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Efficacy of Prophylactic Aerosol Amphotericin B Lipid Complex in a Rat Model of Pulmonary Aspergillosis

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Invasive pulmonary aspergillosis remains an important cause of morbidity and mortality among transplant recipients and patients receiving cancer chemotherapy. The lipid-associated formulation of amphotericin B (AmB), AmB lipid complex (ABLC), was evaluated for its prophylactic efficacy when it was administered as an aerosol in a rat model of pulmonary aspergillosis. Aerosol ABLC (aero-ABLC), in doses from 0.4 to 1.6 mg/kg of body weight given 2 days before infection, significantly delayed mortality compared to the mortality of rats given placebo ($P < 0.001$). At day 10 postinfection, 50% of rats in the 0.4-mg/kg group and 75% of rats in the 1.6-mg/kg group were alive, while all control animals had died. In a second trial aero-ABLC was more effective than an equivalent dose of aerosol AmB (aero-AmB) in prolonging survival, with 100% survival at day 14 postinfection in the ABLC group, compared to 62.5% survival in the AmB group. Mean concentrations of AmB in lungs were 3.7 times higher at day 1 ($P < 0.002$) and almost six times higher at day 7 ($P < 0.001$) after treatment with aero-ABLC than after treatment with a similar dose of aero-AmB. We conclude that aero-ABLC provided higher and more prolonged levels of the parent compound in the lungs than aero-AmB and was more effective in delaying mortality from aspergillosis in this model.

Invasive pulmonary aspergillosis remains a significant complication of bone marrow transplantation and of cancer chemotherapy in patients with hematologic neoplasms (10, 13). Intravenous amphotericin B (AmB) is the standard treatment for invasive pulmonary aspergillosis, but the drug is toxic and is often poorly tolerated, and treatment failures are common. Inhalation of airborne *Aspergillus* spores with deposition in the lungs is thought to be the initial step leading to infection. Yet, in a study of the distribution of intravenous AmB, the highest concentrations of the drug were found in the liver, spleen, and kidneys (4). Inhalation of drug aerosols, by yielding high drug concentrations in the lungs and decreasing the systemic drug burden, should maximize effectiveness and limit systemic toxicity. Study of the pharmacokinetics of aerosol AmB (aero-AmB) in rats has shown that this route efficiently delivered AmB to the lungs, while it limited accumulation of the drug in other organs (12). In a rat model of pulmonary aspergillosis, prophylactic administration of aero-AmB provided high concentrations of the drug in the lungs and significantly prolonged survival (14).

Lipid-associated formulations of AmB, when administered intravenously, have been reported to be less toxic than and as effective as AmB desoxycholate in the therapy of fungal infections in experimental animals (6) as well as in humans (2, 9). One of these, AmB lipid complex (ABLC), which is composed of AmB and the phospholipids dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol, had therapeutic efficacy comparable to that of AmB in an immunocompromised mouse model of aspergillosis (5).

The therapeutic application of liposomal aerosols has been extensively reviewed (15). Uptake of liposomes by the alveolar macrophages with a subsequent slow release of the drug is thought to occur. Therefore, the use of an aerosolized lipid formulation should result in prolonged pulmonary residence of the associated drug. In a rat model of pulmonary aspergillosis (14), we studied the pharmacokinetics of aerosol ABLC (aero-ABLC) and its prophylactic efficacy compared to that of aero-AmB.

MATERIALS AND METHODS

Animal model of pulmonary aspergillosis. The animal research procedures were approved by the Institutional Animal Care and Use Committee of Memorial Sloan-Kettering Cancer Center. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) were used for all experiments.

The rats were fed a low-protein diet (8% protein; Dyet, Bethlehem, Pa.) and were given tetracycline (250 mg dissolved in 750 ml of drinking water) to prevent bacterial infection. Under light anesthesia with enflurane (Ethrane; Ohmeda, Madison, Wis.), the rats were treated thrice weekly with subcutaneous injections of cortisone acetate (150 mg/kg of body weight). The injections were given for 2 weeks until the day of infection (trial 1) or throughout the experiment (trial 2).

