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Efficacies of Liposome-Encapsulated Clarithromycin and Ofloxacin against *Mycobacterium avium-M. intracellulare* Complex in Human Macrophages

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The therapeutic efficacies of liposome-encapsulated ofloxacin and clarithromycin against *Mycobacterium avium-M. intracellulare* (MAI) were evaluated in a model of intramacrophage infection. Liposome encapsulation was found to markedly enhance the uptake of each of the drugs by human macrophages. The human blood-derived macrophages were infected at day 7 of culture with MAI. Treatment was initiated 24 h after the infection, and the number of intracellular bacteria was determined at days 2, 3, and 4. Liposome entrapment of either ofloxacin or clarithromycin significantly ($P < 0.005$) enhanced the activities of the drugs when compared with the antimycobacterial effects of equivalent concentrations of the free (unentrapped) drugs. The drugs were used at concentrations close to their clinically achievable peak levels. The efficacy of clarithromycin, either in the free or liposome-entrapped form, was markedly higher than that of ofloxacin. Liposome-encapsulated ofloxacin or clarithromycin plus ethambutol was, in each case, more effective in organism eradication ($P < 0.005$) than each agent used singly. These results suggest that liposome-encapsulated clarithromycin may be more effective than the free form of the drug against MAI infections in vivo, and the use of a combination therapy with ethambutol could further enhance the efficacy.

Mycobacterium avium-M. intracellulare (MAI), a primarily intracellular organism that multiplies within phagocytic cells, frequently causes opportunistic infections in patients with AIDS (32, 33, 35). The treatment of MAI infections has been difficult (5) because the organism has an intrinsic resistance to most antimicrobial agents, presumably because of the lack of permeation of these agents (26) into this bacterium. Although clarithromycin exhibits high degrees of activity in vitro and in vivo against MAI infections (13, 25), resistant organisms have been observed after monotherapy with the drug in a mouse model (3) and in humans as well (19). Ofloxacin has also been shown to have activity against some MAI strains in vitro (12). Generally, different combinations of commercially available drugs have not been capable of efficiently eradicating MAI infections (1, 18). Thus, there is a need to find new drugs for the treatment of the infection as well as to explore ways of enhancing the efficacies of currently available drugs.

Since liposomes are avidly taken up by phagocytic cells of the reticuloendothelial system and can release their contents intracellularly (2), the liposome encapsulation of an antimicrobial agent has the potential of resulting in enhanced efficacy of the agent against intraphagocytic MAI. Increased therapeutic efficacy has been demonstrated following liposome encapsulation of streptomycin (31), amikacin (11), rifampin (29), and kanamycin (30). It was therefore thought that it would be of interest to know whether the therapeutic efficacies of clarithromycin and ofloxacin could be improved by liposome encapsulation. In the study described here, an in vitro cell model which permits the evaluation of the activities of antimicrobial agents against MAI when multiplying within human macrophages (4, 7, 25) was used.

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MATERIALS AND METHODS

Organism. *M. avium* ATCC 49601 was used after culturing on Mycobacteria 7H11 agar (Difco Laboratories, Detroit, Mich.) supplemented with Middlebrook OADC enrichment (Difco). The transparent colony was isolated and cultivated at 37°C in Middlebrook 7H9 broth (Difco) supplemented with ADC enrichment (Difco). After 14 days of incubation, the bacterial suspension was adjusted to 10^8 CFU/ml by using a McFarland standard. Aliquots of the mycobacterial suspension were frozen at −70°C.

Antimicrobial agents. Clarithromycin was supplied by Abbott Laboratories (Abbott Park, Ill.), and ofloxacin was obtained from R. W. Johnson Pharmaceutical Research Institute. Ethambutol was purchased from Sigma Chemical Co. (St. Louis, Mo.). Stock solutions of each drug were prepared by following the manufacturers' instructions. Ethambutol was dissolved in distilled water. The MICs of these drugs for MAI ATCC 49601 were determined previously (9) and were reported as 4, 4, and 16 μg/ml for clarithromycin, ethambutol, and ofloxacin, respectively. The MIC of clarithromycin was determined with modified Mueller-Hinton broth (pH 7.4) supplemented with 10% Middlebrook OADC enrichment. The MICs of the other agents were determined with modified Middlebrook 7H10 broth (pH 6.6) supplemented with 10% OADC.

