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# Ciprofloxacin in Polyethylene Glycol-Coated Liposomes: Efficacy in Rat Models of Acute or Chronic *Pseudomonas aeruginosa* Infection

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**In a previous study in experimental *Klebsiella pneumoniae* pneumonia, the therapeutic potential of ciprofloxacin was significantly improved by encapsulation in polyethylene glycol-coated ("pegylated") long-circulating (STEALTH) liposomes. Pegylated liposomal ciprofloxacin in high doses was nontoxic and resulted in relatively high and sustained ciprofloxacin concentrations in blood and tissues, and hence an increase in the area under the plasma concentration-time curve (AUC). These data correspond to data from animal and clinical studies showing that for fluoroquinolones the AUC/MIC ratio is associated with favorable outcome in serious infections. Clinical failures and the development of resistance are observed for marginally susceptible organisms like *Pseudomonas aeruginosa* and for which sufficient AUC/MIC ratios cannot be achieved. In the present study the therapeutic efficacy of pegylated liposomal ciprofloxacin was investigated in two rat models of *Pseudomonas aeruginosa* pneumonia. In the acute model pneumonia developed progressively, resulting in a rapid onset of septicemia and a high mortality rate. Ciprofloxacin twice daily for 7 days was not effective at doses at or below the maximum tolerated dose (MTD). However, pegylated liposomal ciprofloxacin either at high dosage or given at low dosage in combination with free ciprofloxacin on the first day of treatment was fully effective (100% survival). Obviously, prolonged concentrations of ciprofloxacin in blood prevented death of the animals due to early-stage septicemia in this acute infection. However, bacterial eradication from the left lung was not effected. In the chronic model, pneumonia was characterized by bacterial persistence in the lung without bacteremia, and no signs of morbidity or mortality were observed. Ciprofloxacin administered for 7 days at the MTD twice daily resulted in killing of more than 99% of bacteria in the lung; this result can also be achieved with pegylated liposomal ciprofloxacin given once daily. Complete bacterial eradication is never observed.**

Many in vitro studies as well as studies in animal models of infection and human infections have investigated the efficacy of fluoroquinolones. The area under the plasma concentration-time curve (AUC) and the peak plasma drug concentration, both in relation to the MIC of the pathogen, have been demonstrated to be of primary importance for successful outcome (33). For example, in several clinical trials in patients with nosocomially acquired pneumonia, a 24-h AUC/MIC ratio of at least 100 to 125 (14, 15, 19) or a peak plasma drug concentration/MIC ratio of 10 or more (26) was closely linked to clinical and microbiological cure in seriously ill patients treated with intravenous ciprofloxacin. Most treatment failures with ciprofloxacin were a consequence of high MIC, low AUC, or both. A peak plasma drug concentration/MIC ratio of 10 or 20 has been shown both in vitro and in vivo to prevent the emergence of resistant mutants during therapy with fluoroquinolones.

These observations in clinical studies are supported by data obtained in in vitro pharmacokinetic models in which bacteria were exposed to changing concentrations of fluoroquinolones mimicking human pharmacokinetics (11, 13, 17, 20). A number of studies in animal models of infection also revealed that the AUC/MIC ratio was the most important predictor of therapeutic efficacy for fluoroquinolones (10, 19). The difficulty lies in achieving AUC/MIC ratios of 125 to 250 in serious infections caused by pathogens such as *Staphylococcus* and *Pseudomonas* species that are only marginally susceptible to fluoroquinolones (MICs of 0.5 μg/ml and above). The drugs may need to be administered in relatively high doses, which might prove to be toxic. As a result, treatment failure frequently occurs.

In our previous study, improvement of the therapeutic potential of ciprofloxacin was achieved by encapsulation in polyethylene glycol (PEG)-coated ("pegylated"), long-circulating, sustained-release (STEALTH) liposomes (5). The liposomes had a PEG coating on the surface that provided a steric barrier against opsonization, thereby reducing the interaction with the mononuclear phagocyte system. Consequently, these "sterically stabilized" liposomes exhibit a prolonged circulation time (24). The pegylated liposomes protect the encapsulated ciprofloxacin, which facilitates use of high doses of the drug without toxic side effects. Administration of ciprofloxacin in the pegy-

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lated liposomal form resulted in delayed ciprofloxacin clearance and increased and prolonged ciprofloxacin concentrations in blood and tissues, thereby increasing the AUC (5). In our rat model of unilateral pneumonia caused by *Klebsiella pneumoniae* (MIC of ciprofloxacin, 0.1 μg/ml) the therapeutic efficacy of pegylated liposomal ciprofloxacin was superior to that of ciprofloxacin in the free form. When administered in the liposomal form, lower daily doses of ciprofloxacin were effective. Pegylated liposomal ciprofloxacin was well tolerated in relatively high doses, permitting once-daily administration without loss in therapeutic efficacy.

