ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1993, p. 2293–2297
0066-4804/93/112293-05$02.00/0

Vol. 37, No. 11

Liposome-Incorporated Ciprofloxacin in Treatment of Murine Salmonellosis

MARCUS MAGALLANES,[1] JAN DIJKSTRA,[2] AND JOSHUA FIERER[1,3]*

Departments of Medicine and Pathology, University of California San Diego School of Medicine, La Jolla, California 92093[1]; EntreMed, Inc., Rockville, Maryland 20850[2]; and the Department of Veterans Affairs Medical Center, San Diego, California 92161[3]

Received 17 July 1992/Returned for modification 23 October 1992/Accepted 11 August 1993

We used a dehydration-rehydration procedure in order to efficiently incorporate ciprofloxacin into phospholipid vesicles (liposomes), which we then used to treat BALB/c mice that had been infected per os with *Salmonella dublin*. A single injection of liposome-incorporated ciprofloxacin (LIC) was 10 times more effective than a single injection of free drug at preventing mortality. When free ciprofloxacin was administered twice daily for 5 days, it was more effective than when it was administered as a single dose. Treatment with LIC produced dose-dependent decreases in bacterial counts in spleen, stool, and Peyer's patches, indicating that the drug had distributed to all areas of inflammation, not just to the major reticuloendothelial system organs. Although LIC was cleared rapidly from the blood, drug persisted in the liver and spleen for at least 48 h after administration of a dose of LIC. We attribute the enhanced activity of LIC to the concentration and persistence of active drug in the infected organs. Our results confirm the fact that ciprofloxacin is effective therapy for systemic salmonella infection and show that LIC is even more effective than aqueous ciprofloxacin in our model.

Liposomes, or artificial phospholipid vesicles, have been used to deliver various drugs, including antibiotics (21), to experimental animals. Systemically administered liposomes are rapidly cleared from the circulation by macrophages in the reticuloendothelial system; hence, the liposomes, and any drug contained within them, accumulate in tissues rich in reticuloendothelial cells such as liver, spleen, lymph nodes, lung, and bone marrow (1). Liposomes also accumulate preferentially at sites of inflammation and tumor growth (23). Because liposomes enhance delivery of drugs to macrophages, liposome-incorporated antibiotics have great promise for treating organisms which survive intracellularly, within the macrophages (15). Examples of such use in experimental animals include treatment of *Listeria monocytogenes* with ampicillin, murine cryptococcoses with amphotericin B, *Mycobacterium avium-M. intracellulare* with amikacin, and murine salmonellosis with streptomycin or gentamicin (3, 8, 9, 12, 14, 26). In general, the liposome-incorporated antibiotics have proven superior to the plain form of the drugs (21).

Ciprofloxacin is a quinolone antibiotic that has excellent in vitro activity against *Salmonella* species (13). It has been used in the treatment of individuals with *Salmonella* infections, including those with typhoid fever and chronic typhoid carriers (19, 25). In previously published studies of animal models of *S. typhimurium* infection, both oral and subcutaneous administration of ciprofloxacin resulted in prolongation of survival and reductions in bacterial counts in liver and spleen compared with those in untreated controls and those after treatment with either ampicillin or chloramphenicol (5).

In the study described here, we investigated the therapeutic value of liposome-incorporated ciprofloxacin (LIC) in a murine model of fatal *S. dublin* infection. Untreated, the infection begins in Peyer's patches, spreads to the regional lymph nodes, and then disseminates to the liver and spleen, leading to bacteremia and death 10 to 14 days after infection (16). In essence, we attempted to determine whether LIC would prove to be superior to aqueous ciprofloxacin.

MATERIALS AND METHODS

Mice. Virus-free female BALB/cJ mice were purchased from Jackson Laboratory, Bar Harbor, Maine. Animals were housed at four to six per cage and were allowed free access to food and water. Mice weighed approximately 20 g when infected. BALB/c mice carry the *ity*^{s} allele, which makes them susceptible to *Salmonella* infections (24).