Preparation of liposomes containing ofloxacin or clarithromycin. Ofloxacin or clarithromycin was encapsulated in liposomes composed of egg yolk phosphatidylcholine, dicetylphosphate, and cholesterol (all from Sigma) in a molar ratio of 7:2:1 by following the method of Tomioka et al. (30). Multilamellar vesicles were prepared from a mixture of the lipids in chloroform; the mixture was evaporated to dryness in a test tube (15 by 125 mm). The resultant dried lipid film was dispersed by sonication into 1 ml of phosphate-buffered saline (PBS) con-

taining 1 mg of either ofloxacin or clarithromycin per ml. Encapsulated vesicles were separated by centrifuging three times (6,000 × *g* for 20 min at 4°C) accompanied by washes with PBS. The final pellets were resuspended in 1 ml of PBS.

The amount of encapsulated ofloxacin was determined by a high-performance liquid chromatographic (HPLC) method after lysis of an aliquot of the liposome suspension with 10-fold excess methanol. The HPLC mobile phase consisted of acetonitrile and 0.01 M phosphate buffer containing 0.005 M tetrabutylammonium hydrogen sulfate (9:91 [vol/vol]; pH 2.8) and was pumped at a rate of 1 ml/min through a 10-μm C_{18} column (300 by 3.9 mm). The chromatographic system comprised a sample processor (WISP 710A; Waters Associates, Milford, Mass.) coupled to a pump (Waters model 6000A), a 254-nm fixed-wavelength detector (Waters model 440), and a HP 3396 series 11 integrator (Hewlett-Packard, Avondale, Pa.). The detection limit of the assay was 0.05 μg/ml. The intra- and interday coefficients of variation were less than 4% at 1.0 μg/ml. The entrapment of ofloxacin into liposomes was 10 to 16%.

Intraliposomal amounts of clarithromycin were determined following the lysis of the vesicles in a 10-fold excess of 0.05% Triton X-100 (Sigma). A validated bioassay method was used for the analysis, and *Micrococcus luteus* ATCC 9341 was the indicator organism (14). The limit of detection of the assay was 0.05 μg/ml. The intra- and interday coefficients of variation were less than 3 and 4%, respectively, at a concentration of 0.1 μg/ml. The entrapment of clarithromycin into liposomes was 22 to 30%.

Human monocyte-derived macrophages. Human blood was obtained from healthy donors, heparinized, and mixed with an equal volume of Hank's balanced salt solution (HBSS; Sigma). The suspension was centrifuged on Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) and was washed as described by Crowle et al. (8) to obtain purified monocytes and lymphocytes. This was adjusted to yield a suspension of 10^7 mononuclear cells per ml in RPMI 1640 medium (Sigma) which was supplemented with 10% fetal bovine serum (Sigma), 2 mM L-glutamine (GIBCO Laboratories, Grand Island, N.Y.), and 50 μg of penicillin per ml. This was then distributed in 1-ml aliquots into 35-by-10-mm petri dishes (Baxter, McGaw Park, Ill.), and the mixture was incubated for 3 h at 37°C (5% CO_2 and humidified air). The supernatant containing nonadherent cells was removed, and the adherent monocyte monolayer was washed twice with 1 ml of HBSS. This was incubated in 1.5 ml of tissue culture medium, which was the supplemented RPMI 1640 medium. The adherent monocytes were cultured for 7 days to obtain macrophages. The macrophage viability was ascertained by trypan blue exclusion to be >97%.

Uptake of liposome-entrapped drugs by macrophages. To the resultant macrophage monolayer was added 1.5 ml of the culture medium containing 200 μg of either free or liposome-entrapped ofloxacin or clarithromycin per ml. The preparation was incubated at 37°C for 3 h in a CO_2 incubator. After rinsing five times with HBSS, the macrophage monolayer was treated with 0.5 ml of distilled water at 37°C for 20 min to lyse the cells. The concentrations of ofloxacin in the resultant lysate were determined by the HPLC method, while clarithromycin concentrations were evaluated by the bioassay procedure described above.