The present study was performed to investigate whether the superior therapeutic potential of pegylated liposomal ciprofloxacin as observed in the rat model of *K. pneumoniae* pneumonia (5) could also be obtained in difficult-to-treat infection caused by *Pseudomonas aeruginosa* with moderate susceptibility to ciprofloxacin. We developed two models of *P. aeruginosa* infection: an acute pneumonia-septicemia and a chronic *P. aeruginosa* pneumonia in rats. In the acute model, pneumonia developed progressively, resulting in a rapid onset of septicemia and death of almost all animals. In the second model, chronic pneumonia was characterized by persistence of *P. aeruginosa* in the left lung without bacteremia. The course of infection revealed no signs of morbidity or mortality.

## MATERIALS AND METHODS

**Animals.** Female RP/AEur/RijHsd strain albino rats (age, 18 to 25 weeks; body weight, 185 to 225 g; Harlan, Horst, The Netherlands) with a specified pathogen-free status were used. The experiments were approved by the ethical committee of the Erasmus University Medical Center Rotterdam.

**Bacteria.** A mucoid strain of *Pseudomonas aeruginosa*, originally isolated from a patient with cystic fibrosis, was used to infect the rats. The MIC and minimum bactericidal concentration of ciprofloxacin for this strain were 0.4 and 0.8 μg/ml, respectively, as determined by the tube dilution test in Mueller-Hinton broth supplemented with $Ca^{2+}$ (25 mg/liter) and $Mg^{2+}$ (12.5 mg/liter) (Difco Laboratories, Detroit, Mich.).

**Infection models of acute *P. aeruginosa* pneumonia-septicemia and chronic *P. aeruginosa* pneumonia.** A left-sided pneumonia was induced, as described in detail elsewhere (2), by intubation of the left primary bronchus followed by inoculation of the left lung with 20 μl of a saline suspension of *P. aeruginosa* bacteria in the logarithmic phase of growth. To establish the acute *P. aeruginosa* pneumonia-septicemia, $7 \times 10^8$ viable *P. aeruginosa* bacteria were inoculated into the left lung. To establish the chronic *P. aeruginosa* pneumonia, the inoculum consisted of $2 \times 10^8$ viable *P. aeruginosa* bacteria. Inocula were prepared as follows: *P. aeruginosa* was cultured in Todd-Hewitt broth (Difco Laboratories) at 37°C for 14 h. This bacterial suspension (end-log phase) had an optical density at 660 nm of 0.23 to 0.24. Bacteria were washed and concentrated appropriately by centrifugation for 10 min at 10,000 × *g* at 4°C.

Blood samples were obtained by retro-orbital bleeding under $CO_2$ anesthesia to assess the course of infection at various intervals after inoculation. Then, animals were sacrificed, the weight of the infected left lung was determined, and the left and right lungs were homogenized (VirTis homogenizer; Virtis, Gardiner, N.Y.) in 20 ml of phosphate-buffered saline for 30 s at 10,000 rpm. Tissue homogenates and blood were serially diluted and plated on tryptone soy agar (Unipath Ltd., Basingstoke, United Kingdom). After dilution, the remaining homogenates were subjected to the pour plate method, and all remaining blood volume (around 4 to 5 ml) was cultured. Plates were incubated overnight at 37°C.

**Liposomes.** Polyethylene-glycol-coated liposomes containing ciprofloxacin consisted of the PEG 2000 derivative of distearoylphosphatidylethanolamine, hydrogenated soybean phosphatidylcholine, and cholesterol in a molar ratio of 5:50:45. Pegylated liposomes were kindly supplied by ALZA Corporation (Mountain View, Calif.). Mean particle size was determined by dynamic light scattering (4700 system; Malvern Instruments, Malvern, United Kingdom). Liposomes containing ciprofloxacin, used in the model of acute *P. aeruginosa* pneumonia-septicemia, had a particle size of 126 ± 11 nm and contained 322 ± 52 μg of ciprofloxacin/μmol of total lipid (means ± standard deviations [SD] of five preparations). Liposomes containing ciprofloxacin used in the model of chronic *P. aeruginosa* pneumonia had a particle size of 107 ± 7.2 nm and contained 256 ± 51 μg of ciprofloxacin/μmol of total lipid (mean ± SD of 10 preparations).

