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Oral Ciprofloxacin Treatment for *Salmonella typhimurium* Infection of Normal and Immunocompromised Mice

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Oral treatment of *Salmonella typhimurium* infection with ciprofloxacin was compared with conventional chemotherapy with ampicillin or chloramphenicol in normal (CFW1) and immunocompromised (C57BL/6) mice. Administration of the antibiotics for 12 days reduced the number of bacteria in livers and the mortality of C57BL/6 mice significantly. Ciprofloxacin was considerably more effective than ampicillin in prolongation of the mean survival time of these mice. Similar to conventional chemotherapeutic agents, ciprofloxacin did not prevent fatal disease in most C57BL/6 mice when the treatment lasted 12 days only. On the other hand, ciprofloxacin cured lethal *S. typhimurium* illness in immunocompromised mice after long-term oral chemotherapy for 26 days at a dosage of 100 mg/kg twice a day. This was not achieved by either ampicillin or chloramphenicol. In normal mice, 12 days of therapy with ciprofloxacin was sufficient for a significant decrease in both the number of viable bacteria in livers and the mortality of lethally infected mice. The results provide a basis for an alternative antibiotic treatment by the oral route in immunocompromised hosts with systemic infections.

Murine typhoid, caused by *Salmonella typhimurium*, resembles infection of humans with *Salmonella typhi* in many of its features (8, 19, 23, 34, 40). This experimental disease has therefore been well accepted for studies on the in vivo efficacy of chemotherapeutic drugs (1, 5, 6, 38). *S. typhimurium* is considered to be a facultative intracellular bacterium in the mouse, because the organisms multiply within macrophages until T-cell-mediated immunity is established during the course of the infection (8, 16, 23). Successful treatment of *S. typhimurium* infection meets with difficulties especially in immunocompromised hosts (3, 4, 12). It is therefore not surprising that increased rates of salmonella infections have been reported for patients with impaired host defense (42).

Ciprofloxacin is an antibacterial agent developed for oral and parenteral use in the treatment of bacterial diseases, including severe systemic infections (7, 27, 32, 35, 36, 41, 44). Because it has recently been reported that ciprofloxacin penetrates phagocytic cells, the drug appears to be well suited for treatment of infections with intracellular pathogens (13, 14).

It has been well established for many decades that susceptibility to salmonella infections in mice is genetically controlled (18, 22, 29, 37, 40). C57BL/6 mice have been reported to be susceptible (*Ity*^{s}) to this infection and were therefore used as immunocompromised hosts in the experiments described here. Because the in vitro antimicrobial properties of ciprofloxacin are rather promising, we considered it worthwhile to study the efficacy of this compound in vivo after oral administration in normal as well as immunocompromised mice and to compare its activity with those of ampicillin and chloramphenicol, which are conventionally used for treatment of typhoid fever in humans. In a previous report by Easmon and Blowers (12), it had been shown only that short-term subcutaneous administration of ciprofloxacin results in the cure of mice with normal host defense mechanisms. We therefore asked whether long-term oral treatment would cure immunocompromised mice.

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MATERIALS AND METHODS

Mice. Specific-pathogen-free, randomly selected female mice (8 to 12 weeks old) were used. C57BL/6 mice were obtained from B. Bomholtgard, Ry, Denmark. These mice are genetically susceptible (*Ity*^{s}) to *S. typhimurium* infection. Outbred CFW1 (SPF) mice came from F. Winkelmann, Versuchstierzucht, Borchen, Federal Republic of Germany. CFW1 mice possess unimpaired defense mechanisms against *S. typhimurium*.

Organisms and culture conditions. The *S. typhimurium* 25268 used in these studies was an isolate from a human stool specimen. The bacteria were at a low-passage level (less than five passages) on artificial medium to avoid significant loss of virulence properties. The strain was a gift from P. Naumann, Institute for Medical Microbiology and Virology, University of Düsseldorf, Düsseldorf, Federal Republic of Germany. After intraperitoneal inoculation in a mouse and reisolation from the spleen, the organisms were grown in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.), distributed in small quantities, and stored under liquid nitrogen.