Infection of macrophage monolayer by MAI and drug treatment. An aliquot of the frozen suspension of MAI was thawed in warm water and was diluted with the tissue culture medium to obtain a concentration of 3 × 10^6 CFU/ml. The macrophage monolayer was inoculated with 2 ml of this MAI suspension and was incubated for 24 h at 37°C in a 5% CO_2 and humidified atmosphere. Extracellular bacteria were removed by three washings with HBSS, and the infected monolayer was treated with 1.5 ml of the culture medium containing the following: free ofloxacin (4 μg/ml), free clarithromycin (4 μg/ml), liposome-entrapped clarithromycin (4 μg/ml), liposome-entrapped ofloxacin (4 μg/ml), free ethambutol (5 μg/ml), and different combinations of the free or liposome-encapsulated drugs at the same concentrations as those used for the single drugs. Enough petri dish cultures of macrophages were used to provide for triplicate determinations immediately after and at 2, 3, and 4 days following treatment.

Evaluation of CFU. The numbers of CFU of MAI for each treatment were determined after discarding the tissue culture medium from the petri dish and washing with Middlebrook 7H9 broth. The macrophages were lysed by adding 1.5 ml of 0.25% sodium dodecyl sulfate (SDS) lysing solution, which was prepared as described by Crowle et al. (8). The plates were allowed to stand for 10 min at room temperature and were then vigorously scraped. The lysates were resuspended in 0.5 ml of 20% bovine serum albumin (Sigma) in sterile water to neutralize the SDS. The suspension was vortexed for 1 min, and a 10-fold serial dilution was made in Middlebrook 7H9 broth and the diluted mixture was plated onto Mycobacteria 7H11 agar plates. The colonies were counted after 2 weeks of incubation at 37°C.

The data were expressed as means ± standard deviations of $\log_{10}$ CFU per milliliter of lysate and were compared between groups by using Student's *t* test. Analysis of variance was used to compare the effects of the various treatments and the combination treatments. A *P* value of <0.05 was regarded as significant.

RESULTS

Uptake of ofloxacin and clarithromycin in free and liposome-entrapped forms by macrophages. Entrapment of ofloxacin and clarithromycin into liposomal vesicles considerably enhanced the incorporation of the drugs by macrophages (Fig. 1). The differences in the uptakes of free and liposome-entrapped drugs were highly statistically significant ($P < 0.005$) for both drugs.

Antimycobacterial effects of free and liposome-encapsulated ofloxacin. The effects of liposome-entrapped ofloxacin were compared with those of the nonencapsulated drug against MAI inside human macrophages at a clinically achievable concentration (Fig. 2). The results indicate that at day 2 following the treatment of the infected macrophages, the free ofloxacin and liposome-encapsulated ofloxacin produced about a 0.28- and 1.0-log-unit decreases in the CFU of MAI, respectively, in comparison with that in the untreated control. The CFU counts following treatment with liposome-encapsulated ofloxacin at day 2 were 0.75 log units lower than those in cell cultures treated with the free drug ($P < 0.0005$). At day 4 following treatment, liposome-encapsulated ofloxacin reduced the CFU of MAI by more than 0.93 log units in comparison with the effect of the free drug ($P < 0.0005$). The addition of liposome-encapsulated phosphate buffer at lipid concentrations corresponding to those in the ofloxacin-containing liposomes had no effect on the CFU compared with that on the CFU of the untreated control.

Antimycobacterial effects of free and liposome-encapsulated clarithromycin. Figure 3 shows the effects of free and liposome-encapsulated clarithromycin on the intracellular survival of MAI. Liposome-encapsulated clarithromycin produced a decrease of about 0.4 log units in the CFU counts when compared with the effect of the free drug at either day 2 or day