**Antimicrobial treatment.** In the model of acute *P. aeruginosa* pneumonia-septicemia, antibiotic was administered as bolus intravenous injections over a 1-min period via the tail vein. Treatment was started 16 h after bacterial inoculation of the left lung and continued for 7 days or was given for only 1 day at the start of treatment. Ciprofloxacin in the free form (CIP) in 5% glucose, pH 7.0, or in the pegylated liposomal form (PL Cipro) in 10% sucrose–10 mM histidine, pH 6.5, was given. Various dosages ranging from 20 to 160 mg of CIP or PL Cipro/kg of body weight/day alone or in combination were administered ($n = 5$ to 15 per dosage). The injection frequency was 12 h. Therapeutic efficacy was assessed by rat survival at day 21 after bacterial inoculation.

In the model of chronic *P. aeruginosa* pneumonia, treatment with CIP or PL Cipro was started 4 days after inoculation of the left lung and continued for 3 or 7 days. Various dosages ranging from 40 to 160 mg/kg/day were administered ($n = 4$ to 9 per dosage). The injection frequency was 12 or 24 h. Therapeutic efficacy was assessed by quantification of bacterial numbers in the left lung, right lung, and blood.

To prevent carryover of ciprofloxacin or liposomal ciprofloxacin (if still present in the lung) to the subculture plates, charcoal (Carbomix; Norit N.V., Amersfoort, The Netherlands) was added to the homogenate suspensions (15 g/100 ml) before the homogenization procedure for immediate inactivation of ciprofloxacin.

In both models, the susceptibility of the *P. aeruginosa* to ciprofloxacin was evaluated in rats that died and in rats sacrificed early.

**Toxicity.** Acute toxicity was characterized in terms of seizures, irritability, and an apparent dazed state. Chronic long-term toxicity was assessed in terms of a significant change in renal or hepatic function. Renal function abnormalities were determined by measuring blood urea nitrogen and serum creatinine; hepatic function abnormalities were detected by measuring the serum aspartate aminotransferase and alanine aminotransferase by established tests (Merck Diagnostica, Darmstadt, Germany).

**Concentrations of ciprofloxacin in tissue after administration of CIP or PL Cipro.** CIP or PL Cipro at the maximal tolerated dose (MTD) (40 or 160 mg/kg, respectively, as a single dose) was injected into rats with chronic *P. aeruginosa* pneumonia 4 days after bacterial inoculation. Total ciprofloxacin concentrations in infected left lung and right lung tissues were measured at 1 h after injection. Rats were sacrificed, and then the infected left lung and right lung were removed and homogenized in 20 ml of phosphate-buffered saline (4°C). Tissue homogenates from rats that received PL Cipro were incubated in 0.1% Triton X-100 (Janssen Chimica, Geel, Belgium) for 30 min at 25°C to disrupt the liposomes in order to determine total (free plus encapsulated) drug concentrations. In the supernatants (obtained after centrifugation of the samples for 5 min at 12,000 × *g*) ciprofloxacin concentrations were determined by the agar diffusion test using a diagnostic sensitivity test agar (Oxoid, Basingstoke, United Kingdom) with *Escherichia coli* as the indicator organism and standards ranging from 0.1 to 1.6 μg of ciprofloxacin/ml in 5% glucose (5, 6). The strain is susceptible to 0.025 μg of ciprofloxacin per ml. Samples of 100 μl were assayed in large agar plates containing wells. The coefficient of variation of 15 determinations of solutions containing 0.1 to 1.6 μg of ciprofloxacin per ml was 1 to 3%.

**Antibacterial effect of ciprofloxacin against *P. aeruginosa* in vitro.** Ciprofloxacin concentrations of 0.5, 1.0, and 2.0 μg/ml were used with final inocula of $10^7$ CFU/ml of logarithmic-phase *P. aeruginosa* or $2.2 \times 10^9$ CFU/ml of stationary-phase *P. aeruginosa*. Cultures in Mueller-Hinton broth (Difco Laboratories) supplemented with $Ca^{2+}$ (25 mg/liter) and $Mg^{2+}$ (12.5 mg/liter) were incubated at 37°C on a shaker for 3 h. Bacterial survival was then determined over 6 h by plating 10-fold serial dilutions of the washed specimens on tryptone soy agar plates.

**Statistical evaluation.** In the model of acute *P. aeruginosa* pneumonia-septicemia, survival rates were compared using the log-rank test. In the model of chronic *P. aeruginosa* pneumonia, results from quantitative cultures were compared using one-way analysis of variance corrected for multiple comparisons using the Bonferroni method.

