ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2008, p. 1573–1576 Vol. 52, No. 4
0066-4804/08/$08.00+0 doi:10.1128/AAC.01488-07

Comparison of Lipid Amphotericin B Preparations in Treating Murine Zygomycosis[▽]

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Received 15 November 2007/Returned for modification 13 January 2008/Accepted 20 January 2008

We compared the efficacies of liposomal amphotericin B (LAmB) and an amphotericin B lipid complex (ABLC) in diabetic ketoacidotic (DKA) or neutropenic mice with disseminated zygomycosis. ABLC was as effective as LAmB in neutropenic but not DKA mice. Low-dose ABLC was less effective than LAmB at reducing brain fungal burdens in both models.

Case reports and case series have documented successful outcomes after the treatment of zygomycosis with either liposomal amphotericin B (LAmB) or an amphotericin B lipid complex (ABLC) (1, 4, 14, 15). To date, there have been no head-to-head preclinical or clinical studies comparing the efficacy of LAmB to that of ABLC for zygomycosis. However, relevant to the treatment of central nervous system (CNS) zygomycosis, a previous rabbit study demonstrated that LAmB penetrates the brain parenchyma at levels more than fivefold above those of ABLC (6). In fact, in that study, the levels of ABLC in the brain were less than or equal to the levels of amphotericin B deoxycholate, despite the fact that ABLC was administered at a fivefold-higher dose. Conversely, recent data demonstrated the efficacy of ABLC therapy in treating experimental CNS aspergillosis (3) and rabbit coccidioidal meningitis (2), suggesting the ability of ABLC to penetrate the brain-blood barrier. A recent retrospective review of 120 cases of zygomycosis in patients with hematological malignancies demonstrated that treatment with LAmB was associated with a 67% survival rate, compared to a 39% survival rate when patients were treated with amphotericin B deoxycholate ($P = 0.02$; χ^2 test) (5). No comparable data set from a review of the effect of ABLC in this setting has been published. Therefore, we sought to compare the efficacy of LAmB to that of ABLC in treating zygomycosis.

(This work was presented in part at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 17 to 20 September 2007 [12].)

To define the relative efficacies of ABLC and LAmB against zygomycosis, we utilized our diabetic ketoacidotic (DKA) mouse model of disseminated zygomycosis, in which heavy infection occurs both systemically and specifically in the CNS (7–10). BALB/c male mice (20 to 23 g) were rendered diabetic with a single intraperitoneal injection of 210 mg of streptozotocin/kg of body weight in 0.2 ml of citrate buffer 10 days prior to fungal challenge, as we have previously described (7–10). Glycosuria and ketonuria were confirmed in all mice 7 to 10 days after streptozotocin treatment. Diabetic mice were infected via the tail vein with *Rhizopus oryzae* 99-880, a clinical brain isolate known to be virulent and tropic toward the brain in our model and susceptible to amphotericin B (MIC = 0.25 μg/ml) (8). LAmB or ABLC (7.5 or 15 mg/kg/day) was diluted in 5% dextrose water and administered intravenously (i.v.) in 0.2 ml for 4 days starting 24 h after infection. These doses were selected based on results from prior studies demonstrating efficacy with a lack of toxicity in uninfected mice when survival was used as an end point (7, 11). Control groups received a 5% dextrose water vehicle (placebo). Treatment with either LAmB or ABLC at either dose significantly improved survival compared to treatment with a placebo (Fig. 1A). However, LAmB at 15 mg/kg/day significantly improved survival compared to ABLC at either dose (Fig. 1A). Of note, no mortality was seen among uninfected animals treated with 15 mg of ABLC or LAmB/kg (data not shown). LAmB at 7.5 mg/kg/day resulted in more surviving animals than ABLC at either dose but did not significantly improve the time to death compared to the ABLC treatments ($P = 0.1$).

