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On the formulation of pH-sensitive liposomes with long circulation times

Sérgio Simões
Universidade de Coimbra, Faculdade de Farmácia

João Nuno Moreira
Universidade de Coimbra, Faculdade de Farmácia

Cristina Fonseca
University of Coimbra, Center for Neuroscience and Cell Biology

Nejat Düzgüneş
University of the Pacific Arthur A. Dugoni School of Dentistry, nduzgunes@pacific.edu

Maria C. Pedroso De Lima
University of Coimbra, Center for Neuroscience and Cell Biology

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Treatment of Intracellular *Mycobacterium avium* Complex Infection by Free and Liposome-Encapsulated Sparfloxacin

NEJAT DÜZGÜNEŞ,[1,2]* DIANA FLASHER,[1] M. VENKATA REDDY,[3] JULIETA LUNA-HERRERA,[3] AND PATTISAPU R. J. GANGADHARAM[3]

Department of Microbiology, School of Dentistry, University of the Pacific, San Francisco, California 94115[1]; Department of Biopharmaceutical Sciences, School of Pharmacy, University of California, San Francisco, California 94143[2]; and Section of Infectious Diseases, Department of Medicine, College of Medicine, University of Illinois at Chicago, Chicago, Illinois 60612[3]

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***Mycobacterium avium-M. intracellulare* complex (MAC) is the most frequent cause of opportunistic bacterial infection in patients with AIDS. Previous studies have indicated that liposome-encapsulated aminoglycosides are highly effective in treating MAC infections in mice. We investigated whether the fluoroquinolone sparfloxacin is effective in treating MAC infection in the murine macrophage-like cell line J774. Sparfloxacin was encapsulated in the membrane phase of multilamellar liposomes composed of phosphatidylglycerol-phosphatidylcholine-cholesterol (1:1:1 molar ratio). MAC-infected macrophages were treated for either 24 h or 4 days with free or liposome-encapsulated sparfloxacin. Treatment with free or liposome-encapsulated sparfloxacin (6 μg/ml) for 24 h resulted in the reduction of the growth index to 25 and 30% of that of untreated controls, respectively. When cultures were treated for 4 days, free sparfloxacin reduced the growth index to 6% of that of the untreated control, while liposome-encapsulated sparfloxacin reduced it to 8% of that of the control.**

Mycobacterium avium-M. intracellulare complex (MAC) causes serious pulmonary and disseminated infection and is the most frequent species of bacteria cultured from patients with AIDS (15, 18, 33, 36). MAC infections contribute substantially to morbidity and death in patients with AIDS (7, 17, 18). MAC is resistant to most antituberculosis drugs (14, 16). The drug susceptibility patterns of MAC isolates from patients with AIDS differ from those of strains isolated from patients without AIDS (19). An additional problem in the therapy of MAC infections is that many drugs that are effective against MAC in vitro are not effective in vivo (25). Four- or five-drug regimens appear to be necessary to control MAC infections (5, 18, 36). However, Agins et al. (1) have reported that although multidrug treatment can clear bacteremia, it perhaps does not prolong the survival of patients with disseminated disease. In contrast, Chin et al. (7) have found that patients with MAC bacteremia who were treated had a longer median survival time than those who were not.

MAC replicates within macrophages in lung, liver, and spleen tissues, bone marrow, lymph nodes, and the intestines and is also found in the blood (2, 18). Since both in vitro and in vivo studies have shown that liposomes are phagocytosed by macrophages (8, 9, 23, 30, 32), and because they are naturally targeted to the liver and spleen, the major organs infected with MAC, they may be ideal vehicles for directing antibiotics to sites of infection. In animal models for MAC infection, liposome-encapsulated amikacin (6, 11), gentamicin (6, 21), streptomycin (10, 13), and kanamycin (34) show efficacies higher than those of the free antibiotics. Liposome-encapsulated amikacin, streptomycin, ciprofloxacin, ofloxacin, clarithromycin, and azithromycin are also more effective than the unencapsulated drugs against MAC in macrophages (3, 20, 24, 27, 28). Sparfloxacin has been identified as an effective antibiotic against several strains of MAC in vitro (22, 35) and inside human monocyte-derived macrophages (12, 29, 31). We examined the efficacy of liposome-encapsulated sparfloxacin against intracellular MAC infection, as a primer for the eventual use of this formulation in vivo.

