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Enhanced Effect of Liposome-Encapsulated Amikacin on *Mycobacterium avium-M. intracellulare* Complex Infection in Beige Mice

NEJAT DÜZGÜNEŞ,[1,2]* VELUCHAMY K. PERUMAL,[3] LAKSHMYYA KESAVALU,[3] JAYNE A. GOLDSTEIN,[1] ROBERT J. DEBS,[1] AND PATTISAPU R. J. GANGADHARAM[3]

Cancer Research Institute[1] and Department of Pharmaceutical Chemistry,[2] University of California, San Francisco, California 94143-0128, and Mycobacteriology Research Laboratory, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206[3]

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We examined the therapeutic effects of free and liposome-encapsulated amikacin on *Mycobacterium avium-M. intracellulare* complex infection by using the beige-mouse model of the disease. In the first series of studies, intravenous administration of four weekly doses of 5 mg of amikacin per kg encapsulated in large (approximately 0.4-μm diameter), unilamellar liposomes arrested the growth of *M. avium-M. intracellulare* complex organisms in the liver, as measured by CFU counts. *M. avium-M. intracellulare* complex levels in untreated animals and in those treated with the same dose of free amikacin increased by several orders of magnitude over 8 weeks. Liposome-encapsulated amikacin was also effective against *M. avium-M. intracellulare* complex organisms in the spleen and kidneys, reducing the CFU counts by about 1,000-fold compared with those of both untreated controls and free-drug-treated mice. In the lungs, a slight reduction in CFU was observed in the liposome-encapsulated-amikacin-treated group, but only at the 8-week point. Neither free nor liposome-encapsulated amikacin reduced the colony counts in the lymph nodes compared with those of control animals. Reductions in CFU in all organs greater than those caused by the liposome preparation could be achieved by intramuscular administration of free amikacin, but only at a 10-fold-higher dose given 6 days a week for 8 weeks. In the second series of studies, we investigated the effects of (i) doubling the dose of liposome-encapsulated amikacin and (ii) increasing the size of the liposomes and prolonging the treatment to five injections. Administration of 10 mg of amikacin per kg in liposomes 2 to 3 μm in diameter was more effective in the liver than 5 or 10 mg of amikacin per kg in liposomes 0.2 μm in diameter. A slight reduction in the CFU levels in the lungs was observed with the higher dose, irrespective of liposome size. Our results indicate that liposome-based delivery of amikacin enhances its anti-*M. avium-M. intracellulare* complex activity, particularly in the liver, spleen, and kidney, and may therefore improve the therapy of this disease.

Mycobacterium avium-M. intracellulare complex infection is a serious acquired immunodeficiency syndrome (AIDS)-related opportunistic infection which is diagnosed in more than 50% of AIDS patients (2, 19, 23, 36, 40). The organism multiplies primarily in resident macrophages of the lungs, spleen, liver, and lymph nodes of infected individuals (19, 23, 36, 39, 40). Anti-*M. avium-M. intracellulare* complex therapy with the available antimycobacterial drugs remains ineffective in both AIDS and non-AIDS patients (4, 20, 22, 38). Many drugs that have high in vitro activity against the *M. avium-M. intracellulare* complex (21, 37) have limited efficacy in vivo (28, 38; P. R. J. Gangadharam, V. K. Perumal, N. R. Podapati, K. Parikh, and M. D. Iseman, 3rd Int. Conf. AIDS, abstr. no. THP147, p. 188, 1987). Inability of the drugs to achieve bactericidal levels at the sites of infection without producing unacceptable toxicity limits the efficacy of current anti-*M. avium-M. intracellulare* complex therapy. Thus, targeting of drugs to sites of *M. avium-M. intracellulare* complex infection is imperative if in vivo efficacy is to be enhanced.

In contrast to several drugs with limited in vivo activity in animal models and in patients, amikacin has been shown to be effective against the *M. avium-M. intracellulare* complex both in vitro and in vivo. Amikacin has anti-*M. avium-M. intracellulare* complex activity in vitro at concentrations achievable in serum (37) and under simulated in vivo conditions in which the drug concentration is decreased over time (15). In the acute-infection model of the disease in beige mice (13), amikacin has been demonstrated to have high activity by itself or in combination with clofazimine or rifabutine or both (17). However, to be effective in vivo, amikacin has to be administered intramuscularly (i.m.) at high doses (50 mg/kg) daily for prolonged periods (6 to 8 weeks in the beige-mouse model). It is therefore necessary to develop methods to reduce the amount of the drug given and decrease the number of administrations without decreasing the in vivo activity. In this report, we describe the use of liposomes for therapy of *M. avium-M. intracellulare* complex infections in mice, both as a means to target antibiotics to infected organs and as a method to decrease the total dose of the drug that must be administered.