Antimicrobial agents. Ciprofloxacin (Bayer AG), ampicillin (Bayer AG), and chloramphenicol (Boehringer GmbH, Mannheim, Federal Republic of Germany) were used. For oral administration, the compounds were dissolved in sterile distilled water and diluted in water containing 5% glucose.

Bacterial susceptibility testing. MICs were determined by the method of Ericsson and Sherris (15). A standard inoculum of approximately 10^5 CFU was used for determination of MICs.

Pharmacokinetic studies. Levels of antimicrobial agents in sera and livers were determined by standard procedures at day 4 after subcutaneous inoculation of 10^3 *S. typhimurium* CFU into C57BL/6 mice (2). Oral administration of the drug was started in the morning of day 3 after infection and took

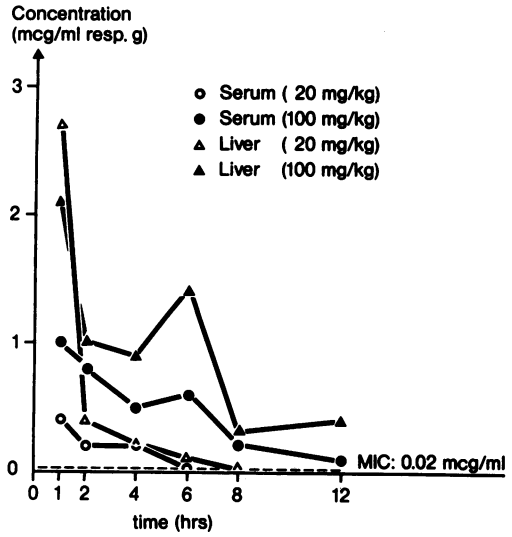

FIG. 1. Concentrations of ciprofloxacin in sera and livers during infection of C57BL/6 mice with *S. typhimurium* at various time intervals after oral administration of the antimicrobial agent. Concentrations are shown in micrograms per milliliter or per gram.

place again 12 and 24 h later. After the third application, pharmacokinetic studies were performed by a plate diffusion assay with *Escherichia coli* ICB 4004 as the test organism. The lower limit of test sensitivity for determination of ciprofloxacin levels in sera was less than 0.01 μg/ml and for ampicillin was less than 0.05 μg/ml.

Determination of LD_{50}s. The 50 percent lethal doses (LD_{50}s) of *S. typhimurium* for various strains of mice were determined by the method of Reed and Muench (31).

Therapeutic trials. The model of mouse typhoid has been described in detail previously (1, 5, 6, 8, 23, 34, 38, 40). Mice were infected subcutaneously or orally with lethal doses of the virulent strain of *S. typhimurium*. The therapeutic regimens were selected to ensure that peak levels of drugs in sera exceeded the MICs, and if possible MBCs, for the challenge strains and to provide levels in serum consistent with those achieved in clinical practice (10).

Quantitation of bacteria in mouse organs. Livers of mice were removed aseptically on different days after infection as indicated in Results. Organs were weighed, and Mueller-Hinton broth was added to yield a 10% suspension (wt/vol) after homogenization in a Stomacher (Kleinfeld; Hannover, Federal Republic of Germany). Serial 10-fold dilutions were plated in duplicate on Mueller-Hinton agar. After incubation for 18 h at 37°C, colonies were counted and the number of CFU was calculated per gram of tissue. Isolates from animal organs were identified as *S. typhimurium* by slide agglutination with the use of commercially available antisera to somatic (O) and flagellum (H) antigens (Behring-Werke, Marburg, Federal Republic of Germany).

Statistical analysis. Analysis of variance, Student's *t* test, and Fisher's exact test were used for evaluation of the data.

RESULTS

Antibiotic susceptibility. The MICs for *S. typhimurium* 25268 were as follows: ciprofloxacin, 0.02 μg/ml; ampicillin, 0.5 μg/ml; and chloramphenicol, 2.0 μg/ml. The low MIC of ciprofloxacin for *S. typhimurium* is in accordance with data on 673 *Salmonella* species (MIC, 0.002 to 0.5 μg/ml; MIC for 50% of strains, 0.023 μg/ml; MIC for 90% of strains 0.064 μg/ml) (44; H. J. Zeiler, unpublished observations, data on file of Bayer AG, Wuppertal, Federal Republic of Germany). The MICs of the other antibiotics are within previously published ranges (1, 5, 6, 9, 26, 39).