* Corresponding author. Mailing address: Department of Microbiology, School of Dentistry, University of the Pacific, 2155 Webster St., San Francisco, CA 94115. Phone: (415) 929-6565. Fax: (415) 929-6564.

MATERIALS AND METHODS

Reagents. Sparfloxacin was a gift from Parke Davis (Ann Arbor, Mich.). Egg yolk phosphatidylcholine and phosphatidylglycerol derived from phosphatidylcholine by transphosphatidylation were from Avanti Polar Lipids (Alabaster, Ala.), and cholesterol was from Calbiochem (La Jolla, Calif.). Bovine serum albumin (BSA), sodium dodecyl sulfate (SDS), NaCl, KCl, sucrose, dimethylsulfoxide (DMSO), ammonium sulfate, and HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) were obtained from Sigma (St. Louis, Mo.), and Sephadex G-75 was from Pharmacia (Piscataway, N.J.). Solutions were prepared in distilled water further purified in a Barnstead Nanopure filtration apparatus.

Preparation of liposomes. Since the aqueous solubility limit of sparfloxacin is very low (0.24 mg/ml, according to the manufacturer's specifications), it was initially dissolved together with the phospholipids and cholesterol in chloroform ($CHCl_3$). Following the evaporation of the solvent and hydration of the dried lipid in HEPES-buffered saline, sparfloxacin was incorporated in the membrane phase of the liposomes. Multilamellar liposomes were prepared as follows. Chloroform solutions of phosphatidylglycerol, phosphatidylcholine, cholesterol, and sparfloxacin were mixed at a 1:1:1:0.4 molar ratio in a glass tube (total phospholipid, 40 to 80 μmol), dried to a thin film on a Büchi rotary evaporator, and then placed in a vacuum oven at room temperature to remove any residual $CHCl_3$. The dried film was hydrated with 1 to 2 ml of HEPES buffer (140 mM NaCl, 10 mM KCl, 10 mM HEPES [pH 7.4]) by vortexing under an argon atmosphere. In some experiments, 132 mM ammonium sulfate was used as the hydration buffer and the molar ratio of sparfloxacin was reduced to 0.2 in the mixture described above. Unincorporated sparfloxacin was removed by centrifugation of the liposomes at 10,000 rpm in an Eppendorf centrifuge, equilibrated at 4°C, followed by resuspension in ice-cold HEPES buffer (this process was repeated four times). The amount of encapsulated sparfloxacin was quantitated by its A_{304} after an aliquot of the liposomes was dissolved in a 10-fold excess of methanol. The encapsulation efficiency of sparfloxacin in these multilamellar liposomes, following three washes in HEPES-buffered saline, was between 45 and 62%. Control liposomes were prepared similarly, but without the sparfloxacin. The lipid concentration was determined by phosphate assay (4).

Microorganisms. MAC 101, originally provided by C. Inderlied (University of California, Los Angeles), and MAC SK12, obtained from the Centers for Disease Control and Prevention, were propagated in beige mice to sustain the virulence of the bacteria. The spleens of the infected mice were homogenized, and MAC was cultured on 7H11 plates. Smooth transparent colonies were isolated, grown in 7H9 broth, and stored in 20% (vol/vol) glycerol in 7H9 in polypropylene vials