Infection. *S. dublin* Lane pSD6 is a kanamycin-resistant derivative of the parent Lane strain in which Tn*5* is inserted in the cryptic plasmid outside the *vir* region; it is fully virulent (6). Bacteria were suspended in 0.1 M $NaHCO_3$ solution to 5×10^8 CFU/ml. A total of 0.2 ml of the suspension was delivered into the stomach by gavage. The MIC of ciprofloxacin for *S. dublin* was 0.03 μg/ml.

Cultures. Organs and tissues were removed aseptically and homogenized in 2 ml of sterile saline as described previously (16). The homogenates were serially diluted 10-fold in saline solution. Appropriate dilutions of the spleen and lymph node homogenates were cultured on Trypticase soy agar; Peyer's patch homogenates were cultured on Hectone-Enteric (HE) agar with kanamycin sulfate (20 μg/ml of agar) to select for *S. dublin* Lane pSD6. Stool was obtained by emptying ceca and mixing feces with 0.5 ml of 0.9% saline. The stool suspension was serially diluted in saline and was subsequently cultured on HE agar with kanamycin. After overnight incubation, we counted the colonies and determined the number of CFU in the specimen.

Preparation of liposomes. The liposomes consisted of dipalmitoyl-phosphatidylcholine (Avanti Polar Lipids, Inc., Alabaster, Ala.), dipalmitoyl-phosphatidylglycerol (Avanti), and cholesterol (type C-8253; Sigma Chemical Co., St. Louis, Mo.) in a molar ratio of 4.1:0.9:4. For the incorporation of ciprofloxacin into phospholipid vesicles, the dehydra-

* Corresponding author.

tion-rehydration procedure of Kirby and Gregoriadis (18) was followed. The liposome-lipid mixture in chloroform (128 μmol of total lipid) was dried by rotary evaporation in pyrogen-free (180°C/6 h), 19-mm-diameter borosilicate-glass culture tubes with a Teflon-lined screw cap. The lipids were further exposed to high vacuum for 1.5 h. The dried material was resuspended at 45°C in 4 ml of sterile water by vortexing, after flushing the tubes with nitrogen gas. Subsequently, the hydrated lipids were sonicated at 45°C for two 15-min periods, with intermittent vortexing (bath-type sonicator; Laboratory Supplies Company, Inc., Hicksville, N.Y.). Four milliliters of sterile ciprofloxacin · HCl (Miles Pharmaceuticals, West Haven, Conn.) in water (10 to 20 mM; 3.9 to 7.8 mg/ml) was added to the resulting opalescent suspension, and the mixture was lyophilized overnight. Next, the dry material was rehydrated at 45°C in 0.4 ml of sterile water by vortexing, after the addition of a few sterile glass beads. The suspension was further incubated at 45°C and was vortexed once every 10 min. After 30 to 45 min, 0.4 ml of sterile sodium-acetate buffer (20 mM; pH 5.0) was added and the suspension was left for another 30 to 45 min at 45°C. The vesicles were subsequently transferred to 26-ml polycarbonate centrifuge tubes with screw-on caps (type 60Ti rotor; Beckman) which had been soaked overnight in 70% ethanol and rinsed with sterile buffer. After the addition of acetate buffer and mixing, the tubes were centrifuged for 15 min at 20°C (25,000 rpm). The liposomal pellet was resuspended in acetate buffer by vigorous vortexing, and the vesicles were centrifuged again. After resuspension in acetate buffer, samples were taken for determination of phospholipid phosphorus and ciprofloxacin levels, and the suspension was centrifuged once again. The final pellet was resuspended in 2.5 ml of sterile, Ca- and Mg-free phosphate-buffered saline (pH 7.4).

Phospholipid phosphorus was assessed by the procedure of Böttcher et al. (4). The amounts of liposome-associated ciprofloxacin were determined as follows. A sample of 0.2 ml (50- to 100-fold diluted liposome suspension in water) was mixed with 0.85 ml of chloroform-methanol (5:12; vol/vol), and the A_{325} of the resulting clear solution was measured. Standard preparations (2 to 32 μg of ciprofloxacin in 0.2 ml of water) were measured similarly after the addition of the chloroform-methanol mixture.

