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TITLE

Efficacy of Liposomal Antibotic Therapy in a Rat Infusion Model of Escherichia Coli Peritonitis

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Efficacy of liposomal antibiotic therapy in a rat infusion model of *Escherichia coli* peritonitis

Lucie Martineau, PhD; Pang N. Shek, PhD

Objective: **To compare the potential therapeutic effect of liposomal vs. free cefoxitin.**

Design: **Randomized, controlled study, using a rat model of peritonitis.**

Setting: **Government research facility.**

Subjects: **Male Sprague-Dawley rats.**

Interventions: **Rats were infused intraperitoneally with 6.5 × 10^8 colony forming units of *Escherichia coli* over 12 hrs. Animals were then randomized to receive intravenous saline, free cefoxitin, liposomal cefoxitin, or plain liposomes twice daily until they were killed.**

Measurements and Main Results: **Free cefoxitin significantly reduced the number of *E. coli* after 24 hrs compared with saline treatment in both liver and spleen. However, liposomal cefoxitin further decreased the bacterial content by five-fold to ten-fold in these organs. Minimal bactericidal effect was observed in animals injected with plain liposomes. Although administration of liposomal cefoxitin for 7 days further reduced bacterial counts in liver and spleen, there was no apparent beneficial bactericidal effect of free cefoxitin over saline at 7 days. There was approximately a ten-fold reduction in bacterial content in the lungs after 24 hrs in all three treatments, but no further reduction was observed after 7 days. There was no difference in 7-day survival rate in animals treated with plain liposomes or saline (45% vs. 39%). Although survival tended to increase with free cefoxitin treatment (64%), this outcome was significantly improved with the use of liposomal cefoxitin (82%).**

Conclusions: **Liposomal cefoxitin enhanced bacterial killing in liver and spleen in this model of *E. coli* peritonitis. It also improved survival outcome relative to no treatment but not compared with free cefoxitin. (Crit Care Med 1999; 27:1153–1158)**

Key Words: sepsis; animal model; peritonitis; bacterial infection; liposomes; antibiotics; cefoxitin; *Escherichia coli*

Improved therapy of Gram-negative intra-abdominal sepsis remains a clinical challenge despite aggressive treatment with antibiotics. Although most antimicrobial agents used in critical care units to treat infections can rapidly generate high drug levels in the systemic circulation, they have a relatively short half-life. This characteristic, together with the finding that most antibiotics poorly penetrate reticuloendothelial cells such as those in the liver and spleen (1), may limit their bactericidal effects in the reticuloendothelial system. Several animal studies have shown that liposomal entrapment of antibiotics improves survival outcome in infections caused by various pathogens (2, 3), likely by enhancing intracellular drug targeting to reticuloendothelial cells, where the offending pathogens reside and persist (4–7).

Most animal studies have assessed the therapeutic efficacy of various free or liposomal antimicrobial agents either prophylactically or soon after induction of peritonitis (i.e., <4 hrs), because further delay of antibiotic treatment has been shown to correlate with increased mortality (8) or enhanced bacterial resistance to the antibiotic being tested (9). However, in a large number of clinical cases, patients with slow-onset peritonitis may not be treated for at least 8 to 12 hrs, partly because of the absence of clinical signs of ongoing abdominal infection. Thus, by the time of diagnosis, the infection may be firmly established within the cells of the reticuloendothelial system in these patients, and therapeutic interventions may not be as effective as expected from promising experimental results. Therefore, delay in the treatment of patients with generalized peritonitis (i.e., resuscitation, mechanical control of the source of contamination, and appropriate antimicrobial therapy) has been shown almost invariably to lead to multiple organ failure and death (10, 11).