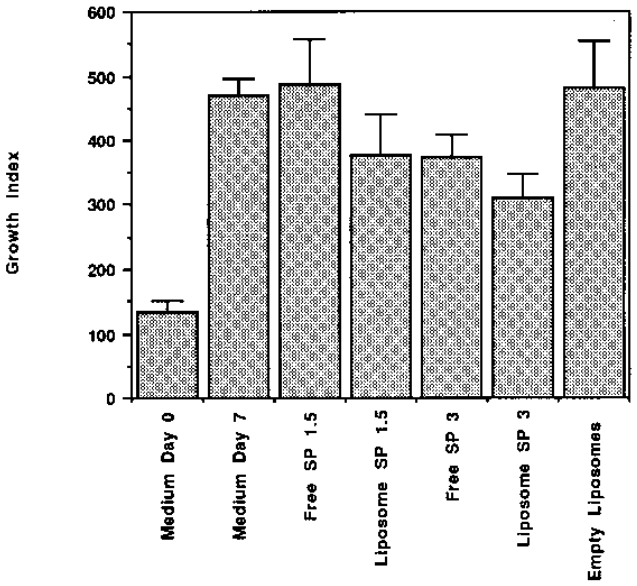

FIG. 1. Treatment of MAC SK12-infected macrophages with free or liposome-encapsulated sparfloxacin. Following infection, the cells were treated for 24 h with free sparfloxacin (Free SP) or sparfloxacin encapsulated in multilamellar liposomes (Liposome SP) (1.5 or 3 μg of sparfloxacin per ml in both cases). The macrophages were lysed on day 7, and the growth index was determined as described in Materials and Methods. Medium Day 0, growth index of MAC obtained from macrophages immediately following the infection period; Medium Day 7, untreated control macrophages; Empty Liposomes, macrophages treated with liposomes containing no drug, at the same lipid concentration as that used for liposome-encapsulated sparfloxacin (3 μg/ml). The data from a representative experiment are expressed as the means ± standard deviations (error bars) of growth indices obtained from three wells for each condition.

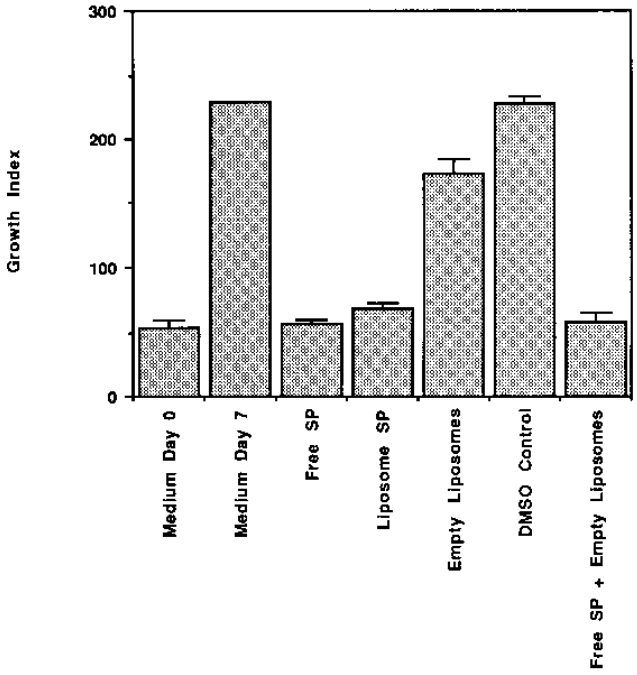

FIG. 2. Short-term treatment of MAC 101-infected macrophages with free or liposome-encapsulated sparfloxacin. Following infection, the cells were treated for 24 h with free sparfloxacin (Free SP) or sparfloxacin encapsulated in multilamellar liposomes (Liposome SP) (6 μg of sparfloxacin per ml in both cases). The macrophages were lysed on day 7, and the growth index was determined as described in Materials and Methods. Medium Day 0, growth index of MAC obtained from macrophages immediately following the infection period; Medium Day 7, untreated control macrophages; Empty Liposomes, macrophages treated with liposomes containing no drug, at the same lipid concentration as that used for liposome-encapsulated sparfloxacin. The data from a representative experiment are expressed as the means ± standard deviations (error bars) of growth indices obtained from three wells for each condition.

at −70°C until use. The MICs of sparfloxacin for these strains were determined by the BACTEC method in Middlebrook 7H12 broth.

Infection and treatment of macrophages. The murine macrophage-like cell line J774 was maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 50 μg of gentamicin per ml in a 5% CO_2 incubator at 37°C. MAC was added to the cells at a ratio between 10 and 20 to 1 and incubated for 2 h at 37°C. The cells were washed three times with Hanks balanced salt solution to remove free bacteria and incubated further in Dulbecco's modified Eagle medium containing only 1% fetal bovine serum to minimize the growth of the cells. Free or liposome-encapsulated sparfloxacin was added to the medium for either 24 h or 4 days and then was removed by washing. Seven days after the initial infection, the macrophages were lysed with 0.5 ml of 0.25% SDS for 10 min, and after this the lysate was neutralized by the addition of 0.5 ml of BSA. The viable bacteria were quantitated by the radiometric BACTEC method using Middlebrook 7H12B growth medium. The amount of $^{14}CO_2$ generated by the metabolism of [^{14}C]palmitic acid within a 24-h period was quantitated and expressed as the growth index. The data are expressed as means ± standard deviations.

