg/kg/day combined with FC at 100 or 250 mg/kg/day (AMB 0.25 + FC 100 and AMB 0.25 + FC 250, respectively), and AMB at 0.5 mg/kg/day combined with FC at 100 or 250 mg/kg/day (AMB 0.5 + FC 100 and AMB 0.5 + FC 250, respectively). Preliminary studies showed that the dosages used for FC were not associated with hematological toxicity (data not shown). Animals were checked once daily for

mortality and abnormal clinical signs suggesting cranial hypertension. Animals were weighed once a day to adjust AMB dosage. Drinking water uptake was measured daily for mice treated with FC either alone or in combination to ensure that mice were given the appropriate dose of the drug. Although mice infected with the FC-resistant isolate and treated with FC alone had a lower water uptake, for all other groups and for both isolates, levels of FC consumption were similar (data not shown). Mice were observed for 4 weeks in total in order to obtain 100% mortality in untreated control mice and to assess the efficacy of therapy for a sufficient period of time after the last dose of treatment. Survivors were sacrificed 29 days postinfection.

**CFU studies.** Since death started to occur in the control group at day 6 postinfection, we modified the treatment regimen for the CFU studies. Treatment was begun 24 h after infection and was continued for 5 days. Preliminary studies have shown that the model was reproducible and that a low variability was seen between CFU data obtained from individual mice. For this reason, for both isolates, each treatment group and each control group contained five mice. Regimen and treatment groups were similar to those used for the mortality study. On day 6 postinfection (24 h after the last dose of AMB), mice were euthanized by $CO_2$ asphyxiation, and brain, spleen, and lungs were removed aseptically, weighed, and homogenized in a tissue grinder in 2 ml of 0.9% NaCl. Several 10-fold dilutions were made, and 100 μl of each suspension was plated onto Sabouraud chloramphenicol agar plates in duplicate. This procedure was used to allow a washout period of at least one half-life for each drug and a minimum dilution of the residual drug concentration of at least 50- to 100-fold before plating in order to get rid of a possible drug carryover. After incubation for 48 h at 30°C, the CFU were counted and the CFU per gram of tissue were calculated.

**Statistical analysis.** Mortality, median survival time, and fungal burden in both control groups were comparable, and results of the two groups were pooled. Mortality data were compared by the log rank test. CFU data of all groups were log transformed and compared by the Kruskal-Wallis test followed by a comparison between two groups by the Mann-Whitney test. Statistical analyses were performed using GraphPad Prism version 4.0 for Windows (GraphPad Software Inc., San Diego, CA). Statistical significance was assumed when $P$ was $\leq 0.05$.

## RESULTS

**Mortality studies. (i) FC-susceptible isolate.** Survival curves and mortality data for the FC-susceptible *C. neoformans* isolate are presented in Fig. 1 and Table 1, respectively. The infection with the FC-susceptible isolate led to 100% mortality on day 18 of the experiment in the control group, with a median survival time of 14 days. FC and AMB monotherapies at both dosages were effective compared to controls ($P < 0.0001$). FC 100 and FC 250 alone increased the median survival time for 6 and 7.5 days, respectively. Treatment with AMB 0.25 and AMB 0.5 alone increased the median survival time for 4 and 5 days compared to controls, respectively. Combination therapy with AMB 0.25 and FC at either dosage did not improve survival compared to that using monotherapies (Fig. 1A and B). Combination therapy with AMB 0.5 + FC 100 increased the median survival by 2.5 days compared to FC monotherapy and by 5 days compared to AMB alone (Fig. 1C). AMB 0.5 + FC 250 increased the median survival time by 4 days and 6.5 days compared to FC and AMB alone at the same dosages, respectively ($P < 0.05$) (Fig. 1D).

