ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1992, p. 1466–1471
0066-4804/92/071466-06$02.00/0

Vol. 36, No. 7

Aerosolized Liposomal Amphotericin B for Treatment of Pulmonary and Systemic *Cryptococcus neoformans* Infections in Mice

BRIAN E. GILBERT,* PHILIP R. WYDE, AND SAMUEL Z. WILSON

Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030

Received 8 January 1992/Accepted 1 May 1992

Cryptococcus **infections of the lung and central nervous system have become major problems in immunocompromised patients, leading to the need for additional treatment protocols. We have utilized a *Cryptococcus*-mouse model that mimics human cryptococcal disease to evaluate the efficacy of amphotericin B-liposomes (AmpB-Lip) when delivered by small-particle aerosol (SPA). In the model, initial intranasal inoculation leads to a pulmonary infection that spreads after 2 to 3 weeks to distant organs, including the brain. Aerosols of AmpB-Lip that were generated by a Collison nebulizer had mass median aerodynamic diameters of 1.8 μm and contained 10.3 μg of AmpB per liter. When AmpB-Lip SPA was begun at 24 h postinoculation, a single 2-h treatment (0.3 mg of AmpB per kg of body weight) was effective in reducing pulmonary *Cryptococcus* infection. This regimen was more effective than intravenous administration of AmpB-Lip given for 3 continuous days. This single 2-h exposure to AmpB-Lip also was effective in reducing pulmonary *Cryptococcus* infection when treatment was delayed for 7 or 14 days. At day 21, when organisms had spread to the brain in all animals, the single 2-h aerosol treatment reduced the number of cryptococci in the brain as well as in the lungs. AmpB-Lip SPA administered once for 2 h on days 7, 14, and 21 also was effective in increasing the duration of survival of infected animals. These results demonstrate that aerosolized AmpB-Lip can be effective in treating both local, pulmonary *Cryptococcus* disease and systemic disease.**

Opportunistic mycotic infections of immunocompromised individuals have become a major problem in recent years. In AIDS patients, they are an important cause of morbidity and mortality. While some forms of mycotic infections have remained relatively rare, the incidences of progressive pulmonary and disseminated forms of cryptococcosis, histoplasmosis, and coccidioidomycosis all have increased in AIDS patients (reviewed in reference 2). In fact, often when *Pneumocystis carinii* pneumonia fails to improve with appropriate therapy, a concomitant mycotic infection is present.

The Centers for Disease Control has reported a 7 to 8% prevalence of cryptococcosis among AIDS patients in the United States between 1981 and 1987; a much higher incidence has been reported in specific areas of the country (2). Since the lungs are the natural portal of entry for cryptococci, pulmonary lesions may be the initial presenting feature. On lung biopsy, a characteristic interstitial pattern of encapsulated yeast cells in the alveolar space with little inflammation and no well-formed granulomas has been reported (3). In 75 to 90% of cases of cryptococcosis in AIDS patients, a further involvement of the central nervous system is manifested primarily as meningoencephalitis.

The treatment of systemic fungal infections with amphotericin B (AmpB) has been effective, although this antifungal agent is often quite toxic. The association of AmpB with liposomes does not diminish the antifungal activity but does reduce the drug's cytotoxicity (1), although cardiopulmonary toxicity after liposomal-AmpB (AmpB-Lip) infusion has been reported (11). In an experimental *Candida* model, AmpB-Lip could be administered intravenously (i.v.) at 10 times the free-AmpB 50% lethal dose without any apparent toxicity (1, 12, 13). The problem with systemic treatment with AmpB-Lip is the rapid clearance of the liposomes by the reticuloendothelial system. This requires administration of higher doses to obtain sufficient levels of drug in the lungs and may lead to side effects.

To better target AmpB to the lungs, aerosolized Fungizone (AmpB in deoxycholate [AmpB-DOC]) has been tested in the rat model (14, 15). AmpB was shown to have a half-life of elimination of 4.8 days and to accumulate following multiple treatments. Treatment with 60 mg/kg of body weight for 6 h was well tolerated, and no histopathological changes in the lungs were noted. Prophylaxis and treatment of pulmonary aspergillosis with aerosolized AmpB-Lip (1.6 mg/kg/day) significantly prolonged the duration but not the rate of survival.

Combining aerosolization to specifically target the lungs and AmpB-Lip to reduce toxicity, we have shown in a *Cryptococcus*-mouse model the efficacy of a small-particle aerosol (SPA) of AmpB-Lip in the treatment of both pulmonary and systemic diseases.