Our laboratory recently developed a rat model of intra-abdominal infection that simulates the clinical condition of a trauma-induced, slow, sustained bacterial release from the gut (12). We have shown that this model reproduces many of the immunologic, biochemical, and pathologic characteristics observed in human sepsis (12, 13). The objective of the present study was to assess the effect of delayed systemic antibiotic treatment in our rat model of intra-abdominal infection. More specifically, we determined the effect of intravenous free and liposome-encapsulated cefoxitin treatment on both survival outcome and bacterial sequestration in different organs and body fluids. The second-generation cephalosporin, cefoxitin, was selected for the present study because it remains one of the antimicrobial agents recommended clinically to treat intra-abdominal infections (14).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (n = 173) with a mean body weight of 350 ± 5 g (mean ± SEM) were obtained from Charles River (St. Constant, PQ, Canada). The animals were housed individually, allowed to adapt to

From the Operational Medicine Sector, Defence and Civil Institute of Environmental Medicine, West Toronto, ON, Canada

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Address for reprints to Dr L Martineau, DCIEM/OM, 1133 Sheppard Avenue, West Toronto, ON, M3M 3B9, Canada E-mail lucie@dciem.dnd.ca

the environmental conditions (22°C [71 6°F], 12-hr light/dark cycle), and handled daily before undergoing surgery 7 days later. All animals had free access to standard rodent chow and water at all times during the experimental period All procedures described in this study were performed in adherence with the Canadian Council on Animal Care regulations for the use of experimental animals (15) and were reviewed and approved by the institutional animal ethics committee.

Intra-Abdominal Infection Protocol. The experimental procedures for the induction of intra-abdominal infection in our model have been described in detail elsewhere (12). Briefly, all surgical procedures were performed under aseptic conditions, with the animals under general anesthesia (1.5% halothane, 1:1 oxygen/nitrous oxide). A sterile, self-made intraperitoneal cannula (Silastic tubing; 0.76 mm inner diameter, 1.65 mm outer diameter; Dow Corning Medical, Mississauga, ON, Canada) was inserted into the abdominal cavity. The distal end of the cannula was tunneled subcutaneously along the spine to emerge through a 1-cm skin incision at the nape of the neck, and then was led through a stainless steel tether. Another self-made venous cannula (16) was then introduced into the right jugular vein using standard surgical procedures. All wounds were closed in two layers with nonabsorbable 3–0 silk sutures (Johnson & Johnson, Mississauga, ON, Canada), and topical antibiotic (Hibitane, Ayerst Laboratories, Montréal, PQ, Canada) was applied. The cannulated animals received a subcutaneous injection of sterile saline (30 mL/kg body weight) and were given analgesics (intramuscular buprenorphine, 0.05 μg/g body weight).

Each tether was connected to a temperature-controlled (3°C–5°C [37.4°F–41°F]) fecal inoculum infusion system via a swivel to which the intraperitoneal cannula was attached. The infusion of 2 mL of either a bacterial inoculum or a control inoculum was initiated 8 to 10 hrs later. The bacterial inoculum consisted of a volume of *Escherichia coli* (ATCC 25922; PML Microbiologics, Mississauga, ON, Canada) culture containing approximately 6.5×10^8 colony forming units (CFU), 1.5 mL of sterile saline, and 0.5 mL of a sterile rat fecal suspension. The same volume of sterile saline was used to substitute for the *E. coli* culture in the control inoculum.

Experimental Drug Regimen At the end of the 12-hr infusion period, the infected animals were randomly assigned to a treatment group receiving twice daily an intravenous injection of saline, free cefoxitin (30 μg/g body weight/dose), liposomal cefoxitin (30 μg/g body weight/dose), or plain liposomes, noninfected control animals received the same volume (500 μL) of saline twice daily. This dose of free cefoxitin was selected based on preliminary work demonstrating that it was the lowest dose allowing maximal bactericidal effects to be achieved in the liver and spleen at 24 hrs under our experimental conditions (our unpublished data).

Each cannulated rat (i.e., control or infected) was then anesthetized, a small (500-μL) blood sample was obtained, and an intravenous dose (in 500 μL) of the appropriate drug was administered. A midline laparotomy was performed; 5 mL of warm, sterile saline was injected into the abdominal cavity and thoroughly mixed with the peritoneal fluid, which was then sampled The laparotomy and lavage procedures were performed to mechanically reduce the bacterial burden, as recommended in clinical practice (14) The animals received a subcutaneous injection of sterile saline (30 mL/kg body weight), and topical antibiotic was applied to all wounds. Blood and peritoneal fluid were serially diluted in sterile phosphate-buffered saline, plated onto tryptic soy agar enriched with 5% sheep blood, and incubated overnight at 37°C (98.6°F) to determine bacterial content. Analgesia and intravenous drugs were administered twice daily, until the animals were killed. The animals received the last dose of antibiotics (i.e., 30 μg/g body weight) approximately 12 hrs before they were killed and tissue cultures were taken.