Liposomes are phospholipid bilayer vesicles that can encapsulate a variety of drugs and macromolecules. They are avidly phagocytosed by macrophages both in vivo and in vitro and are naturally targeted to the major organs involved in disseminated infection with the *M. avium-M. intracellulare* complex (9, 31–33). Thus, liposomes are potentially ideal vehicles for directing drugs to sites of *M. avium-M. intracellulare* infection. Administration of liposome-encapsulated antibiotics and antiviral agents has resulted in enhanced efficacy against a number of infections localized in the reticuloendothelial system, including *Leishmania dono-*

* Corresponding author.

vani, *Listeria monocytogenes*, *Candida albicans*, and Rift Valley fever virus (1, 3, 25, 27). In this study, we compared the efficacies of free and liposome-encapsulated amikacin on the multiplication of *M. avium-M. intracellulare* complex organisms in beige mice.

MATERIALS AND METHODS

Drugs and reagents. Amikacin sulfate was obtained from Sigma Chemical Co. (St. Louis, Mo.). Phosphatidylglycerol and phosphatidylcholine, both prepared from egg yolk, were obtained from Avanti Polar Lipids (Birmingham, Ala.). Cholesterol was purchased from Behring Diagnostics and recrystallized twice from methanol. NaCl was from Mallinckrodt Inc. (St. Louis, Mo.), KCl was from Fisher Scientific Co. (Pittsburgh, Pa.), and HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and glycine were from Sigma.

Strain of *M. avium-M. intracellulare* complex and infection of animals. A single-cell suspension of a predominantly (>95%) transparent colony type of the *M. avium-M. intracellulare* complex (strain 101; serotype 1) was used for the experiments. The strain was originally isolated from the blood of a patient with AIDS in California and was obtained from Clark Inderlied. The organisms were maintained by repeated passages through beige mice to retain their virulence (14, 18). Each animal received approximately 10^6 to 10^7 viable units of the organism by intravenous (i.v.) injection. Five- to seven-week-old male beige (C57BL/6/bg^J/bg^J) mice were used for the experiments. The animals were kept five to a cage in a biohazard room. Food and water were given ad libitum.

Preparation of liposomes. Amikacin was encapsulated in liposomes composed of phosphatidylglycerol-phosphatidylcholine-cholesterol (1:1:1 molar ratio) by reverse-phase evaporation followed by extrusion through polycarbonate membranes (10, 34). A sterile 50-mg/ml solution of amikacin in 10 mM KCl–5 mM glycine, pH 9.6, adjusted to an osmolality of 300 mosM with NaCl, was added to a solution of the lipids in diethyl ether (50 μmol of lipid in 2 ml) at a 1:3 ratio, and the mixture was sonicated under argon for 5 min. The resulting emulsion was placed in a rotary evaporator and evaporated under controlled vacuum as described previously (10). To eliminate any residual ether, the suspension was supplemented with 1.32 ml of the amikacin solution and placed in the evaporator for an additional 20 min. The liposomes were then extruded through polycarbonate membranes with a 0.2-μm pore diameter (Nuclepore Corp., Pleasanton, Calif.) under argon pressure. Unencapsulated amikacin was eliminated by chromatographing the liposome suspension on sterilized Sephadex G-75 with 140 mM NaCl–10 mM KCl–10 mM glycine, pH 9.6, as the elution buffer. To ensure the sterility of the liposome suspension, the latter was filtered through a 0.22-μm (pore size) filter (Schleicher & Schuell, Inc., Keene, N.H.). The size distribution of the liposomes was ascertained in an NP-4 dynamic light-scattering instrument (Coulter Electronics, Inc., Hialeah, Fla.). The mean diameter of the vesicles was in the range of 400 nm, with a standard deviation of 140 nm. In the second series of experiments, large, multilamellar vesicles were prepared by drying the lipid from a chloroform solution onto the sides of a glass tube as a thin film and hydrating the lipid with the amikacin solution by slow rotation for several hours. The unencapsulated material was separated from the liposomes by repeated centrifugation and washing with elution buffer. The size distribution of these vesicles was in the range of 2 to 3 μm, as estimated by a Coulter Channelyzer with latex particles as standards.

The amount of encapsulated amikacin was determined by an enzyme-linked immunosorbent assay after the liposomes were lysed with 0.5% Triton X-100 by using an aca discrete clinical analyzer (Du Pont Co., Wilmington, Del.). The assay is based on the activity of glucose-6-phosphate dehydrogenase conjugated to amikacin. The enzyme is inhibited when the conjugate is bound to anti-amikacin antibody. The amount of bound conjugate is determined by the concentration of free amikacin, which competes for the antibody. Phospholipid concentrations were determined by phosphate analysis (5). Control liposomes containing buffer only, without amikacin, were prepared with 140 mM NaCl–10 mM KCl–5 mM glycine, pH 9.6, as the aqueous medium.