By using the indicated amounts of the antibiotic, 50 to 70 μg of ciprofloxacin per μmol of total lipid was found to be associated with the liposomes (incorporation efficiency, 30 to 40%, respectively). The recovery of the liposomal phospholipids amounted to over 90%. A preparation containing 3.4 mg of ciprofloxacin per ml and 49 μmol of total liposomal lipid per ml was used as a stock for the experiments described here. Control liposomes not containing ciprofloxacin were prepared similarly, and they also contained 49 μmol of total lipid per ml. Dilutions of the stock suspensions were prepared as needed by adding 0.9% NaCl solution.

Treatment. Infected mice received their initial treatment on day 4 of infection (the day of infection being designated day 1). Treatment regimens involved a single intravenous (i.v.) tail vein injection of 0.2 ml of appropriately diluted LIC, a single subcutaneous (s.c.) injection of 0.2 ml of aqueous ciprofloxacin, or subcutaneous injections of 0.2 ml of aqueous ciprofloxacin every 12 h for 5 days. Aqueous ciprofloxacin · HCl (Miles) in a stock solution of 2 mg/ml was diluted in 0.9% NaCl solution, as needed, for the various dosing regimens tested.

Assay of ciprofloxacin in sera and organs. Uninfected BALB/c mice weighing approximately 20 g each were injected either with an i.v. dose of LIC of 20 mg/kg of body weight or an s.c. dose of aqueous ciprofloxacin of 20 mg/kg for kinetics studies. The mice were subsequently bled to obtain serum samples. Spleens and liver segments were removed aseptically and were homogenized in 2 ml of saline solution. These homogenates were frozen at −70°C and were later thawed to accomplish cell lysis to release any intracellular antibiotic. After freezing-thawing, the homogenates were then centrifuged at 10,000 × g for 10 min and the supernatant was assayed for ciprofloxacin.

The ciprofloxacin concentration was determined by reverse-phase high-pressure liquid chromatography (HPLC) analysis by a previously described method (28). This method used a C18 radial pack column (Waters Associates) and a mobile phase consisting of 35% HPLC-grade methanol in phosphate buffer (67 mmol/liter) delivered through a Beckman HPLC system (model 334). UV absorption was monitored at 277 nm. The ciprofloxacin (peak height) retention time was consistently between 6.0 and 6.5 min. Ciprofloxacin concentrations in the sample unknowns were determined by comparison of the peak heights of the unknowns with the standard curves made by adding ciprofloxacin to normal mouse serum, spleen, and liver.

The standards contained 0, 200, 400, 500, 750, and 1,000 ng of ciprofloxacin per ml. At least two separate samples were run for each known concentration tested. Computer-generated best-fit curves were constructed by linear regression analysis with the following R values for each resultant standard curve: serum, 0.98; liver, 1.00; spleen, 0.99.

The lower limit of detection was 200 ng/ml for serum and 400 ng/ml for both liver and spleen. Quality assurance samples were run prior to the beginning of each day's run of sample unknowns. Determination of ciprofloxacin levels in serum by HPLC has been shown to have very good correlation with bioassay results but is more rapid and has the added advantage of increased sensitivity (17).

Statistics. The significance of differences between drugs was assessed by analysis of variance to control for the effects of the doses of drug administered. Chi-square analysis with Yates' correction was used to compare the results of a single dose of different forms of ciprofloxacin.

RESULTS

Kinetics. Levels of ciprofloxacin in serum were determined with sera obtained after s.c. injection of aqueous ciprofloxacin (20 mg/kg). The results are shown in Fig. 1, with the best-fit curve being the exponential function $y = 6821\, e^{-0.0120t}$ ($R = 0.95$), where t is time. This is of the form $y = C_0 e^{-kt}$ (where C_0 is the concentration at time zero, k is a constant, and t is time), which approximates first-order kinetics. By these equations, the half-life of aqueous ciprofloxacin after subcutaneous injection was found to be 58 min in a mouse that weighs approximately 20 g. Recently published data have shown a half-life in serum of 1.6 h for ciprofloxacin after s.c. administration to uninfected C57BL/6 mice (2). (The dose tested previously [2] was 100 mg/kg, and antibiotic concentrations were determined by bioassay.).