Other groups of mice were euthanized after 72 h of infection, prior to the initiation of death from infection in the placebo group, for the determination of tissue fungal burdens by quantitatively culturing gently homogenized organs (10) and for the determination of amphotericin B concentrations in harvested tissues by a bioassay as described previously (13). All antifungals reduced kidney fungal burdens compared to those in the controls (Fig. 1B). ABLC at 7.5 mg/kg/day did not reduce the brain fungal burdens compared to those in the controls, but higher-dose ABLC and both doses of LAmB did reduce brain fungal burdens. Both drugs were equally effective in reducing kidney fungal burdens. ABLC at 7.5 mg/kg/day achieved low or undetectable levels in the kidneys, whereas high-dose ABLC and both doses of LAmB achieved significantly higher levels in the kidneys (Fig. 1B). Levels of drugs in the brains were generally below the level of detectability (~0.5 μg/g of tissue). Of note, measuring amphotericin B concentra-

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▽ Published ahead of print on 28 January 2008.

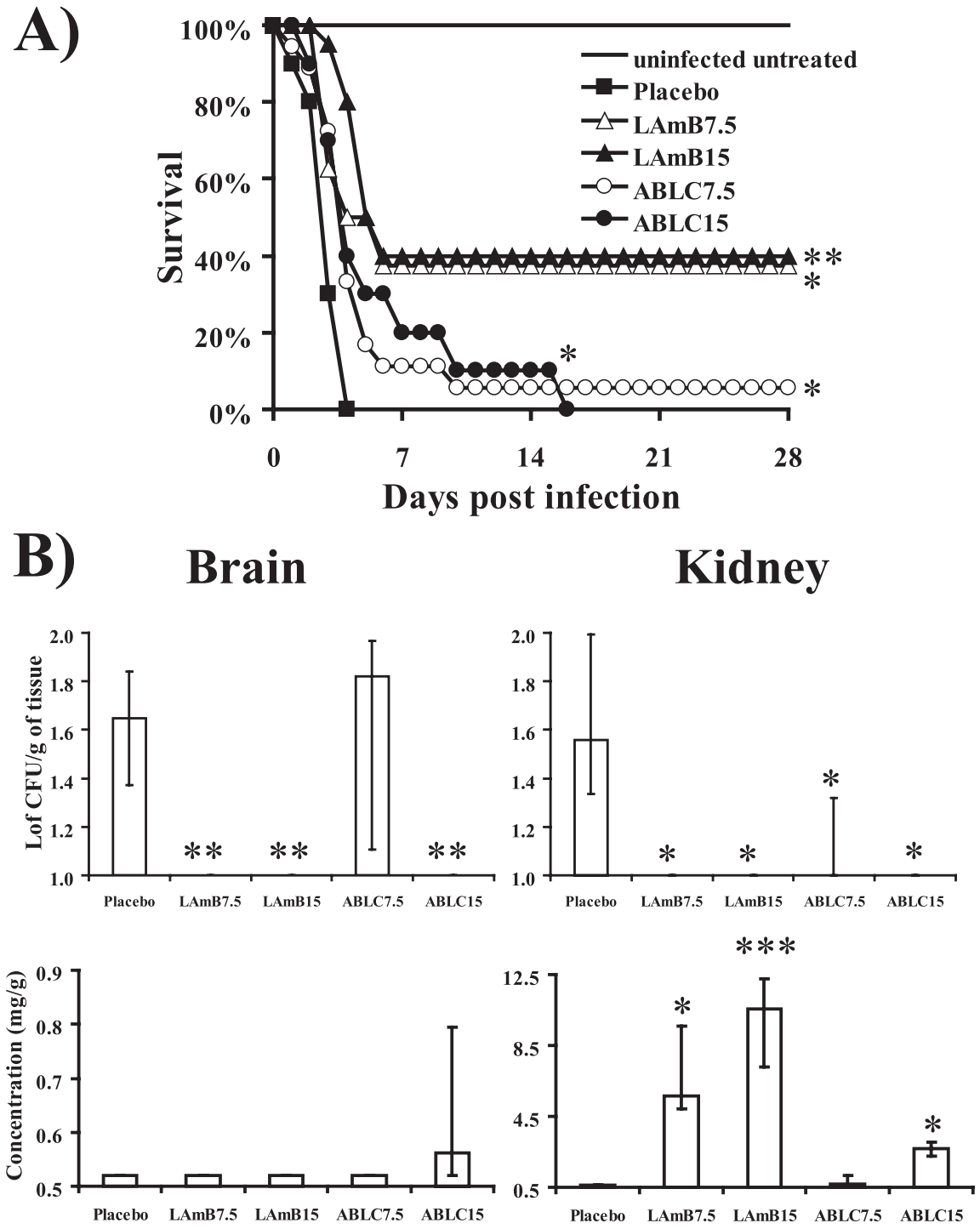