**(ii) FC-resistant isolate.** Survival curves and mortality data for the FC-resistant *C. neoformans* isolate are presented in Fig. 2 and Table 1, respectively. The mortality rate and median survival time without antifungals were comparable to those recorded for infection with the FC-susceptible isolate. At both given dosages, FC monotherapy was ineffective against the FC-resistant isolate. AMB 0.5 showed efficacy against the FC-resistant isolate compared to the control ($P < 0.0001$), with an increase of the median survival time of 9.5 days. AMB 0.25 was less effective than AMB 0.5 but increased the median survival by 6.5 days compared to untreated controls ($P < 0.001$). The combination of AMB 0.25 and FC 100 or FC 250 (Fig. 2A and B) or the combination of AMB 0.5 and FC 100 (Fig. 2C) was not statistically more effective than AMB alone. AMB 0.5 + FC 250 increased the median survival time by 2 days compared to AMB (Fig. 2D), but the difference was not statistically significant.

TABLE 1. Survival times of mice infected with an FC-susceptible or an FC-resistant isolate of *C. neoformans* and treated with FC and AMB alone or in combination[a]

| Treatment group[b] | FC-susceptible isolate | | FC-resistant isolate | |
|---|---|---|---|---|
| | % Survival | Median (range) survival time (days) | % Survival | Median (range) survival time (days) |
| Control | 0 | 14 (6–18) | 0 | 15 (11–27) |
| AMB 0.25 | 0 | 18 (6–26)[c] | 7 | 21.5 (15–29)[d] |
| AMB 0.5 | 7 | 19 (6–29)[c] | 29 | 24.5 (14–29)[c] |
| FC 100 | 0 | 20 (18–22)[c] | 0 | 15.5 (10–26) |
| FC 250 | 0 | 21.5 (8–26)[c] | 7 | 15 (11–29) |
| AMB 0.25 + FC 100 | 0 | 20 (6–25) | 7 | 20 (7–29) |
| AMB 0.25 + FC 250 | 0 | 22 (6–26) | 21 | 23 (16–29) |
| AMB 0.5 + FC 100 | 7 | 24 (6–29) | 29 | 20.5 (8–29) |
| AMB 0.5 + FC 250 | 21 | 25.5 (6–29)[e] | 43 | 26.5 (14–29) |

[a] Treatment was started 6 days postinfection and was continued for 5 days. For statistical analysis, drugs alone were compared to controls and combinations were compared to monotherapies.
[b] FC 100 and FC 250, flucytosine at 100 and 250 mg/kg/day; AMB 0.25 and AMB 0.5, amphotericin B at 0.25 and 0.5 mg/kg/day.
[c] $P < 0.0001$ (compared to controls).
[d] $P < 0.001$ (compared to controls).
[e] $P < 0.05$ (compared to FC and AMB alone).

**CFU experiments. (i) FC-susceptible isolate.** Results for the FC-susceptible isolate are presented in Fig. 3. Mice in the control group were heavily infected with a mean fungal burden of 6.0 to 6.7 $\log_{10}$ CFU/g in the three organs studied. FC was effective in all three organs, decreasing fungal burden by approximately 1 to 2 $\log_{10}$ CFU/g compared to controls ($P < 0.01$). The highest efficacy of FC was achieved in the brain and spleen ($P < 0.001$ compared to controls). There was no dose-dependent effect, with FC 100 being as effective as FC 250. AMB was effective in spleen and in lungs, with a decrease of approximately 1 to 2 $\log_{10}$ CFU/g ($P < 0.005$), but was ineffective in the brain. Nevertheless, the addition of either FC 100 or FC 250 to AMB significantly decreased the fungal burden in the brain compared to AMB alone. When AMB 0.5 was used in combination with FC 250 but not with FC 100, a significant decrease of fungal burden was achieved in all three organs compared to monotherapies and controls ($P < 0.05$).

**(ii) FC-resistant isolate.** Results for the FC-resistant isolate are presented in Fig. 4. Fungal loads in brain and lungs after inoculation by the FC-resistant isolate in control mice (6.2 to 6.8 $\log_{10}$ CFU/g) were comparable to that caused by the FC-susceptible isolate, while in the spleen the fungal burden was 1.3 $\log_{10}$ CFU/g lower than that for the FC-susceptible isolate. FC 100 or FC 250 did not reduce fungal burden in all three organs. AMB 0.25 or AMB 0.5 was ineffective in the brain but was effective in the spleen and in the lungs, with a decrease of about 0.4 or 1.5 $\log_{10}$ CFU/g compared to controls ($P$ value of $<0.05$ to $<0.005$). AMB 0.5 in combination with FC significantly reduced organ fungal burden, of about 1 $\log_{10}$ CFU/g, in the brain (FC 100, $P < 0.01$; FC 250, $P < 0.05$) and in the