Sera were obtained from two mice per interval after i.v. injection of LIC. The results are shown in Fig. 2, with the best-fit curve being the function $y = (2.953 \times 10^5)\, t^{-1.1884}$ ($R = 0.98$). As expected, the initial peak concentration was very high, and drug was rapidly cleared to levels comparable to those found after the s.c. administration of aqueous ciprofloxacin. The area under the curve (AUC) was calculated by using the best-fit curves and revealed similar values

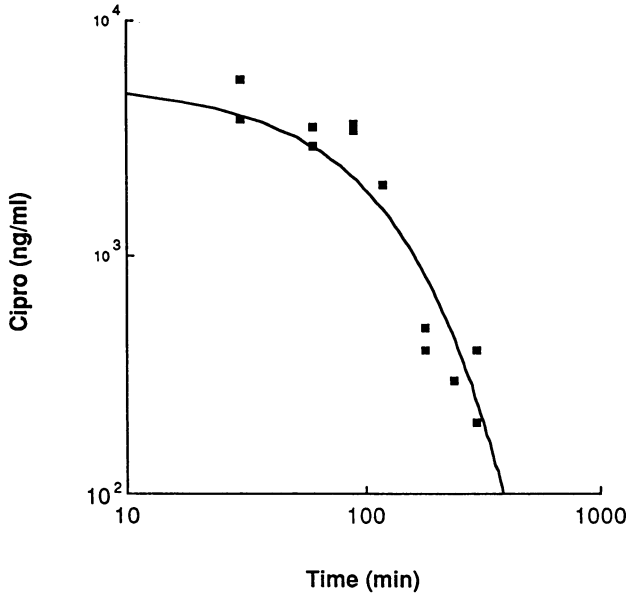

FIG. 1. Levels of ciprofloxacin (Cipro) in serum after s.c. injection of aqueous ciprofloxacin (20 mg/kg).

for both modes of antibiotic administration. The AUC from 10 min to 10 h for LIC administered i.v. was approximately 5.5×10^5 ng · min/ml, and for aqueous ciprofloxacin administered s.c. the AUC was 5.0×10^5 ng · min/ml; the AUC from 30 min (the first time point after s.c. injection of aqueous ciprofloxacin) to 10 h for LIC administered i.v. was 3.6×10^5 ng · min/ml, and for aqueous ciprofloxacin administered s.c., the AUC was 3.9×10^5 ng · min/ml.

Levels of ciprofloxacin in the spleen and liver were obtained from two mice at various time points up to 1 week after i.v. injection of LIC (ciprofloxacin at 20 mg/kg). The results are shown in Table 1. There was no measurable

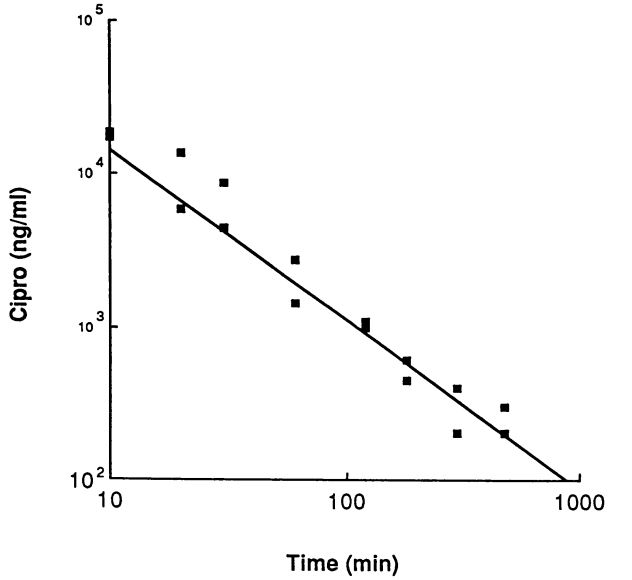

FIG. 2. Levels of ciprofloxacin (Cipro) in serum after i.v. injection of LIC (20 mg/kg).