FIG. 1. Efficacy of LAmB and ABLC in the DKA mouse model of zygomycosis. (A) Survival of DKA mice ($n \geq 10$ mice per group from two independent experiments). The fungal inoculum was 1.5×10^3 spores administered i.v. Numbers after the drug names indicate the doses in milligrams per kilogram. *, $P < 0.05$ versus a placebo; **, $P < 0.05$ versus a placebo, ABLC at 15 mg/kg, and ABLC at 7.5 mg/kg by the log rank test. (B) Tissue fungal burdens and amphotericin B concentrations in organs harvested from DKA mice ($n = 10$ per group) 72 h postinfection. Data are displayed as medians with interquartile ranges. The *y* axes reflect the lower limits of detection by the assay. *, $P < 0.05$ versus a placebo; **, $P < 0.05$ versus a placebo and ABLC at 7.5 mg/kg; ***, $P < 0.05$ versus a placebo, ABLC at 7.5 mg/kg, and ABLC at 15 mg/kg by the nonparametric Mann-Whitney test for multiple comparisons.

tions in mouse brains is technically challenging due to the small volumes of tissue, and to date no studies quantifying amphotericin B levels in rodent brains have been published.

We next tested the efficacies of the same treatment regimens in mice made neutropenic by a single intraperitoneal dose of 200 mg of cyclophosphamide/kg on day −2 relative to infection. Both drugs were administered i.v. for 4 days starting 24 h after infection with *R. oryzae* 99-880. ABLC or LAmB at both