## RESULTS

**Rat model of acute *P. aeruginosa* pneumonia-septicemia.** An inoculum of $7 \times 10^8$ *P. aeruginosa* bacteria was used to establish the left lung infection. The resulting infection was characterized by a rapid increase in bacterial numbers in the left lung

TABLE 1. Course of infection in rats with acute *P. aeruginosa* pneumonia-septicemia[a]

| Time (h) after inoculation | Mean wt (g) of left lung ± SD | Mean log no. of bacteria ± SD in: | | Median log no. of bacteria/ml of blood (range) |
|---|---|---|---|---|
| | | Left lung | Right lung | |
| 16 | 1.3 ± 0.15 | 9.6 ± 0.24 | 5.9 ± 1.4 | 0 (0–2.6) |
| 24 | 1.9 ± 0.30 | 10.6 ± 0.2 | 7.4 ± 0.84 | 2.6 (0–3.5) |

[a] Left-sided pneumonia was produced by inoculation of the rats with $7 \times 10^8$ CFU of *P. aeruginosa*. At various intervals after inoculation, rats were sacrificed, the weight of the infected left lung was determined, and quantitative cultures of infected organs were performed. In untreated infection all rats died between 24 and 48 h after bacterial inoculation. Data are based on results for seven to nine rats.

TABLE 3. Course of infection in rats with chronic *P. aeruginosa* pneumonia

| Time (days) after inoculation | Mean wt (g) of left lung ± SD | Mean log no. of bacteria ± SD in: | | Median log no. of bacteria/ml of blood (range) |
|---|---|---|---|---|
| | | Left lung | Right lung | |
| 1 | 0.90 ± 0.14 | 8.2 ± 0.51 | 2.7 ± 0.86 | 0 (0–1.6) |
| 4 | 1.6 ± 0.25 | 7.8 ± 0.37 | 3.5 ± 0.39 | 0 |
| 7 | 1.1 ± 0.30 | 8.2 ± 0.72 | 2.2 ± 1.4 | 0 |
| 11 | 0.78 ± 0.09 | 7.1 ± 0.24 | 3.3 ± 1.38 | 0 |

[a] Left-sided pneumonia was produced by inoculation of the rats with $2 \times 10^8$ CFU of *P. aeruginosa*. At various intervals after inoculation, rats were sacrificed, the weight of the infected left lung was determined, and quantitative cultures of infected organs were performed. Data are based on results for nine rats.

up to sixfold within 16 h after bacterial inoculation (Table 1). A small variation between the individual animals was observed in quantitative cultures of *P. aeruginosa* from the infected left lung. The acute infectious process in the left lung was reflected by a substantial increase in the weight of the left lung up to fourfold, compared with the normal weight of the uninfected left lungs (0.3 to 0.4 g). The infection rapidly spreads to the right lung. Three of nine rats developed positive blood cultures.

At 24 h after inoculation, bacterial numbers in the left lung, the weight of the left lung, and bacterial numbers in the right lung had further increased. Four of seven rats developed septicemia. The course of infection revealed a high mortality rate: Most rats died between 24 and 48 h after bacterial inoculation. Three of 27 untreated rats (11%) survived.

**Therapeutic efficacy of PL Cipro versus that of CIP in rats with acute *P. aeruginosa* pneumonia-septicemia.** In untreated rats, acute pneumonia developed progressively, resulting in a rapid onset of septicemia and death of most of the animals, mortality being 89% (Table 2).

At 16 h after bacterial inoculation, the time that in untreated rats the bacterial numbers in the left lung had increased approximately sixfold and three of nine rats had developed septicemia, antimicrobial treatment with CIP and PL Cipro alone or in combination was started. Results of treatment are shown in Table 2. Treatment with CIP alone at dosages below the MTD was not effective: Survival of rats was not significantly increased compared with controls at a CIP dosage of 40 mg/kg/day twice daily. At a CIP dosage of 80 mg/kg/day twice daily, survival of rats was increased (although not significantly) to 38%. However, acute toxicity following administration of CIP was observed in 8 of 15 rats. When a single 40-mg/kg dose of PL Cipro was added on the first day of the 7-day treatment with CIP at 40 mg/kg/day twice daily, toxic side effects were not observed. This dosage regimen resulted in 80% survival, a significant ($P = 0.03$) increase over that in controls.

TABLE 2. Therapeutic efficacy of treatment at 12-h intervals of rats with acute *P. aeruginosa* pneumonia-septicemia[a]

| Treatment | % Survival (no.) |
|---|---|
| None | 11 (27) |
| CIP | |
| 40 (7) | 17 (12) |
| 80 (7) | 38[b] (15) |
| PL Cipro | |
| 40 (7) | 25 (12) |
| 80 (7) | 73 (11) |
| 160 (7) | 100 (7) |
| CIP + PL Cipro | |
| 40 (7) + 40 (1) | 80 (5) |
| 20 (1) + 40 (7) | 60 (5) |
| 40 (1) + 40 (7) | 100 (5) |

[a] Treatment is given as dosage (milligrams per kilograms per day), with duration (in days) shown in parentheses. Treatment was started at 16 h after inoculation of *P. aeruginosa* into the left lung and continued for 7 days or was administered only 1 day at the start of treatment. Various doses of CIP or PL Cipro alone or in combination were administered. The injection frequency was 12 h. Survival of rats was assessed for 21 days.

