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Pharmacokinetics, Toxicity, and Efficacy of Liposomal Capreomycin in Disseminated *Mycobacterium avium* Beige Mouse Model

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Capreomycin was incorporated into multilamellar vesicles of pure dipalmitoylphosphatidylcholine. The pharmacokinetics and nephrotoxicity of capreomycin in the free and liposomal forms were studied in normal mice. The efficacies of the two forms were evaluated by using the *Mycobacterium avium* complex beige mouse model. Approximately 10^7 viable *M. avium* cells were injected intravenously. Seven days later, treatment with either liposomal or free capreomycin at 60 or 120 mg/kg of body weight was administered daily for 5 days. Mice were sacrificed 5 days after the end of treatment, and the viable bacteria in liver, spleen, lungs, and blood were counted. After 5 days of treatment with dosages of 60 or 120 mg/kg/day, the level of blood urea nitrogen increased in the group treated with free capreomycin but not in the group treated with liposomal capreomycin. After intravenous injection of 120 mg/kg, liposomes enhanced the diffusion of capreomycin in the spleen, lungs, and kidneys and increased the half-life in serum. The 120-mg/kg dose of liposomal capreomycin significantly reduced the number of viable mycobacteria in the liver, spleen, and blood compared with those in the controls. Although these results are promising, further studies are needed to assess the efficacy of liposomal capreomycin for the treatment of *M. avium* complex infections.

Since the appearance of the human immunodeficiency virus (HIV), the incidence of *Mycobacterium avium* complex (MAC) infections has increased considerably and has involved between 18 and 50% of HIV-infected patients (17, 24, 33). Treatment is difficult because of the weakened immune defense system of the host and the resistance of MAC strains to many classic antituberculous agents. Moreover, the phenotypes of resistance of these strains are very heterogeneous (25, 26, 49).

Experimental infection models involving animal or human macrophage cultures and acute or chronic infections in the mouse have been developed to assess the activities of antiinfectious agents against MAC (32). Numerous studies have been performed either in vitro (25, 28, 36, 49) or on macrophages (46) and in animal models (6, 21, 34, 38, 39) with classic or more recent antituberculous agents (ciprofloxacin, sparfloxacin, azithromycin, and clarithromycin) alone or in combination.

Amikacin and clarithromycin have proved to be the most active agents both in vitro and in vivo (18, 21, 34, 46). However, to date only clarithromycin has been demonstrated to have curative efficacy in humans in a controlled clinical trial (12). Nonetheless, there is no standardized therapy for MAC infections, and the curative treatments used associate four or five agents. Furthermore, potentially more active forms of drugs have been developed by encapsulating the drugs in liposomes (2).

Liposomes can encapsule drugs either in their lipid bilayer, if the drugs are liposoluble, or in the internal aqueous compartment, if they are hydrosoluble (1, 41). After intravenous (i.v.) injection, they are rapidly eliminated from the bloodstream, taken up by cells of the reticuloendothelial system (1, 41), and then concentrated in the liver, spleen, and lung macrophages. They are thus of particular interest as vectors for agents intended to attack MAC, which reproduces in macrophages and is subsequently disseminated in the organs involved.

Liposomes containing aminoglycosides (amikacin, gentamicin, streptomycin, kanamycin) used in a mouse model of disseminated MAC infection have proved to be more efficient than free aminoglycosides in reducing the number of viable MAC organisms in organs (2, 10, 11, 15, 16, 19, 37). Liposome encapsulation reduces acute antibiotic toxicity and improves therapeutic activity by increasing the drug concentration at the infection site. In this context, we investigated the therapeutic potential of an antituberculous agent, capreomycin (4, 14), which is used as a secondary-line drug against *Mycobacterium tuberculosis* infections because of its toxicity and which has only been tested against MAC in in vitro studies. Our purpose was to assess the in vivo activities of the free and liposomal forms of capreomycin in a mouse model of systemic MAC infection.