TABLE 1. Ciprofloxacin levels in liver and spleen of mice treated with LIC

Time (h)	Ciprofloxacin concn in[a]	
	Spleen (ng/spleen)	Liver (ng/g)
8	5,800	22,100
24	2,650	16,650
48	2,300	4,600
160	NMA[b]	NMA

[a] Values are means for three mice.
[b] NMA, no measurable amount.

ciprofloxacin in the liver or spleen at 8 or 24 h after s.c. injection of aqueous drug. As expected, LIC accumulated in the liver and spleen, and high, measurable levels persisted for 2 days. However, by 1-week, levels were no longer detectable.

Treatment experiments. Control mice received either no treatment or empty liposomes at the stock concentration, a 1/4 dilution, or a 1/70 dilution. All control mice (9 mice given no treatment, 10 mice given empty liposomes) died by day 12. There was no apparent difference in the duration of survival between the untreated mice and those treated with empty liposomes.

We compared the mortality rates of infected mice treated with single doses of aqueous ciprofloxacin or LIC; one group of mice received a single s.c. injection of ciprofloxacin, and another group received the same amount i.v. as LIC. The third group received aqueous ciprofloxacin, but in 10 equal doses that were administered twice daily for 5 days, beginning on the same day that LIC was given. The results are shown in Table 2. A single injection of LIC was significantly more effective at preventing death caused by *S. dublin* than was a single injection of the same amount of aqueous ciprofloxacin (50% effective dose, 0.5 versus 5.0 mg/kg; $F = 9.4$; $P = 0.005$). However, when we administered 2.0 mg of aqueous ciprofloxacin per kg in divided doses over a 5-day period, it was as effective as a single injection of LIC ($P = 0.1$), and at a dose of 8.0 mg/kg, all mice in both treatment groups survived.

Another index of the effectiveness of treatment is the reduction in viable bacterial counts in the spleen or liver. However, because we were concerned that LIC might distribute differently than aqueous ciprofloxacin, we also determined the number of viable *S. dublin* organisms in mesenteric lymph nodes, Peyer's patches, and feces, sites of infection that might receive less LIC than the major organs

TABLE 2. Survival of mice treated with LIC or aqueous ciprofloxacin

Dose (mg/kg)	No. of survivors/no. of treated mice[a]		
	LIC	Aqueous	
		Single dose	Daily dose[b]
0.2	0/4	0/4	ND[c]
0.5	3/5	0/4	ND
2.0	5/6	ND	4/12
8.0	6/6	3/5	6/6
10.0	ND	ND	6/6

[a] Determined 18 days after infection.
[b] The total dose was divided and was given every 12 h for 5 days.
[c] ND, not done.