Administration of drugs. In the first series of experiments, free or liposome-encapsulated amikacin was administered to mice i.v. via a caudal vein at a dose of 100 μg per mouse per week (5 mg/kg once a week). Treatment was started 1 day after infection and was continued for 3 weeks (a total of four injections). In addition, one group of animals received a daily dose of 1 mg per mouse (50 mg/kg daily) i.m. 6 days a week for the entire 8-week period of the experiment. Two control groups were included, one receiving empty (buffer-loaded) liposomes administered at the same lipid concentration as the amikacin-liposome group and the other receiving buffer. In the second series of experiments, the same protocol was followed, except that two doses of liposome-encapsulated amikacin (5 and 10 mg/kg) were administered in five weekly injections, and another group of mice was given 10 mg of amikacin per kg in large, multilamellar vesicles. Free amikacin was given i.v. at a dose of 10 mg/kg. In these experiments, the effect of i.m. amikacin was not investigated. Control groups were given buffer or buffer-loaded liposomes at the same lipid concentration as the liposomes used for the 10-mg/kg dose in unilamellar vesicles.

Assessment of results. Three randomly selected mice were killed on day 1 following infection (base line) and at 2-week intervals thereafter for up to 8 weeks. The CFU counts were determined in the liver, spleen, lungs, kidneys, and pooled lymph nodes (superficial inguinal, mesenteric, superficial deep cervical, and renal), following homogenization and plating on 7H11 agar medium.

Statistics. Standard randomization procedures were used in selecting animals to be killed at scheduled intervals. To avoid bias, the identity of the group of animals was concealed from the observer until the CFU counts were enumerated. Student's *t* test and analysis of variance were used in individual experiments to analyze the data.

RESULTS

Effect of amikacin treatment on the progress of *M. avium-M. intracellulare* complex disease. The effect of treatment with the free and liposome-encapsulated forms of amikacin on the progress of *M. avium-M. intracellulare* complex disease over a period of 8 weeks is discussed separately for each tissue type.

(i) Liver. The progress of *M. avium-M. intracellulare* complex infection in the liver over an 8-week period and the effect of treatment are shown in Fig. 1. Administration (i.v.) of four weekly low doses of amikacin (5 mg/kg) encapsulated in liposomes prevented in vivo multiplication of the *M. avium-M. intracellulare* complex. In contrast, the CFU counts increased monotonically in control animals that received injections of buffer of liposomes containing buffer

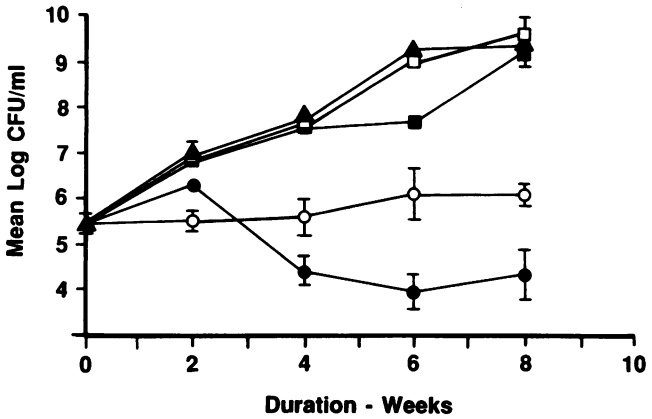

FIG. 1. Progression of *M. avium-M. intracellulare* complex infection in the livers of beige mice (□) and effects of weekly injections of free (■) or liposome-encapsulated (○) amikacin or buffer-loaded liposomes (▲). The data are presented as mean log CFU counts of *M. avium-M. intracellulare* complex organisms per milliliter of tissue. Amikacin was administered in four weekly i.v. injections, starting 1 day after infection. The effects of i.m. injections of 50 mg of free amikacin per kg 6 days a week for 8 weeks are also shown (●). Each point represents the mean of three animals. The bars represent standard deviations. For points without a bar, the standard deviation is within the magnitude of the symbol.