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MATERIALS AND METHODS

Antibiotic and reagent sources. Capreomycin sulfate was provided in titrated powder form (Capastat) by the Eli Lilly Laboratory (Saint Cloud, France). Dipalmitoylphosphatidylcholine (DPPC), Triton X-100, and chloroform (Sigma, St. Louis, Mo.), phosphate-buffered saline (PBS; Biomérieux, Marcy l'Etoile, France), and a phospholipid colorimetric assay kit (Phospholipid-B kit; Unipath, Dardilly, France) were also used.

Microorganisms. (i) Isolation. Thirteen MAC strains isolated at the Nantes University Hospital in hemocultures from HIV-infected patients and identified by nucleic acid hybridiza-

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tion analysis (GenProbe Inc., San Diego, Calif.) (43) were tested to determine the MICs of capreomycin for the strains.

(ii) Susceptibility. These strains were selected because of their susceptibilities to amikacin (MIC, ≤4 μg/ml), and one strain (MAC 92-169) was used for the experimental infection. The MICs of the different antibiotics for this strain were as follows: amikacin, ≤2 μg/ml; ethambutol, ≤2 μg/ml; rifampin, 4 μg/ml; clarithromycin, 4 μg/ml; ciprofloxacin, 4 μg/ml; sparfloxacin, 8 μg/ml; and capreomycin, 20 μg/ml.

(iii) Inoculum preparation. After growth in Middlebrook 7H11 agar medium (Difco, Detroit, Mich.) supplemented with 10% OADC (oleic acid, albumin, dextrose, and catalase; Difco), transparent MAC colonies were cultured for 7 days at 37°C in Middlebrook 7H9 broth (Difco) supplemented with 10% OADC. A suspension containing 5×10^8 CFU/ml was then divided into aliquots and deep frozen at −70°C in 7H9 broth for later use in different stages of the study.

The size of the inoculum used for each experiment was determined by dilution, resuspension in 7H11 medium supplemented with OADC, and measurement of CFU counts after 10 days of incubation at 37°C under a 5% CO_2 atmosphere.

Mice. Female C57BL/6 J-*bg*^{j}/*bg*^{j} mice (age, 6 to 8 weeks; weight, 20 g) obtained from Harlan Laboratories (Gannat, France) were used in the study. These mice, commonly known as beige mice, present with a syndrome similar to the human Chédiak-Higashi syndrome in humans. The animals were housed at five to eight mice per cage and received food and water ad libitum.

In vitro studies. (i) Capreomycin susceptibility tests. MIC determinations for the different MAC strains were performed by a radiometric method in 7H12 broth (25, 27, 40, 42, 47). The MICs of the two forms of capreomycin for the MAC strain used in vivo were determined.

(ii) Liposome preparation. The suspension of liposomal capreomycin was performed by a multilamellar vesical preparation process (23, 44).

DPPC was dissolved in chloroform, evapored with a vacuum pump at room temperature, and deposited as a phospholipid film on the walls of a 50-ml glass flask. A solution of capreomycin sulfate dissolved in PBS (10 mM; pH 7.4) was added to the glass flask to obtain a phospholipid concentration of 40 mM (29.4 mg/ml) and a final antibiotic concentration of 6, 12, 20, 24, 36, or 48 mg/ml. The preparation was then strongly shaken in a stream of water at 50°C until the phospholipids were resuspended and a homogeneous emulsion was obtained. This emulsion was used extemporaneously.

To evaluate the encapsulation of capreomycin into the liposomes, 1 ml of a suspension containing 20 mg of liposomal capreomycin per ml was deposited on a gel permeation chromatography column (Sephadex G25 PD-10 column; Pharmacia, Uppsala, Sweden) and eluted with PBS.

The eluate was collected in 500-μl fractions. For each fraction, phospholipids were assayed by an enzymatic method with colorimetric detection and spectrophotometric readings at 505 nm. The capreomycin concentration was determined after rupture of lipid membranes by the addition of 0.5% Triton X-100.