A spore suspension of *Aspergillus fumigatus* H11-20, isolated from a steroid-treated rat dying of spontaneously acquired pulmonary aspergillosis, was used for infection. Spores were obtained after subculturing the organism on Sabouraud dextrose agar for 4 to 5 days; they were harvested by using a 0.02% Tween 80 solution and were washed twice in sterile saline. A final suspension of 10^7 spores/ml was prepared by counting with a hemacytometer. Hemacytometer counts were confirmed by culturing dilutions of the spore suspension on Sabouraud dextrose agar.

While the rats were under general anesthesia induced by inhaled enflurane, their tracheas were exposed and 0.1 ml of the *Aspergillus* spore suspension (10^6 spores) was injected with a tuberculin syringe. The wound was closed with surgical staples (Autoclip; Clay Adams, Parsipanny, N.J.).

Administration of aerosols. AmB desoxycholate (Fungizone) was purchased from Squibb & Sons, Princeton, N.J. A 5.0-mg/ml solution was prepared with distilled water immediately prior to its use. ABLC (ABELCET; 5 mg/ml) was obtained from The Liposome Co., Princeton, N.J., and was stored in a refrigerator. At the time of administration, the stock solution was diluted in sterile water to the final concentrations.

The rats were treated 48 h before infection. They were placed in a glass chamber which was swept with a stream of aerosolized drug solution. The aerosol was generated by air flowing at 8 liters/min through a nebulizer (Cadema Medical Products, Middletown, N.Y.). The estimated amount of AmB or ABLC inhaled by the rats was calculated from the product of the concentration of the drug in the chamber, the minute volume of the rats (lung volume times respiratory rate), and the time of exposure (14). To deliver a dose of 1.6 mg of

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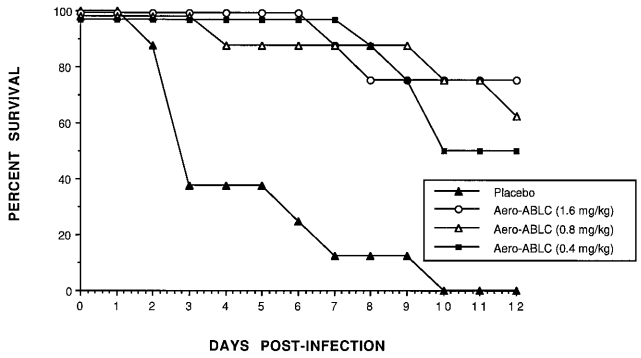

FIG. 1. Survival of animals in trial 1 in which corticosteroid treatment was discontinued at the time of infection (day 0). Treatment was administered 2 days before infection.

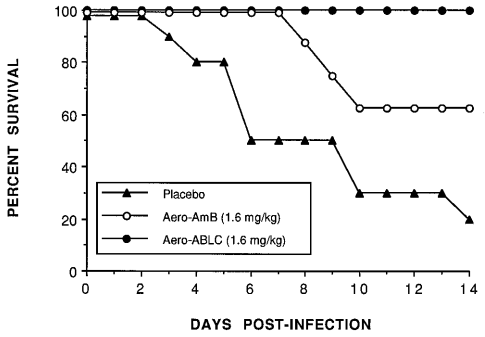

FIG. 2. Survival of animals in trial 2 in which corticosteroid treatment was continued throughout the experiment. Treatment was administered 2 days before infection.

aero-AmB or aero-ABLC per kg, the nebulizer was charged with 4.5 ml of a 5-mg/ml stock solution, which was aerosolized for 15 min.

In trial 1, rats in groups of eight rats each were treated with either placebo (sterile water) or three different doses of aero-ABLC once, 2 days before infection. The doses administered were 0.4, 0.8, and 1.6 mg of ABLC per kg. The immunosuppressive treatments with cortisone acetate were discontinued at the time of infection. The animals were monitored for survival daily for 7 days.

In trial 2, rats in groups of 8 to 10 rats each received either placebo (sterile water), a single dose of 1.6 mg of aero-AmB per kg, or 1.6 mg of aero-ABLC per kg 2 days before infection. Immunosuppressive treatment with cortisone acetate was continued through 14 days postinfection.