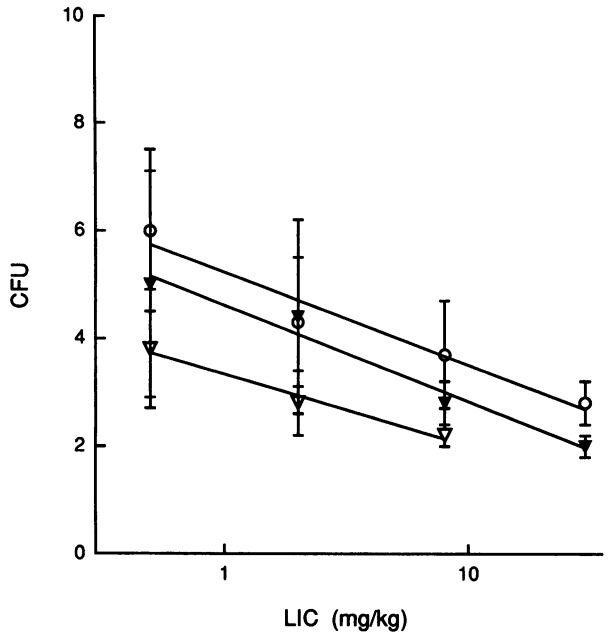

FIG. 3. Viable counts of *S. dublin* in various organs 18 days after infection. Mice were treated with LIC on day 4. Each point is the mean for six mice except for the 0.5-mg/kg group, which was three mice. ○, spleen; ▼, stool; ▽, Peyer's patches. Cultures of Peyer's patches were not done for the mice treated with 30 mg of LIC per kg.

of the reticuloendothelial system. We sacrificed the mice 15 to 18 days after infection (11 to 14 days after treatment) and determined viable bacterial counts in the selected organs. As shown in Fig. 3, there was a dose-related decrease in the number of viable bacteria in spleen, stool, and Peyer's patches. Similar results were found with cultures of mesenteric lymph nodes and livers (data not shown). For comparison, we treated another group of mice for 5 days with 10 mg of aqueous ciprofloxacin per kg given in divided doses. The average number of viable bacteria at each site (spleen, 3.9 $\log_{10}$; Peyer's patch, 2.3 $\log_{10}$) was nearly identical to the numbers of bacteria recovered from mice treated with a single 8-mg/kg dose of LIC (Fig. 3). These results suggest that LIC and aqueous ciprofloxacin distribute in therapeutic concentrations to the organs primarily involved in salmonella infections.

DISCUSSION

In the study described here, we showed that LIC is superior to aqueous ciprofloxacin when each is administered as a single injection. This result cannot be explained simply by the difference in the mode of administration (i.v. versus s.c.). Although the initial level of ciprofloxacin in the blood of LIC-treated mice was higher, both groups of mice had comparable levels in blood after 30 min (the initial time point blood was assayed for the s.c. aqueous ciprofloxacin group), and both treatments resulted in similar AUCs. The improved survival may have been due to the persistence of ciprofloxacin in the liver and spleen for at least 2 days after a single injection of 20 mg of LIC per kg (Table 1). This had the effect of prolonging therapy well beyond the time when the drug had disappeared from the circulation. In contrast, at 8 h after administration of the same dose of aqueous ciprofloxacin, there was no measurable drug in the spleen. Although the aqueous form of the drug reportedly distributes well to body tissues, including the liver and spleen, it does not preferentially concentrate in these organs and it is in equilibrium with extracellular drug (10, 20). To achieve a comparable therapeutic effect with aqueous ciprofloxacin, drug had to be administered twice daily.

Majumdar et al. (22) recently showed that liposome-encapsulated ciprofloxacin has 10 times the antimicrobial activity of free drug against *Mycobacterium intracellulare* grown in human monocytes. Although some of the difference can be attributed to inactivation of free ciprofloxacin in the tissue culture medium, their results support our contention that liposomal ciprofloxacin is taken up by macrophages, in which it is active against ingested bacteria.

We were concerned that by using survival as a measure of efficacy we might be overestimating the therapeutic efficacy of LIC. Untreated mice infected with virulent *Salmonella* species die when bacterial counts reach 10^7 in the spleen, at which time they have overwhelming bacteremia (7, 11). Since LIC accumulates preferentially in the liver and spleen, we considered it possible that LIC-treated animals might survive, but the treatment might not eradicate the infection in organs that are not thought of as part of the reticuloendothelial system, such as Peyer's patches. This was not the case. We found a dose-related decrease in bacterial counts in all organs in LIC-treated mice, and the result was nearly identical to the one that we achieved with comparable doses of aqueous ciprofloxacin given twice daily for 5 days (Fig. 3). By this measure, a single injection of 8 mg of LIC per kg was equivalent to 10 mg of aqueous ciprofloxacin per kg given in divided doses over 5 days. A single dose of LIC was also equivalent to 5 days of aqueous ciprofloxacin therapy in terms of preventing mortality (Table 2).