(iii) Capreomycin assay. Capreomycin was assayed by a microbiological method in AM5 agar medium (pH 8; Difco), with *Bacillus subtilis* ATCC 6633 used as the bacterial indicator organism. Linearity limits were 2.5 to 160 μg/ml.

In vivo studies. (i) Toxicities of free and liposomal capreomycin. Mouse tolerance to the i.v. injection of capreomycin was tested at doses of 30, 60, 120, 180, and 240 mg/kg of body weight. Each group of seven or eight animals was treated by a daily injection of 0.1 ml of a solution containing 6, 12, 24, 36, or 48 mg of capreomycin per ml in free or liposomal form. Acute toxicity (100% lethal dose) was defined as the first dose that caused 100% mortality within 1 min after i.v. administration. For lower dosages, treatment was continued for 5 days, and nephrotoxicity was estimated by measuring blood urea levels by an enzymatic method at the end of treatment and comparing the results with the mean level in a group of untreated control mice.

(ii) Pharmacokinetics in serum and tissue. A single 120-mg/kg dose of free or liposomal capreomycin in a 0.1-ml i.v. injection titrated at 24 mg/ml was administered into the lateral tail veins of five mice per group. Mice were sacrificed by chloroform inhalation at 0.25, 0.5, 0.75, 1, 2, 4, and 6 h.

Blood was collected by cardiac puncture and was immediately centrifuged, and the serum was stored at −20°C until antibiotic assay by the microbiological method.

The spleens, kidneys, and lungs were removed, weighed, frozen at −20°C, and subsequently homogenized in 7H9 broth, and the capreomycin was assayed. For each type of tissue assay, an antibiotic calibration range was determined with the organ involved (6, 45). The elimination half-life ($t_{1/2\beta}$) was calculated according to a single-compartment model by exponential regression of the concentrations in serum from 0.25 to 2 h. Areas under the curve (AUCs) from 0 to 6 h were calculated by the trapezoidal rule method.

(iii) In vivo activity in beige mouse infection model. The disseminated MAC infection model in the beige mouse has been described in detail (3, 20). Mice were infected by injecting approximately 10^7 CFU of MAC in 0.1 ml of 7H9 broth into the tail vein.

Curative treatment was initiated 7 days after infection. The animals received a daily i.v. injection of 60 or 120 mg of free or liposomal capreomycin per kg for 5 days in a volume of 0.1 ml (37).

Prophylactic treatment consisting of a daily injection of 120 mg of free or liposomal capreomycin per kg for 4 days was begun on the day before infection (11, 37).

For both curative and prophylactic procedures, the animals were sacrificed by chloroform inhalation 5 days after receiving the final treatment dose. For each experiment, groups of infected and untreated control mice were sacrificed at the same times as the treated ones. Each experimental or control group included five to eight mice. Empty liposomes were given to a control group to determine whether the liposomes had an intrinsic effect on the course of mycobacterial infection.

To obtain MAC counts, blood samples were collected by heart puncture just before sacrifice, and 0.1 ml was inoculated into flasks containing BACTEC 7H12 medium. The quantity of MAC in blood was determined by a previously described T_{100} method (2, 34–36). T_{100} was defined as the time required to reach a growth index of 100 in a flask containing 7H12 medium. Because T_{100} is inversely proportional to the $\log_{10}$ of the MAC concentration inoculated into the flask, it was possible to determine the number of CFU per milliliter by extrapolation from a standard established with our MAC strain. The liver, spleen, and lungs of the mouse were removed by sterile means at the conclusion of each experiment. The organs were weighed and homogenized with a Thomas Teflon pestle tissue homogenizer in a determined volume of 7H9 broth. Series of homogenate dilutions were obtained and plated onto Middlebrook 7H11 agar medium supplemented with OADC by using a Spiral System (Interscience) (22). After incubation for 10 days at 37°C under 5% CO_2, the colonies were counted and the number of CFU per gram of tissue was calculated. The determination of counts with 1:100 and 1:1,000

