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Efficacies of Liposome-Encapsulated Streptomycin and Ciprofloxacin against *Mycobacterium avium-M. intracellulare* Complex Infections in Human Peripheral Blood Monocyte/Macrophages

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Current treatments of disseminated infection caused by the *Mycobacterium avium-M. intracellulare* complex (MAC) are generally ineffective. Liposome-mediated delivery of antibiotics to MAC-infected tissues in vivo can enhance the efficacy of the drugs (N. Düzgüneş, V. K. Perumal, L. Kesavalu, J. A. Goldstein, R. J. Debs, and P. R. J. Gangadharam, Antimicrob. Agents Chemother. 32:1404–1411, 1988; N. Düzgüneş, D. A. Ashtekar, D. L. Flasher, N. Ghori, R. J. Debs, D. S. Friend, and P. R. J. Gangadharam, J. Infect. Dis. 164:143–151, 1991). We investigated the therapeutic efficacies of liposome-encapsulated streptomycin and ciprofloxacin against growth of the MAC inside human peripheral blood monocyte/macrophages. Treatment was initiated 24 h after infection of macrophages with the MAC and stopped after 20 h, and the cells were incubated for another 7 days. The antimycobacterial activity of streptomycin was enhanced when the drug was delivered to macrophages in liposome-encapsulated form, reducing the CFU about threefold more than the free drug did throughout the concentration range studied (10 to 50 μg/ml). With 50 μg of encapsulated streptomycin per ml, the CFU were reduced to 11% of the initial level of infection. Liposome-encapsulated ciprofloxacin was at least 50 times more effective against the intracellular bacteria than was the free drug: at a concentration of 0.1 μg/ml, liposome-encapsulated ciprofloxacin had greater antimycobacterial activity than the free drug at 5 μg/ml. With liposome-encapsulated ciprofloxacin at 5 μg/ml, the CFU were reduced by more than 1,000-fold at the end of the 7-day incubation period, compared with untreated controls. These results suggest that liposome-encapsulated ciprofloxacin or other fluoroquinolones may be effective against MAC infections in vivo.

The incidence of *Mycobacterium avium-M. intracellulare* complex (MAC) infection has increased significantly in the past decade, primarily in patients with AIDS. The MAC is the most frequent species of bacteria cultured from AIDS patients (36). This organism can invade and replicate within monocyte/macrophages (6), causing disseminated infection in the lungs, liver, spleen, bone marrow, lymph nodes, and blood (2, 23). The prognosis for MAC-infected patients is very poor, owing in part to the resistance of this organism to available antimicrobial agents, including those used for antituberculosis therapy (28). In addition, severe toxicity is associated with many of these compounds when they are used at clinically useful levels (20, 34). Complex mixtures of available drugs given at their maximum tolerated doses have little effect on the progress of the disseminated disease (1), although recent studies have indicated that clarithromycin, in monotherapy and in combination with four other drugs, can clear bacteremia in patients with AIDS (10).

Since the MAC is an intracellular pathogen, the ability to treat MAC infections effectively may depend critically on the concentration of the drugs inside macrophages. Liposomes are potentially ideal vehicles for homing antibacterial agents to macrophages, because they are avidly taken up by phagocytic cells of the reticuloendothelial system and can release their contents intracellularly (27, 29). Liposome-encapsulated amikacin (7, 13, 18), gentamicin (7, 24), and rifampin (31) have been demonstrated to have better efficacy than the free drugs in animal models of MAC infection. It is important to establish an in vitro system to screen conveniently and reliably the efficacies of liposome-encapsulated antibiotics against intracellular MAC organisms. In this study, we investigated the efficacies of liposome-encapsulated streptomycin and ciprofloxacin against intracellular growth of the MAC in macrophages derived from human peripheral blood monocytes.

We have shown recently that the efficacy of streptomycin against MAC infections in beige mice was improved by delivery in liposomes (12, 17). Liposome-encapsulated streptomycin was also more effective against the MAC in murine peritoneal macrophages (3). As a primer to the eventual administration of liposome-encapsulated streptomycin to patients with MAC disease, we investigated its efficacy against the MAC in human peripheral blood monocyte/macrophages. Previous studies have shown that combined use of ethambutol and streptomycin has a synergistic effect against the MAC in vitro (22, 26). Although in vitro (bacterial culture) studies indicated synergism when ethambutol was used in combination with rifampentine or rifabutine, no synergistic effect was noted in vivo (in beige mice) (26). To

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ascertain whether streptomycin enhances the effect of ethambutol against the MAC, we used the MAC-infected macrophage system as a model for the in vivo action of the two antibiotics.

The quinolone antibiotic ciprofloxacin has been shown to have activity against some MAC strains in vitro (15, 19). We developed a stable liposome-encapsulated formulation of ciprofloxacin and investigated its efficacy against the MAC inside human macrophages in comparison with that of the free drug. The efficacy of ciprofloxacin was enhanced drastically by administration in liposome-encapsulated form.