In a previously published study (5), C57BL/6 mice were infected s.c. with *S. typhimurium* and were then treated for 5 days with aqueous ciprofloxacin administered s.c. The median survival time was increased from 11 days (untreated controls) to 18 days after infection, but all mice died after treatment was stopped. When mice were treated for 12 days with 100 mg of ciprofloxacin per kg twice daily, the investigators were able to sterilize the mouse livers (5). In our experiments, treated BALB/c mice did not have relapses after 5 days of treatment. The in vitro MIC for our *S. dublin* strain was comparable to the MIC for the *S. typhimurium* strain used in the earlier study (5). However, in mice *S. typhimurium* is more virulent than *S. dublin*, which may account for the increased efficacy of ciprofloxacin in our study (27).

We have previously compared liposome-incorporated gentamicin with aqueous gentamicin in the same animal model of infection (12). In that comparison, the liposomal drug was very effective and the aqueous drug was essentially inactive. This is in contrast to the modest but definitely beneficial effect of a single dose of aqueous ciprofloxacin demonstrated in the present study. The superiority of ciprofloxacin in the present model of invasive *Salmonella* infection is consistent with the reports of ciprofloxacin's efficacy in human *Salmonella* infections and is most likely due to the intracellular penetration of ciprofloxacin (10).

ACKNOWLEDGMENTS

This work was supported in part by the MRS, Department of Veterans Affairs; NIH grant DK35108; and a grant from ImClone Systems, Inc., New York, N.Y.