MATERIALS AND METHODS

Materials. AmpB (80% pure) was purchased from Sigma Chemical Co., St. Louis, Mo. Egg yolk phosphatidylcholine was obtained from Avanti Polar Lipids, Inc., Pelham, Ala. Chemicals for high-pressure liquid chromatographic (HPLC; Waters, Milford, Mass.) analysis were HPLC grade or as pure as possible. DOC, sodium salt, was purchased from Calbiochem, San Diego, Calif.

Cryptococcus **isolate.** A clinical isolate of *Cryptococcus neoformans* was obtained from the Clinical Microbiology Laboratory, The Methodist Hospital, Houston, Tex. This isolate was passaged in mice to increase its virulence. Microscopic examination revealed that the yeast cells were

* Corresponding author.

encapsulated. The 50% inhibitory concentrations for the liposomal and DOC AmpB preparations against this strain of *Cryptococcus* were similar and in the range of 0.1 to 0.6 μg/ml.

Mice. Six- to eight-week-old (23- to 28-g), random-bred, CD-1 mice obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass., were used in all experiments. The animals were housed in cages covered with barrier filters and had mouse chow and water ad libitum. Mice lightly anesthetized with CO_2 were inoculated intranasally with *C. neoformans* in 50 μl of saline (day 0). Three to five mice per group were used in all experiments (see figure legends) except the survival study, which used 15 mice per group. Day 0 values represent the initial pulmonary inoculum without the presence of organisms in any other tissue.

Preparation of AmpB-Lip. Multilamellar liposomes were prepared as previously described (4). Briefly, powdered AmpB (2 mg/ml final concentration) was added to egg yolk phosphatidylcholine (30 mg/ml final concentration) in chloroform solution. This solution was diluted with absolute methanol (four times the volume of the chloroform solution) to give a uniform film on drying. The solvent was removed by rotoevaporation (Buchi 421 rotavapor) at 52°C under vacuum. Liposomes were then formed by mechanical shaking of the dried residue in sterile water and sonication in a water bath. Control liposomes were prepared similarly but without AmpB. Liposome preparations were kept at 4°C for up to 3 days.

SPA treatment. Mice were placed in sealed plastic cages and exposed to aerosols as previously described (5, 17). The Collison aerosol generator was the same as previously described (9, 17) except that the shape of the reservoir was modified to allow collection of liposomes at the bottom. This change yields a more uniform aerosol. The estimated dose of drug retained by each mouse was calculated from the amount of drug in the aerosol, the minute volume, a retention factor of 0.3, and the duration of treatment, as previously described (5, 17).

Characterization of the AmpB-Lip aerosol. The concentration of AmpB generated in the aerosol and the particle size distribution were determined by using an all-glass impinger and an Andersen sampler, respectively, as previously described (6). Aerosol samples were taken at the 1-h midpoint of the treatment period. Samples from the all-glass impingers were analyzed directly, while those collected by the Andersen sampler were eluted from the filters by soaking the filters in 20 ml of absolute methanol overnight. AmpB was measured by HPLC as described below.

i.v. administration. Mice (five per group) were inoculated i.v. in the tail vein with 0.1 ml of AmpB-Lip or AmpB in 0.2 mg of DOC per ml.

Concentration of AmpB in lung tissue. Mice were given two 2-h treatments of AmpB-Lip (2 mg of AmpB per mL; 40 mg of egg yolk phosphatidylcholine per ml) by SPA using a Collison nebulizer with 0.3, 0.43, and 2 mg of AmpB per ml in the reservoir. These treatments gave estimated retained doses of 0.09, 0.12, and 0.58 mg/kg/day, respectively. For comparison, animals were given a single i.v. injection. At the end of the second SPA treatment period or 1 h after the i.v. injection, mice were euthanized with CO_2, lungs were removed and homogenized, and AmpB was extracted and analyzed by HPLC. AmpB (98 to 99% pure) used for these experiments was a gift of Gabriel Lopez-Berestein, M. D. Anderson Cancer Center, Houston, Tex.