RESULTS

The MICs of sparfloxacin for both MAC strains, SK12 and 101, were 0.5 μg/ml. Higher concentrations of sparfloxacin were then tested in the MAC-infected macrophage model. J774 cells infected with MAC SK12 were treated for 24 h with free or liposome-encapsulated sparfloxacin at concentrations of 1.5 or 3 μg/ml (Fig. 1). Free sparfloxacin at 1.5 μg/ml had no effect on the growth of MAC, while at the same concentration the liposome-encapsulated drug was slightly effective, reducing the growth index to 80% of that of the untreated control. At 3 μg/ml, free and liposome-encapsulated sparfloxacin reduced the growth index to 79.6 and 66.2% of that of the untreated control, respectively. The reduction in growth index by free drug was not statistically significant, while that caused by liposomal sparfloxacin was significant ($P < 0.05$). Treatment of the macrophages with liposomes without sparfloxacin did not affect the growth of MAC.

Because of the rather limited effect of sparfloxacin on the SK12 strain even at 3 μg/ml, a concentration well above the MIC, the experiments with strain 101 were performed with a 6-μg/ml concentration of sparfloxacin. Treatment of infected macrophages for 24 h with either free or encapsulated sparfloxacin resulted in a pronounced reduction of the growth index (Fig. 2). The reductions were to 24.5 and 29.7% of the growth index of the untreated controls in the cases of free sparfloxacin ($P < 0.001$) and the liposome-encapsulated drug ($P < 0.001$), respectively. The difference between the reductions by the two types of treatment was statistically significant ($P < 0.05$). Control treatment of infected macrophages with DMSO at the same concentration (0.1%) as that used for free sparfloxacin did not affect the growth index compared with that of the untreated controls. In this experiment, treatment with liposomes without sparfloxacin, at the same lipid concentration as that of drug-containing liposomes, reduced the growth index slightly. The addition of free sparfloxacin plus liposomes without the drug reduced the growth index to the same level as that with free sparfloxacin alone.

The effect of extending the treatment time to 4 days was also investigated (Fig. 3). Free sparfloxacin reduced the growth index to 6.0% of that of the untreated control, while liposome-encapsulated sparfloxacin reduced it to 8.3% of that of the control ($P < 0.001$ for both cases). The small difference be-