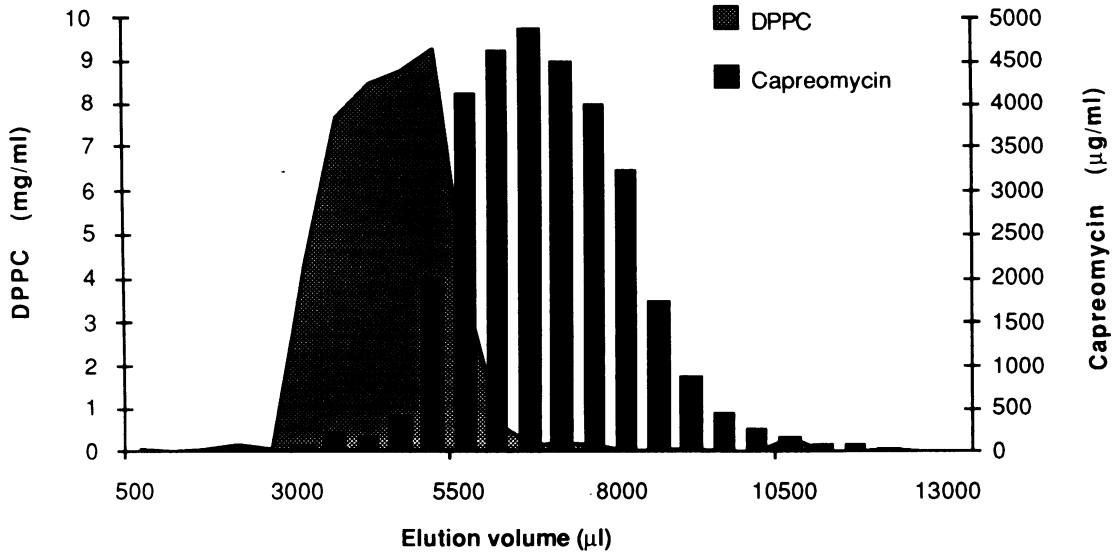

FIG. 1. Gel permeation chromatography of multilamellar vesicles containing 20 mg of capreomycin per ml and 29.4 mg of DPPC per ml.

dilutions 5 days after the end of treatment eliminated the carryover phenomenon.

Statistical analysis. Statgraphics software (STSC, Inc., Rockville, Ill.) was used for statistical analysis. After analysis of variance, mean concentrations of urea in serum as well as the AUC for capreomycin concentrations were compared by Student's *t* test. Bacterial counts, expressed in decimal logarithms, were compared by the nonparametric Mann-Whitney test. For all results, $P < 0.05$ was considered significant.

RESULTS

In vitro study. (i) Capreomycin susceptibility tests. The MICs of free capreomycin, as determined by a radiometric method for 13 MAC strains isolated from HIV-infected patients, ranged from 20 μg/ml for 6 strains to 40 μg/ml for 4 strains to 80 μg/ml for 3 strains. The MIC of both the free and liposomal forms of capreomycin for MAC 92-169 was 20 μg/ml. Lipid did not interfere with the capreomycin assay, and vice versa.

(ii) Evaluation of liposome-encapsulated capreomycin. The elution patterns of capreomycin and DPPC in the form of multilamellar vesicles after passage through the gel permeation chromatography column are shown in Fig. 1. The antibiotic pattern corresponded to 22% of the DPPC pattern. The elution pattern for free capreomycin was the same as that for liposomal capreomycin.

In vivo study. (i) Toxicities of free and liposomal forms of capreomycin. The immediate toxicity obtained with 180 mg of free or liposomal capreomycin per kg (100% lethal dose) caused the immediate deaths of the mice upon injection. For doses of 30, 60, and 120 mg/kg, the renal toxicity of capreomycin was estimated by assay of urea in serum after 5 days of treatment (Table 1). At 30 mg/kg, no modifications in urea concentrations in blood were noted with either form, whereas at 60 and 120 mg/kg the concentrations of urea in blood were significantly increased with free capreomycin but remained comparable to those in controls with the liposomal form.