Pharmacokinetics. The levels of ciprofloxacin and ampicillin in serum and liver samples after oral administration and during infection as determined by bioassay can be seen in Fig. 1 and 2. Concentrations of ciprofloxacin in livers exceeded the levels in sera at all points of time tested. After administration of 20 mg/kg, concentrations in sera and livers were above the MIC until 4 to 6 h, and in animals which had received 100 mg/kg, until 12 h after drug application (Fig. 1). In animals treated with ampicillin, levels in sera were usually higher than the concentrations in livers (Fig. 2). Ampicillin levels above or approximately at the MIC were observed up to 2 h after administration of 20 mg/kg and up to 8 h after application of 100 mg/kg. Concentrations of chloramphenicol in mouse sera have been determined with chemical procedures by Reiche and Frey (33). The authors found levels of chloramphenicol above or at 2 μg/ml (MIC for *S. typhimurium*) for 3 h after oral administration of 50 mg/kg. At 4 h after administration, chloramphenicol levels in sera were still detectable but below 2 μg/ml.

Virulence of *Salmonella typhimurium*. The LD_{50}s of *S. typhimurium* 25268 for immunocompromised as well as for normal mice are shown in Table 1. As expected, the LD_{50} of the virulent strain was less than 10 CFU per mouse for C57BL/6 mice, whereas a much higher inoculum level was required to establish a lethal infection in CFW1 mice. Fatal results could also be observed for C57BL/6 mice after oral inoculation of this virulent strain of *S. typhimurium* (Table 1).

Effect of short-term therapy with ciprofloxacin on infection of normal mice. In a first series of experiments, the efficacy

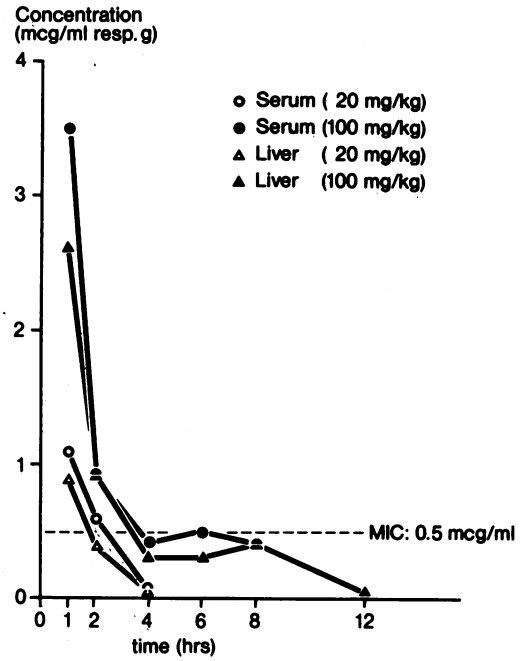

FIG. 2. Levels of ampicillin in sera and livers of infected C57BL/6 mice after oral treatment. Concentrations are shown in micrograms per milliliter or per gram.

TABLE 1. LD_{50}s of *S. typhimurium* 25268 for susceptible (C57BL/6) and normal (CFW1) mice

Route of administration	LD_{50} (CFU/mouse) for:	
	C57BL/6	CFW1
Intraperitoneal	$<10^{1.0}$	$10^{2.3}$
Subcutaneous	$<10^{1.0}$	$10^{5.3}$
Oral	$10^{2.2}$	$10^{7.0}$

of ciprofloxacin was studied for mice with unimpaired host defense mechanisms. Therapy was started on day 3 after subcutaneous infection with an inoculum of 10 times the LD_{50} ($10^{6.3}$ CFU per mouse). At this time after inoculation, symptoms had not developed but the spread of infection was obvious (mean ± standard deviation of $\log_{10}$ CFU per gram of liver: 3.3 ± 0.5 [n = 5]). The drug was administered to groups of 10 mice subcutaneously for 2 days. Thereafter, treatment was continued orally for an additional 10 days. Thus, the antibacterial agent was administered for a total of 12 days, i.e., until day 14 after infection.