[b] Toxic side effects (acute toxicity) were observed in 8 of 15 rats.

Treatment with PL Cipro alone at 40 mg/kg/day twice daily in two divided doses did not significantly increase rat survival. Increasing the daily dosage of PL Cipro did not result in toxic side effects, and 100% survival was achieved ($P < 0.0001$ compared with controls) when PL Cipro was administered at 160 mg/kg/day twice daily. A significant increase in the survival was also effected by the addition of CIP only at the first day of treatment to a 7-day treatment of PL Cipro at 40 mg/kg/day twice daily: 100% survival was obtained when CIP at 40 mg/kg was added on the first day ($P < 0.0001$). Toxic side effects were not observed with this dosage schedule. In the surviving rats the bacterial numbers in the left lung were determined at day 21. *P. aeruginosa* organisms were always cultured, numbers ranging from $10^2$ to $10^7$ cells.

Previous experiments showed that the administration of placebo liposomes with a mean diameter of 105 ± 6.4 nm had no effect on the survival of rats.

The ciprofloxacin susceptibility of *P. aeruginosa* recovered from dead or sacrificed rats was never changed compared with that of the inoculated bacteria.

**Rat model of chronic *P. aeruginosa* pneumonia.** To establish the left lung infection, *P. aeruginosa* was used at an inoculum of $2 \times 10^8$ bacteria. The resulting infection was characterized by bacterial persistence in the left lung for at least 11 days (Table 3). A small variation between individual animals was seen. Bacterial persistence in the right lung at a relatively low level was also observed. Blood cultures of rats were always negative from day 4. The course of infection revealed no signs of morbidity or mortality.

**Therapeutic efficacy of PL Cipro versus that of CIP in rats**

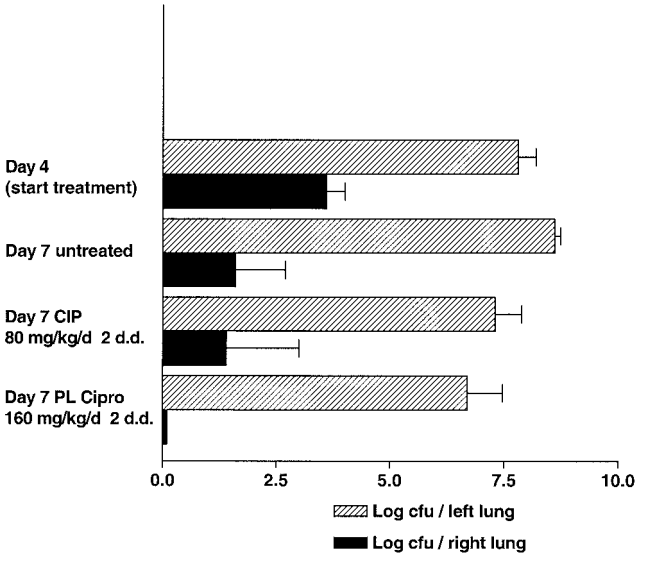


FIG. 1. Therapeutic efficacy of treatment at 12-h intervals for 3 days of rats with chronic *P. aeruginosa* pneumonia. Treatment was started at 4 days after bacterial inoculation in the left lung ($n = 4$ to 9 per dosage). At the end of treatment (7 days after bacterial inoculation), rats were sacrificed, and quantitative cultures of left lung, right lung, and blood were performed. Results are expressed as means + SD (error bars).