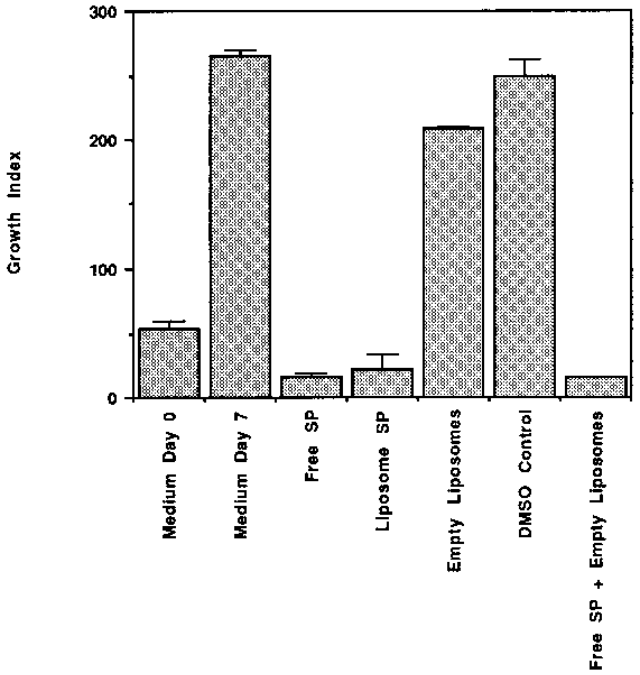

FIG. 3. Long-term treatment of MAC 101-infected macrophages with free or liposome-encapsulated sparfloxacin. Following infection, the cells were treated for 4 days with free sparfloxacin (Free SP) or sparfloxacin encapsulated in multilamellar liposomes (Liposome SP) (6 μg of sparfloxacin per ml in both cases). The macrophages were lysed on day 7, and the growth index was determined as described in Materials and Methods. Medium Day 0, growth index of MAC obtained from macrophages immediately following the infection period; Medium Day 7, untreated control macrophages; Empty Liposomes, macrophages treated with liposomes containing no drug, at the same lipid concentration as that used for liposome-encapsulated sparfloxacin; DMSO Control, cells treated with the same concentration of DMSO as in the free sparfloxacin wells, because of the dilution of the stock sparfloxacin dissolved in DMSO. The data from a representative experiment are expressed as the means ± standard deviations (error bars) of growth indices obtained from three wells for each condition.

tween the reductions by the two treatments was statistically significant ($P = 0.001$). The effects on the growth index of DMSO and liposomes without sparfloxacin were minimal. Treatment of the infected cells with empty (drug-free) liposomes plus free sparfloxacin produced the same reduction in the growth index as did free sparfloxacin. The effect of sparfloxacin treatment of MAC-infected macrophages on the viability of the macrophages was also investigated (Table 1). In an experiment involving a 24-h treatment period, the viability of cells treated with free or liposomal sparfloxacin was slightly higher than that of untreated controls at the end of 7 days of incubation (Table 1). The difference in viability between untreated controls and the treated macrophages was particularly apparent in an experiment involving a treatment period of 4 days (Table 1). This observation suggests that the treatment of the infected macrophages, and the resulting decrease in the growth index of the MAC, reduced the toxicity caused by the intracellular MAC bacilli.

TABLE 1. Viability of J774 macrophages infected with MAC 101 and effect of treatment with free or liposome-encapsulated sparfloxacin[a]

Treatment	24-h exposure		4-day exposure	
	GI[b] (%)	Viability[c] (%)	GI (%)	Viability (%)
Day 0 control		100		100
Day 7 control	100	56	100	75
Liposome-encapsulated sparfloxacin	30.3	63	19.6	92
Free sparfloxacin	18.2	60	7.8	89

[a] Infected cells were treated for either 24 h or 4 days with free or liposome-encapsulated sparfloxacin at a concentration of 6 μg/ml.
[b] Growth index, given as the percentage of the growth index of day 7 controls. The growth index was determined by lysing the macrophages after 7 days in culture.
[c] The viability was determined by trypan blue exclusion. For each condition, 300 cells were counted.

DISCUSSION

Our results indicate that free and liposome-encapsulated sparfloxacin have similar effects on the growth of intracellular MAC, particularly at the higher drug concentrations used. This observation is in contrast to our previous results with ciprofloxacin, streptomycin, and amikacin, indicating that the liposome-encapsulated drugs were more effective than the free drug against MAC inside murine peritoneal or human monocyte-derived macrophages (3, 20, 24). Thus, it is likely that both free sparfloxacin and encapsulated sparfloxacin are taken up by the cells and that they localize to similar extents inside intracellular vacuoles containing MAC. This is expected on the basis of the lipophilic nature of the drug. The slightly greater anti-MAC effect of liposomal sparfloxacin at the lowest concentration used, compared with that of the free antibiotic (Fig. 1), could be the result of a greater accumulation and availability of the drug in the compartments containing MAC. The enhanced uptake of liposome-encapsulated antibiotics by J774 macrophages compared with that of the free drug was demonstrated in the case of another fluoroquinolone antibiotic, ciprofloxacin, encapsulated in the aqueous phase of liposomes composed of distearoylphosphatidylglycerol-distearoylphosphatidylcholine-cholesterol (1:1:1) (27). Another antibiotic, azithromycin, encapsulated in the membrane phase of such liposomes showed enhanced efficacy against MAC inside J774 macrophages compared with the free drug, particularly at high molar ratios of distearoylphosphatidylglycerol to distearoylphosphatidylcholine (27). In the present study, however, sparfloxacin encapsulated in the membrane phase of similar liposomes (composed of analogs of the phospholipids used for azithromycin [see above]) did not show an enhancement in efficacy. The lack of enhancement in the case of liposome-encapsulated sparfloxacin is most likely due to the efficient uptake of the free drug by the infected macrophages, thereby obviating the dependence on liposome-mediated uptake (Fig. 1).