Study I. Bactericidal Effects of Drug Regimen. Intra-abdominal infection was induced in 85 rats, and eight noninfected animals received the sterile inoculum (control). Eight nonsurgical, healthy animals were also included in the study to provide baseline values for the different variables measured (i.e., whole body weights, bacterial counts of body fluids and organs). The animals that had not died of the sequelae of infection 24 hrs after initiation of intravenous treatment were killed We have shown that maximal bacterial sequestration occurred at this time in untreated infected rats (12). The surviving infected animals (n = 41) and all noninfected control and healthy animals were anesthetized, and samples of blood and peritoneal fluid were collected and plated as described previously. Lungs, liver, and spleen were dissected rapidly and surface-sterilized in 70% ethyl alcohol. Sterility was verified by taking a swab of the surface and streaking it onto tryptic soy agar enriched with 5% sheep blood. The tissues were then weighed, homogenized in phosphate-buffered saline, serially diluted, and plated onto tryptic soy agar enriched with 5% sheep blood to assess the extent of bacterial sequestration. The minimal threshold of detection for bacteria was 50 CFU per organ For purposes of statistical analysis, sterile tissues were assigned a value of zero.

Study II· Assessment of Drug Regimen on Survival Outcome. Intra-abdominal infection was induced in another group of 55 rats to assess the effects of the different drug regimens on survival outcome over 7 days. Nine noninfected control and eight nonsurgical, healthy animals were also included in the study to provide baseline values for the different variables measured (i.e., whole body weights, bacterial counts of body fluids and organs) Blood samples were taken at the time of surgery, at the end of the 12-hr infusion period (i.e., at the time of laparotomy and peritoneal lavage), and on day 7 and assayed as described previously Peritoneal fluid and organs of all noninfected control and healthy animals, and those of the infected rats that survived the 7-day study (n = 30), were collected at 7 days and processed as described previously to determine the bactericidal effect of the different drug regimens (see below).

Preparation of Liposomes Liposomes were prepared in our laboratory as described previously (17) Briefly, palmitoyloleylphosphatidylcholine and cholesterol (Avanti Polar Lipids, Alabaster, AL) were dissolved in chloroform at a molar ratio of 55·45 and dried by rotary evaporation into a thin film under a stream of helium in a controlled vacuum The lipid film was rehydrated at room temperature with sterile saline containing cefoxitin (Merck Sharp & Frosst Canada, Kirkland, PQ, Canada) at an initial drug-to-lipid ratio of 710 mg cefoxitin/mmol total lipid in a final volume of 5 to 7 mL Cefoxitin-free plain liposomes, also containing 20 mg lipid/mL, were prepared similarly. The multilamellar liposomes were then subjected to repeated extrusion through polycarbonate filters of 100 nm (Nuclepore Corp., Pleasanton, CA) in a Lipex Biomembranes (Vancouver, BC, Canada) extrusion apparatus. The final working concentration of the liposomal cefoxitin preparation was then adjusted with sterile saline, as required by the experimental protocol. The mean vesicle diameter was 152 ± 7 nm. Liposomal cefoxitin contained a mixture of liposome-entrapped and nonentrapped drug, the extent of drug entrapment being approximately 50%. Liposome suspensions were kept at 4°C (39.2°F) and used within 24 hrs of preparation.

Statistics. Within each group, data were analyzed using the Kruskal-Wallis test When the p value proved significant ($p < .05$), Mann-Whitney tests were used to compare the individual time points (i.e., 24 hrs or 7 days) with baseline values for each of the variables measured (i e., whole body weights, bacterial counts of body fluids and organs). Mann-Whitney tests were also used to compare intergroup differences for each of those variables. The chi-square test was used to determine whether there were differences in survival outcomes in the study. When the chi-square value proved significant ($p < 05$), all possible combinations of Fisher's exact tests were completed to determine which group(s) differed from which other group(s). All data are expressed as mean ± SEM.