MATERIALS AND METHODS

Antimicrobial agents and reagents. Ciprofloxacin was a gift from Miles Inc. (West Haven, Conn.) or purchased from Sigma Chemical Co. (St. Louis, Mo.). Streptomycin sulfate was obtained from Pfizer (New York, N.Y.). Ethambutol hydrochloride was from Sigma. Egg yolk phosphatidylglycerol and phosphatidylcholine were purchased from Avanti Polar Lipids (Alabaster, Ala.), and cholesterol was from Calbiochem (La Jolla, Calif.). Bovine serum albumin, sodium dodecyl sulfate, NaCl, KCl, and HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) were obtained from Sigma. Modified 7H9 broth and 7H10 agar plates were obtained from the Media Preparation Center, San Francisco General Hospital (San Francisco, Calif.). Where indicated, solutions were prepared in pyrogen-free water obtained from the University of California San Francisco Cell Culture Facility. Otherwise, solutions were prepared in distilled water further purified in a Barnstead Nanopure filtration apparatus.

Preparation of liposomes containing streptomycin. Streptomycin was encapsulated in unilamellar liposomes composed of phosphatidylglycerol-phosphatidylcholine-cholesterol (molar ratio, 1:9:5) prepared by reverse-phase evaporation followed by extrusion through polycarbonate membranes (0.2-μm pore diameter) (12, 13, 32). Streptomycin was dissolved at a concentration of 140 mg/ml in pyrogen-free water containing 10 mM HEPES, pH 7.4. The osmolality of the solution was adjusted to 290 mosM with NaCl by using a vapor pressure osmometer from Wescor (Logan, Utah). The solution was then sterilized by passage through a 0.22-μm Millex filter. Lipids (10 to 20 μmol) were dried from a chloroform solution and redissolved in 1 ml of diethyl ether (which was washed with water or HEPES-buffered saline just before use to eliminate any peroxidation products). An aliquot (0.34 ml) of the streptomycin solution was added to the lipid and sonicated for 2 min under an argon atmosphere in a sealed glass tube (for details, see reference 14). The emulsion was placed in a rotary evaporator under a controlled vacuum to achieve a liposome suspension. An additional 0.66 ml of streptomycin solution was added to the liposomes, and the mixture was vortexed and further evaporated for 20 min to eliminate any residual ether. The liposomes were extruded through polycarbonate membranes (Nuclepore, Pleasanton, Calif.) under argon pressure in a high-pressure extrusion apparatus (Lipex Biomembranes, Vancouver, British Columbia, Canada). The unencapsulated drug was separated by passing the liposome preparation through a sterile Sephadex G-75 (Pharmacia, Piscataway, N.J.) column eluted with 140 mM NaCl–10 mM KCl–10 mM HEPES buffer, pH 7.4. The preparation was then filter sterilized by passage through a 0.22-μm Millex filter. The amount of encapsulated streptomycin was determined by a fluorescence assay as described earlier (12, 21), after lysis of an aliquot of the liposome suspension with 10-fold excess methanol. The lipid concentration in the preparation was determined by phosphate analysis (5).

Preparation of liposomes containing ciprofloxacin. Ciprofloxacin was encapsulated in multilamellar vesicles composed of phosphatidylglycerol-phosphatidylcholine-cholesterol (molar ratio, 1:9:5). The lipids were first dried from chloroform onto the sides of a glass tube as a thin film by using a rotary evaporator and then hydrated with a sterile ciprofloxacin solution. Since ciprofloxacin crystallizes at a neutral pH but is soluble at an acidic pH, solutions of the antibiotic at 25 mg/ml were prepared in either (i) unbuffered water, (ii) 40 mM glycine buffer at pH 3.5, or (iii) 10 mM acetate buffer at pH 5.6. These solutions were adjusted to an osmolality of 290 mosM by addition of sucrose crystals. It was found to be important to avoid use of NaCl to adjust the osmolality, since ciprofloxacin precipitated in the presence of this salt. These solutions were sterilized by filtration through 0.22-μm Millex syringe filters.

The hydrated lipid was mixed thoroughly by vortexing, and the mixture was subjected to four cycles of freezing (using an ethanol-dry ice bath) and thawing (using a water bath at room temperature). Because of the presence of sucrose in the antibiotic solution, the prepared liposomes were initially diluted 30-fold in either (i) 140 mM NaCl–10 mM KCl, (ii) 101 mM NaCl–10 mM KCl–40 mM glycine, pH 3.5, or (iii) 140 mM NaCl–10 mM KCl–10 mM acetate, pH 5.6 (dilution media), to enable pelleting and washing of the liposomes. Dilution of the ciprofloxacin solution outside the liposomes prevented precipitation of the antibiotic in the salt solution. The unencapsulated drug was eliminated by repeated (three times) centrifugation at 15,000 rpm in an SS-34 rotor in a Sorvall RC-5B centrifuge for 15 min each time at 4°C and washing with the respective dilution medium. The liposomes were then suspended in 140 mM NaCl–10 mM KCl–10 mM HEPES buffer, pH 7.4. Sterile conditions were maintained throughout these procedures. The glass tubes used for liposome preparation, centrifuge tubes, and Pasteur pipets had been previously autoclaved. The amount of encapsulated ciprofloxacin was determined by measuring the absorbance of methanol-lysed vesicles at 276 nm in a Perkin Elmer Lambda 4B spectrophotometer by using a calibration curve made with 1 to 25 μg of ciprofloxacin per ml.