Quantification of AmpB by HPLC. AmpB was quantified by HPLC with a linear gradient (60 to 95%) of methanol in 0.01 M acetate buffer, pH 4, and monitoring at 407 nm. All measurements were made at ambient temperature on a Microsorb C18 stainless-steel HPLC column (particle size, 5 μm; length, 25 cm; inner diameter, 4.6 mm; Rainin Instrument Co., Emeryville, Calif.). Lung tissue was homogenized in 1 ml of HPLC-grade water by shaking with glass beads. An aliquot (ca. 300 μl) was removed and mixed with an equal volume of 100% ethanol. Following centrifugation, 100 μl of the extract was injected. As standards, AmpB was extracted from lung tissue and quantified by HPLC using homogenized tissue samples with known amounts of AmpB and processed in the same manner.

Quantitation of cryptococci in organs. Organs were removed, rinsed of any adhering blood, and homogenized in 1.0 ml of minimal essential medium supplemented with 2% fetal calf serum in a Mini-Beadbeater (Biospec Products, Bartlesville, Okla.). Serial 10-fold dilutions of the homogenates were plated (10 μl) on petri dishes containing Sabouraud agar. Following incubation at 35°C for 2 to 3 days, colonies were quantitated. Initial inocula (day 0) of cryptococci were quantified in the same manner. The minimal level of detection was 100 CFU; for statistical purposes, the value 50 CFU was used when no organisms were isolated from a tissue. The average standard deviation of CFU was about ±50% of the mean values. Significant differences between all groups and time points were determined by standard statistical methods (see below). Results are presented in the text as means ± standard deviations.

Statistical analysis. Data analysis was performed with the True Epistat statistical package from Epistat Services, Richardson, Tex. *P* values (two tailed) were based on analysis of these data by Student's *t* test with analysis of variation, Fisher's exact test, or Life Table Analysis.

RESULTS

Characterization of AmpB-Lip aerosol. The aerosol characteristics of AmpB-Lip were similar to those of other liposome preparations (6) and of aqueous aerosols (8) generated from the Collison nebulizer. Following 1 h of aerosolization, the mass median aerodynamic diameter of the heterodispersed aerosol particles was 1.8 ± 0.2 μm, with a geometric standard deviation of 3.4 ± 0.6 μm. The aerosol contained 10.3 ± 2.4 μg of AmpB and 295 ± 84 μg of phosphatidylcholine per liter. Judging from this value, the estimated retained dose of AmpB with a twice-daily 2-h treatment was 0.58 mg/kg/day (5, 17).

Model of pulmonary and systemic *C. neoformans* infections in mice. A model of pulmonary cryptococcosis was developed by using a mouse-adapted clinical isolate of *C. neoformans*. Following intranasal inoculation, organisms replicated in the lungs of mice for 2 to 3 weeks (Fig. 1A). Although the total number of organisms in the lungs was dependent on the size of the initial inoculum, the greatest increase in growth took place with smaller inocula during the first 7 to 10 days. Lungs appeared to become saturated with organisms by about 2 to 3 weeks, with most alveoli containing one or more yeast cells when examined histologically. At this time, cryptococci could be found in other organs, especially the brain (Fig. 1B), liver (Fig. 1C), and kidneys (Fig. 1D). Over the 4-week period, the level of cryptococci in the lungs, brain, and liver with the two largest inocula increased 10- to 100-fold or more. Animals given limited treatment with different preparations of AmpB-Lip or AmpB-DOC (see below) still harbored organisms, developed symptoms, and died weeks later.