The observation that the anti-MAC effect of liposome-encapsulated sparfloxacin is similar to that of the free antibiotic is in apparent contradiction to results obtained by directly incubating the liposomes with MAC in broth (26). In the study by Mehta et al. (26), the MICs of liposomal sparfloxacin at which 50 and 90% of the isolates were inhibited were four times higher than those of the free antibiotic. The ability of liposomes to deliver encapsulated antibiotics to MAC bacteria most likely depends on the microenvironment of both the MAC and the liposomes, as well as their local concentration. In macrophages, liposomes deliver their contents locally inside endosomes or in MAC-containing phagosomes, while in broth, the contents are diluted into the test tube.

Although liposome-encapsulated aminoglycosides show a modest increase in efficacy against MAC in macrophages in culture (3, 20, 24), they are effective at much lower doses in

vivo than that required for the free drug (10, 11, 13). Our results indicate that free and liposome-encapsulated sparfloxacin are about equally effective against MAC in macrophages in culture. In analogy with the aminoglycosides, it is likely that liposome-encapsulated sparfloxacin will have an enhanced effect against MAC infection in vivo because of its ability to localize in the mononuclear phagocyte (reticuloendothelial) system. Nevertheless, additional factors, such as the stability of sparfloxacin in liposomes during circulation in the bloodstream, can influence the efficacy of liposomal sparfloxacin in vivo.