REFERENCES

1. **Alving, C. R.** 1983. Delivery of liposome-encapsulated drugs to macrophages. Pharmacol. Ther. **22**:407–424.
2. **Azoulay-Dupuis, E., J. P. Bedos, E. Vallee, D. J. Hardy, R. N. Swanson, and J. J. Pocidalo.** 1991. Antipneumococcal activity of ciprofloxacin, ofloxacin, and temafloxacin in an experimental mouse pneumonia model at various stages of the disease. J. Infect. Dis. **163**:319–324.
3. **Bakker-Woudenberg, I., A. F. Lokerse, F. H. Roerdink, and M. F. Michel.** 1985. Free versus liposome-entrapped ampicillin in treatment of infection due to *Listeria monocytogenes* in normal and athymic (nude) mice. J. Infect. Dis. **151**:917–924.
4. **Böttcher, C. J. F., C. M. Van Gent, and C. Fiers.** 1961. A rapid and sensitive sub-micro phosphorus determination. Anal. Chim. Acta **24**:203–204.
5. **Brunner, H., and H. J. Zeiler.** 1988. Oral ciprofloxacin treatment for *Salmonella typhimurium* infection of normal and immunocompromised mice. Antimicrob. Agents Chemother. **32**:57–62.
6. **Chikami, G. K., J. Fierer, and D. G. Guiney.** 1985. Plasmid-mediated virulence in *Salmonella dublin* demonstrated by use of a Tn*5-oriT* construct. Infect. Immun. **50**:420–424.
7. **Collins, F. M.** 1974. Vaccines and cell-mediated immunity. Bacteriol. Rev. **38**:371–402.
8. **Desiderio, J. V., and S. G. Campbell.** 1983. Liposome-encapsulated cephalothin in the treatment of experimental murine salmonellosis. RES J. Reticuloendothel. Soc. **34**:279–287.
9. **Düzgüneş, N., V. K. Perumal, L. K. Kesavalu, J. A. Goldstein, R. J. Debs, and P. R. J. Gangadharam.** 1988. Enhanced effect of liposome-entrapped amikacin on *Mycobacterium avium-M. intracellulare* complex infection in beige mice. Antimicrob. Agents Chemother. **32**:1404–1411.
10. **Easmon, C. S. F., and J. P. Crane.** 1985. Uptake of ciprofloxacin by macrophages. J. Clin. Pathol. **38**:442–444.
11. **Easmon, C. S. F., J. P. Crane, and A. Blowers.** 1986. Effect of ciprofloxacin on intracellular organisms: *in vitro* and *in vivo* studies. J. Antimicrob. Chemother. **18**(Suppl. D):43–48.
12. **Fierer, J., L. Hatlen, J. Lin, D. Estrella, P. Mihalko, and A. Yau-Young.** 1990. Successful treatment using gentamicin liposomes of *Salmonella dublin* infections in mice. Antimicrob. Agents Chemother. **34**:343–348.
13. **Goodman, L. J., R. M. Fliegelman, G. M. Trenholm, and R. L. Kaplan.** 1984. Comparative in vitro activity of ciprofloxacin against *Campylobacter* spp. and other bacterial enteric pathogens. Antimicrob. Agents Chemother. **25**:504–506.
14. **Graybill, J. R., P. C. Craven, R. L. Taylor, D. M. Williams, and W. E. Magee.** 1982. Treatment of murine cryptococcosis with liposome-associated amphotericin B. J. Infect. Dis. **145**:748–752.
15. **Groisman, E. A., and M. H. Saier.** 1990. *Salmonella* virulence: new clues to intramacrophage survival. Trends Biochem. Sci. **15**:30–33.
16. **Heffernan, E. J., J. Fierer, G. Chikami, and D. Guiney.** 1987. Natural history of oral *Salmonella dublin* infection in BALB/c mice: effect of an 80-kilobase-pair plasmid on virulence. J. Infect. Dis. **155**:1254–1259.
17. **Joos, B., B. Ledergerber, M. Flepp, J. D. Bettex, R. Luthy, and W. Siegenthaler.** 1985. Comparison of high-pressure liquid chromatography and bioassay for determination of ciprofloxacin in serum and urine. Antimicrob. Agents Chemother. **27**:353–356.
18. **Kirby, C. J., and G. Gregoriadis.** 1984. A simple procedure for preparing liposomes capable of high encapsulation efficiency under mild conditions, p. 19–27. *In* G. Gregoriadis (ed.), Liposome technology, Vol. 1. Preparation of liposomes, CRC Press, Inc., Boca Raton, Fla.
19. **Lahdevirta, J.** 1989. Ciprofloxacin in the elimination of enteric *Salmonella* carriage stage. Scand. J. Infect. Dis. Suppl. **60**:112–115.
20. **Limon, L.** 1989. Ciprofloxacin: a fluoroquinolone antimicrobial. Part I. Am. Pharm. New Ser. **29**:42–45.
21. **Lopez-Berenstein, G.** 1987. Liposomes as carriers of antimicrobial agents. Antimicrob. Agents Chemother. **31**:675–678.
22. **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.
23. **Ostro, M. J., and P. R. Cullis.** 1989. Use of liposomes as injectable-drug delivery systems. Am. J. Hosp. Pharm. **46**:1576–1587.
24. **Plant, J., and A. A. Glynn.** 1976. Genetics of resistance to infection with *Salmonella typhimurium* in mice. J. Infect. Dis. **133**:72–78.
25. **Rodriguez-Noriego, E., J. Andrade-Villanueva, and G. Amaya-Tapia.** 1989. Quinolones in the treatment of *Salmonella* carriers. Rev. Infect. Dis. Suppl. **11**:1179–1186.
26. **Tadakuma, T., N. Ikewaki, T. Yasuda, M. Tsutsumi, S. Saito, and K. Saito.** 1985. Treatment of experimental salmonellosis in mice with streptomycin entrapped in liposomes. Antimicrob. Agents Chemother. **28**:28–32.
27. **Valtonen, V. V.** 1970. Mouse virulence of *Salmonella* strains: the effect of different smooth-type O side-chains. J. Gen. Microbiol. **64**:255–268.
28. **Weber, A., D. Chaffin, A. Smith, and K. Opheim.** 1985. Quantitation of ciprofloxacin in body fluids by high-pressure liquid chromatography. Antimicrob. Agents Chemother. **27**:531–534.