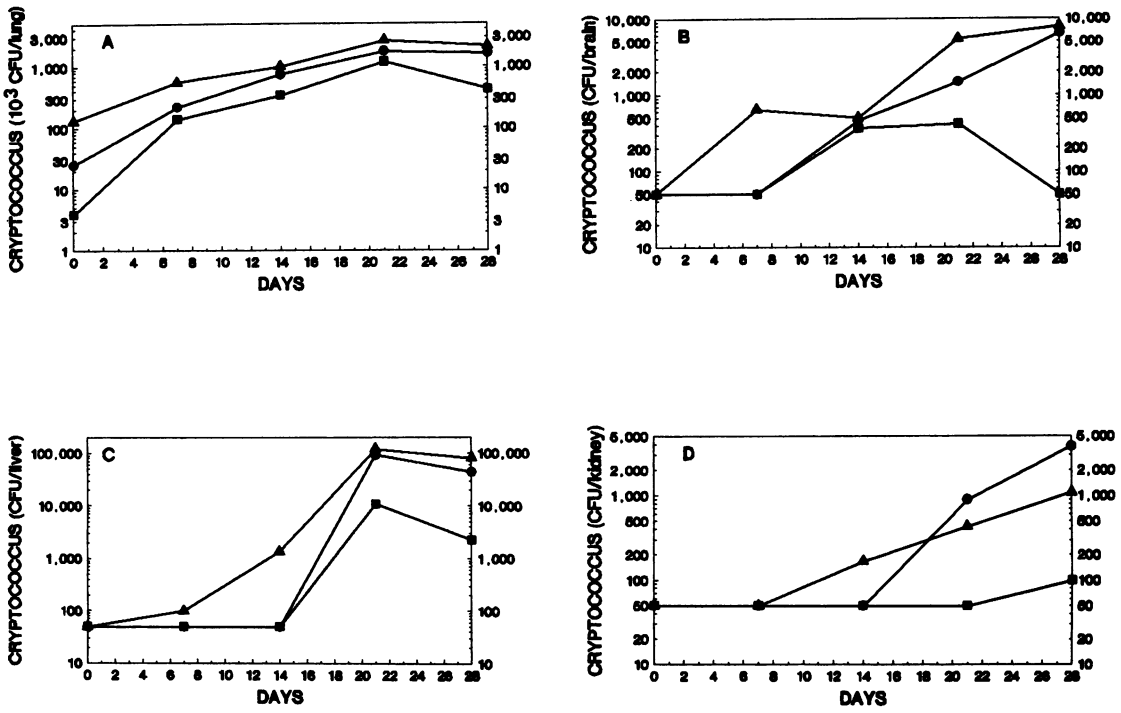

FIG. 1. Kinetics of *C. neoformans* growth in mouse lungs, brain, liver, and kidneys. An intranasal inoculum containing 3.9×10^3 (■), 2.5×10^4 (●), or 1.3×10^5 (▲) cryptococcal cells in 0.05 ml was given to mice (three per group) on day 0. On the days indicated, organs were removed and homogenized, and cryptococci were quantitated on Sabouraud agar. Day 0 values represent the initial inoculating dose. (A) Lungs; (B) brain; (C) liver; (D) kidneys.

Effectiveness of AmpB-Lip SPA for the treatment of pulmonary and systemic cryptococcal diseases. We have evaluated the efficacy of AmpB-Lip administered by aerosol. When AmpB-Lip SPA treatment was begun at 24 h postinoculation, a single 2-h treatment (0.3 mg of AmpB per kg) was effective in reducing numbers of pulmonary cryptococci compared with numbers in the control ($[4.9 \pm 5.2] \times 10^3$ versus $[542 \pm 386] \times 10^3$ CFU per lung; $P = 0.0001$); this regimen was more effective than i.v. administration of drug for three consecutive days (0.6 mg/kg/day; $[4.9 \pm 5.2] \times 10^3$ versus $[228 \pm 114] \times 10^3$ CFU per lung; $P = 0.002$) (Fig. 2). SPA regimens given for multiple days were also effective although not statistically better than the single treatment on day 1. Three days of i.v. treatment was better statistically than a single day of treatment ($[228 \pm 114] \times 10^3$ versus $[486 \pm 219] \times 10^3$ CFU per lung; $P = 0.039$).

To better mimic the slow spread of *C. neoformans* from its natural portal of entry, the lungs, to other organs, especially the brain, AmpB-Lip SPA treatment was delayed 7 to 21 days postinoculation. As seen with the early-treatment protocol, a single 2-h treatment (0.3 mg/kg) on day 7 significantly reduced the pulmonary titer of *C. neoformans* on day 14 ($[10 \pm 3] \times 10^5$ versus $[1.2 \pm 0.8] \times 10^5$ CFU per lung; $P = 0.014$); i.v. administration of AmpB-Lip again failed to have a statistically significant effect (Fig. 3, left panel). At this time, however, there was little spread to the brain (only one of five mice in the control group and none of the treated animals had a high titer of *C. neoformans*; the difference was not significant [$P = 0.840$]) (Fig. 3, right panel).