RESULTS

Gross Effect of Surgical Procedures and Peritonitis. There were no apparent signs of stress or distress in any of the noninfected animals after the 12-hr infu-

sion period. In contrast, infected rats showed piloerection, diarrhea, and nasal and/or ocular discharge. As reported previously in our model (12), the intraperitoneal cannulae in the noninfected animals were relatively free from fibrinous adhesions at the end of the infusion period; in contrast, the intraperitoneal cannulae of infected animals were sometimes loosely wrapped in loops of omentum or epididymal fat infiltrated with inflammatory cells. However, this phenomenon was severe enough to completely occlude the tip of the intraperitoneal cannulae in 11 of the 140 animals that received the bacterial challenge (i.e., seven and four cannulae in study I and study II, respectively); these animals were killed at 12 hrs and their data were excluded from the analysis. Data from all other infected animals, regardless of whether they were bacteremic at the end of the infusion period, were included in the data analysis.

Survival Outcome. All control animals that received a sterile inoculum survived. The survival rates for the different groups of infected rats are shown in Figure 1. Eleven animals underwent peritonitis in each group, except for the group receiving saline, in which 18 rats were infected. There was no difference in 7-day survival rates between untreated infected rats (39%) and rats treated with plain liposomes (45%). Furthermore, survival was not significantly improved by administration of free cefoxitin (64%) compared with saline. In contrast, liposomal delivery of cefoxitin significantly ($p < .05$) improved this outcome (82%). However, there was no difference in the 7-day survival outcome between animals treated with free cefoxitin and animals treated with liposomal cefoxitin.

Whole Body Weights. Cumulative changes in body weight from baseline (i.e., before cannulation) for each experimental group are shown in Figure 2. Surgical procedures or infusion of a sterile inoculum had no or minimal effect on the body weight in control compared with healthy, nonsurgical animals during the study period. In contrast, there was a small but significant decrease ($p < .05$; $-7.4\% \pm 0.4\%$) in body weight in all infected animals after 24 hrs. Although body weight loss was further exacerbated in saline- and plain liposome-treated animals at 7 days ($p < .05$; $-10.9\% \pm 1.1\%$), there was no further change in this variable for the remainder of the study period with free cefoxitin or liposomal cefoxitin.

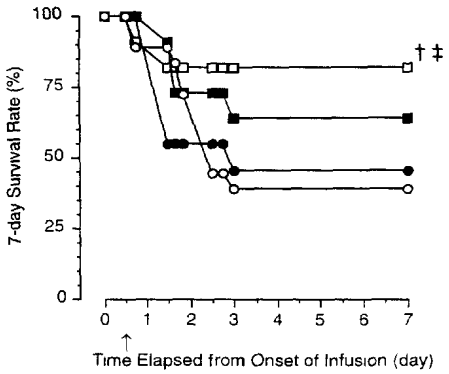


Figure 1. Survival outcome in infected rats administered saline (n = 18, ○), free cefoxitin (n = 11, ■), liposomal cefoxitin (n = 11, □), or plain liposomes (n = 11, ●) for 7 days. The *arrow* indicates the time at which the intravenous treatment was started. †Significantly different from saline treatment, ‡significantly different from plain liposome treatment.

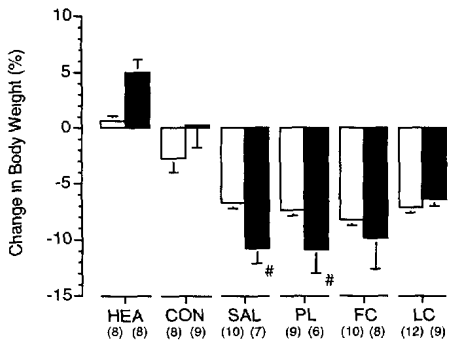


Figure 2. Cumulative changes in body weight in rats administered saline (*SAL*), free cefoxitin (*FC*), liposomal cefoxitin (*LC*), or plain liposomes (*PL*) for 24 hrs (*white bars*) or 7 days (*black bars*). Baseline values obtained from healthy, nonsurgical animals (*HEA*) and from control rats (*CON*) are also indicated. The number of experimental animals killed at a given time is indicated in parentheses. Values for any group of infected rats were significantly different ($p < 05$) from those of CON and HEA throughout the study. #Significantly different from the preceding time ($p < 05$).