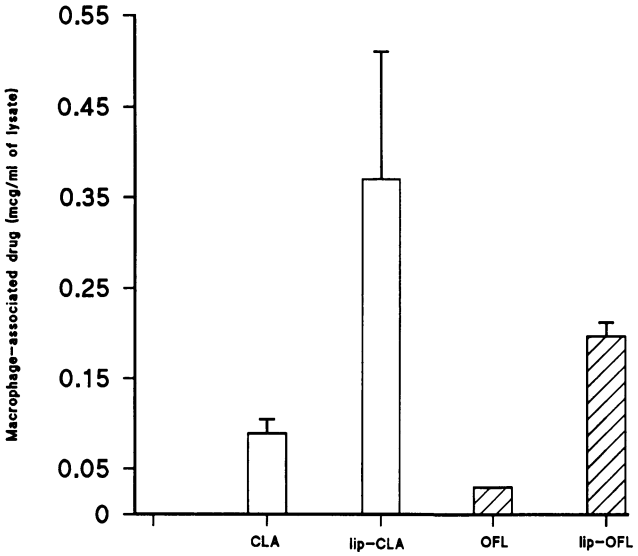

FIG. 1. Uptake of free ofloxacin (OFL), liposome-encapsulated ofloxacin (lip-OFL), free clarithromycin (CLA), and liposome-encapsulated clarithromycin (lip-CLA) by human blood-derived macrophages. The macrophage monolayer was incubated in medium for 3 h with or without 200 μg of ofloxacin or clarithromycin per ml in free or liposome-entrapped form. The amounts of macrophage-associated ofloxacin and clarithromycin were determined as described in the text. Bars represent means ± SDs of three determinations. Nothing was detected in the lysates from the macrophage monolayer exposed to the medium containing no drugs.

4 following treatment. This decrease was statistically significant ($P < 0.0005$).

Effect of ethambutol in combination with free and liposome-encapsulated ofloxacin or clarithromycin. Figure 4 shows the

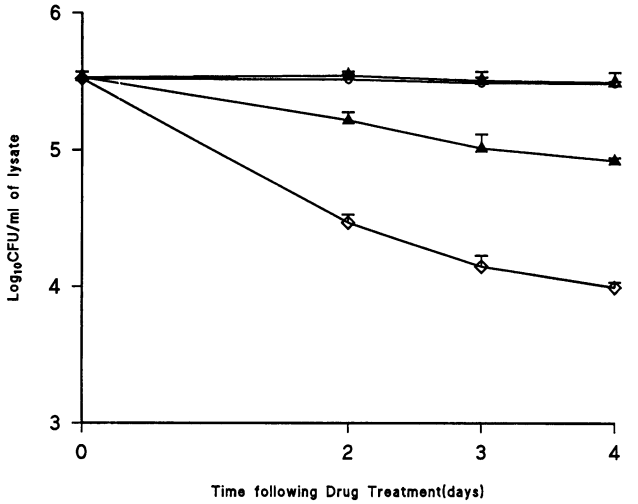

FIG. 2. Effects of free ofloxacin and liposome-encapsulated ofloxacin on the survival of MAI ATCC 49601 in human macrophages. The CFU of the lysate was determined as described in the text. The data are presented as the mean ± SD CFU obtained from triplicate determinations by using macrophages derived from a single donor. ○, untreated control; △, buffer-loaded liposomes; ▲, free ofloxacin (4 μg/ml); ◇, liposome-encapsulated ofloxacin (4 μg/ml).

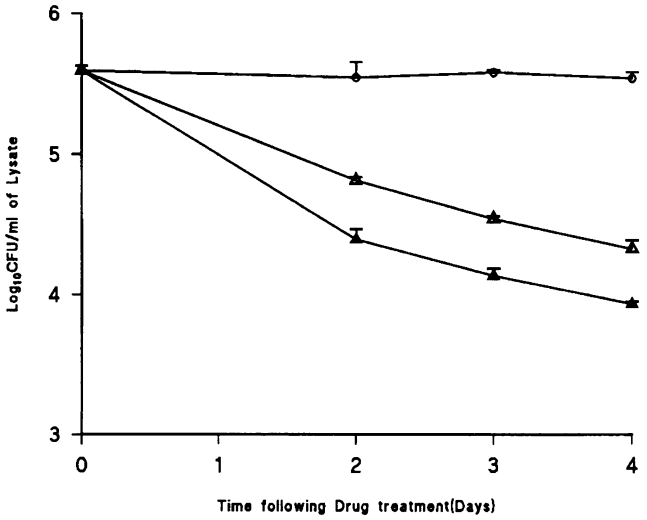

FIG. 3. Effects of free and liposome-encapsulated clarithromycin against MAI ATCC 49601 growth inside human macrophages. The macrophages were lysed to determine the CFU of MAI following the procedure described in the text. The bars represent means ± SDs of triplicate determinations. ○, untreated control; △, free clarithromycin (4 μg/ml); ▲, liposome-encapsulated clarithromycin (4 μg/ml).