When the start of AmpB-Lip aerosol treatment was delayed for 21 days, a single 2-h treatment still reduced pulmonary *Cryptococcus* titers ($[2.6 \pm 0.9] \times 10^6$ versus $[0.5 \pm 0.8] \times 10^6$ CFU per lung; $P = 0.021$); i.v. AmpB-Lip was also effective ($[2.6 \pm 0.9] \times 10^6$ versus $[0.01 \pm 0.01] \times 10^6$ CFU per lung; $P = 0.010$), but AmpB-DOC was not (Fig. 4, left panel). By day 21, the organisms had spread to the brain, and they continued to increase in number through day 28 (Fig. 4, right panel). As with pulmonary disease, AmpB administered by either SPA ($[15.7 \pm 5.9] \times 10^3$ versus $[0.9 \pm 1.5] \times 10^3$ CFU per brain; $P = 0.018$) or i.v. injection ($[15.7 \pm 5.9] \times 10^3$ versus $[0.13 \pm 0.05] \times 10^3$ CFU per brain; $P = 0.021$) was effective in reducing the number of cryptococci in the brain. There was no statistically significant difference in

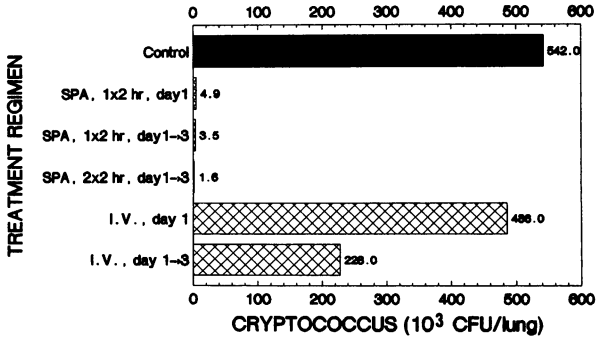

FIG. 2. Effect of routes of administration and dosing schedule of AmpB-Lip on pulmonary *C. neoformans* in mice. Mice (five per group) were inoculated with 1.6×10^5 organisms on day 0 and treated on day 1 or on days 1 to 3 by SPA or i.v. administration. Lungs were harvested on day 4. Dosages: SPA once every 2 h, 0.3 mg/kg/day; SPA twice every 2 h, 0.6 mg/kg/day; i.v., 0.6 mg/kg/day. None of the SPA treatment groups was statistically different. Numbers next to bars are exact values.

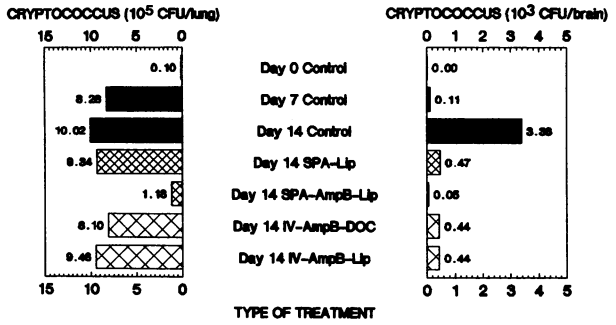

FIG. 3. Effect of routes of administration of AmpB-Lip, liposomes, and AmpB-DOC on *C. neoformans* in the lungs and brain of mice treated once for 2 h at 7 days after inoculation. Mice (five per group) were inoculated with 10^4 organisms on day 0 and treated on day 7 by SPA or i.v. administration. Lungs and brain were harvested on day 14. Dosages: SPA once every 2 h, 0.3 mg/kg/day; i.v., 0.3 mg/kg/day. Numbers next to bars are exact values.

the efficacy of SPA or i.v. AmpB-Lip treatment of the lungs or brain.

Concentration of AmpB in lung tissue. The effectiveness of AmpB-Lip SPA may be related to the levels of drug obtained in the lungs. At the end of a second 2-h treatment, pulmonary concentrations of AmpB as measured by HPLC varied according to the dose administered by aerosol and were higher on a milligram-per-kilogram-per-day basis than when administered i.v. (Fig. 5). The concentration of AmpB in the lungs of mice following a single 2-h SPA administration of AmpB (0.3 mg/kg/day) in DOC was at the limit of detection (25 to 50 ng per lung), while peak levels of 320 ng per lung were detected in the lungs of mice exposed to AmpB-Lip SPA (data not shown). When lung concentrations following the second 2-h treatment with an aerosol reservoir containing 0.3 mg of AmpB-Lip per ml are estimated, 0.19 μg of AmpB in the lungs of a 25-g mouse would be about 7.6 μg of AmpB per ml of respiratory secretions (7). This is well above the 50% inhibitory concentration for *C. neoformans* (ca. 0.1 μg/ml as either AmpB-DOC or AmpB-Lip).