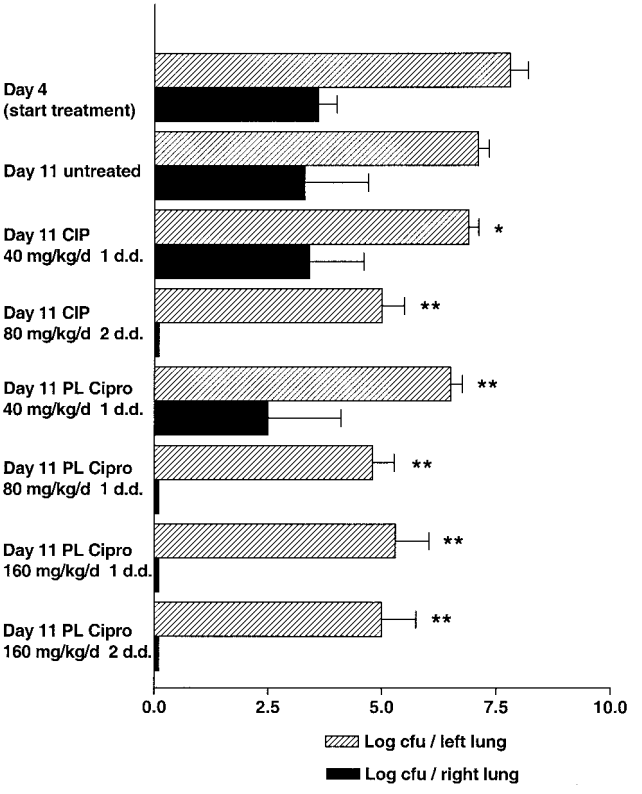


FIG. 2. Therapeutic efficacy of treatment at 12- or 24-h intervals for 7 days of rats with chronic *P. aeruginosa* pneumonia. Treatment was started at 4 days after bacterial inoculation in the left lung ($n = 4$ to 8 per dosage). At the end of treatment (11 days after bacterial inoculation), rats were sacrificed, and quantitative cultures of left lung, right lung, and blood were performed. Results are expressed as means + SD (error bars). Significant differences against the bacterial numbers at the start of treatment (day 4) are noted (**, $P < 0.001$; *, $P = 0.003$).

**with chronic *P. aeruginosa* pneumonia.** At 4 days after bacterial inoculation, the time that in untreated rats the bacterial numbers in the left lung and right lung were 7.8 ± 0.37 and 3.5 ± 0.39, respectively (Fig. 1 and 2), and blood was sterile, antimicrobial treatment with CIP or PL Cipro was started. Results of treatment are shown in Fig. 1 and 2. A 3-day treatment with CIP at 80 mg/kg/day twice daily (MTD) was not effective: bacterial numbers in the left lung (7.3 ± 0.6) were not significantly decreased compared with bacterial numbers at the start of treatment (Fig. 1). Similarly, a 3-day treatment with PL Cipro at 160 mg/kg/day (MTD) twice daily was not effective: Although the bacteria in the right lung were killed, bacterial numbers in the left lung were 6.7 ± 0.8. Blood cultures of rats treated with CIP or PL Cipro remained negative.

Increasing the length of treatment up to 7 days resulted in therapeutic efficacy (Fig. 2). At all dosage schedules of CIP and PL Cipro tested, bacterial numbers in the left lung were significantly ($P \leq 0.003$) decreased compared with bacterial numbers at the start of treatment. Significant bacterial killing in the left lung of >99% could be achieved with CIP at the MTD provided it was administered twice daily, i.e., at a dosage of 80 mg/kg/day. Bacterial numbers in the left lung were 5.0 ± 0.5 ($P < 0.001$). Right lungs were sterile. Once-daily administration of CIP at 40 mg/kg/day did not result in >99% bacterial killing: bacterial numbers in the left lung were 6.9 ± 0.2, and right lungs were not sterile. PL Cipro at 80 mg/kg/day once daily also effected >99% killing of bacteria in the left lung and sterilization of the right lung. Bacterial numbers in the left lung were 4.8 ± 0.5 ($P < 0.001$). Complete bacterial eradication in the left lung could not be achieved even with intensive dosage schedules of PL Cipro at 160 mg/kg/day (MTD) administered either twice daily or once daily. Under these dosage regimens bacterial numbers in the left lung were 5.0 ± 0.8 ($P < 0.001$) and 5.3 ± 0.7 ($P < 0.001$), respectively.

The administration of placebo liposomes had no effect on the infectious process: at day 7 after bacterial inoculation the weight of the left lung and the bacterial loads in the left lung and right lung and blood were not significantly different compared to values for untreated controls.

The ciprofloxacin susceptibility of *P. aeruginosa* recovered from dead or sacrificed rats was never changed compared with that of the inoculated bacteria.