Infection of human peripheral blood monocyte/macrophages by the MAC. Human peripheral blood mononuclear cells were isolated by Histopaque-1077 (Sigma) density gradient centrifugation (8) followed by adherence on 100-mm-diameter plastic cell culture dishes (Falcon) for 1 h. Nonadherent cells were removed by repeated washing with phosphate-buffered saline (PBS). Monocytes were collected by treating the adherent cells with cold PBS containing 0.2% EDTA and 10% heat-inactivated fetal bovine serum for 1 h at 4°C. The cells were then washed twice with PBS, suspended in RPMI 1640 medium containing 10% fetal bovine serum, and cultured at a density of 10^6 per well in 24-well tissue culture plates (Falcon) at 37°C and 5% CO_2 in a humidified cell culture incubator (Forma). After 5 days, the cells were exposed for 24 h to a single-cell suspension of the MAC (serovar 4) at a multiplicity of infection of 10:1. The MICs of ciprofloxacin, ethambutol, and streptomycin for this MAC strain determined previously (by using the procedure described in reference 35) were 0.5 (reference 35), 16, and >16 μg/ml (35a), respectively. Extracellular bacteria were removed by washing with PBS, and the cells were incubated for another 24 h before any treatment. The viability of the cells was ascertained by Trypan blue exclusion to be >90%.

The cultures were monitored for cell loss by visual inspection under a phase-contrast microscope and counting of macrophages in random fields. No significant differences between untreated control cultures and treated cultures were noted. The small standard deviations of triplicate wells also attested to the uniformity of the cultures.

Treatment of infected macrophages with antimicrobial agents and evaluation of CFU. Infected monolayers were treated with either free or liposome-encapsulated antibiotics for 20 h. The medium was then removed, and the cells were washed three times with PBS and incubated for 7 days in RPMI 1640–10% fetal bovine serum without antibiotics. The number of MAC CFU was determined after lysis of the macrophages with 0.25% sodium dodecyl sulfate, which was subsequently neutralized with 10% bovine serum albumin. The lysate was diluted serially (10^4- to 10^7-fold) and plated in duplicate on 7H10 agar plates. The colonies were enumerated after 2 to 3 weeks of incubation at 37°C. The initial infection of the macrophages was assessed after the 24 h of incubation with the MAC, following removal of extracellular bacteria. Triplicate wells were used for each experimental condition tested. The data are expressed as means ± standard deviations and were compared between groups by using Student's *t* test with the aid of the Statview program (Abacus Concepts, Inc.) for the Macintosh computer.

Electron microscopy. Macrophages were infected as described above and treated with free or liposome-encapsulated ciprofloxacin (or buffer-loaded liposomes) for 20 h. The cells were fixed in 1.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, containing 1% sucrose at 4°C for 2 h, stained with 0.5% uranyl acetate in 0.1 M acetate Veronal buffer, dehydrated with ethanol, removed from culture plates with propylene oxide, and collected by centrifugation. The resulting pellet was embedded in Epon, and ultrathin sections were cut and mounted on nickel grids. The samples were observed in a Siemens 101 electron microscope.

RESULTS

Antimycobacterial effects of free and liposome-encapsulated streptomycin. The effect of streptomycin encapsulated in liposomes was compared with that of nonencapsulated streptomycin against the MAC inside human monocyte-derived macrophages in a concentration range achievable in the serum of treated patients. As shown in Fig. 1, the number of MAC CFU decreased with the increase in streptomycin concentration, in comparison with untreated controls (i.e., 0 μg/ml on the abscissa), at the end of the 7-day incubation period following the treatment. Liposome-encapsulated streptomycin at 20 μg/ml reduced the CFU by about 2 orders of magnitude from the value obtained for untreated controls, and at 50 μg/ml the decrease was about 3 orders of magnitude. The difference in CFU between encapsulated streptomycin at 10 versus 40 or 50 μg/ml was statistically significant ($P \leq 0.0005$). The CFU counts in cells treated with liposome-encapsulated streptomycin were consistently lower than those in cells treated with free streptomycin, in the concentration range of 10 to 40 μg/ml ($P \leq 0.0005$). Liposomal streptomycin at 10 μg/ml was as effective in reducing the MAC CFU as free streptomycin at 30 μg/ml. At 50 μg/ml, CFU counts in cells treated with liposome-encapsulated streptomycin were more than 0.5 log unit lower than in cells treated with the free drug, and this difference was statistically significant ($P \leq 0.0005$).