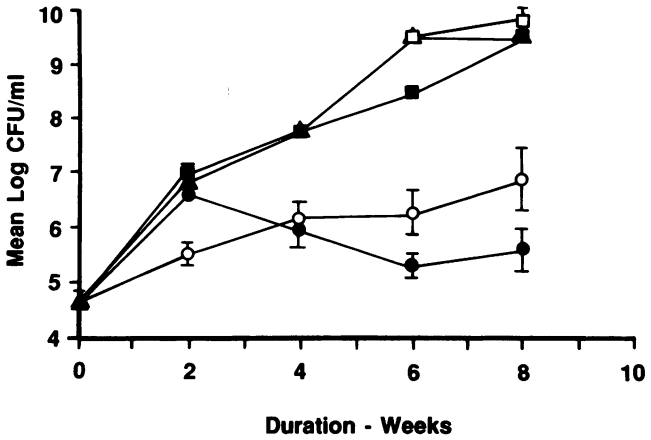

FIG. 2. Time course of *M. avium-M. intracellulare* complex infection in the spleen and effect of amikacin therapy. The results are given as mean log *M. avium-M. intracellulare* complex CFU per milliliter of tissue. The symbols are defined in the legend to Fig. 1.

only (empty liposomes). The same dose of the free drug given under the same conditions did not arrest the growth of the organisms. At the 8-week point, CFU counts in the liposome-encapsulated amikacin group were more than 3 orders of magnitude below those of the buffer or empty-liposome controls and the free-amikacin group. The effect of liposome-encapsulated amikacin persisted for at least 5 weeks after the termination of treatment. Although amikacin targeted to the liver via liposomes was effective in arresting *M. avium-M. intracellular* complex growth, it did not reduce the number of viable organisms.

Previous studies (17) on the therapeutic effect of amikacin have used i.m. administration of the drug at a daily dose of 50 mg/kg. We compared this i.m. dose of amikacin with the i.v. dose under the same experimental conditions (Fig. 1). Amikacin administered i.m. 6 days a week for the entire 8 weeks showed the greatest reduction in CFU counts (Table 1). However, at the 2-week point, the CFU counts were significantly higher than those of the liposome-encapsulated amikacin group ($P < 0.0005$), even though the total i.m. dose of amikacin was 60-fold higher.

(ii) Spleen. Essentially similar results were obtained in the spleen and the liver. Liposome-encapsulated amikacin was more effective than the free drug given i.v. (Fig. 2). By 2 weeks, the CFU counts in animals treated with liposomal amikacin were about 1.5 orders of magnitude lower than the CFU in untreated animals and those that received the free

TABLE 1. Comparison of the reduction in CFU counts in beige mice treated with free or liposome-encapsulated amikacin administered i.v. or amikacin given i.m.

Time and treatment	Total dose (μg)	Reduction of mean log CFU relative to control				
		Liver	Spleen	Lungs	Kidneys	Lymph nodes
2 Wk						
Empty liposomes	0	−0.07	0.11	0.03	−0.10	1.18
Amikacin-liposomes	200	1.37	1.46	0.08	1.11	0.85
Free amikacin	200	−0.10	0.00	0.03	−0.11	−0.44
Amikacin i.m.	12,000	0.62	0.32	1.10	1.15	1.16
4 Wk						
Empty liposomes	0	−0.04	−0.02	0.05	0.71	−0.29
Amikacin-liposomes	400	2.12	1.56	0.21	1.57	0.13
Free amikacin	400	0.10	−0.06	−0.10	0.73	−0.24
Amikacin i.m.	24,000	3.34	1.77	2.76	3.43	1.95
6 Wk						
Empty liposomes	0	−0.20	−0.03	−0.15	−0.10	−0.32
Amikacin-liposomes	400	2.97	3.17	0.66	1.98	0.71
Free amikacin	400	1.36	0.99	0.80	0.78	0.08
Amikacin i.m.	36,000	5.12	4.14	4.71	4.18	4.78
8 Wk						
Empty liposomes	0	0.10	0.25	−0.21	1.16	0.24
Amikacin-liposomes	400	3.45	2.95	0.57	3.14	0.24
Free amikacin	400	0.26	0.28	−0.22	0.86	0.54
Amikacin i.m.	48,000	5.23	4.23	4.59	6.01	4.54

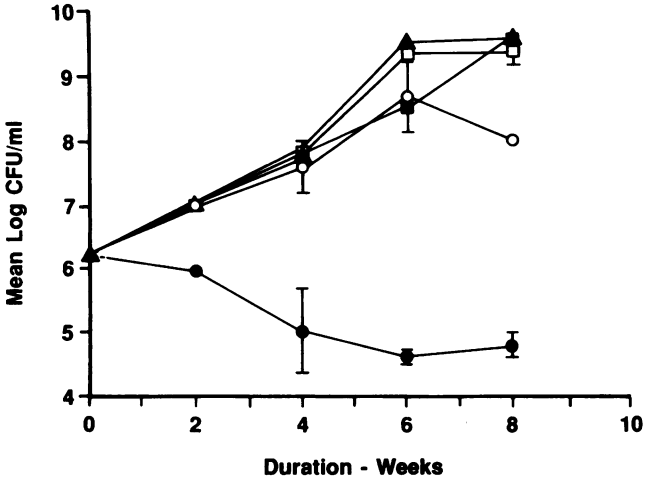

FIG. 3. Progression of *M. avium-M. intracellulare* complex infection in the lungs, presented as log *M. avium-M. intracellulare* complex CFU per milliliter of tissue, and effect of amikacin treatment. The symbols are given in the legend to Fig. 1.