Concentration of AmB in lung tissue. At 1 and 7 days after the administration of a single 4.8-mg/kg dose of either aero-ABLC or aero-AmB, the rats were euthanized with carbon dioxide. The lungs, liver, kidneys, and spleen of each rat were removed and homogenized in distilled water (3 ml/g of tissue). After ethanol extraction, AmB levels were determined by high-performance liquid chromatography (4). All samples were assayed in duplicate. The concentrations of AmB were determined from a linear regression analysis of the peak areas obtained with standard solutions of AmB in water. Results were expressed in micrograms of AmB per gram of tissue. The lowest detectable concentration of AmB was 0.125 μg/g. The elimination half-life of AmB from the lungs was determined by using a semilogarithmic plot of the concentrations of the drug in the lungs at 1 and 7 days posttreatment.

Statistical analysis. Survival analysis comparing treatments was performed by the log-rank test. Survival curves were generated by the method of Kaplan and Meier. Student's *t* test was used to assess significant differences in lung tissue drug concentrations.

RESULTS

Prophylactic efficacy of aero-ABLC: trial 1. Control animals were moribund as early as 2 days following infection, with an overall survival rate of less than 12.5% at 7 days and a survival rate of 0% at 10 days. On the other hand, 50% of the rats in the 0.4-mg/kg group and 75% of the rats in the 1.6-mg/kg group were alive at day 10 (Fig. 1). All treatment groups exhibited better survival than the control group ($P < 0.001$). Although efficacy in prolonging survival appeared best at the highest dose tested (1.6 mg/kg), the survival times among the three treatment groups were not significantly different ($P < 0.7$).

Prophylactic efficacy of aero-ABLC compared to that of aero-AmB: trial 2. Up until day 7 postinfection, aero-AmB and aero-ABLC appeared to be equally effective in delaying mortality compared with that of the placebo (Fig. 2). However, aero-ABLC was 100% effective in prolonging survival through day 14, while the survival rate declined to 62.5% in the aero-AmB group and to 30% in the placebo group by day 10. Although aero-AmB was marginally better than the placebo in delaying mortality ($P < 0.067$), its efficacy was less prolonged than that of ABLC, which was significantly better than that of the placebo ($P = 0.001$). Aero-ABLC was marginally better than aero-AmB in delaying mortality, although the difference did not reach statistical significance ($P = 0.063$).

Treatments with aero-ABLC were well tolerated by the animals. While rats treated with aero-ABLC or placebo appeared undisturbed, rats in the aero-AmB group showed consistent huddling during treatment. They also displayed signs of eye irritation in the sense that they kept their eyes closed during treatment, whereas the rats in the ABLC group kept their eyes open during treatment. Also, nebulization of AmB desoxycholate resulted in significant foaming in the reservoir compared to nebulization of ABLC.

Pulmonary deposition and elimination of aero-ABLC compared to those of aero-AmB. The levels of AmB in the lungs were 3.7 times higher at 24 h ($P < 0.002$) and almost 6 times higher at 7 days ($P < 0.001$) after treatment with aero-ABLC than after treatment with the same dose of aero-AmB (Table 1). A more prolonged half-life of elimination of AmB from the lungs was noted after the administration of aero-ABLC compared to that after the administration of aero-AmB (9.8 versus 5.8 days). AmB was undetectable in the liver, spleen, or kidneys at 24 h after the administration of either aero-AmB or aero-ABLC.

DISCUSSION

Invasive fungal infection due to *Aspergillus* spp. remains an important cause of morbidity and mortality in patients with prolonged granulocytopenia and is still too often an autopsy discovery. Once established, the infection will usually progress, despite the use of high-dose AmB, unless the predisposing immunosuppression is reversed. Therefore, preventing established infection is desirable.