Compared with the level of the initial infection achieved at the end of the first 24 h of incubation with the MAC (log

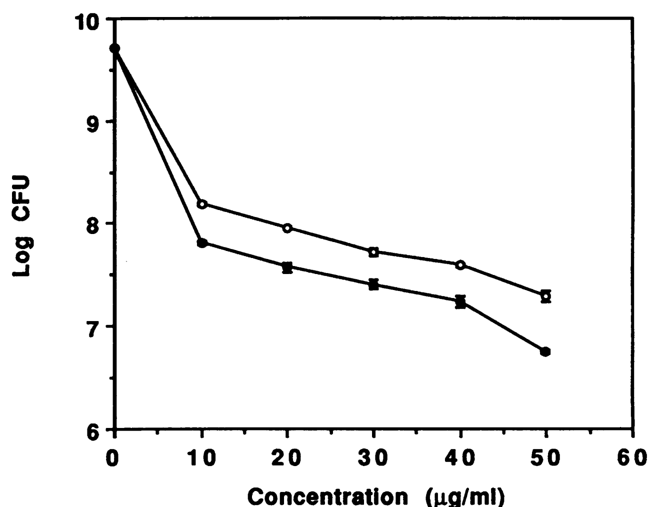


FIG. 1. Effect of liposome-encapsulated streptomycin against intracellular MAC growth. Human monocyte-derived macrophages were infected with the MAC as described in Materials and Methods. At this stage, the MAC CFU were 7.73 log units. The infected cells were incubated for 24 h without treatment and then for 20 h with the indicated concentrations of free (○) or liposome-encapsulated (●) streptomycin. After removal of the drug, cells were further incubated for 7 days in drug-free medium and lysed and the lysate was plated for CFU determination as described in Materials and Methods. The data are presented as means ± standard deviations of CFU obtained from triplicate wells containing macrophages isolated from a single donor. The standard deviation for any point where the error bars do not show up in the figure is within the size of the symbol. The untreated control is represented by 0 μg/ml on the abscissa.

CFU, 7.73), liposome-encapsulated streptomycin at 50 μg/ml reduced the CFU by 89.1% ($P \leq 0.0005$). Control additions of buffer-loaded liposomes at lipid concentrations corresponding to those in the streptomycin-containing liposomes had no effect on the CFU, except at the highest lipid concentration tested (corresponding to 50 μg of streptomycin per ml), which caused a reduction of about 0.2 log unit compared with untreated samples.

Effect of ethambutol in combination with free or liposome-encapsulated streptomycin. We examined whether streptomycin would enhance the effect of ethambutol in inhibiting MAC growth inside macrophages. Infected macrophages were incubated either with ethambutol alone (at 10 or 20 μg/ml) or in combination with free or liposome-encapsulated streptomycin (10 μg/ml) for 20 h and incubated without the antibiotics for another 7 days, and then the MAC CFU were determined. The presence of free or liposome-encapsulated streptomycin did not significantly alter the level of intracellular infection obtained with free ethambutol (data not shown).

Antimycobacterial effects of free and liposome-encapsulated ciprofloxacin. The effects of different concentrations of ciprofloxacin, administered either as the free drug or encapsulated in liposomes, on intracellular MAC growth were examined (Fig. 2). These concentrations are in a range achievable in the serum of treated patients. For these experiments, ciprofloxacin was dissolved in 40 mM glycine-HCl buffer at pH 3.5 and either used to prepare liposomes as described in Materials and Methods or diluted as the free drug into the culture medium of infected cells. A ciprofloxacin concentration as low as 0.1 μg/ml, when administered in liposome-encapsulated form, reduced the CFU by 2 log units

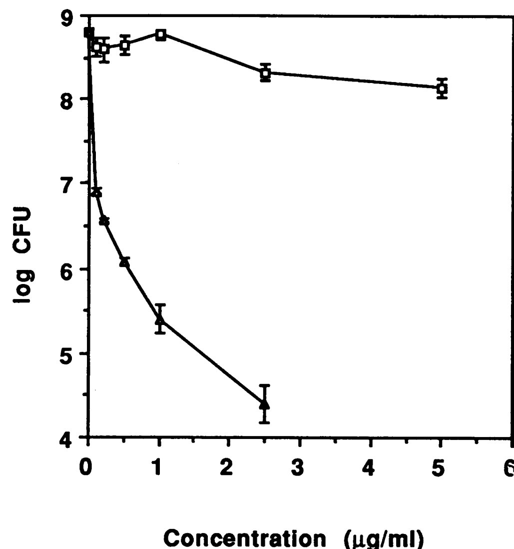


FIG. 2. Effects of free and liposome-encapsulated ciprofloxacin against MAC growth inside human macrophages. Infected cells were incubated with free (□) or liposome-encapsulated (△) ciprofloxacin for 20 h. After 7 days, cells were lysed to determine MAC CFU. The bars indicate standard deviations. The untreated controls are represented by 0 μg/ml on the abscissa. The CFU counts at 5 μg of the liposome-encapsulated drug per ml were below 4 log units.