Effect of AmpB-Lip on survival rates. AmpB-Lip aerosol treatment was effective in increasing survival rates. A single 2-h SPA administration of AmpB-Lip once a week for 3

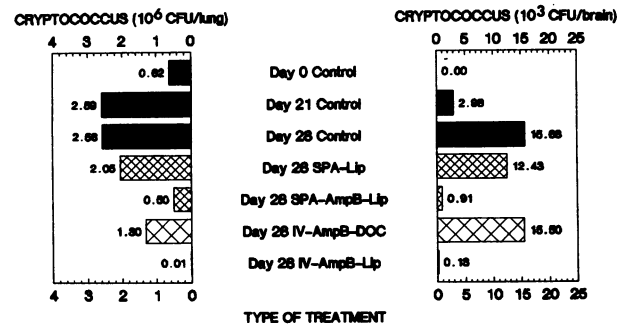

FIG. 4. Effect of routes of administration of AmpB-Lip, liposomes, and AmpB-DOC on *C. neoformans* in the lungs and brain of mice treated once for 2 h at 21 days after inoculation. Mice (five per group) were inoculated with 6.2×10^4 organisms on day 0 and treated on day 21 by SPA or i.v. administration. Lungs and brain were harvested on day 28. Dosages: SPA once every 2 h, 0.3 mg/kg/day; i.v., 0.3 mg/kg/day. Numbers next to bars are exact values.

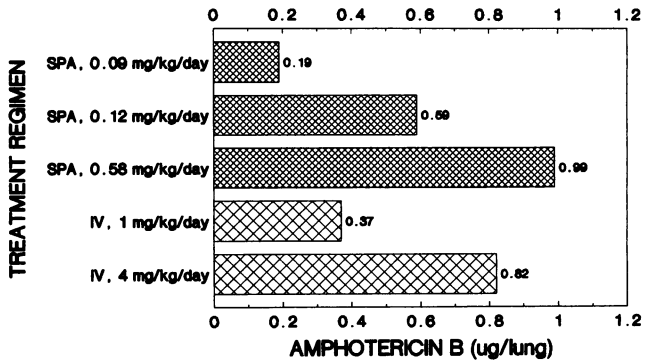

FIG. 5. Concentration of AmpB in mouse lungs following AmpB-Lip treatment. Mice (three to five per group) were given two 2-h treatments of AmpB-Lip by SPA (0.3, 0.43, and 2 mg of AmpB per ml in the reservoir, giving estimated retained doses of 0.09, 0.12, and 0.58 mg/kg/day, respectively) or were given a single i.v. injection. At the end of the second SPA treatment or 1 h after the i.v. injection, lungs were removed and homogenized, and AmpB was extracted and analyzed by HPLC as described in Materials and Methods. Numbers next to bars are exact values.

consecutive weeks was effective in increasing the duration of survival of mice infected with *C. neoformans* ($P = 0.010$) (Fig. 6). These data indicate that this regimen was very effective but that 3 weeks of treatment offered limited protection. Three weeks after the last treatment in week 3, animals began dying, presumably because of residual cryptococci. Delaying treatment until day 21 did not protect the mice ($P = 0.095$), while the single treatment on day 7 had only limited effectiveness ($P = 0.053$).

DISCUSSION

The particle characteristics of aerosolized AmpB-Lip were similar to those previously reported for aerosolized enviroxime (an antiviral compound effective against picornaviruses) incorporated into liposomes composed only of egg yolk phosphatidylcholine (6). This liposome composition gave us a preparation that appeared to have the proper aerosol particle characteristics following the nebulization process. While liposomes of more-complex composition

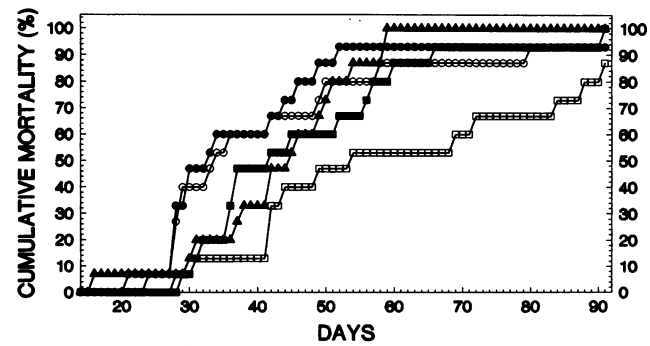

FIG. 6. Effect of aerosol AmpB-Lip on survival following pulmonary *Cryptococcus* infection in mice. Mice (15 per group) were inoculated with 2×10^5 organisms (day 0) and treated by SPA administration once for 2 h on day 7, day 21, or days 7, 14, and 21. Symbols: ●, control; ○, liposome-only treatment on days 7, 14, and 21; ■, AmpB-Lip treatment on day 7; ▲, AmpB-Lip treatment on day 21; □, AmpB-Lip treatment on days 7, 14, and 21.