(ii) Pharmacokinetics in serum and tissue. The concentrations of free or liposomal capreomycin in the sera of mice after a single injection of 120 mg/kg as well as $t_{1/2\beta}$ and AUC are given in Table 2. Free capreomycin, unlike the liposomal form, was not detectable in serum 2 h after administration. Moreover, $t_{1/2\beta}$ and AUC were significantly greater for liposomal capreomycin than free capreomycin.

The corresponding concentrations in tissues are given in Table 3. Comparison of the AUC show that the distribution of liposomal capreomycin in tissues was significantly greater in the lungs (2.1-fold), spleen (2.5-fold), and kidneys (1.6-fold).

With either injected form, capreomycin was almost entirely eliminated from the lungs in 6 h, whereas it accumulated in the kidneys and the concentrations in the spleen remained practically constant and relatively high.

(iii) In vivo activity in beige mouse infection model. To determine the effects of empty liposomes on MAC infection, the curative procedure was applied by treating mice with empty liposomes after they were infected with 10^7 viable MAC. No significant differences were found between treated and untreated control mice.

To determine the effect of treatment with free or liposomal capreomycin in the curative procedure, mice were infected with 6×10^6 viable MAC in the procedure with 60 mg of capreomycin per kg and 1×10^7 viable MAC in the procedure with 120 mg/kg. The results for curative treatment at the two doses are indicated in Table 4. No deaths of control or treated animals occurred during the study.

TABLE 1. Blood urea nitrogen levels after 5 days of treatment with free and liposomal capreomycin

Formulation (no. of mice)	Blood urea nitrogen level (mmol/liter [mean ± SD]) after 5 days of treatment by with capreomycin at (mg/kg/day):			
	0	30	60	120
Control (n = 5)	10.8 ± 0.9			
Free capreomycin (n = 8)		10.6 ± 1.7	12.7 ± 1.2[a]	13.3 ± 1.7[b]
Liposomal capreomycin (n = 7)		9.7 ± 0.6	10 ± 1.4	10.5 ± 1.2

[a] $P = 0.01$ versus control; $P = 0.001$ versus liposomal form (Student's *t* test after analysis of variance).

[b] $P = 0.004$ versus control and versus liposomal form.

TABLE 2. Pharmacokinetics of capreomycin in serum after one i.v. injection of 120 mg of free or liposomal capreomycin per kg

Formulation	Capreomycin concn (μg/ml [mean ± SD])							$t_{1/2\beta}$ (h)	AUC (μg · h/ml)
	0.25 h	0.5 h	0.75 h	1 h	2 h	4 h	6 h		
Free capreomycin (n = 5)	94 ± 19	41 ± 12	11.5 ± 5.4	4.2 ± 1.6	<2.5	<2.5	<2.5	0.18 ± 0.05	34 ± 6.4
Liposomal capreomycin (n = 5)	130.8 ± 23	41.8 ± 10.4	18.8 ± 3.1	11 ± 2.4	2.8 ± 0.5	<2.5	<2.5	0.40 ± 0.05[a]	50.4 ± 8.8[b]

[a] $P < 0.001$ versus free form (Student's t test after analysis of variance).
[b] $P < 0.01$ versus free form.

After treatment with 60 mg of liposomal capreomycin per kg, the number of CFU per gram of organ was significantly reduced in the liver and spleen compared with those in the organs of controls ($P < 0.05$), whereas no difference was found for the lungs. Free capreomycin at 60 mg/kg caused no significant reduction in the number of viable MAC except in the liver ($P < 0.05$). There were no significant differences in bacterial counts in any of these organs after treatment with either form.

After the administration of 120 mg of liposomal capreomycin per kg, there was a reduction in the number of CFU per gram of organ in the liver ($P = 0.002$), spleen ($P = 0.003$), lungs ($P = 0.008$), and blood ($P = 0.02$), whereas no changes occurred with the free form. Bacterial counts in the spleen were significantly lower with the liposomal form ($P < 0.01$).