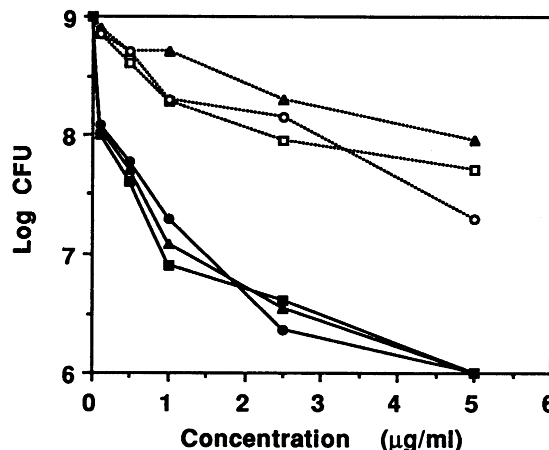


FIG. 3. Effects of various preparations of free and liposome-encapsulated ciprofloxacin against intracellular MAC growth inside human macrophages. Infected cells were incubated with free or liposome-encapsulated ciprofloxacin for 20 h and then for another 7 days in the absence of drugs. The cells were then lysed to determine the CFU. The untreated controls are represented by 0 μg/ml on the abscissa. The CFU counts at 5 μg of the liposome-encapsulated drug per ml were below 6 log units. Symbols: open, free ciprofloxacin; filled, liposome-encapsulated ciprofloxacin. The antibiotic was dissolved either in unbuffered saline (squares) or in saline buffered with glycine (triangles) or acetate (circles).

compared with untreated controls (0 μg/ml on the abscissa; $P \leq 0.0005$), while the free drug was ineffective in inhibiting growth at this concentration (Fig. 2). Liposome-encapsulated ciprofloxacin at 2.5 μg/ml reduced the CFU by 4 orders of magnitude compared with the CFU obtained with the free drug. The CFU were reduced below 4 log units at a concentration of 5 μg of the encapsulated antibiotic per ml (Fig. 2; this was the lowest dilution used for enumeration of CFU). Buffer-loaded (empty) liposomes caused no significant reduction in CFU compared with untreated controls, while incubation of infected macrophages with empty liposomes plus 5 μg of free ciprofloxacin per ml reduced the CFU to 0.27 log unit below the value obtained with the same concentration of the drug alone (data not shown).

Ciprofloxacin, initially soluble in solutions with acidic or mildly acidic pHs, may become ineffective when it crystallizes at a neutral pH. We therefore tested the effects of liposome-encapsulated ciprofloxacin initially dissolved in either unbuffered saline or saline buffered to pH 3.5 or 5.6 (Fig. 3). Differences in CFU counts on the order of 0.4 to 0.65 log unit were observed between the different preparations of free ciprofloxacin. The differences in the CFU counts obtained with liposome-encapsulated ciprofloxacin prepared with the different solutions were less pronounced. The reduction in CFU, relative to the untreated controls (i.e., 0 μg/ml), obtained when infected macrophages were treated with free glycine-buffered ciprofloxacin at a dose of 5 μg/ml was similar to that achieved with only 0.2 μg of liposome-encapsulated ciprofloxacin per ml. For ciprofloxacin in acetate buffer, the same reduction in CFU as that caused by the free drug at 5 μg/ml was achieved with 1 μg of encapsulated ciprofloxacin per ml. At the dilutions of cell lysates used for this experiment, no CFU were observed when infected macrophages were incubated with 5 μg of liposome-encapsulated ciprofloxacin per ml. This finding reflects at least a 1,000-fold reduction of CFU compared with the untreated control.

Electron microscopy of ciprofloxacin-treated MAC-infected macrophages. Infected macrophages were incubated for 24 h without any treatment and then incubated for 20 h with either free or encapsulated ciprofloxacin (5 μg/ml) or only buffer-loaded liposomes. Figure 4 shows that the bacteria localized in phagosomes in the macrophages, with a characteristic electron-transparent area (16) between the bacterium and the phagosome membrane (Fig. 4c). This region is thought to be composed of glycopeptidolipid (30). Some of the bacteria inside macrophages treated with free or liposome-encapsulated ciprofloxacin showed signs of degradation (Fig. 4d and e, compare the texture and vacuolation of the bacterial cytoplasm with those of that shown in panel a). Thus, ciprofloxacin can manifest its antimycobacterial effect within 20 h of incubation of macrophages with the antibiotic. At this stage of the infection, it was not possible to discern differences between mycobacteria inside macrophages treated with free ciprofloxacin and those inside macrophages treated with liposome-encapsulated ciprofloxacin. Treatment of macrophages with buffer-loaded liposomes (at the same lipid concentration as those containing ciprofloxacin) did not appear to cause bacterial degradation (Fig. 4f). It is difficult to discern individual liposomes in such experiments without using histochemical markers, since degradation products inside vacuoles can also have a lamellar appearance.

DISCUSSION

We investigated the effects of liposome-encapsulated streptomycin and ciprofloxacin against the MAC in an in vitro human monocyte-macrophage model. Electron microscopy indicated that the macrophages were invaded profusely by bacteria. Treatment of MAC-infected macrophages with