Bacteriology. Cultures of body fluids and organ homogenates were negative in all control animals. Bacteremia was observed in 85% of infected animals at 12 hrs, the systemic bacterial counts averaging $4.2 \pm 2.4 \times 10^4$ CFU. Injection of saline or plain liposomes for 24 hrs had no effect on the systemic bacterial counts. However, bacteria remained detectable ($9.2 \pm 1.4 \times 10^2$ CFU) in the blood of only 13% of free cefoxitin- and liposomal cefoxitin-treated animals at 24 hrs. None of the liposomal cefoxitin- and free cefoxitin-treated rats was bacteremic at 7 days, whereas 14% of the saline- and plain liposome-treated animals had systemic bacterial counts averaging $5.4 \pm 2.4 \times 10^2$ CFU.

Bacterial counts in the peritoneal fluid collected at the end of the infusion period were comparable in all infected rats, averaging $8.8 \pm 2.8 \times 10^7$ CFU/mL (Fig. 3). These bacterial counts were reduced ($p < .05$) in all four experimental groups 24 hrs after the onset of treatment. However, this bactericidal effect was greater ($p < .05$) in the peritoneal fluid of cefoxitin-treated rats than in that of either saline- or plain liposome-treated animals; there was no difference in bactericidal effect in peritoneal fluid between free cefoxitin and liposomal cefoxitin treatment (approximately 590-fold) or between saline and plain liposome treatment (approximately four-fold) at 24 hrs (Fig. 3). Bacteria remained detectable in the peritoneal fluid of only 40% of infected animals at 7 days, regardless of the experimental group. The bacterial counts in the peritoneal fluid of rats that received free cefoxitin for 7 days were comparable to those of saline- and plain liposome-treated animals; however, liposomal delivery of cefoxitin further reduced ($p < .05$) these counts by approximately five-fold (Fig. 3).

Figure 4 shows the changes in tissue bacterial counts throughout the experiment, expressed as CFU per wet organ. Bacterial sequestration in the different organs was observed in all infected animals at 24 hrs. Free cefoxitin significantly reduced ($p < .05$) the number of *E. coli* after 24 hrs compared with saline in both liver (nine-fold) and spleen (14-fold). However, liposomal cefoxitin decreased ($p < .05$) the bacterial content by an additional five-fold to ten-fold compared with free cefoxitin in these organs at 24 hrs. Administration of liposomal cefoxitin for 7 days further reduced (approximately five-fold; $p < .05$) bacterial counts in liver and spleen; however, there was no apparent beneficial bactericidal effect of free cefoxitin over saline or plain liposomes in these organs during that period (Fig. 4, *top* and *middle*). It is noteworthy that bacterial clearance was observed ($p < .05$) in saline- and plain liposome-treated rats in liver and spleen at 7 days, but the magnitude of this bactericidal effect was comparable in these groups. Bacterial sequestration in liver and spleen at 7 days was observed in all saline- and plain liposome-treated rats, in

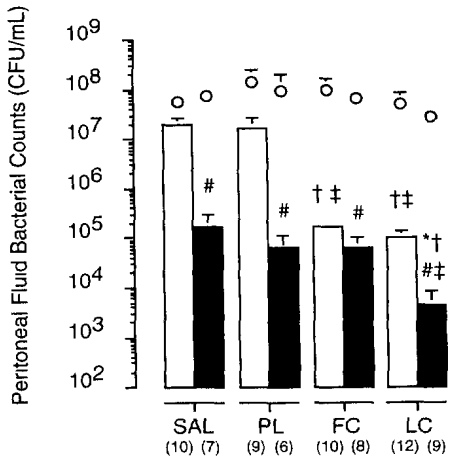


Figure 3. Bacterial counts in peritoneal fluid in infected rats administered saline (*SAL*), free cefoxitin (*FC*), liposomal cefoxitin (*LC*), or plain liposomes (*PL*) for 24 hrs (*white bars*) or 7 days (*black bars*) ○, bacterial counts in peritoneal fluid at the time of laparotomy. The number of experimental animals killed at a given time is indicated in parentheses. CFU, colony forming units #significantly different from the preceding time ($p < 05$); †significantly different from SAL treatment ($p < .05$), *significantly different from FC treatment ($p < 05$), ‡significantly different from PL treatment ($p < .05$)