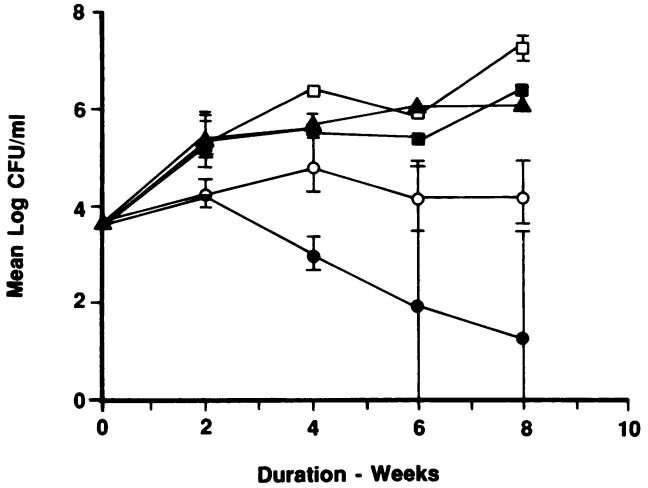

FIG. 4. Effect of amikacin therapy on *M. avium-M. intracellulare* complex infection in the kidneys, shown as log CFU per milliliter of tissue. The symbols are the same as in Fig. 1.

drug. At 8 weeks, i.e., 5 weeks after the end of treatment, the CFU counts were approximately 3 orders of magnitude lower in liposome-encapsulated-amikacin-treated animals than in controls or those receiving free amikacin i.v. The CFU in the spleens of untreated or free-amikacin-treated mice increased by 5 orders of magnitude in 8 weeks, whereas in liposome-encapsulated-amikacin-treated animals the increase was only 2 orders of magnitude. It is of interest that animals treated with liposome-encapsulated amikacin showed a considerable reduction in CFU compared with controls at the 2-week point, at which time the daily i.m. treatment with the high dose did not show any effect ($P < 0.0005$; Table 1 and Fig. 2). At 4 weeks, the CFU counts in the two groups were similar, despite the 60-fold-higher dose of i.m. amikacin.

(iii) Lungs. CFU levels in lungs were not affected by i.v. administration of free or liposome-encapsulated amikacin over the 6-week period. At 8 weeks, the liposome-encapsulated-amikacin-treated group exhibited a slight reduction in CFU counts compared with those of the untreated control group ($P < 0.025$; Fig. 3). Administration of amikacin i.m. at high doses did reduce the CFU counts below the base-line (day 1) level, starting at the 2-week point, reaching 1.6 orders of magnitude below this level by week 6.

(iv) Kidneys. In the kidneys, liposome-encapsulated amikacin prevented an increase of CFU counts (Fig. 4). At the end of the 8-week period, the CFU in mice treated with liposome-encapsulated amikacin were 1,000-fold lower than those of untreated animals ($P < 0.025$), whereas in mice given free amikacin i.v. the reduction in CFU was less than 10-fold (Fig. 4 and Table 1). The results with liposome-encapsulated amikacin are similar to those for liver and spleen; however, unlike those tissues, no difference in CFU counts between the liposome-encapsulated-amikacin and i.m. treatment groups was observed at 2 weeks in the kidneys. At later times, the CFU counts in the i.m. treatment group were reduced considerably (Table 1).

(v) Lymph nodes. In the lymph nodes, the results were essentially the same as with the lungs, in that neither free nor encapsulated amikacin was effective for 8 weeks (Fig. 5). The i.m. treatment group, at a dose 60-fold higher than that of the free- or liposome-encapsulated-amikacin-treatment group, showed a reduction in CFU counts below the base-line (day 1) level after 4 weeks ($P < 0.005$).