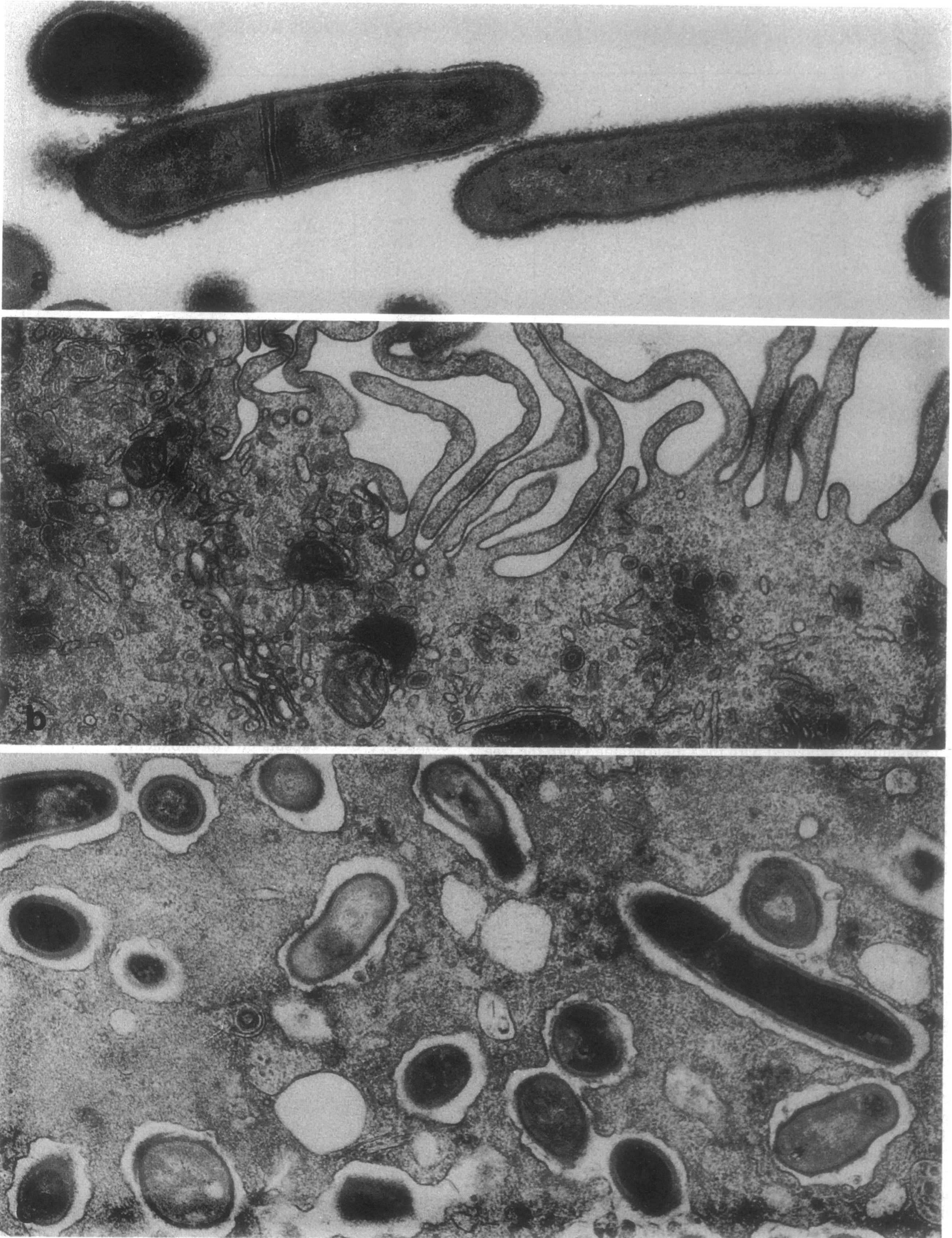
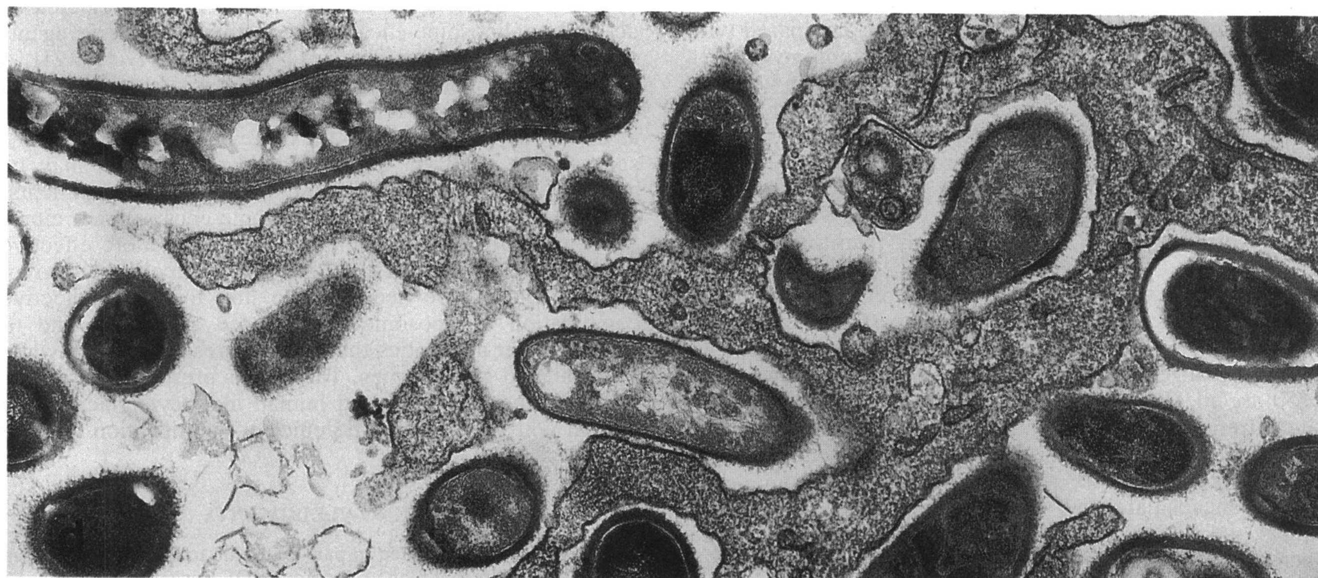