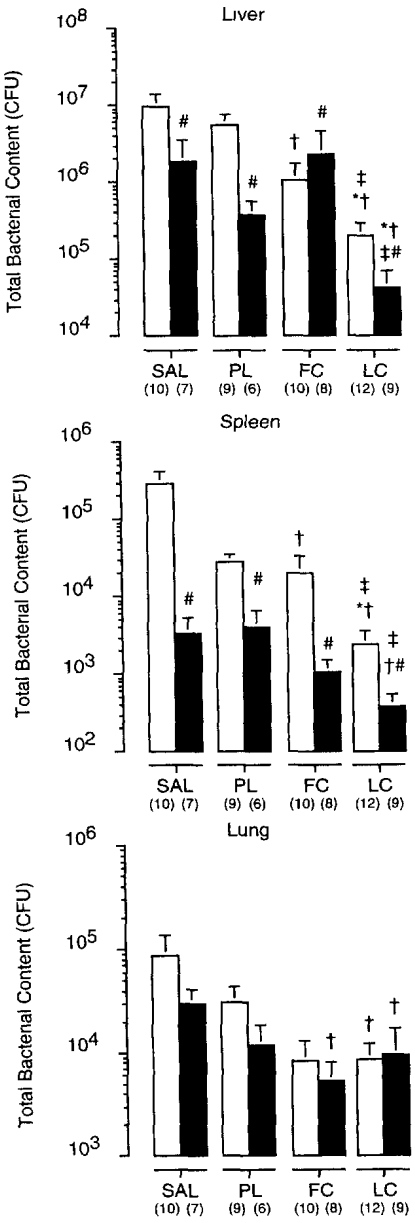


Figure 4. Bacterial counts in spleen, lung, and liver in infected rats administered saline (*SAL*), free cefoxitin (*FC*), liposomal cefoxitin (*LC*), or plain liposomes (*PL*) for 24 hrs (*white bars*) or 7 days (*black bars*). The number of experimental animals killed at a given time is indicated in parentheses CFU, colony forming units #Significantly different from the preceding time ($p < 05$); †significantly different from SAL treatment ($p < 05$), *significantly different from FC treatment ($p < 0\ 05$); ‡significantly different from PL treatment ($p < .05$).

66% of rats that received free cefoxitin, and in 44% of liposomal cefoxitin-treated rats. Recalculation of the results excluding the negative tissue cultures in the cefoxitin-treated animals did not alter the statistical conclusions. There was an approximately ten-fold reduction ($p < .05$) in bacterial content in the lungs of liposomal cefoxitin-treated rats after 24 hrs compared with saline-treated rats. A similar reduction in bacterial content in the lungs was also observed with free cefoxitin, but this trend did not achieve statistical significance ($p < .06$). This bactericidal effect was maintained after 7 days in all cefoxitin-treated animals (Fig. 4, *bottom*). There was no significant bactericidal effect in the lungs with plain liposomes or saline during the study period.

DISCUSSION

We have described the effects of systemic administration of free and liposomal cefoxitin on survival outcome and tissue bacterial content in a rat model of Gram-negative peritonitis involving the slow, continuous infusion of an *E. coli* bacterial inoculum over a 12-hr period. It is noteworthy that the data presented reflect the combined effects of surgical and antibiotic treatment in the absence of supportive fluid management. Nevertheless, our results indicate that delivery of antimicrobial agents with liposomes may be useful in treating intra-abdominal infection, even when treatment is delayed for 12 hrs after infection.

Liposomal administration of cefoxitin improved survival outcome compared with control infected animals. This survival benefit was associated with lower bacterial counts in most organs as well as in peritoneal fluid. Our findings are consistent with the suggestion that mortality in rats with abdominal sepsis is proportional to the peritoneal concentration of *E. coli* (17). The survival benefit in the present study was likely attributable to the drug encapsulation *per se*, and not to an adjuvant effect of the plain liposomes on the free cefoxitin. Indeed, it has been observed that the 7-day mortality rate of infected rats receiving a mixture of free cefoxitin and plain liposomes was similar to that of saline-treated animals (A. Kresta, unpublished data).