Comparison of i.v. and i.m. doses. Table 1 summarizes the changes in CFU counts in all of the tissues of *M. avium-M. intracellulare* complex-infected beige mice treated with the three amikacin regimens. For comparison, the total doses of the drug administered are also included. A considerable reduction in CFU counts compared with those of controls was observed in the i.m. and liposome-encapsulated-amikacin treatment groups. At 2 weeks, the liposome group showed a greater reduction than the i.m. group in both the liver and the spleen. If the reduction in CFU counts is expressed with respect to the unit dose of the drug administered, the group treated with liposome-encapsulated amikacin showed a remarkable efficiency of therapeutic response. For example, at 2 weeks, the reduction in CFU in the spleen per unit dose of liposome-encapsulated amikacin was 1,280-fold greater than in the i.m. amikacin group and 390-fold greater in the liver. At the 4-week point, these

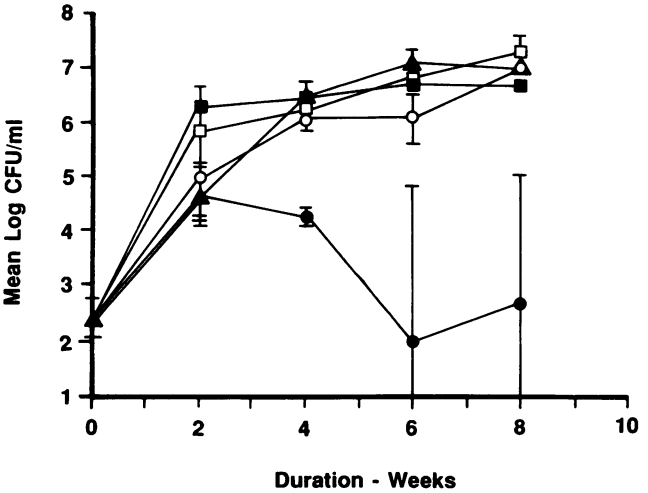

FIG. 5. Effects of free and liposome-encapsulated amikacin on *M. avium-M. intracellulare* complex CFU counts in lymph nodes. The symbols are defined in the legend to Fig. 1.

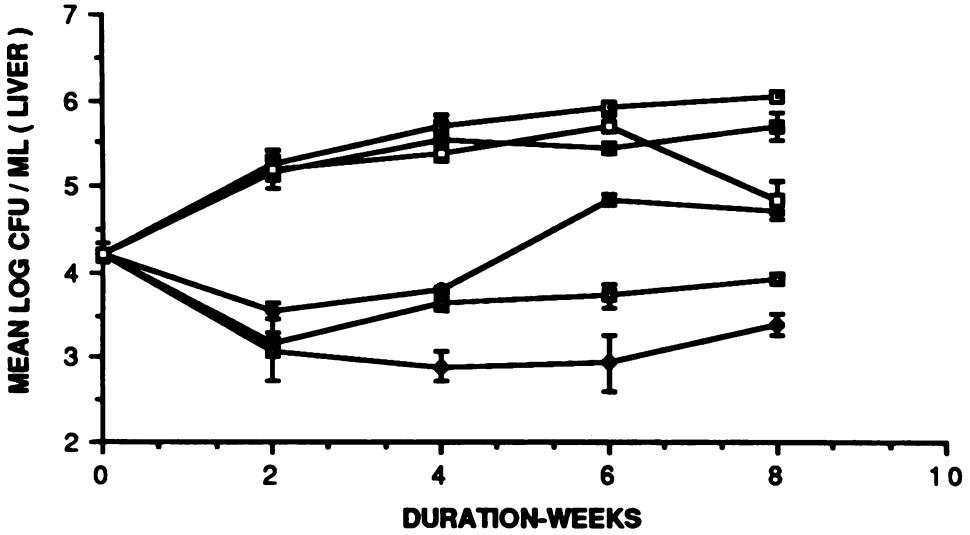

FIG. 6. Effect of 10 mg of amikacin per kg encapsulated in unilamellar vesicles (◆) or large, multilamellar vesicles (◇) on the progression of *M. avium-M. intracellulare* complex infection in the livers of beige mice. The drug was given by weekly i.v. injections for 5 weeks. The effect of 5 mg of amikacin per kg in unilamellar vesicles was also determined in these experiments (◘). Free amikacin was administered i.v. at 10 mg/kg (□). The effect of injections of buffer (▩) or empty liposomes (■) is also shown.

values were 3.6-fold in the liver and 38-fold in the spleen. Although the CFU counts in the i.m. group were reduced below the base-line (day 1) levels over the 8-week period, the reduction of CFU in the liver and spleen per unit drug dose was still greater in the liposome-encapsulated-amikacin treatment group than in the i.m. treatment group (2- and 6.3-fold, respectively).

Effects of increasing the dose of liposome-encapsulated amikacin, prolonging the period of treatment, and increasing the liposome size. In the second series of studies, we investigated the effects on *M. avium-M. intracellulare* complex infection of (i) increasing the dose of amikacin from 5 to 10 mg/kg, (ii) increasing the number of injections from four to five, i.e., at day 1 and at weekly intervals for up to 4 weeks, and (iii) using large, multilamellar vesicles with the intention of increasing lung localization (8). In view of the large number of animals involved in testing the effects of the above-described variables, the i.m. high dose of free amikacin was not examined in these experiments.