FIG. 4. Electron micrographs of MAC organisms in human macrophages either untreated or treated with free or liposome-encapsulated ciprofloxacin. (a) Extracellular bacteria (magnification, ×34,968). (b) Uninfected macrophage (magnification, ×21,297). (c) Infected macrophage (magnification, ×30,597). (d) Bacteria inside a macrophage treated with free ciprofloxacin (magnification, ×31,700). (e) Infected macrophage treated with liposome-encapsulated ciprofloxacin (magnification, ×38,400). (f) Infected macrophage treated with buffer-loaded liposomes (magnification, ×38,400).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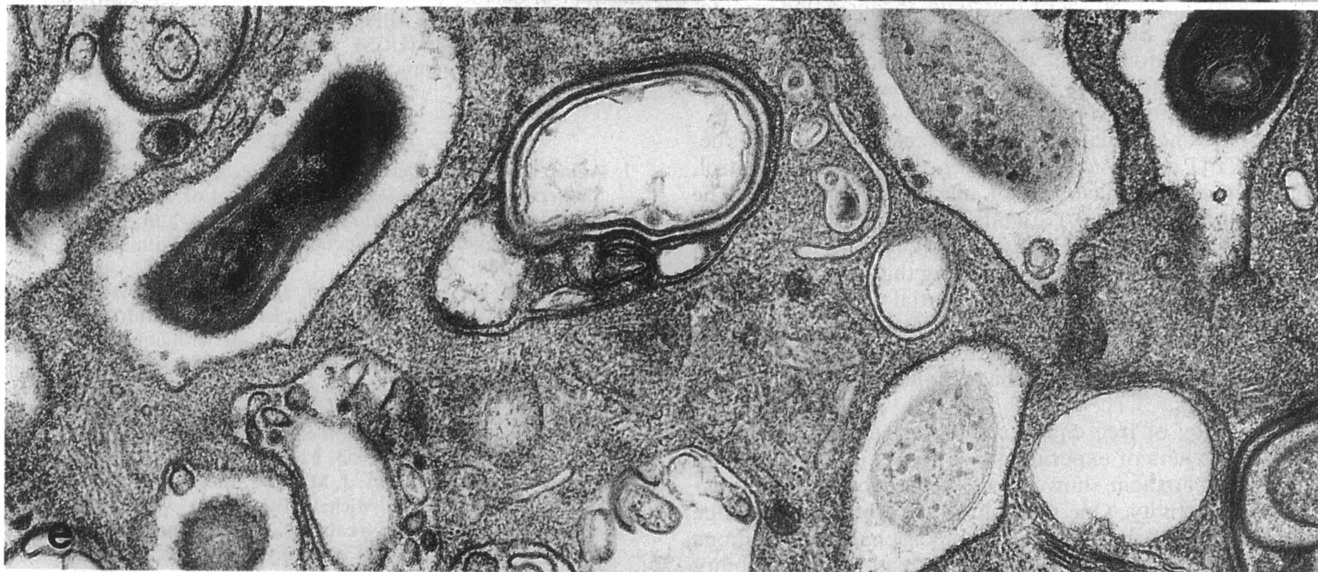
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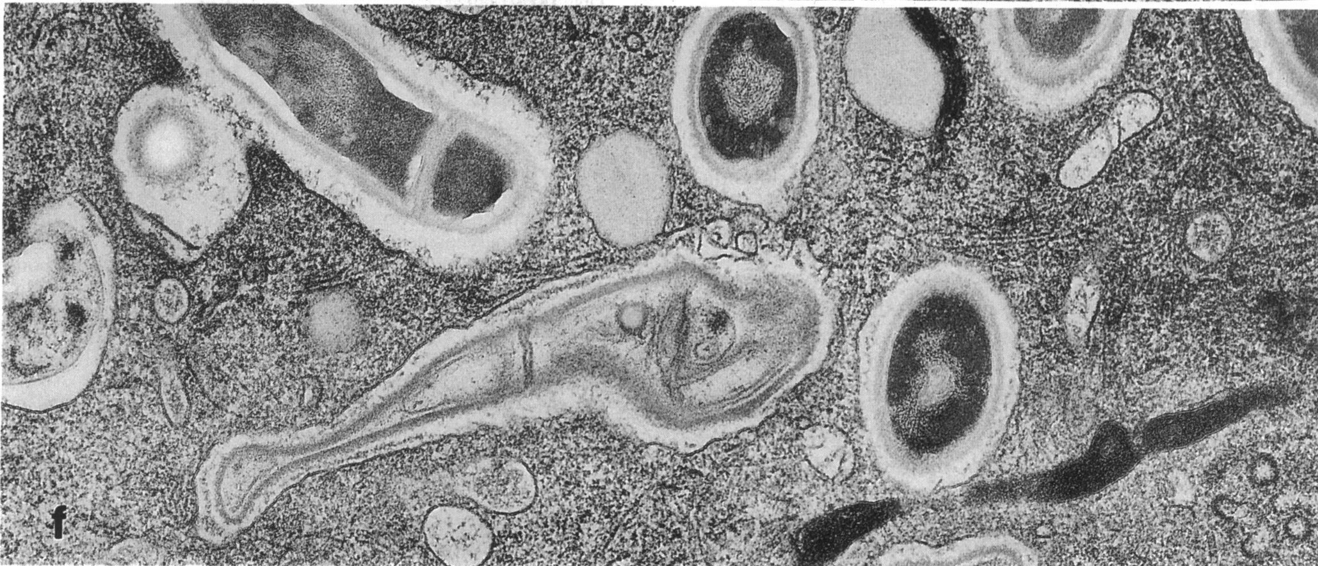
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liposome-encapsulated streptomycin resulted in a concentration-dependent decrease in viable bacteria, compared with untreated controls. Liposome-entrapped streptomycin was more active against intracellular MAC growth than was free streptomycin over the concentration range tested. These results are similar to those obtained with mouse peritoneal macrophages infected with MAC strain 101 and treated with one concentration of streptomycin (3). Liposome-encapsulated streptomycin was also shown to reduce MAC CFU in the beige mouse model more effectively than the free drug (12, 17).