To determine the effect of treatment with free or liposomal capreomycin in the prophylactic procedure, mice were infected by inoculating them with 7×10^6 viable MAC and the mice were treated with 120 mg of capreomycin per kg (Table 5). The liposomal form significantly reduced the number of viable MAC in the liver ($P = 0.003$), spleen ($P = 0.005$), and blood ($P = 0.04$) but not the lungs. Similarly, the free form was active in the liver ($P = 0.03$), spleen ($P = 0.01$), and blood ($P = 0.03$) but not the lungs. There were no significant differences between bacterial counts in the spleen and liver after treatment with the free and liposomal forms.

DISCUSSION

Capreomycin has been used as a secondary antituberculous agent for 20 years, but the renal and auditory toxicities (of various frequencies [7, 13, 31]) have limited its use. The resistance of MAC to numerous antibiotics and the heterogeneity of this resistance were noted in 1970 by Burjanová and Urbancik (8), who emphasized the need to evaluate treatments in animal models. The results obtained by those investigators, who treated MAC-infected mice with capreomycin, showed that capreomycin was beneficial for the general course of the disease, survival, and pulmonary necrosis. In this context, we studied the activity of liposome-encapsulated capreomycin in a beige mouse model of disseminated MAC infection. The encapsulated form carries the antibiotic to its site of action, where liposomes injected i.v. can be rapidly phagocytized by macrophages, and reduces the toxicity of the drug, thereby increasing the dose that can be used.

Our results for the elution profile showed that 22% of the antibiotic was superposed on DPPC, although it could not be proved that all of this amount was actually encapsulated in liposomes. Nevertheless, several comparative studies of the activity of streptomycin encapsulated in unilamellar and multilamellar vesicles suggest that there is little difference in the in vivo results obtained with these preparations (15, 19). In all studies, both types of liposomes reduced the number of CFU similarly, although the concentrations in tissues were different. Furthermore, a study performed with amikacin and the same type of liposomes used in the present study reported 23% encapsulation but did not clearly specify the measurement method used (11).

The studies performed by Heifets and colleagues (25, 29) with 31 and 100 MAC strains, respectively, showed 30 to 40% susceptibility at a MIC of ≤5 μg/ml and 60 to 70% resistance at a MIC of ≥10 μg/ml. No differences were found in the MIC distributions for 50 strains isolated from HIV-infected patients and 50 strains isolated from patients not infected HIV. According to the criteria of Heifets and colleagues (25, 29), the 13 strains tested by us were all resistant to capreomycin; the MICs for the strains ranged from 20 to 80 μg/ml.

The renal toxicity suggested by the significantly increased urea level in serum after a 5-day treatment with free capreomycin was not documented by a renal pathologic study. Nevertheless, it is highly probable that the mechanism of this toxicity is similar to those observed with other aminoglycosides. Similar reductions in the toxicity of the anti-infectious agents after liposome encapsulation have been reported previously, particularly for amphotericin B (1, 41). However, it is impossible to conclude if encapsulation of capreomycin into liposomes reduces the long-term nephrotoxicity.

TABLE 3. Concentrations of capreomycin in tissues after one i.v. injection of 120 mg of free or liposomal capreomycin per kg