(e.g., addition of cholesterol, use of defined fatty acids) might work as well or better, the effect of the rigorous aerosolization process on aerosolized liposome particles with increased rigidity, higher transition temperatures, etc., would need to be evaluated.

The AmpB-Lip particles generated by the Collison nebulizer in this study were within the range that would be deposited throughout the respiratory tract (8, 10). With an average AmpB concentration in the aerosol of 10.3 μg/liter when the nebulizer reservoir contained 2 mg/ml, a twice-a-day 2-h treatment would produce an estimated 0.99 μg of AmpB per lung (Fig. 5) or an estimated concentration in the respiratory secretions of a 25-g mouse of 39.6 μg/ml (7). Even at the lowest concentration tested (0.3 mg of AmpB per ml in the reservoir), the estimated concentration of AmpB in respiratory secretions was well above the inhibitory concentration for *C. neoformans*.

The *Cryptococcus*-mouse model used in these studies appears to mimic the natural course of infection. Intranasal inoculation (natural port of entry) of a mouse-adapted, encapsulated, clinical strain of *Cryptococcus* led initially to proliferation of the organism in the lungs (acute-disease model). In general, the rapidity of pulmonary growth and systemic spread was a function of the inoculum size. After about 2 weeks, cryptococci were detected in other organs, indicating systemic spread. By 4 weeks, the lungs were grossly nodular by visual observation, had dramatically increased in size, and contained encapsulated yeast cells histologically visible throughout the alveoli (chronic-disease model). After about 4 to 5 weeks, animals began to die, with mortality continuing for 2 to 3 months. This systemic spread of cryptococci from the lungs to the brain makes this model attractive for the evaluation of antifungal compounds that might be useful in the treatment of AIDS patients, in whom cryptococcal central nervous system involvement is common. In this study, i.v. treatment with AmpB-Lip on day 21 following infection but not on day 7 was effective in reducing the number of cryptococci in the lungs and brain. The effectiveness of i.v. AmpB-Lip at this time may have been due to enhanced trapping of liposomes in the highly diseased lung and in the brain.

SPA administration of AmpB-Lip was effective in treating pulmonary *Cryptococcus* infection in mice. With acute infection, early SPA treatment begun on day 1 postinfection was very effective in reducing the number of cryptococci in the lungs and was better than i.v. administration even when the latter was given for three consecutive days. Aerosol delivery specifically targets the lungs, so there are high concentrations of AmpB in the lungs. The estimated concentration of AmpB in respiratory secretions of mice is many-fold greater than the 50% inhibitory concentration for *C. neoformans* or other pulmonary fungi. Thus, early treatment may effectively kill the organisms in the lung, preventing their replication and spread to other organs.

Even when SPA treatment was delayed for 7 to 21 days, it was still effective in reducing pulmonary *Cryptococcus* infection and preventing dissemination to the brain. A single weekly 2-h SPA treatment during weeks 1 to 3 was effective in increasing the duration of survival. With this regimen, only two mice died through day 41 postinfection, 3 weeks after the last treatment. These data suggest that limited aerosol treatments spread over many (6 to 8) weeks may be a more-effective administration of AmpB-Lip. Accumulation of AmpB in the lungs following aerosolization of AmpB-Lip should be similar to that seen for AmpB-DOC (14) and should effectively suppress the replication of cryptococci.

While high concentrations of AmpB were detected in the lungs of these mice (Fig. 5), some drug must have reached the systemic circulation, since aerosol administration reduced the number of organisms systemically. Thus, AmpB delivered to the lungs as a liposomal aerosol can reach both sides of the lung-blood barrier. The mechanism by which AmpB gets from the lung to the systemic circulation is not known. Following aerosolization in mice, the phosphatidylcholine of similar liposomes which contained enviroxime was shown to be intimately associated with the cells lining the bronchi and bronchioles (16). Perhaps phospholipid exchange with cellular membranes or lipoprotein transport is responsible for the release of AmpB in and from the lungs.