Enhancement of the antimycobacterial effect of liposome-encapsulated streptomycin, compared with the same concentration of free streptomycin, was similar over the concentration range of 10 to 40 μg/ml but was more pronounced at 50 μg/ml. It is possible that the higher intracellular concentration of liposomal streptomycin achieved with this dose reached a critical value for higher antibacterial activity. Another explanation for this finding is that liposomes that did not encapsulate any streptomycin (buffer-loaded liposomes) were also found to inhibit MAC growth inside macrophages when added at a lipid concentration corresponding to that of 50 μg of liposome-encapsulated streptomycin per ml. Some lipids have a potent macrophage-activating function (11); thus, it is possible that the liposomes enhance host resistance to bacteria through an immunomodulatory effect.

Liposome-encapsulated ciprofloxacin dramatically decreased CFU counts compared with the untreated control, while free ciprofloxacin had a much smaller effect. The lowest concentration (0.1 μg/ml) of the liposome-encapsulated drug showed much greater activity than the highest concentration (5 μg/ml) of the free drug that was used for this study (Fig. 2), indicating the differential availability of the active unit of the drug inside the cells. Our results thus point to the need to evaluate the antimycobacterial activity of antibiotics in infected macrophages following initial in vitro determinations.

The efficacy of free ciprofloxacin varied considerably in two different sets of experiments, whereas liposome-encapsulated ciprofloxacin showed little difference in its antimycobacterial activity. One possible source for this difference in the activity of the free drug is macrophage preparations from different donors. Another source may be the solubility of ciprofloxacin. Ciprofloxacin is soluble in water at acidic pH but forms microcrystals at neutral pH. Addition of ciprofloxacin to the culture medium may have resulted in formation of microcrystalline ciprofloxacin, thereby altering its availability inside the cells. Liposomal delivery of ciprofloxacin may be one method to protect the active unit from the extracellular environment, so that the drug will accumulate inside infected cells. Studies with radiolabeled ciprofloxacin will be necessary to investigate the differential uptake of the drug in free and liposome-encapsulated forms. Radioactive ciprofloxacin is not available (27a).

MAC infection is one of the most frequent opportunistic infections seen in patients with AIDS. Because of the inherent resistance of the bacteria to most of the available antimycobacterial agents, successful treatment of this disease is difficult. Selective delivery of drugs to reticuloendothelial cells by liposomes has resulted in greater efficacy against a number of intracellular pathogens. Delivery of ampicillin (4), streptomycin (33), and amphotericin B (25) in liposomes markedly enhanced the activities of these drugs against experimental infections induced by *Listeria monocytogenes*, *Salmonella enteritidis*, and *Candida albicans*, respectively. Several laboratories have reported increased activities of liposome-encapsulated antimycobacterial agents against MAC infections in vivo (7, 9, 12, 13, 17, 18, 24). A liposome formulation of gentamicin is already in phase II clinical trials (25a). Our experiments reported here, as well as our previous studies with the beige mouse model (12, 17), suggest that liposome-encapsulated streptomycin may be used effectively against MAC infections in AIDS patients. Our results also suggest that liposome-encapsulated ciprofloxacin or other fluoroquinolones could be more effective than the free antibiotics against MAC infections in vivo. Further studies are necessary to find the most effective combination of liposome-encapsulated antibiotics and to identify other antibiotics that show increased efficacy when encapsulated in liposomes. The results presented here indicate that the MAC-infected human monocyte/macrophage system will be useful in the efficient identification of such antibiotics.

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