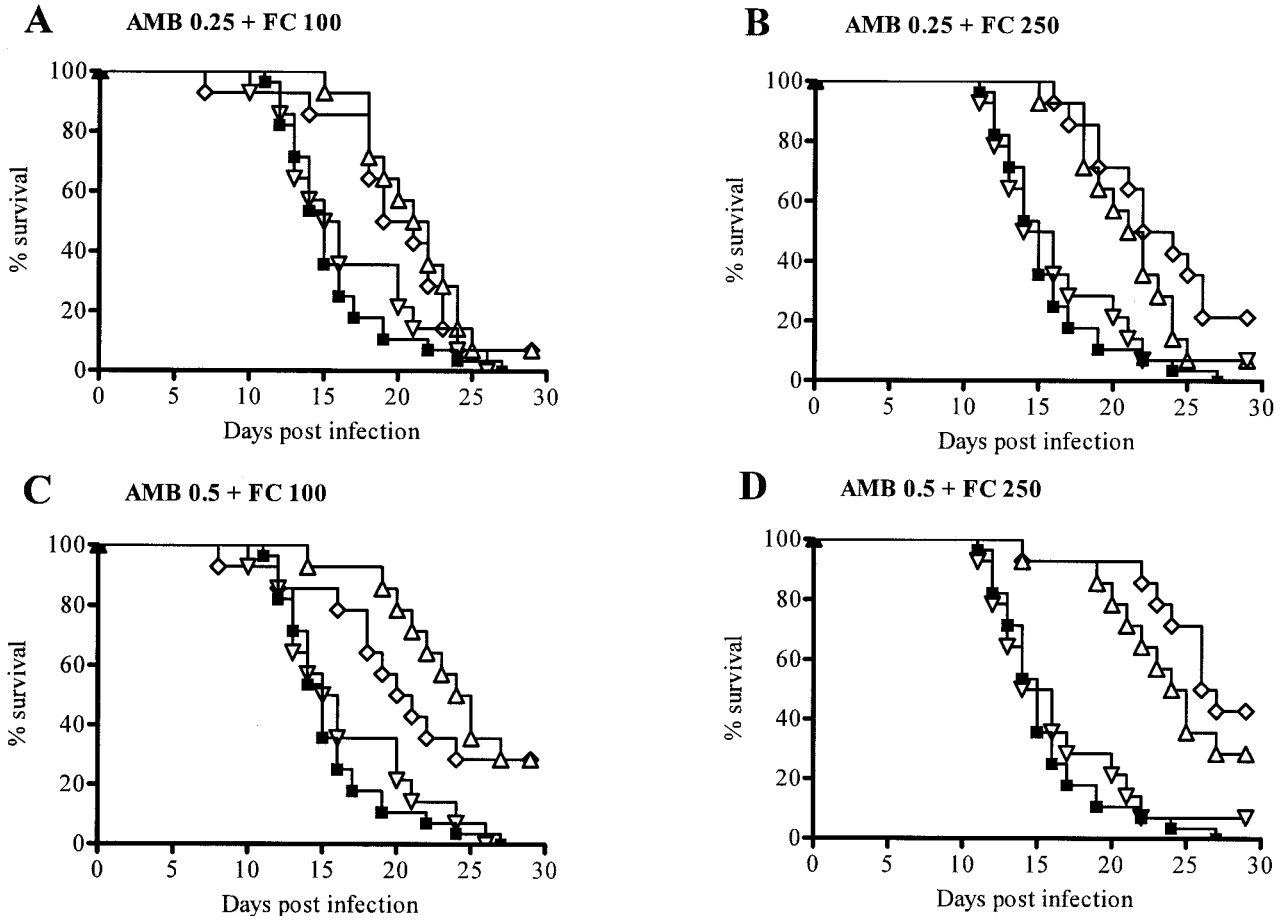


FIG. 2. Survival curves of mice infected with a flucytosine-resistant isolate of *C. neoformans*. Mice were treated with AMB given either at 0.25 mg/kg/day (AMB 0.25) or at 0.5 mg/kg/day (AMB 0.5) or with FC given either at 100 mg/kg/day (FC 100) or at 250 mg/kg/day (FC 250) alone or in combination. ■, control; △, AMB alone at 0.25 mg/kg/day (A and B) or at 0.5 mg/kg/day (C and D); ▽, FC alone at 100 mg/kg/day (A and C) or at 250 mg/kg/day (B and D); ◇, AMB combined with FC at the indicated dosages.

spleen (FC 250, $P < 0.05$) compared to AMB alone. In the lungs, the combination of FC and AMB failed to reduce fungal burden compared to the group treated by AMB monotherapy.

## DISCUSSION

Combination therapy with AMB and FC is the recommended first-line treatment for disseminated cryptococcosis in both immunocompetent and immunosuppressed patients (28). In HIV-negative patients with cryptococcal meningitis, Bennett et al. demonstrated that among 66 patients, 68% were cured or improved with the combination compared to 47% in the AMB treatment group (2). Similarly, in cryptococcal meningitis in patients with AIDS, a multivariate analysis showed that the addition of FC to initial therapy with AMB was independently associated with early cerebrospinal fluid sterilization (32). Patients infected with FC-resistant isolates are generally treated with AMB monotherapy. Indeed, it is assumed that for these patients, combination therapy with AMB and FC will not be useful or may even be