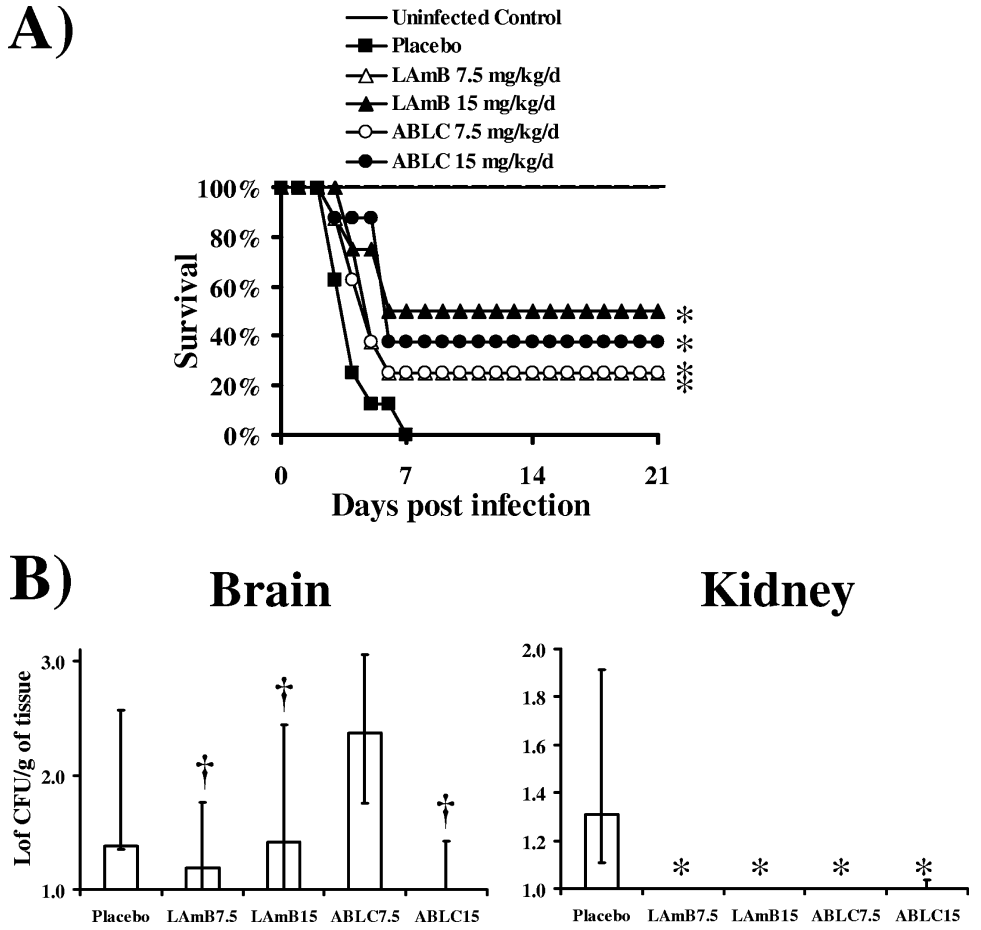

FIG. 2. Efficacy of LAmB and ABLC in the neutropenic mouse model of zygomycosis. (A) Survival of mice ($n = 8$ per group) infected i.v. with 1.8×10^3 spores of *R. oryzae*. Numbers after the drug names indicate the doses in milligrams per kilogram. *, $P < 0.05$ versus a placebo by the log rank test. (B) Fungal burdens of organs harvested from mice ($n = 10$ per group) 72 h postinfection. Data are displayed as medians with interquartile ranges. The *y* axes reflect the lower limits of detection by the assay. *, $P < 0.025$ versus a placebo; †, $P < 0.05$ versus ABLC at 7.5 mg/kg by the nonparametric Mann-Whitney test for multiple comparisons.

doses improved survival compared to the placebo (Fig. 2A). There was no significant difference in survival among the mice treated with the different antifungals at the different doses, although, again, the highest number of surviving mice was in the group treated with LAmB at 15 mg/kg/day. All antifungal treatments reduced kidney fungal burdens compared to those in placebo-treated mice (Fig. 2B). In contrast, brain fungal burdens were not reduced compared to those in placebo-treated mice by any antifungal treatment. However, among the antifungal-treated groups, the brain fungal burdens were highest in the group treated with ABLC at 7.5 mg/kg/day.

LAmB and ABLC for the treatment of zygomycosis had not been previously compared head to head in either preclinical or clinical studies. Our data from the murine DKA model demonstrate that LAmB at 15 mg/kg/day was superior to ABLC at either dose. In contrast, in neutropenic mice, LAmB and ABLC were similarly effective at improving survival rates, although ABLC at 7.5 mg/kg/day was again inferior at clearing brain fungal burdens. LAmB at either dose reduced brain fungal burdens versus those in mice treated with the placebo or ABLC at 7.5 mg/kg/day. Of note, despite its inferiority to LAmB at 15 mg/kg/day at improving survival in the DKA model, ABLC at 15 mg/kg/day did reduce brain fungal burdens versus those in both placebo-treated mice and mice treated with ABLC at 7.5 mg/kg/day. One possible explanation for the lack of an impressive survival benefit in the face of reductions in tissue fungal burdens is that ABLC at 15 mg/kg/day was toxic to infected DKA mice. Further studies are needed to explore this possibility. The difference in the activities of ABLC and LAmB in the brains in the two models may be due to diminished meningeal inflammation in neutropenic animals compared to DKA mice, resulting in lower drug penetration through the blood-brain barrier. This possibility merits additional investigation.

In summary, our data demonstrate that ABLC was as effective as LAmB in the treatment of zygomycosis in neutropenic but not DKA mice.

This work was supported by Public Health Service grants R01 AI063503 and R21 AI064716 and research and educational grants from Astellas Pharmaceuticals, Enzon Pharmaceuticals, and Gilead Sciences Inc. to A.S.I. B.S. is supported by Public Health Service grant K08 AI060641 and American Heart beginning grant-in-aid 0665154Y and is a Liu Young Investigator in Biomedical Research. Research described in this report was conducted in part at the research facilities

of the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center.

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