Interestingly, this enhanced survival in our study was achieved despite the use of a dose of cefoxitin lower than the recommended range in patients with moderately severe infections (i.e., 60 vs. 100 mg/kg) and with a longer dosing interval than in a clinical setting (i.e., 12 vs. 6–8 hrs). The distribution of free and liposome-encapsulated cefoxitin in various tissues was not assessed in the present study. However, we have shown that tissue and body fluid levels of free cefoxitin were almost negligible only 5 hrs after intraperitoneal injection of a single dose (45 mg/kg body weight) in rats with intra-abdominal infection (5). In contrast, only 84% of the total drug injected was eliminated after 10 hrs in liposomal cefoxitin-treated rats, this proportion being marginally increased at 24 hrs. It is difficult to compare the adequacy of the cefoxitin regimen between rodents and humans because of interspecies differences in pharmacokinetics. Nevertheless, our data suggest that subtherapeutic dosing of a liposomal antibiotic may not necessarily lead to clinical failure, as would be expected if low doses of the conventional drug were administered. Furthermore, low dosing of certain antibiotics may even be advantageous in reducing their potential nephrotoxicity and ototoxicity.

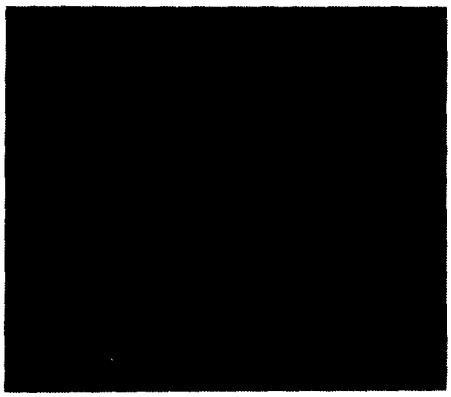
In the present study, fewer animals died in the liposomal cefoxitin group than in the free cefoxitin group, but this trend did not achieve statistical significance. Bohnen (18) has reported that administration of intraperitoneal liposomal cefoxitin for only 24 hrs (total dose of 45 mg/kg body weight) significantly improved the 7-day survival outcome in rats

with peritonitis compared with the same dose of free antibiotic. This discrepancy with our results may be partly attributable to differences in the route of drug administration and the severity of intra-abdominal infection between the experimental models. Intrinsic differences in the mortality rates between the two groups of cefoxitin-treated animals in the present study are unlikely, because we have shown that the lethality of our model was very consistent between groups of untreated animals infected on different days (12). Nevertheless, a larger-scale investigation is required to determine whether the trend that we observed will prevail.

Free cefoxitin treatment had no effect on survival outcome in our model of peritonitis. This finding is in agreement with those of several studies in which a comparable regimen of various cephalosporins was delayed for up to 14 hrs after fecal peritonitis in rats (19, 20). However, one may argue that the lack of efficacy of conventional cefoxitin in reducing the 7-day mortality rate (as well as in enhancing the innate bacterial elimination from the host) in the present study may be partly the result of our use of a subtherapeutic dose and dosing interval. Nevertheless, survival outcome after cecal ligation and puncture in rats was improved when three intramuscular doses of cefoxitin (80 mg/kg) were administered for 7 days (21). Interestingly, though, administration of massive doses (200 mg/kg, three times per day) of cefoxitin starting 2 hrs before infection had no effect on the 7-day survival rates when mice were infected with a bolus intraperitoneal dose of *E. coli* comparable to that used in our model of peritonitis (22); however, the efficacy of this antibiotic was greatly improved when the severity of the infection was reduced (22, 23). Clearly, the efficacy of conventional cefoxitin in improving survival outcome in rodent models of intra-abdominal infection is a complex issue. Although the bactericidal effect of conventional cefoxitin has been shown in patients with intra-abdominal infections (14), there are no clinical data to suggest that conventional cefoxitin *per se* has a significant effect on survival outcome.