antimycobacterial effects of various treatments and combination treatments on the intracellular growth of MAI. Free ethambutol and ofloxacin, acting singly, significantly ($P < 0.05$) reduced the CFU of MAI in comparison with that in the untreated control. Free ethambutol was slightly more effective, but there was no significant difference between the effects of

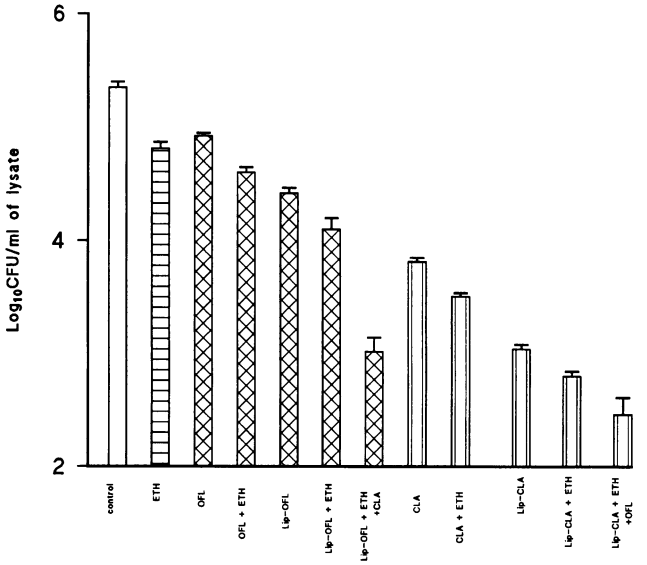

FIG. 4. Effects of various treatments on the survival of MAI ATCC 49601 inside human macrophages. In the drug combinations, the concentration of each drug was the same as that in the treatment with the single drug. The lysate was obtained as described in the text following exposure of the infected macrophages to drugs for 6 days. The bars represent means ± SDs of triplicate determinations. OFL, ofloxacin (4 μg/ml); CLA, clarithromycin (4 μg/ml); ETH, ethambutol (5 μg/ml); Lip, liposome-encapsulated form.

both drugs ($P > 0.05$). However, a combination of both drugs produced a significant ($P < 0.05$) reduction in the CFU in comparison with that from either drug acting singly. Liposome-encapsulated ofloxacin caused a 35% decrease in the CFU of MAI when compared with that from the combination treatment of ofloxacin plus ethambutol ($P > 0.05$). The addition of ethambutol to liposome-encapsulated ofloxacin significantly increased the efficacy of the liposome-encapsulated ofloxacin preparation ($P < 0.05$).

Free clarithromycin significantly reduced the CFU more than the treatment regimen of combination of liposome-encapsulated ofloxacin plus ethambutol did ($P < 0.05$). The addition of ethambutol to free clarithromycin further enhanced the efficacy ($P < 0.05$). Liposome-encapsulated clarithromycin significantly decreased the CFU ($P < 0.05$) in comparison with the treatment with the combination of free clarithromycin plus ethambutol. The addition of ethambutol to liposome-encapsulated clarithromycin further enhanced the efficacy of the liposomal preparation ($P < 0.05$). The triple combination of liposome-encapsulated ofloxacin, free ethambutol, and free clarithromycin produced an effect comparable ($P > 0.05$) to that of liposome-encapsulated clarithromycin acting alone. On the other hand, the combination of liposome-encapsulated clarithromycin plus ethambutol and ofloxacin was the most effective of the various treatments tested.

DISCUSSION

Liposome encapsulation of ofloxacin and clarithromycin was shown to significantly ($P < 0.005$) enhance the delivery of drugs into macrophages when compared with the uptake of the free forms of the drugs by macrophages. This is not surprising since liposomes are known to be endocytosed by phagocytic cells, leading to the intracellular delivery of the encapsulated agent (28). It has been reported that the macrolide antibiotic clarithromycin is markedly transported into human polymorphonuclear leukocytes, in which it is concentrated 16-fold (21). The present study demonstrates that liposome encapsulation of the drug further enhances intracellular drug incorporation. Similar increases in drug uptake by macrophages after liposomal drug encapsulation have been reported for kanamycin (30) and rifampin (29).