Thus, pulmonary and systemic *Cryptococcus* infections can be effectively treated with aerosolized AmpB-Lip. The advantage of this treatment is the selective deposition of drug throughout the respiratory tract without the use of invasive procedures. Weekly 2-h aerosol treatments of AIDS patients with *Cryptococcus* infections or other systemic fungal infections while the patients are waiting to undergo other medical procedures may be convenient and effective. In addition, these models can serve as alternative treatment protocols for evaluating potential antifungal compounds, especially those with limited solubility in aqueous solutions.

ACKNOWLEDGMENTS

These studies were supported by funds from the Clayton Foundation for Research, Houston, Tex.

We thank Yee Chan-Li for her technical assistance.

REFERENCES

1. **Brajtburg, J., W. G. Powderly, G. S. Kobayashi, and G. Medoff.** 1990. Amphotericin B: delivery systems. Antimicrob. Agents Chemother. **34**:381–384.
2. **Diamond, R. D.** 1991. The growing problem of mycoses in patients infected with the human immunodeficiency virus. Rev. Infect. Dis. **13**:480–486.
3. **Gal, A. A., M. N. Koss, J. Hawkins, S. Evans, and H. Einstein.** 1986. The pathology of pulmonary cryptococcal infections in the acquired immunodeficiency syndrome. Arch. Pathol. Lab. Med. **110**:502–507.
4. **Garcon, N. M., H. R. Six, J. Frazer, C. Hazlewood, B. E. Gilbert, and V. Knight.** 1989. Liposomes of enviroxime and phosphatidylcholine: definition of the drug-phospholipid interactions. Antiviral Res. **11**:89–98.
5. **Gilbert, B. E., and V. Knight.** 1986. Biochemistry and clinical applications of ribavirin. Antimicrob. Agents Chemother. **30**: 201–205.
6. **Gilbert, B. E., H. R. Six, S. Z. Wilson, P. R. Wyde, and V. Knight.** 1988. Small particle aerosols of enviroxime-containing liposomes. Antiviral Res. **9**:355–365.
7. **Gilbert, B. E., and P. R. Wyde.** 1988. Pharmacokinetics of ribavirin aerosol in mice. Antimicrob. Agents Chemother. **32**: 117–121.
8. **Knight, V., and B. Gilbert.** 1988. Antiviral therapy with small particle aerosols. Eur. J. Clin. Microbiol. Infect. Dis. **7**:721–731.
9. **Knight, V., B. E. Gilbert, and S. Z. Wilson.** 1986. Ribavirin small particle aerosol treatment of influenza and respiratory syncytial virus infections, p. 37–56. *In* T. Stapleton (ed.), Studies with a broad spectrum antiviral agent. Royal Society of Medicine Series, New York.
10. **Knight, V., C. P. Yu, B. E. Gilbert, and G. W. Divine.** 1988. Estimating the dosage of ribavirin aerosol according to age and other variables. J. Infect. Dis. **158**:443–448.
11. **Levine, S. J., T. J. Walsh, A. Martinez, P. Q. Eichacker, G. Lopez-Berestein, and C. Natanson.** 1991. Cardiopulmonary toxicity after liposomal amphotericin B infusion. Ann. Intern. Med. **114**:664–666.
12. **Lopez-Berestein, G.** 1988. Liposomes as carriers of antifungal

drugs. Ann. N.Y. Acad. Sci. **544**:590–597.
13. **Lopez-Berestein, G., R. Mehta, R. L. Hopfer, K. Mills, L. Kasi, K. Mehta, V. Fainstein, M. Luna, E. M. Hersh, and R. Juliano.** 1983. Treatment and prophylaxis of disseminated infection due to Candida albicans in mice with liposome-encapsulated amphotericin B. J. Infect. Dis. **147**:939–945.
14. **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.
15. **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.
16. **Wyde, P. R., H. R. Six, S. Z. Wilson, B. E. Gilbert, and V. Knight.** 1988. Activity against rhinoviruses, toxicity, and delivery in aerosol of enviroxime in liposomes. Antimicrob. Agents Chemother. **32**:890–895.
17. **Wyde, P. R., S. Z. Wilson, B. E. Gilbert, and R. H. A. Smith.** 1986. Protection of mice from lethal influenza virus infection with high dose-short duration ribavirin aerosol. Antimicrob. Agents Chemother. **30**:942–944.