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REFERENCES

1. **Agins, B. D., D. S. Berman, D. Spicehandler, W. El-Sadr, M. S. Simberkoff, and J. J. Rahal.** 1989. Effect of combined therapy with ansamycin, clofazimine, ethambutol, and isoniazid for *Mycobacterium avium* infection in patients with AIDS. J. Infect. Dis. **159:**784–787.
2. **Armstrong, D., J. W. M. Gold, J. Drysanski, E. Whimbey, B. Polsky, C. Hawkins, A. E. Brown, E. Bernard, and T. K. Kiehn.** 1985. Treatment of infections in patients with the acquired immunodeficiency syndrome. Ann. Intern. Med. **103:**738–743.
3. **Ashtekar, D., N. Düzgüneş, and P. R. J. Gangadharam.** 1991. Activity of free and liposome-encapsulated streptomycin against *Mycobacterium avium* complex (MAC) inside peritoneal macrophages. J. Antimicrob. Chemother. **28:** 615–617.
4. **Bartlett, G. R.** 1959. Phosphorus assay in column chromatography. J. Biol. Chem. **234:**466–468.
5. **Benson, C.** 1994. Disseminated *Mycobacterium avium* complex disease in patients with AIDS. AIDS Res. Hum. Retroviruses **10:**913–916.
6. **Bermudez, L. E., A. O. Yau-Young, J.-P. Lin, J. Cogger, and L. S. Young.** 1990. Treatment of disseminated *Mycobacterium avium* complex infection of beige mice with liposome-encapsulated aminoglycosides. J. Infect. Dis. **161:** 1262–1268.
7. **Chin, D. P., A. L. Reingold, E. N. Stone, E. Vittinghoff, C. R. Horsburgh, Jr., E. M. Simon, D. M. Yajko, W. K. Hadley, S. M. Ostroff, and P. C. Hopewell.** 1994. The impact of *Mycobacterium avium* complex bacteremia and its treatment on survival of AIDS patients—a prospective study. J. Infect. Dis. **170:**578–584.
8. **Daleke, D. L., K. Hong, and D. Papahadjopoulos.** 1990. Endocytosis of liposomes by macrophages: binding, acidification and leakage of liposomes monitored by a new fluorescence assay. Biochim. Biophys. Acta **1024:**352–366.
9. **Dijkstra, J., W. J. M. van Galen, C. E. Hulstaert, D. Kalicharan, F. H. Roerdink, and G. L. Scherphof.** 1984. Interaction of liposomes with Kupffer cells *in vitro*. Exp. Cell Res. **150:**161–176.
10. **Düzgüneş, N., D. R. Ashtekar, D. L. Flasher, N. Ghori, R. J. Debs, D. S. Friend, and P. R. J. Gangadharam.** 1991. Treatment of *Mycobacterium avium-intracellulare* complex infection in beige mice with free and liposome-encapsulated streptomycin: role of liposome type and duration of treatment. J. Infect. Dis. **164:**143–151.
11. **Düzgüneş, N., V. K. Perumal, L. Kesavalu, J. A. Goldstein, R. J. Debs, and P. R. J. Gangadharam.** 1988. Enhanced effect of liposome-encapsulated amikacin on *Mycobacterium avium-M. intracellulare* complex in beige mice. Antimicrob. Agents Chemother. **32:**1404–1411.
12. **Fattorini, L., B. Li, C. Piersimoni, E. Tortoli, Y. Xiao, C. Santoro, M. L. Ricci, and G. Orefici.** 1995. In vitro and ex vivo activities of antimicrobial agents used in combination with clarithromycin, with or without amikacin, against *Mycobacterium avium*. Antimicrob. Agents Chemother. **39:**680–685.
13. **Gangadharam, P. R. J., D. A. Ashtekar, N. Ghori, J. A. Goldstein, R. J. Debs, and N. Düzgüneş.** 1991. Chemotherapeutic potential of free and liposome-encapsulated streptomycin against experimental *Mycobacterium avium* complex infections in beige mice. J. Antimicrob. Chemother. **28:**425–435.
14. **Hawkins, C. C., J. W. M. Gold, E. Whimbey, T. E. Kiehn, P. Brannon, R. Cammarata, A. E. Brown, and D. Armstrong.** 1986. *Mycobacterium avium* complex infections in patients with the acquired immunodeficiency syndrome. Ann. Intern. Med. **105:**184–188.
15. **Henderson, H. M., and S. W. Chapman.** 1994. *Mycobacterium avium-intracellulare*. Curr. Opin. Infect. Dis. **7:**225–230.
16. **Horsburgh, C. R., U. G. Mason III, D. C. Farhi, and M. D. Iseman.** 1985. Disseminated infection with *Mycobacterium avium-intracellulare*. A report of 13 cases and a review of the literature. Medicine (Baltimore) **64:**36–48.
17. **Horsburgh, C. R., and R. M. Selik.** 1989. The epidemiology of disseminated nontuberculous mycobacterial infection in the acquired immunodeficiency syndrome (AIDS). Am. Rev. Respir. Dis. **139:**4–7.
18. **Horsburgh, C. R., Jr.** 1991. *Mycobacterium avium* complex infection in the acquired immunodeficiency syndrome. N. Engl. J. Med. **324:**1332–1338.
19. **Horsburgh, C. R., Jr., D. L. Cohn, R. B. Roberts, H. Masur, R. A. Miller, A. Y. Tsang, and M. D. Iseman.** 1986. *Mycobacterium avium-M. intracellulare* isolates from patients with or without acquired immunodeficiency syndrome. Antimicrob. Agents Chemother. **30:**955–957.