With increased uptake of liposome-encapsulated ofloxacin and clarithromycin by macrophages, it was expected that this might translate into higher treatment efficacies compared with those of the treatments with the free drugs. The concentration of each drug was chosen to be close to the peak levels obtained in the sera of humans given the drug orally (6, 16). Although the MIC of ofloxacin for the strain of MAI used in the study was 16 μg/ml, the free drug was found to reduce significantly the CFU counts at a concentration of 4 μg/ml (one-fourth the MIC). This is probably because of the intracellular concentration of the drug, which is reported to concentrate 8.2-fold within human polymorphonuclear leukocytes (24). As predicted from the results showing increased macrophage uptake of the liposome-entrapped ofloxacin, liposome encapsulation markedly enhanced the efficacy of the drug against MAI. Similarly, liposome-encapsulated clarithromycin was significantly ($P < 0.005$) more effective in the intramacrophage killing of MAI than the same concentration of the free drug was. Delivery of drugs to phagocytic cells by liposomes resulted in greater efficacy against a number of intracellular pathogens including *Listeria monocytogenes* (2). Also, several laboratories have reported increased efficacies of liposome-entrapped antimycobacterial agents against MAI infections (11, 29, 30, 31). Free clarithromycin demonstrated activity much greater than that of free ofloxacin, and this is in agreement with results of previous studies that used an in vitro cell model similar to the one used in the present study (7, 25, 27). This also appears to be consistent with the susceptibilities of MAI to the drugs in vitro and the capabilities of the drugs to concentrate within blood cells.

The presence of ethambutol significantly ($P < 0.05$) decreased the intracellular survival of MAI exposed to ofloxacin or clarithromycin, either in the free or liposomal form. The in vitro extracellular bactericidal activities of combinations of antimicrobial agents reveal that ethambutol is frequently synergistic against strains of MAI (20, 23, 34). Also, the intramacrophage killing by free clarithromycin has previously been shown to be enhanced by the addition of ethambutol (27). In fact, it has been proposed that ethambutol has a key role in the treatment of MAI infections because of its interaction with other drugs (22).

Phosphate buffer-loaded liposomes at lipid concentrations corresponding to those in the clarithromycin- or ofloxacin-containing liposomes had no effect on the CFU of MAI. It was necessary to evaluate the effect of the buffer-loaded liposomes since some lipids have been shown to have a potent macrophage-activating function (10), and thus, it is not impossible that some of the lipids used to make the liposomes could enhance bactericidal activity through an immunomodulatory effect.

The results of the present study indicate that liposome encapsulation of ofloxacin and clarithromycin significantly increases the efficacies of the drugs against MAI. The study also suggested that the addition of ethambutol to the liposomal drug preparation leads to a further marked enhancement of the activities of the antimicrobial agents. Selection of resistant mutants of *M. avium* by clarithromycin monotherapy has been observed previously (3, 19), and it is envisaged that increased intracellular concentration of the drug by entrapment in liposomes has the potential of mitigating such a development. A major drawback in the practical applications of liposomal formulations was the concern over its rather short shelf-life. However, progress toward clinical uses of liposomal products has gained tremendous momentum, and new developments such as concentrating aqueous liposome dispersions or freeze-drying of the dispersions have been shown to yield liposomes with pharmaceutically acceptable shelf-lives (17). Liposome-encapsulated gentamicin is already in a stage of clinical trials for the treatment of MAI infections in patients with AIDS (15), and this underscores the potential clinical utility of the liposome-encapsulated drugs described here. Since, in clinical trials for the treatment of MAI infections in patients with AIDS, the results obtained with combinations of antimicrobial agents were often found to be disappointing (1, 18), the use or inclusion of liposome-encapsulated clarithromycin and ofloxacin might improve the clinical response. Thus, further studies are necessary to evaluate the in vivo efficacies of liposome-entrapped clarithromycin and ofloxacin.