**Toxic side effects.** The intravenous administration of CIP and PL Cipro as a bolus injection results in higher concentrations in serum than observed in humans in whom it is administered as bolus infusion. This may increase the acute toxicity compared to that observed in humans. The MTD for CIP was 20 mg/kg/dose in the severely ill rats with acute *P. aeruginosa* pneumonia-septicemia. At higher doses, acute toxicity was observed shortly after the first dose. In rats with chronic *P. aeruginosa* pneumonia, the MTD for CIP was 40 mg/kg/dose. Administration of PL Cipro up to a dosage of 160 mg/kg/day did not result in acute toxic side effects in the models of acute or chronic *P. aeruginosa* infection. In addition, long-term toxicity was not observed. Dosages of PL Cipro of >160 mg/kg/dose were not tested because of the reported side effects of the

TABLE 4. Concentrations of total ciprofloxacin (free plus liposome-encapsulated) in infected lung at 1 h after administration in rats with chronic *P. aeruginosa* pneumonia[a]

| Lung | Mean concn (μg/g of tissue) ± SD (% of injected dose) | |
|---|---|---|
| | CIP | PL Cipro |
| Left | 23.8 ± 6.5 (0.4) | 459 ± 245 (2.0) |
| Right | 38.8 ± 22.3 (0.3) | 1,207 ± 268 (2.7) |

[a] CIP (40 mg/kg) or PL Cipro (160 mg/kg) was injected as a single dose at 4 days after inoculation of rats with *P. aeruginosa*.
[b] Data are based on results for five rats.

relatively high lipid doses that can result from the formulation (4).

**Concentrations of ciprofloxacin in tissue after administration of CIP or PL Cipro at the MTD in rats with chronic *P. aeruginosa* pneumonia.** Total concentrations of ciprofloxacin (free plus liposome-encapsulated) are presented in Table 4. At 1 h after administration of CIP at 40 mg/kg (MTD) as a single dose, recovery of ciprofloxacin from infected left lung and right lung was relatively low, at 0.4 and 0.3% of the injected dose, respectively. Administration of PL Cipro at 160 mg/kg (MTD) as a single dose resulted in increased total ciprofloxacin concentrations in the infected left lung and right lung. At 1 h after injection, ciprofloxacin concentrations in infected left lung and right lung were 19- and 31-fold increased, respectively, compared with the concentrations after administration of CIP at its MTD.

**Effect of ciprofloxacin against *P. aeruginosa* in broth in relation to bacterial growth rate.** Logarithmically growing *P. aeruginosa* organisms in broth were killed by ciprofloxacin dependent on the concentrations used. When the bacteria were in the stationary phase of growth, ciprofloxacin was still able to kill the bacteria effectively. Within 3 h of incubation, ciprofloxacin at the concentration of 0.5 μg/ml killed logarithmically growing bacteria and stationary-phase bacteria efficiently, and the Δlog CFU was 2.1 and 2.7, respectively. At the highest concentration, 2 μg of ciprofloxacin per ml, bacterial killing was increased, and the Δlog CFU was 4.2 and 3.9, respectively.

## DISCUSSION

In previous studies in our laboratory antimicrobial agents were encapsulated in PEG-coated long-circulating liposomes in order to achieve different pharmacokinetics of the agents (2, 3, 5, 27–30). When ciprofloxacin is encapsulated these liposomes act as a sustained-release microreservoir (5). The AUC at 24 h for ciprofloxacin, when administered at similar doses of 20 mg/kg in the liposome-encapsulated (once daily) or free (twice daily) form, are 900 and 74 μg·h/ml, respectively. In the rat model of *K. pneumoniae* pneumonia, it was demonstrated that the increased AUC of ciprofloxacin led to an increased therapeutic efficacy (5). These data agree with the findings of Drusano et al. obtained in a neutropenic rat model of *P. aeruginosa* sepsis, in which the relationship between the plasma concentration-time profile and therapeutic efficacy (survival) of lomefloxacin was investigated (10). AUC/MIC ratios as well as peak concentration in plasma/MIC ratios appeared to be associated with favorable outcome. The clinical relevance of these pharmacodynamic parameters has been examined and confirmed in clinical trials of fluoroquinolones in seriously ill patients (14, 15, 19, 25, 26).

In clinical practice, an optimal dose regimen is particularly important for infections due to microorganisms such as *P. aeruginosa* and *Staphylococcus aureus* for which the MICs of most fluoroquinolones are at or slightly below the breakpoint for susceptibility and for which selection of bacterial resistance has been associated with failure of treatment (25). Sufficient AUC/MIC ratios cannot be achieved (14). Relatively high doses of fluoroquinolones should be avoided due to toxic side effects. The question is whether encapsulation of fluoroquinolones in PEG-coated liposomes that results in a low toxicity profile and relatively high, sustained concentrations in plasma can compensate for the relatively high MIC, resulting in an adequate AUC/MIC ratio. This was investigated in the present study.

We developed two models of *P. aeruginosa* infection in rats and compared the therapeutic efficacies of PL Cipro and CIP. The susceptibility of the *P. aeruginosa* strain, a mucoid strain and clinical isolate from a patient with cystic fibrosis, was relatively low (MIC = 0.5 μg/ml). In the model of acute *P. aeruginosa* pneumonia-septicemia, initially the infection in the lung developed rapidly, resulting in septicemia at an early stage and a high mortality rate. In the model of chronic *P. aeruginosa* pneumonia, bacterial persistence in the lung was observed over a prolonged period, which did not lead to mortality of rats. These infection models have totally different characteristics and are both clinically relevant.