Tissue	Form	Concn (μg/g [mean ± SD]) in groups of five mice each							AUC (μg · h/g)
		0.25 h	0.5 h	0.75 h	1 h	2 h	4 h	6 h	
Spleen	Free capreomycin	17.3 ± 8.7	14.6 ± 7	15.5 ± 20.4	25.1 ± 9.6	41.7 ± 23.2	20.8 ± 31.7	54.7 ± 42.8	184 ± 88
	liposomal capreomycin	80.3 ± 46.4[a]	92.6 ± 50[a]	98.8 ± 38.3[a]	72.6 ± 8.8[a]	89.8 ± 18.1[a]	77.6 ± 20.7[a]	66.5 ± 30.2	459.7 ± 50[a]
Kidney	Free capreomycin	104.5 ± 41	89.9 ± 29	112.7 ± 47	121.2 ± 25	130.6 ± 11	203 ± 44	241.2 ± 19	982.4 ± 93
	liposomal capreomycin	236.1 ± 121[a]	140.2 ± 61	206.8 ± 51[a]	208.8 ± 137	226.4 ± 42[a]	280.5 ± 87	425.6 ± 155[a]	1573 ± 38[a]
Lung	Free capreomycin	67.9 ± 30.3	33 ± 17.7	17.9 ± 10.7	16.5 ± 9.1	8.6 ± 4.7	5.5 ± 2.4	4.3 ± 1.9	59.7 ± 8.6
	liposomal capreomycin	202.9 ± 107[a]	77.2 ± 37.5[a]	47 ± 28.7	41.3 ± 22.3[a]	19.6 ± 11.3	6 ± 2.9	7 ± 4.2	125.7 ± 24[b]

[a] $P < 0.01$ versus free form.
[b] $P < 0.001$ versus free form.

TABLE 4. In vivo activity of five days of treatment of free or liposomal forms of capreomycin in beige mouse model of disseminated MAC infection

Treatment	$\log_{10}$ CFU/g (or CFU/ml) of tissue (or blood) (mean ± SD)			
	Liver	Spleen	Lung	Blood
Control (n = 5)	6.8 ± 0.3	6.9 ± 0.3	4.8 ± 0.3	ND[a]
Free capreomycin at 60 mg/kg (n = 7)	6.3 ± 0.3[b]	6.5 ± 0.3	4.5 ± 0.3	ND
Liposomal capreomycin at 60 mg/kg (n = 7)	6.3 ± 0.2[b]	6.4 ± 0.3[b]	4.6 ± 0.4	ND
Control (n = 6)	7.2 ± 0.2	7.5 ± 0.3	5.3 ± 0.2	3.3 ± 1
Free capreomycin at 120 mg/kg (n = 8)	7.0 ± 0.1	7.3 ± 0.1	5 ± 0.4	2.1 ± 1.3
Liposomal capreomycin at 120 mg/kg (n = 6)	6.6 ± 0.2[c,d]	6.6 ± 0.2[c,d]	4.6 ± 0.4[c]	2 ± 0.5[b]

[a] ND, not done.
[b] $P < 0.05$ versus control (Mann-Whitney test).
[c] $P < 0.01$ versus control.
[d] $P < 0.01$ versus free form.

The $t_{1/2\beta}$ and AUC for concentrations in serum and tissues were greater with the liposomal form than the free form. The greater $t_{1/2\beta}$ and AUC observed with the liposomal form are in agreement with data presented in the literature (11, 15, 48) and can be attributed to the richness of cells in the reticuloendothelial system (which take up liposomes preferentially) in the organs studied (lungs and spleen) (1).

After the intramuscular injection of 14 mg of capreomycin per kg to humans, the AUC from 0 to 6 h for capreomycin in serum calculated from the results of Black et al. (5) was 115 μg · h/ml, whereas in mice after i.v. injection of 120 mg/kg, the AUC from 0 to 6 h for capreomycin in serum was 34 μg · h/ml for the free form and 50 μg · h/ml for the liposomal form.

Thus, the antibacterial activity of 120 mg of free or liposomal capreomycin per kg observed in our mouse model corresponded to AUCs in serum that were two to four times less than those obtained in humans at a dose of 14 mg/kg. The concentrations in serum and especially tissue differed with both forms, tending to be rather stable in spleen and to decrease more progressively in lungs with the liposomal form, as has been reported in other studies in mice in which aminoglycosides were used (11, 15, 48). In the kidney, an increase in the capreomycin concentrations was noted from 0 to 6 h, which may be explained by the renal route of elimination (5).