deleterious (14). The concern that the clinical outcome of treated patients might be hampered if the infecting isolate is resistant to FC explains the need for experimental data using FC-resistant isolates compared to FC-susceptible isolates.

Acquired resistance to FC was a common event when patients were treated with FC monotherapy (12). For this reason, FC is now always used in combination with another antifungal drug (33). In vitro FC resistance in *C. neoformans* has been reported previously in several large surveys that tested clinical isolates (3, 6, 25). Overall, primary FC resistance was more frequent than in vitro resistance to fluconazole or AMB in *C. neoformans*. According to CLSI breakpoints (i.e., susceptible with an MIC of $\leq$4 μg/ml and resistant with an MIC of $\geq$32 μg/ml), primary resistance to FC was noted in up to 7% of the isolates (6). There are few studies evaluating the in vitro combination of AMB and FC against FC-resistant isolates of *C. neoformans* (11, 20, 30), and various results have been observed, including antagonism in some instances when low concentrations of FC were used (11). In our laboratory, we evaluated the combination of AMB and FC by checkerboard studies against 30 clinical isolates of *C. neoformans* including three FC-resistant isolates and found in vitro synergy of the combination in both FC-susceptible and FC-resistant isolates (29). As there is no standardized techniques for testing antifungal combinations in vitro and because pharmacokinetics of drugs and host