20. **Kesavalu, L., J. A. Goldstein, R. J. Debs, N. Düzgüneş, and P. R. J. Gangadharam.** 1990. Differential effects of free and liposome encapsulated amikacin on the growth of *Mycobacterium avium* complex in mouse peritoneal macrophages. Tubercle **71:**215–218.
21. **Klemens, S. P., M. H. Cynamon, C. E. Swenson, and R. S. Ginsberg.** 1990. Liposome-encapsulated-gentamicin therapy of *Mycobacterium avium* complex infection in beige mice. Antimicrob. Agents Chemother. **34:**967–970.
22. **Klopman, G., S. Wang, M. R. Jacobs, S. Bajaksouzian, K. Edmonds, and J. J. Ellner.** 1993. Anti-*Mycobacterium avium* activity of quinolones: in vitro activities. Antimicrob. Agents Chemother. **37:**1799–1806.
23. **Lee, K.-D., S. Nir, and D. Papahadjopoulos.** 1993. Quantitative analysis of liposome-cell interactions in vitro: rate constants of binding and endocytosis with suspension and adherent J774 cells and human monocytes. Biochemistry **32:**889–899.
24. **Majumdar, S., D. Flasher, D. S. Friend, P. Nassos, D. Yajko, W. K. Hadley, and N. Düzgüneş.** 1992. Efficacies of liposome-encapsulated streptomycin and ciprofloxacin against *Mycobacterium avium-M. intracellulare* complex infections in human peripheral blood monocyte/macrophages. Antimicrob. Agents Chemother. **36:**2808–2815.
25. **Masur, H., C. Tuazon, V. Gill, G. Grimes, B. Baird, A. S. Fauci, and H. C. Lane.** 1987. Effect of combined clofazimine and ansamycin therapy on *Mycobacterium avium-Mycobacterium intracellulare* bacteremia in patients with AIDS. J. Infect. Dis. **155:**127–129.
26. **Mehta, R. T., A. Keyhani, T. J. McQueen, B. Rosenbaum, K. V. Rolston, and J. J. Tarrand.** 1993. In vitro activities of free and liposomal drugs against *Mycobacterium avium-M. intracellulare* complex and *M. tuberculosis*. Antimicrob. Agents Chemother. **37:**2584–2587.
27. **Oh, Y. K., D. E. Nix, and R. M. Straubinger.** 1995. Formulation and efficacy of liposome-encapsulated antibiotics for therapy of intracellular *Mycobacterium avium* infection. Antimicrob. Agents Chemother. **39:**2104–2111.
28. **Onyeji, C. O., C. H. Nightingale, D. P. Nicolau, and R. Quintiliani.** 1994. Efficacies of liposome-encapsulated clarithromycin and ofloxacin against *Mycobacterium avium-M. intracellulare* complex in human macrophages. Antimicrob. Agents Chemother. **38:**523–527.
29. **Perronne, C., A. Gikas, C. Truffot-Pernot, J. Grosset, J.-L. Vilde, and J.-J. Pocidalo.** 1991. Activities of sparfloxacin, azithromycin, temafloxacin, and rifapentine compared with that of clarithromycin against multiplication of *Mycobacterium avium* complex within human macrophages. Antimicrob. Agents Chemother. **35:**1356–1359.
30. **Poste, G., C. Bucana, A. Raz, P. Bugelski, R. Kirsh, and I. J. Fidler.** 1982. Analysis of systemically administered liposomes and implications for their use in drug delivery. Cancer Res. **42:**1412–1422.
31. **Rastogi, N., V. Labrousse, K. S. Goh, and J. P. Carvalho de Sousa.** 1991. Antimycobacterial spectrum of sparfloxacin and its activities alone and in association with other drugs against *Mycobacterium avium* complex growing extracellularly and intracellularly in murine and human macrophages. Antimicrob. Agents Chemother. **35:**2473–2480.
32. **Roerdink, F., J. Regts, T. Daemen, I. Bakker-Woudenberg, and G. Scherphof.** 1986. Liposomes as drug carriers to liver macrophages: fundamental and therapeutic aspects, p. 193–206. *In* G. Gregoriadis, J. Senior, and G. Poste (ed.), Targeting of drugs with synthetic systems. Plenum Press, New York.
33. **Spencer, P. M., and G. G. Jackson.** 1989. Fungal and mycobacterial infections in patients infected with the human immunodeficiency virus. J. Antimicrob. Chemother. **23**(Suppl. A)**:**107–125.
34. **Tomioka, H., H. Saito, K. Sato, and T. Yoneyama.** 1991. Therapeutic efficacy of liposome-encapsulated kanamycin against *Mycobacterium intracellulare* infection induced in mice. Am. Rev. Respir. Dis. **144:**575–579.
35. **Yajko, D. M., C. A. Sanders, P. S. Nassos, and W. K. Hadley.** 1990. In vitro susceptibility of *Mycobacterium avium* complex to the new fluoroquinolone sparfloxacin (CI-978; AT-4140) and comparison with ciprofloxacin. Antimicrob. Agents Chemother. **34:**2442–2444.
36. **Young, L. S., C. B. Inderlied, O. G. Berlin, and M. S. Gottlieb.** 1986. Mycobacterial infections in AIDS patients, with an emphasis on the *Mycobacterium avium* complex. Rev. Infect. Dis. **8:**1024–1033.