Figure 3 provides data of a representative experiment which indicate that ciprofloxacin treatment of mice reduced their mortality compared with that of untreated animals. The difference reached statistical significance in the high-dosage group ($P < 0.005$ versus control [Fisher's exact test]). Bacteria were still present in the livers of surviving mice at day 25 after inoculation ($10^{4.2 \pm 0.5}$ CFU/g). This indicates that persistent infection had developed, but despite this chronic carrier state, the mice had survived (Fig. 3). These findings confirm data of Easmon and Blowers (12), who used normal (*Ity*^{r}) CBA/J and A/J mice for their studies on the efficacy of ciprofloxacin for *S. typhimurium* infection, but results on oral treatment were not reported by the authors (12).

Effect of short-term therapy on mortality after infection of immunocompromised mice. Ciprofloxacin was then administered to C57BL/6 mice in the same dosages and for the same time course as was used for the normal mice. Although the median survival time was considerably increased in the treatment groups compared with that of untreated animals, the majority of these immunocompromised mice finally succumbed to the infection (Fig. 4). Whereas the median survival time of controls was 10.5 days, it was 21 days in the lower-dosage group and 22 days for the higher-dosage regimen of ciprofloxacin. Consequently, in further experiments the dosage of ciprofloxacin given orally was increased even more (100 mg/kg twice a day (b.i.d.) and compared with the same dosage of ampicillin. The high dosage of ciprofloxacin was chosen to achieve comparable levels in mouse sera and tissues as had been described for a single oral dose of 500 mg in humans (10). A considerable increase in the median survival time (25.5 days) was observed for ciprofloxacin-treated mice compared with that of animals treated with ampicillin (15.5 days) or controls (11.5 days), demonstrating the superiority of ciprofloxacin over ampicillin for the treatment of this infection.

When ciprofloxacin was compared with chloramphenicol by using the short-term therapeutic regimen (12 days), a difference in favor of ciprofloxacin was not observed. Oral administration of the drugs at the high dosage (100 mg/kg b.i.d.) increased the median survival time in mice infected by either the oral or the subcutaneous route. The median survival times (in days) were 33 for chloramphenicol, 30 for ciprofloxacin, and 12.5 for the controls after oral infection and 20 for chloramphenicol, 24 for ciprofloxacin, and 13 for the controls after subcutaneous administration. Oral infection with *S. typhimurium* was studied in addition to the subcutaneous inoculation because this pathogen enters the host orally under natural conditions. When antibiotic therapy in both the subcutaneously and the orally infected mice was discontinued 2 weeks after infection, most animals did not survive beyond another 3 to 5 weeks.

Effect of short-term therapy on the number of bacteria in livers and on liver weight of immunocompromised mice. When the number of bacteria per gram of liver was determined at the end (i.e., at day 14 after infection) of short-term oral

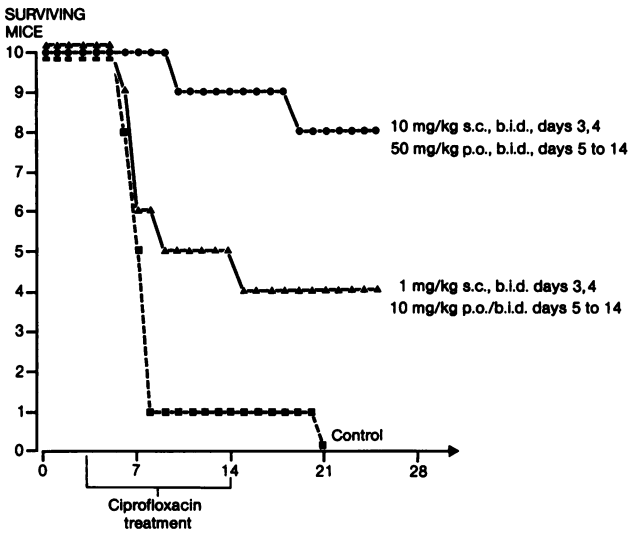

FIG. 3. Effect of ciprofloxacin treatment on mortality in mice (CFW1) with unimpaired host defense after inoculation (10 times LD_{50} [$10^{6.3}$ CFU per mouse]) with *S. typhimurium* (10 mice in each group). Abbreviations: s.c., subcutaneously; p.o., orally.