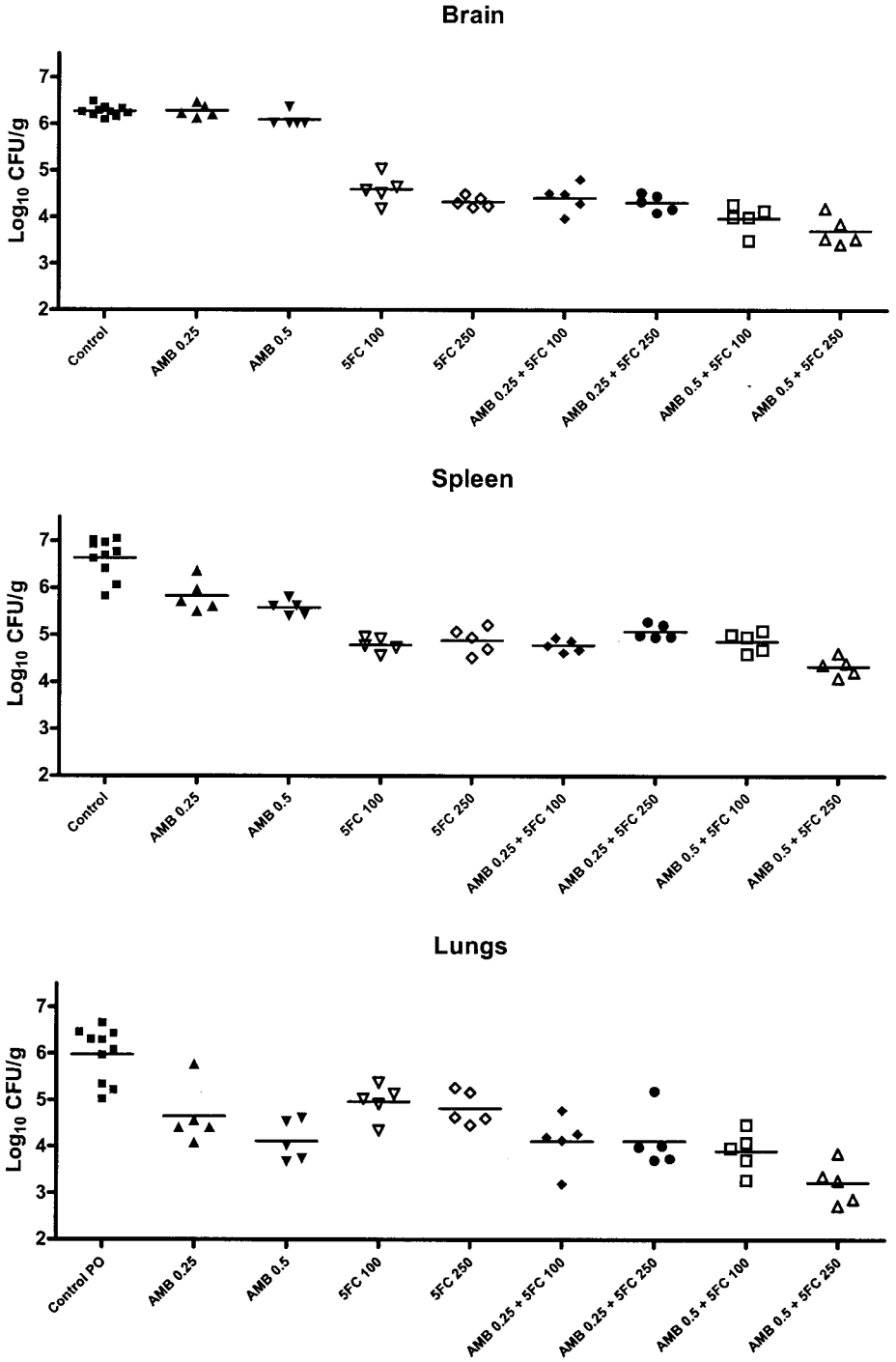


FIG. 3. Fungal burden in brain, spleen, and lungs of mice infected with a flucytosine-susceptible isolate of *C. neoformans*. Mice were treated with AMB given either at 0.25 mg/kg/day (AMB 0.25) or at 0.5 mg/kg/day (AMB 0.5) or with FC given either at 100 mg/kg/day (FC 100) or at 250 mg/kg/day (FC 250) alone or in combination. Bars represent the means.

characteristics could play an important role, animal models of fungal infections are valuable to assess the in vivo interactions between antifungal drugs and to confirm results obtained in vitro.

In the present study, the efficacy of AMB and FC either alone or in combination was tested in a murine model of disseminated cryptococcosis. We used a model that has been shown to closely reproduce the major features of cryptococcosis in humans, particularly in AIDS patients (5, 18). For the evaluation of treatment efficacy, very stringent conditions were used as the antifungal therapy was started on day 6 postinfection for mortality studies. It has been clearly shown, for exam-