Previously, prophylactic aero-AmB delayed mortality in a rat model of pulmonary aspergillosis (14). In the current studies, a single treatment with aero-ABLC 2 days before infection with *Aspergillus* at doses ranging from 0.4 to 1.6 mg/kg significantly

TABLE 1. Concentration of AmB in lungs on days 1 and 7 following administration of a single aerosolized dose of 4.8 mg of either AmB desoxycholate or ABLC per kg

Treatment	Mean ± SD concn (μg/g of tissue)[a]		$t_{1/2}$ (days)[b]
	Day 1	Day 7	
AmB desoxycholate	9.4 ± 3.5 ($n = 2$)	3.9 ± 1.2 ($n = 2$)	5.8
ABLC	35.1 ± 2.0[c] ($n = 3$)	22.6 ± 1.6[d] ($n = 3$)	9.8

[a] n is number of rats.
[b] $t_{1/2}$, elimination half-life.
[c] $P < 0.002$ compared with AmB desoxycholate.
[d] $P < 0.001$ compared with AmB desoxycholate.

prolonged the survival of immunosuppressed rats compared to that of untreated animals, and ABLC was also more effective than AmB in prolonging survival in this model. The levels of the parent compound in the lungs were also significantly higher with ABLC than with AmB 24 h and 7 days after treatment with the aerosolized forms.

Factors that could account for higher levels in tissue after treatment with ABLC include better delivery to the airways and/or higher and more prolonged retention of the drug. AmB desoxycholate tended to foam more than ABLC, which could have affected drug delivery. Sorensen et al. (16) demonstrated in earlier studies that the tendency of AmB desoxycholate to foam resulted in more variable drug concentrations in chamber air compared to that of another lipid-associated AmB preparation, AmBisome. Allen et al. (1) also found that nebulization of lipid-associated AmB was more consistent than that of AmB desoxycholate. The respiration pattern of the animals is also an important factor in determining the actual dose delivered to the lungs. The apparent irritant effect of AmB desoxycholate on the rats in the current study might have adversely affected their respiratory patterns. Another major factor accounting for the higher levels in the lung and the prolonged half-life of elimination of AmB with ABLC is greater entrapment by alveolar macrophages with subsequent slow release. Since the in vitro killing activity of AmB is concentration dependent, efficacy in vivo is likely to correlate with levels in tissue.

This is the first study, to our knowledge, in which a lipid-associated formulation of AmB achieved greater efficacy (as measured by prolonged survival) than an equivalent dose of AmB (on a milligram-per-kilogram basis). Although Allen et al. (1) observed that lipid-associated AmB was more effective than AmB desoxycholate in preventing *Aspergillus* infections, as determined with quantitative lung cultures, no significant difference in survival between the two groups could be demonstrated.

These results have potential implications for immunocompromised persons who could benefit from similar prophylactic measures if efficacy and safety were likewise demonstrated. Studies with humans demonstrated that the use of prophylactic aero-AmB in granulocytopenic patients was safe and well tolerated (3, 7, 8). The major side effects of the treatments were cough and aftertaste, which were believed to be due to the desoxycholate carrier rather than the AmB. Studies with mice (11) and human volunteers (17) provide strong evidence that liposomal aerosols are innocuous, even after prolonged administration. Plans for such studies are under development.

In conclusion, the administration of aero-ABLC provided higher and more prolonged levels of AmB compared with those provided by the administration of aero-AmB. This was associated with improved efficacy in the prophylaxis of experimental aspergillosis, as measured by overall survival.