Thus, liposomal encapsulation increased antibiotic diffusion within the liver, spleen, and lungs. Moreover, the liposomal form of capreomycin had a better in vivo activity than the free form against MAC isolates. The bacterial counts obtained for controls were concordant with those reported in the literature (2, 3, 20) in the beige mouse model. The difference in the mean CFU for each organ was slight (0.6 to 0.9 $\log_{10}$ CFU/g of organ for the 120-mg/kg dose by the curative procedure); thus, the antibacterial activity observed was a bacteriostatic effect. However, these results were obtained after a very short treatment period (5 days) in a rather demanding model, so that the differences in activity should be considered with caution. It would be interesting to evaluate longer periods of treatment to show whether there are more significant differences between the two formulations, but the poor venous tolerance remains a difficult challenge. Thus, the major result of the present work is that although the efficacies of the two forms are quite similar, liposomal capreomycin is less toxic.

Other studies have used this same model and a therapeutic procedure similar to ours for the administration of amikacin and gentamicin at variable doses (10 to 200 and 10 to 50 mg/kg, respectively) after liposome encapsulation (2, 11, 37). The results show slightly greater reductions in CFU (1 to 2 $\log_{10}$) in the liver, spleen, and lungs than we obtained by our curative procedure, whereas the counts in blood were similar (1 $\log_{10}$ CFU). Clarithromycin, which is apparently more bacteriostatic than bactericidal in its in vitro activity (30), induced reductions in CFU within 1 to 2.5 $\log_{10}$ units in the liver, spleen, and lungs (10, 18, 38) at doses ranging from 50 to 300 mg/kg when it was given per os for 10 days. The results obtained with azithromycin were comparable to those obtained with clarithromycin at the same doses (9, 35).

As prophylactic treatment, Cynamon et al. (11) found 3-$\log_{10}$-unit reductions in the liver and spleen when they used liposome-encapsulated amikacin at a dose of 50 mg/kg, whereas the results from Klemens et al. (37) with liposomal gentamicin at a 120-mg/kg dose were comparable to ours (2 $\log_{10}$) except in lungs, where, contrary to the observations of the other investigators (37), we found no reduction in CFU.

The present study shows that the association of capreomycin with phospholipids in the form of multilamellar vesicles reduced the renal toxicity of this antibiotic in mice, increased its diffusion into tissues, and slightly enhanced its activity in the beige mouse model of MAC infection. Because the in vitro and in vivo activities of an antibiotic can vary depending on the

TABLE 5. In vivo activity of prophylactic treatment of free or liposomal capreomycin (120 mg/kg) in beige mouse model of disseminated MAC infection

Treatment	$\log_{10}$ CFU/g (or CFU/ml) of tissue (or blood) (mean ± SD)			
	Liver	Spleen	Lung	Blood
Control (n = 6)	7.5 ± 0.3	7.3 ± 0.3	4.7 ± 0.7	3.4 ± 1
Free capreomycin (n = 7)	6.4 ± 0.7[a]	5.9 ± 0.3[b]	4.4 ± 0.3	2.1 ± 0.8[a]
Liposomal capreomycin (n = 6)	5.5 ± 0.6[b]	5.4 ± 0.7[b]	4.9 ± 0.6	2.2 ± 0.9[a]

[a] $P < 0.05$ versus control (Mann-Whitney test).
[b] $P < 0.01$ versus control.

MAC strain tested (9, 26, 36–38), it would be useful to confirm our results on different strains, particularly those already used in other studies (e.g., strain 101) and with strains that are more susceptible in vitro. The use of antibiotic combinations with capreomycin would also be suitable.

The poor venous tolerance of liposomal capreomycin constitutes a serious limitation for treatment beyond 5 to 10 days in mice. However, the therapeutic challenge of disseminated MAC infections in HIV-infected patients warrants that in vitro and in vivo testing of any potentially active antibiotic be carried out. The results of our study do not indicate whether capreomycin can play such a therapeutic role but suggest that it has a potential role which must be further investigated.

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