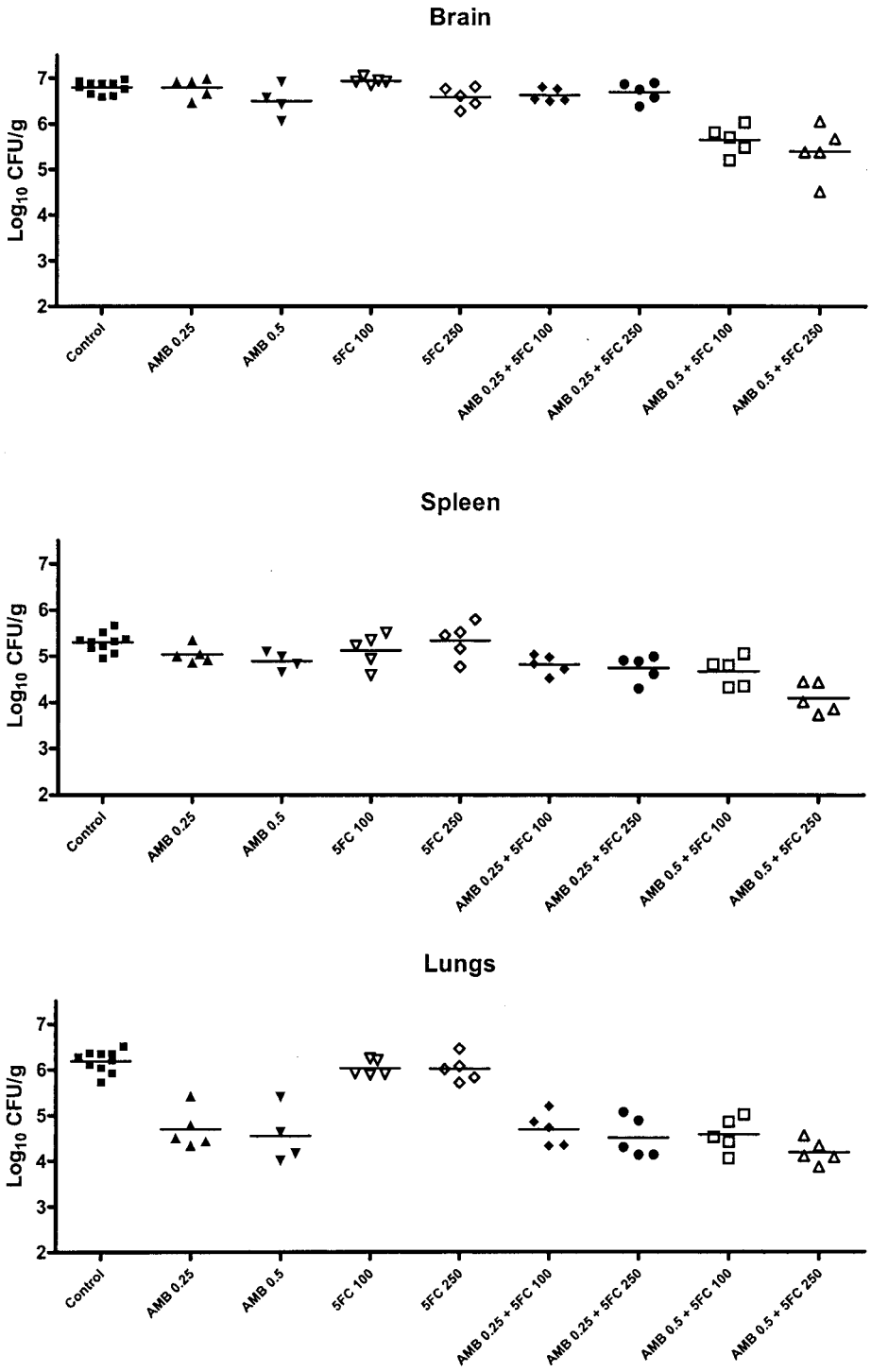


FIG. 4. Fungal burden in brain, spleen, and lungs of mice infected with a flucytosine-resistant isolate of *C. neoformans*. Mice were treated with AMB given either at 0.25 mg/kg/day (AMB 0.25) or at 0.5 mg/kg/day (AMB 0.5) or with FC given either at 100 mg/kg/day (FC 100) or at 250 mg/kg/day (FC 250) alone or in combination. Bars represent the means.