(i) Liver. Liposome-encapsulated amikacin administered i.v. decreased the CFU counts from the values at day 1, indicating the bactericidal effect of the treatment (Fig. 6). Free amikacin (10 mg/kg) administered identically had no effect on *M. avium-M. intracellulare* complex CFU counts. At the 2-week point, all preparations produced similar decreases in CFU. At later time points, amikacin in large (2- to 3-μm-diameter), multilamellar vesicles was more effective than the drug encapsulated in unilamellar vesicles (approximately 0.2 μm in diameter in this series of experiments).

(ii) Spleen. Liposome-encapsulated amikacin given at a dose of 5 mg/kg produced results comparable to those of previous experiments (Fig. 7), except that the bacteriostatic effect of the treatment was more pronounced in the second series of experiments. Increasing the dose to 10 mg/kg was slightly more effective. Administration of 10 mg of amikacin per kg in large, multilamellar vesicles prolonged the bacteriostatic effect of the drug. Buffer-loaded liposomes or free amikacin at the same dose had negligible effects on the growth of the organism.

(iii) Lungs. The use of large, multilamellar vesicles to deliver amikacin was marginally effective in reducing the CFU in the lungs at the 4-week point but did not bring about a significant reduction at later times (Fig. 8). Unilamellar vesicles containing the same amount of amikacin (10 mg/kg) also exhibited a slight CFU-reducing effect.

DISCUSSION

Our results demonstrate that i.v. delivery of amikacin encapsulated in large, unilamellar liposomes arrests the growth of *M. avium-M. intracellulare* complex organisms in the livers and spleens of beige mice infected with *M. avium-M. intracellulare* complex. Liposome-encapsulated amikacin was substantially more effective than comparable amounts of free amikacin administered i.v. This reduction was achieved even with four or five weekly injections of 1/10 to 1/5 of the daily i.m. dose of 50 mg/kg. When the efficacies of the liposome-encapsulated drug given i.v. and the free drug given i.m. were expressed as reductions in CFU per unit amount of drug, the former was severalfold higher than the latter. These observations suggest that the negatively charged liposomes used in our study were taken up by the infected macrophages of the liver and spleen. It is also likely that the liposomes, and hence the encapsulated amikacin, were localized at intracellular sites where the mycobacteria reside, since it is known that liposomes are avidly phagocytosed by macrophages in vitro and in vivo (6, 31, 32). Although the duration of the treatment was 3 weeks, the bacteriostatic effect of liposome-encapsulated amikacin persisted for at least 5 weeks, particularly in the liver and kidneys. At the relatively low dose administered in liposomes, amikacin was not able to reduce the number of viable *M. avium-M. intracellulare* organisms but did arrest the growth of the organism. During the initial 2 weeks of therapy, amikacin in liposomes was more effective in the liver and spleen than a 60-fold higher total i.m. dose of free amikacin. Liposome-encapsulated amikacin also stabilized the number of viable mycobacteria in the kidneys at the base-line level but did not inhibit replication of mycobacteria in the lungs and lymph nodes. Our contention that increasing the liposome size might influence the effectiveness of amikacin in the lungs was not borne out by the results of the second series of experiments.

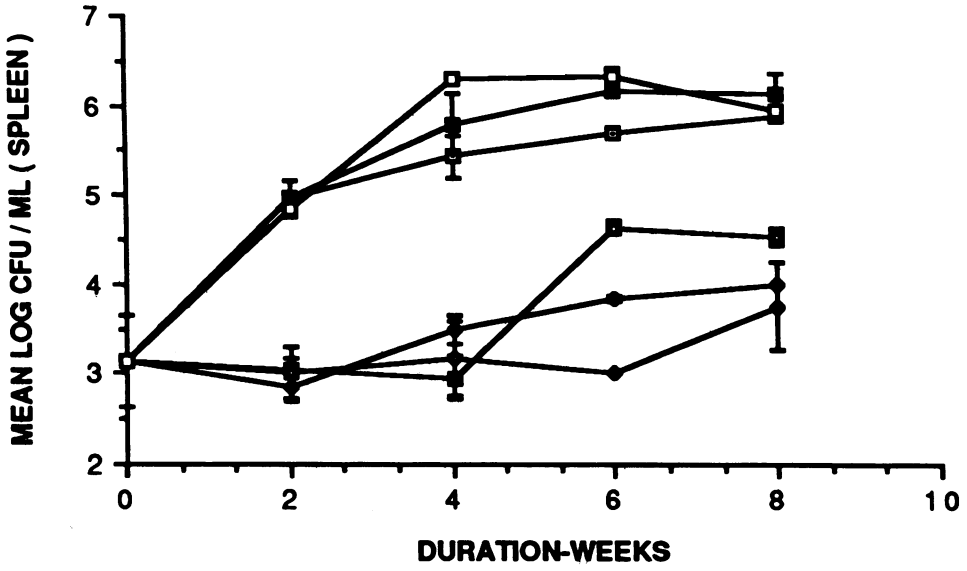

FIG. 7. *M. avium-M. intracellulare* complex CFU counts in the spleens of beige mice treated with 10 mg of amikacin per kg in unilamellar or large, multilamellar vesicles administered i.v. in five weekly injections. The symbols are defined in the legend to Fig. 6.