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REFERENCES

1. **Agins, B. D., D. S. Berman, D. E. Spicehandler, W. Sadr, M. S. Simberkorf, and J. J. J. Rahal.** 1989. Effects of combined therapy with ansamycin, clofazimine, ethambutol and isoniazid for *Mycobacterium avium* infection in patients with AIDS. J. Infect. Dis. **159**:784–787.
2. **Bakker-Woundenberg, I. A. J. M., A. F. Lokerse, J. C. Vink-**

vanderberg, F. N. Roedink, and M. C. Michel. 1986. Effect of liposome-entrapped ampicillin on survival of *Listeria monocytogenes* in murine peritoneal macrophages. Antimicrob. Agents Chemother. **30:**295–300.
3. **Baohong, J. I., N. Lounis, C. Truffot-Pernot, and J. Grosset.** 1992. Selection of resistant mutants of *Mycobacterium avium* in beige mice by clarithromycin monotherapy. Antimicrob. Agents Chemother. **36:**2839–2840.
4. **Bermudez, L. E., M. Wu, and L. S. Young.** 1987. Intracellular killing of *Mycobacterium avium* complex by rifapentine and liposome-encapsulated amikacin. J. Infect. Dis. **156:**510–513.
5. **Brass, J. B.** 1986. *Mycobacterium avium-intracellulare*: rational therapy of chronic pulmonary infection? Am. Rev. Respir. Dis. **134:**431–432.
6. **Chu, S., D. S. Wilson, D. R. Guay, and C. Craft.** 1992. Clarithromycin pharmacokinetics in healthy young and elder volunteers. J. Clin. Pharmacol. **32:**1045–1049.
7. **Crowle, A. J., N. Elkins, and M. H. May.** 1988. Effectiveness of ofloxacin against *Mycobacterium tuberculosis* and *Mycobacterium avium*, and rifampin against *M. tuberculosis* in cultured human macrophages. Am. Rev. Respir. Dis. **137:**1141–1146.
8. **Crowle, A. J., J. A. Sbarbaro, F. N. Judson, G. S. Douvas, and M. H. May.** 1984. Inhibition by streptomycin of tubercle bacilli within human macrophages. Am. Rev. Respir. Dis. **130:**839–844.
9. **Cynamon, M. H., and S. P. Klemens.** 1992. Activity of azithromycin against *Mycobacterium avium* infection in beige mice. Antimicrob. Agents Chemother. **36:**1611–1613.
10. **Dijkstra, J., W. J. M. van Galden, 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.
11. **Düzgünes, N., V. K. Perumal, L. Kesavalu, J. A. Goldstein, R. T. Debs, and P. R. J. Gangadharam.** 1988. Enhanced effect of liposome-encapsulated amikacin on *Mycobacterium avium-M. intracellulare* complex infection in beige mice. Antimicrob. Agents Chemother. **32:**1404–1411.
12. **Fenlon, C., and M. H. Cynamon.** 1986. Comparative in vitro activities of ciprofloxacin and other four-quinolones against *Mycobacterium tuberculosis* and *Mycobacterium intracellulare*. Antimicrob. Agents Chemother. **29:**386–388.
13. **Fernandes, P. B., D. J. Hardy, D. McDaniel, C. W. Hanson, and D. N. Swason.** 1989. In vitro and in vivo activities of clarithromycin against *Mycobacterium avium*. Antimicrob. Agents Chemother. **33:**1531–1534.
14. **Fernandes, P. B., N. Ramer, R. A. Rode, and L. Freibery.** 1988. Bioassay for A-56268 (TE-031) and identification of its major metabolite, 14-hydroxy-6-O-methyl erythromycin. Eur. J. Clin. Microbiol. Infect. Dis. **7:**73–76.
15. **Fielding, R. M.** 1991. Liposomal drug delivery. Advantages and limitations from a clinical pharmacokinetics and therapeutic perspective. Clin Pharmacokinet. **21:**155–164.
16. **Flor, S.** 1989. Pharmacokinetics of ofloxacin. An overview. Am. J. Med. **87**(Suppl. 6c)**:**6c–25s.
17. **Geerdiem, M. Z., and D. J. H. Crommelin.** 1988. Liposome: parenteral administration to man, p. 795–817. *In* G. Gregoriades (ed.), Liposomes as drug carriers. Recent trends and progress. John Wiley & Sons, Inc., New York.