ple, for fluconazole, that the in vivo antifungal efficacy was dependent on the time of initiation of the antifungal therapy, with less efficacy when treatment was delayed (19). Delayed therapy explains the poor efficacy of either AMB or FC alone, in terms of mortality, in our model. A limited efficacy of monotherapies was useful to detect a potential synergy between drugs (10). Flucytosine was given in the drinking water to ensure that the animals received the dose continuously over time as opposed to a daily bolus. Indeed, pharmacokinetic/pharmacodynamic data in a murine model of candidiasis have

shown that time above the MIC is more important to predict efficacy than peak level/MIC (1). Moreover, another study has also demonstrated a better efficacy of FC given by subcutaneous implanted pump compared to bolus injections (13). Recent data from an animal model of aspergillosis showed that the in vivo efficacy depended of the total daily dose of FC and that the best predictors of efficacy were the area under the serum concentration-time curve and MIC ratio (31). FC dosages chosen in the present study were in the same range as those used in previous murine models of cryptococcosis (8, 17). As FC is mainly eliminated by the kidneys, renal insufficiency may alter pharmacokinetics of FC (33). Nevertheless, it has been shown that doses of AMB used in the present studies did not increase creatinin serum levels in mice (15), and it could be assumed that mice that received FC either alone or in combination were exposed to the same level of drug.

We performed mortality studies and fungal burden experiments in mice infected with either an FC-susceptible or an FC-resistant *C. neoformans* isolate. Interestingly, AMB alone significantly prolonged survival of mice but was unable to decrease the fungal burden in the brain significantly, although both isolates exhibited low in vitro AMB MICs. This is not surprising due to the severity of the model and the short course of therapy. Similar results were seen in HIV-infected patients with a slow and moderate decrease of CFU in the cerebrospinal fluid during the first days of therapy with AMB alone (4). For the FC-resistant isolate, the poor in vivo efficacy of FC alone in survival and CFU experiments correlated with the in vitro resistance to the drug. Overall, we demonstrated that combination therapy with AMB and FC was more effective than monotherapies against both the FC-susceptible and -resistant isolates when fungal burden in tissues was analyzed. This combination also showed a better efficacy than monotherapies in the mortality studies, although a statistical significance was only reached for the FC-susceptible isolate. Importantly, antagonism was never observed. One limitation of the present study is to have an in vivo evaluation for only one FC-resistant isolate. For practical reasons, it is difficult to test a large number of isolates in animal models. Alternatively, as the data presented here showed that in vitro results were in accordance with the in vivo efficacy of the combination, it will be possible to evaluate in vitro interactions of FC with AMB against a panel of FC-resistant isolates of *C. neoformans*.

In most of the previous studies in which the antifungal combination of FC with other drugs was evaluated, only FC-susceptible strains were used. The combination of AMB and FC against FC-susceptible isolates of *C. neoformans* showed to be additive to indifferent interactions in vivo (7, 26). Most of the recent studies focused on the double combination of FC with azoles, particularly fluconazole (8, 17, 24), or on triple combinations of FC with AMB and fluconazole (7, 16). Overall, these studies showed that moderate to low doses of FC interacted synergistically with fluconazole (7, 8, 17). In contrast, there are few studies that have evaluated the combination of AMB and FC in vivo against FC-resistant isolates of *C. neoformans* (11, 26), and these studies showed conflicting results. In one study, it was demonstrated that the combination was additive against an FC-resistant isolate (26). Moreover, in the same study, in vivo synergy against one FC-resistant isolate of *Candida albicans* was also demonstrated (26), indicating that FC resistance is not per se an obstacle to the efficacy of the combination.

Hamilton and Elliot (11) previously evaluated the combination of AMB and FC in vivo and reported antagonism for an FC-resistant isolate, while drug interactions for the FC-susceptible isolate were considered to be additive (11). Nevertheless, in this study, endpoints for evaluating the activity of the antifungal treatments were not the same for both isolates, and this makes interpretation of the results confusing.

In conclusion, we were able to demonstrate the higher efficacy of the addition of FC to AMB compared to monotherapies against both FC-susceptible and FC-resistant isolates of *C. neoformans* even for the control of cerebral infection. These results show that a combination of AMB and FC could be beneficial for patients with disseminated cryptococcosis due to FC-resistant isolates of *C. neoformans*.

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