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REFERENCES

1. **Allen, S. D., K. N. Sorensen, M. J. Nejdl, C. Durrant, and R. T. Proffitt.** 1994. Prophylactic efficacy of aerosolized liposomal (AmBisome) and non-liposomal (Fungizone) amphotericin B in murine pulmonary aspergillosis. J. Antimicrob. Chemother. **34:**1001–1013.
2. **Anaissie, E. J., M. White, O. Uzun, C. Singer, G. P. Bodey, D. Matzke, N. Azarnia, and G. Lopez-Berestein.** 1995. Amphotericin B lipid complex (ABLC) versus amphotericin B (AmB) for treatment of hematogenous and invasive candidiasis: a prospective, randomized, multicenter trial, abstr. LM21, p. 330. *In* Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
3. **Beyer, J., G. Barzen, G. Risse, C. Weyer, K. Miksits, K. Dullenkopf, D. Huhn, and W. Siegert.** 1993. Aerosol amphotericin B for the prevention of invasive pulmonary aspergillosis. Antimicrob. Agents Chemother. **37:**1367–1369.
4. **Christiansen, K. J., E. M. Bernard, J. W. M. Gold, and D. Armstrong.** 1985. Distribution and activity of amphotericin B in humans. J. Infect. Dis. **152:** 1037–1043.
5. **Clark, J. M., R. R. Whitney, S. J. Olsen, R. J. George, M. R. Swerdel, L. Kunselman, and D. P. Bonner.** 1991. Amphotericin B lipid complex therapy of experimental fungal infections in mice. Antimicrob. Agents Chemother. **35:**615–621.
6. **Gondal, J. A., R. P. Swartz, and A. Rahman.** 1989. Therapeutic evaluation of free liposome-encapsulated amphotericin B in the treatment of systemic candidiasis in mice. Antimicrob. Agents Chemother. **33:**1544–1548.
7. **Gryn, J., J. Goldberg, E. Johnson, J. Siegel, and J. Inzerillo.** 1993. The toxicity of daily inhaled amphotericin B. Am. J. Clin. Oncol. **16:**43–46.
8. **Heinemann, V. P., P. Scolz, and U. Jehn.** 1992. Inhalation of amphotericin B as prophylactic antifungal treatment during intensive leukemia therapy. Ann. Hematol. **78:**A110. (Abstr. 144.)
9. **Lopez-Berestein, G., G. P. Bodey, V. Fainstein, M. Keating, L. S. Frankel, B. Zeluff, L. Gentry, and K. Metha.** 1989. Treatment of systemic fungal infections with liposomal amphotericin B. Arch. Intern. Med. **149:**2533–2536.
10. **Meyer, R. D., L. S. Young, D. Armstrong, and B. Yu.** 1973. Aspergillosis complicating neoplastic disease. Am. J. Med. **56:**6–15.
11. **Myers, M. A., R. W. Niven, L. Straub, H. Schreier, and R. J. Gonzalez-Rothi.** 1990. Alveolar macrophage profiles in mice chronically exposed to liposome aerosols. Am. Rev. Respir. Dis. **141:**A675.
12. **Niki, Y., E. M. Bernard, H. J. Schmitt, W. P. Tong, F. F. Edwards, and D. Armstrong.** 1990. Pharmacokinetics of aerosol amphotericin B in rats. Antimicrob. Agents Chemother. **34:**29–32.
13. **O'Donnell, M. R., G. M. Schmidt, B. R. Tegtmeier, C. Faucett, J. L. Fahey, J. Ito, A. Nademanee, J. Niland, P. Parker, E. P. Smith, D. S. Snyder, A. S. Stein, K. G. Blume, and S. J. Forman.** 1994. Prediction of systemic fungal infection in allogeneic marrow transplant recipients: impact of amphotericin B prophylaxis in high-risk patients. J. Clin. Oncol. **12:**827–834.
14. **Schmitt, H. J., E. M. Bernard, M. Hauser, and D. Armstrong.** 1988. Aerosol amphotericin B is effective for prophylaxis and therapy in a rat model of pulmonary aspergillosis. Antimicrob. Agents Chemother. **32:**1676–1679.
15. **Schreier, H.** 1992. Liposome aerosols. J. Liposome Res. **2**(2)**:**145–184.
16. **Sorensen, K. N., S. D. Allen, M. J. Nejdl, and R. T. Proffitt.** 1993. Aerosolization of liposomal (AmBisome) and non-liposomal (Fungizone) amphotericin B as a treatment for pulmonary fungal infections, p. 187. *In* Proceedings of the Sixth International Symposium on Recent Advances in Drug Delivery Systems Abstracts. University of Utah, Salt Lake City.
17. **Thomas, D. A., M. A. Myers, B. M. Wichert, H. Schreier, and R. J. Gonzalez-Rothi.** 1991. Acute effects of liposome aerosol inhalation on pulmonary function in healthy human volunteers. Chest **99:**1268–1270.