In another experimental system, liposome-associated amphotericin B has also been shown to be effective against *Candida* infection in the kidneys, as well as in the liver and spleen (27). One of the advantages of administration of liposome-encapsulated drugs is significant reduction of drug toxicity. Administration of both amphotericin and adriamycin in liposomes substantially reduces drug toxicity in patients (12, 26).

Encapsulation in liposomes enhances the antimicrobial activities of a number of antibiotics against infections both in cultured cells and in vivo (for reviews, see references 11 and 30). Vladimirsky and Ladigina (35) have shown that administration of streptomycin in liposomes to mice infected with *M. tuberculosis* causes a significant reduction of colony counts in the spleen and increases survival compared with the free drug. Orozco et al. (29) have also found an enhanced effect of liposome-encapsulated rifampin and isoniazid on reduction of *M. tuberculosis* CFU in the spleen. However, survival was not prolonged in mice that received encapsulated drugs. These two studies used liposomes of different composition and prepared by different methods.

Although the results obtained in this study are encouraging for the use of liposomes in the therapy of *M. avium-M. intracellulare* complex infections, further work is necessary to optimize the targeting of amikacin and other antimycobacterial agents to sites of *M. avium-M. intracellulare* complex infection in vivo. Future experiments will attempt to increase the localization of amikacin in the lungs by aerosol administration (8) and in the lymph nodes by subcutaneous injection (24) or antibody targeting of liposomes (7). It should also be possible to reduce the CFU counts below the initial levels by administering a higher dose of liposome-

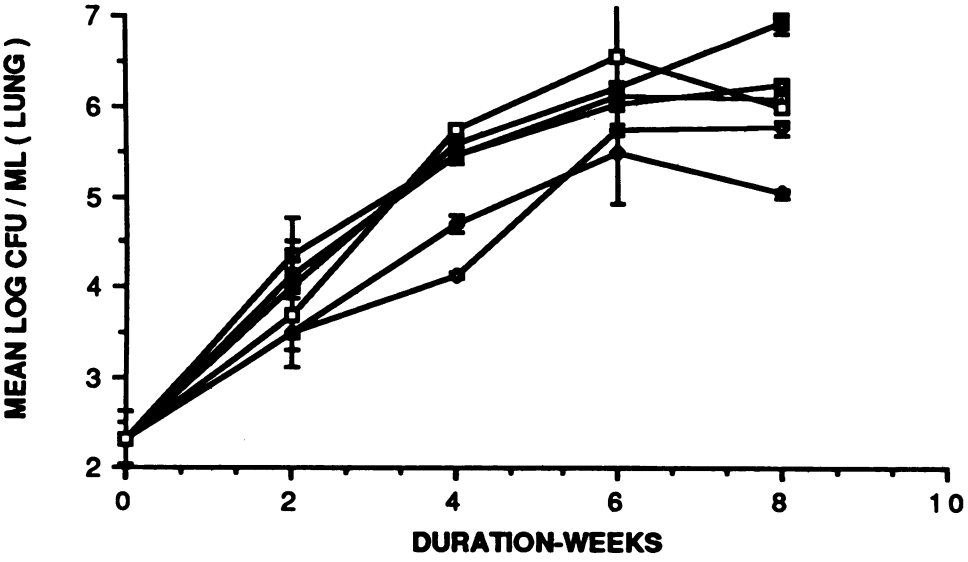

FIG. 8. Effect of 10 mg of amikacin per kg in unilamellar or large, multilamellar liposomes on *M. avium-M. intracellulare* complex CFU counts in the lungs. The symbols and experimental details are given in the legend to Fig. 6.

encapsulated amikacin, since the amount of lipid injected in our experiments was well below the tolerable limit. It may also be possible to develop therapy using both free and liposome-encapsulated drugs or combination chemotherapy. When more effective treatment regimens are developed, it will be necessary to test their effects on other more drug-resistant *M. avium-M. intracellulare* complex strains. Previous experience with the use of liposomal amphotericin B in the treatment of patients with candidiasis (26) and the use of liposome-encapsulated doxorubicin in phase I trials (12) is expected to expedite the application of effective liposome-based therapy regimens in mice to the treatment of patients with *M. avium-M. intracellulare* complex infections.

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