The MTD of CIP in the severely ill rats with acute *P. aeruginosa* pneumonia-septicemia was 20 mg/kg/dose, which was twofold lower than the MTD in rats with chronic *P. aeruginosa* pneumonia. However, in both models PL Cipro was well tolerated in high doses up to 160 mg/kg.

In the acute *P. aeruginosa* pneumonia-septicemia, CIP was not effective whereas PL Cipro in relatively high doses which are well tolerated was effective in all rats. Addition of PL Cipro at low dosage on the first day of treatment with CIP at the MTD resulted in increased therapeutic efficacy without toxicity. Addition of CIP at the MTD on the first day of treatment with PL Cipro at low dosage also effected therapeutic efficacy in all rats without toxic side effects. As the early onset of septicemia in the acute *P. aeruginosa* infection is the primary cause of death in these rats before pneumonia is established, the sustained release of ciprofloxacin from the liposomes resulting in prolonged concentrations in the blood is of major importance for survival of the animals. Sufficiently high and sustained concentrations of ciprofloxacin in plasma are needed due to the moderate susceptibility of the *P. aeruginosa* strain. This can be achieved with a high dosage of PL Cipro, or with a low dosage of PL Cipro in combination with CIP at the start of treatment to increase the bioavailability of ciprofloxacin in the early phase of treatment. These treatment schedules, although resulting in animal survival, did not effect bacterial eradication in the lung.

In the chronic *P. aeruginosa* pneumonia, a treatment period of 3 days using CIP or PL Cipro at their respective MTDs had no effect. After 7 days of treatment, however, both CIP and PL Cipro effected a significant decrease in bacterial count in the chronically infected left lung compared with that at the start of

treatment. If given twice daily CIP at its MTD resulted in killing of >99% of the bacteria, an effect also achieved with PL Cipro administered once daily. In this respect, PL Cipro is superior to CIP. Bacterial eradication was never obtained, even after intensive treatment with PL Cipro. Although ciprofloxacin in the liposomal form can be administered in a relatively high dosage, resulting in a 19-fold increase in concentration of ciprofloxacin at the infected site compared to administration in the free form, this concentration seems to be insufficient for killing all *P. aeruginosa* organisms.

Studies by other investigators using non-PEG-coated liposomal fluoroquinolones in models of intracellular infections revealed an increase in therapeutic effect as a result of liposomal encapsulation (8, 9, 21, 32). To what extent infected tissue targeting of the liposomal antibiotics also contributes to the increased therapeutic efficacy in these animal models was not investigated. Other studies showed an increased intracellular penetrating capacity of liposomal fluoroquinolones compared with drugs in the free form (22, 23). Leitzke et al. compared drug concentrations in a model of *Mycobacterium avium* infection in lung and liver of mice after administration of amikacin or ciprofloxacin in the free or liposomal form (18). Liposomal encapsulation of amikacin, but not of ciprofloxacin, resulted in sustained high drug levels in infected tissues. These comparative data agree with observations obtained for gentamicin and ciprofloxacin in our model of *K. pneumoniae* pneumonia (5, 30).

Also, in patients with respiratory tract infections due to *P. aeruginosa*, in spite of intensive treatment with ciprofloxacin bacterial persistence has been described by various authors (1, 7, 12, 16, 31). Successful eradication of bacteria from lung tissue chronically infected with *P. aeruginosa* is probably hampered by various factors. The mucoid, alginate-producing *P. aeruginosa* bacteria form microcolonies that may impair antibiotic penetration. In addition, the bacteria may be at a low metabolically active state inside the microcolonies, which may further diminish antibiotic killing capacity. However, based on their mode of action, the fluoroquinolones are expected to be active against bacteria in a low metabolically active state, which is also demonstrated in the present study, showing the concentration-dependent killing of ciprofloxacin in vitro against *P. aeruginosa* in the logarithmic phase as well as stationary phase of growth. Another factor contributing to the failure of treatment to fully eradicate the bacteria may be the intracellular localization of a minority of the *P. aeruginosa* organisms. It is not known to what extent the ciprofloxacin concentrations measured in the infected tissue reflect interstitial concentrations or intracellular concentrations. For further improvement of therapeutic efficacy in chronic *P. aeruginosa* infection, the application of fluoroquinolone-containing liposomes that retain their content during circulation, thereby effecting targeting of the drugs to the site of infection, may be of major importance.

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