18. **Hawkins, C. C., J. W. M. Gold, E. Whimbey, T. E. Kiehn, P. Brannon, K. Cammarata, A. E. Brown, and D. Armstrong.** 1986. *Mycobacterium avium* complex infections in patients with acquired immunodeficiency syndrome. Ann. Intern. Med. **105:**184–188.
19. **Heifets, L., N. Mo, and J. Vanderkolk.** 1993. *Mycobacterium avium* strain resistant to clarithromycin and azithromycin. Antimicrob. Agents Chemother. **37:**2364–2370.
20. **Hoffner, S. E., M. Kratz, B. Olsson-Liljequist, S. B. Svenson, and G. Kallenius.** 1989. *In vitro* synergistic activity between ethambutol and fluorinated quinolones against *M. avium* complex. J. Antimicrob. Chemother. **24:**317–324.
21. **Ishiguro, M., H. Koga, S. Kohno, T. Hayashi, K. Yamaguchi, and M. Hirota.** 1989. Penetration of macrolides into human polymorphonuclear leukocytes. J. Antimicrob. Chemother. **24:**719–729.
22. **Kallenius, G., S. B. Svenson, and S. E. Hoffner.** 1989. Ethambutol: a key for *Mycobacterium avium* complex chemotherapy. Am. Rev. Respir. Dis. **140:**264.
23. **Kent, R. J., M. Bakhitiar, and D. C. Shanson.** 1992. The *in vitro* bactericidal activities of combinations of antimicrobial agents against clinical isolates of *Mycobacterium avium-intracellulare*. J. Antimicrob. Chemother. **30:**643–650.
24. **Kogu, H.** 1987. High-performance liquid chromatographic measurement of antimicrobial concentrations in polymorphonuclear leukocytes. Antimicrob. Agents Chemother. **31:**1904–1908.
25. **Perronne, C., A. Gikas, C. Truffot-Pernot, J. Grosset, J. J. Posidalo, and L. J. Vilde.** 1990. Activities of clarithromycin, sulfisoxazole, and rifabutin against *Mycobacterium avium* complex multiplication within human macrophages. Antimicrob. Agents Chemother. **34:**1504–1511.
26. **Rastogi, N., C. Frehel, A. Ryter, H. Ohahon, M. Lesourd, and H. L. David.** 1981. Multiple drug resistance in mycobacterium: is the cell wall architecture responsible for the exclusion of antimicrobial agents? Antimicrob. Agents Chemother. **20:**666–677.
27. **Rastogi, N., and V. Labrousse.** 1991. Extracellular and intracellular activities of clarithromycin used alone and in association with ethambutol and rifampin against *Mycobacterium avium* complex. Antimicrob. Agents Chemother. **35:**462–470.
28. **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. Poster (ed.), Targeting of drugs with synthetic systems. Plenum Press, New York.
29. **Saito, H., and H. Tomioka.** 1989. Therapeutic efficacy of liposome-entrapped rifampin against *Mycobacterium avium* complex infection in mice. Antimicrob. Agents Chemother. **33:**429–433.
30. **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.
31. **Vladimirsky, M. A., and G. A. Ladigina.** 1982. Antibacterial activity of liposome-entrapped streptomycin in mice with *Mycobacterium tuberculosis*. Biomedicine **36:**375–377.
32. **Wallace, R. J., R. O'Brien, J. Glassroth, J. Raleigh, and A. Dutt.** 1990. Official statement of the American Thoracic Society. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Am. Rev. Respir. Dis. **142:**940–953.
33. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119:**107–157.
34. **Yajko, D. M., J. Kirihara, C. Sanders, P. Nassos, and W. K. Hardley.** 1988. Antimicrobial synergism against *Mycobacterium avium* complex strains isolated from patients with acquired immune deficiency syndrome. Antimicrob. Agents Chemother. **32:** 1392–1395.
35. **Young, L. S., C. B. Inderlied, O. G. Berlin, and M. S. Gottlied.** 1986. Mycobacterial infections in AIDS patients, with emphasis on the *Mycobacterium avium* complex. Rev. Infect. Dis. **8:**1024–1033.