Our results demonstrate that although intravenous administration of free cefoxitin for 7 days did not significantly inhibit the growth of *E. coli* in liver and spleen compared with administration of saline, liposomal cefoxitin had a marked bactericidal effect in these organs. Several studies have shown that after intravenous administration, liposomes were rapidly taken up and retained by hepatic and splenic phagocytic cells (6, 16). Intracellular killing of *E. coli* in a macrophage cell line by streptomycin or chloramphenicol was also enhanced by encapsulating the drugs in liposomes (24). We have also shown in another model of fecal peritonitis that liposome encapsulation reduced whole body cefoxitin elimination (5). Although the initial reduction in bacterial counts achieved 24 hrs after administration of free cefoxitin was maintained for 7 days in the liver, the decline continued throughout the experiment in the spleen. This difference in the response to free cefoxitin may be attributable to differential tissue accumulation of the drug after 7 days. A long-term pharmacokinetic study is needed to correlate the efficacy of the free or encapsulated drug with its levels in different tissues.

The comparable bactericidal efficacies of liposomal and free cefoxitin in the lungs of infected rats are in agreement with our previous finding that very little free or liposomal cefoxitin was recovered in the infected lungs of rats after a single intraperitoneal injection with either formulation of antibiotic (5). Vladimirsky and Ladigina (25) have also shown that streptomycin concentrations in the lungs of mice infected with *Mycobacterium* species were independent of the form of the antibiotic. Unlike reticuloendothelial organs such as the liver, in which the discontinuous capillaries allow extravasation of liposomes smaller than 200 nm (26, 27), the lung possesses predominantly continuous capillaries that preclude the transcapillary passage of even smaller liposomes (28). Therefore, in contrast to their counterparts in the liver and spleen, alveolar macrophages are not readily exposed to the bloodstream; thus, these macrophages are less likely to take up liposomes administered intravenously. However, the lack of superiority of liposome-encapsulated antibiotics over the free drugs in the lungs in other models of infections localized in the reticuloendothelial system has also been associated with markedly higher tissue levels of the liposomal drug (4, 29). Whether or not the liposomal antibiotic was localized in the same compartment as the pathogens was not determined in these studies. Nevertheless, our data suggest that complementary methods of targeting the pathogens residing in the lungs need to be assessed in peritonitis.



The significant and comparable reductions in bacterial counts in peritoneal fluid observed at 24 hrs in the cefoxitin-treated animals highlighted the beneficial effect of either formulation of antibiotic administered intravenously. Eyal et al. (30) reported that cephalosporins rapidly permeate the peritoneal cavity of rats with acute polymicrobial peritonitis, reaching concentrations that exceed the minimum inhibitory concentration of *E. coli* and *Bacteroides fragilis* within 1 hr. Furthermore, intravenous free cefoxitin reached the abdominal cavity of pigs with bacterial peritonitis more rapidly and yielded higher concentrations than the liposomal drug; however, the intraperitoneal levels of the two antibiotics were comparable after 3 hrs, thus explaining the similar recovery of *E. coli* from the peritoneal fluid in the two experimental groups (31). This is in contrast to our finding of an enhanced bactericidal efficacy in peritoneal fluid associated with liposomal delivery of cefoxitin for 7 days. Whether this discrepancy might be attributed to interspecies differences in the pharmacokinetics of the two formulations of cefoxitin remains to be determined. Nevertheless, our data suggest that systemic administration of free antibiotics provides a beneficial bactericidal effect in the early stages of infection, whereas liposomal intravenous delivery may enhance local bacterial clearance in chronic infections.

Several experimental studies have shown the beneficial effect of intraperitoneal liposomal delivery of antibiotics in the treatment of peritonitis (3, 5, 18). However, the technical difficulties associated with repeated intraperitoneal administration in patients with intra-abdominal infection raise questions regarding the applica-

bility of these experimental data to the clinical setting. Our finding that liposomal intravenous delivery of cefoxitin can enhance bacterial clearance in the peritoneal cavity, normally a nonreticuloendothelial site, suggests that intravenous liposome-encapsulated antibiotics might have a significant role in the treatment of bacterial peritonitis. This suggestion is consistent with the observation that intravenous liposomal ciprofloxacin was distributed to sites beyond the reticuloendothelial system (e.g., mesenteric lymph nodes, Peyer's patches) in a murine model of salmonellosis (6).

In summary, the present experimental data suggest the therapeutic potential of delayed liposomal antibiotic administration in the treatment of Gram-negative peritonitis. Future studies will investigate the efficacy of liposomal antibiotic encapsulation, in combination with the use of immunomodulators, to treat sepsis in this experimental model.

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