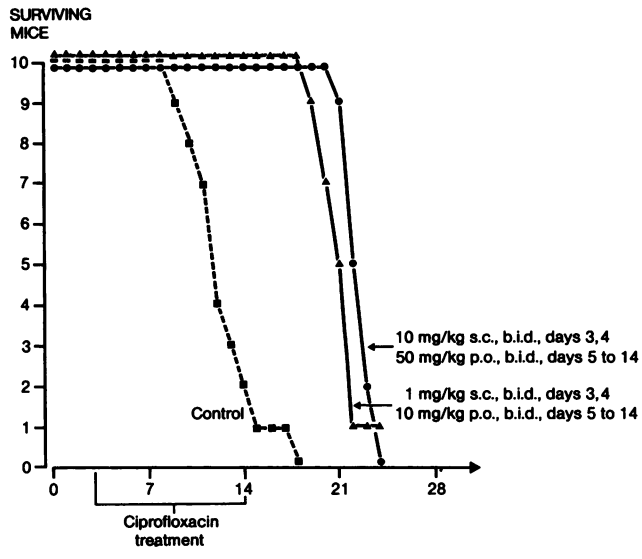

FIG. 4. Effect of ciprofloxacin treatment on mortality of mice (C57BL/6) with a genetic defect in macrophage function after lethal (10^3 CFU per mouse) infection with *S. typhimurium* (10 mice in each group). Abbreviations: s.c., subcutaneously; p.o., orally.

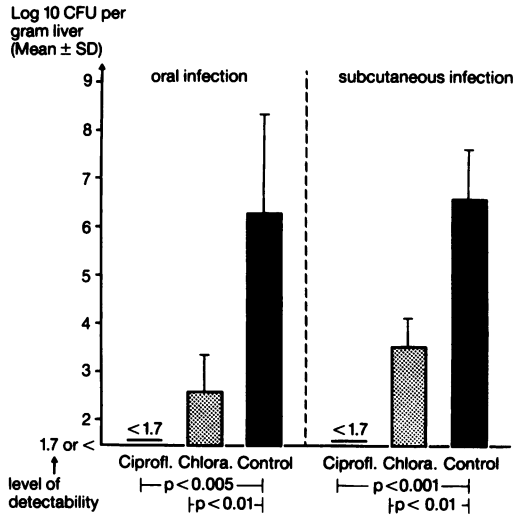

FIG. 5. Efficacy of oral treatment with ciprofloxacin (Ciprofl.) or chloramphenicol (Chlora.) for reducing the number of bacteria in the livers of lethally infected C57BL/6 mice 14 days after infection, i.e., at the end of therapy (100 mg/kg b.i.d. for 12 days). *P* values were calculated by Student's *t* test (five mice per group).

therapy (100 mg/kg b.i.d. for 12 days), a significant reduction in the mean number of organisms was seen for ciprofloxacin- and chloramphenicol-treated animals (Fig. 5). The number of organisms was below the level of detectability in ciprofloxacin-treated mice, whereas in chloramphenicol-treated mice bacteria could still be detected. As expected, the mean liver weight was significantly reduced in ciprofloxacin-treated mice (0.70 ± 0.06 g for subcutaneous infection as compared with 1.11 ± 0.28 g for untreated infected animals), indicating a less-severe inflammatory response during treatment with ciprofloxacin. A significant reduction in the liver weight of chloramphenicol-treated mice (1.14 ± 0.13 g) compared with liver weights of untreated controls was not seen. The mean ± standard deviation of liver weight for uninfected mice of the same age was 0.76 ± 0.03 g.

Long-term oral chemotherapy and mortality of immunocompromised mice. Because eradication of bacteria and cure of disease were not achieved by the above-mentioned 12-day chemotherapy, the antibiotics were administered in subsequent experiments from day 3 until day 28 after lethal infection. Therapy with low (20 mg/kg b.i.d.) and high (100 mg/kg b.i.d.) dosages of ampicillin or chloramphenicol increased the mean survival time significantly, but after 13 weeks most animals finally succumbed to the infection (Fig. 6). In contrast, ciprofloxacin at a high dosage (100 mg/kg b.i.d.) cured the disease in 80% of the animals ($P < 0.001$ [Fisher's exact test]). Of the animals of the low-dose ciprofloxacin group, 33% ($P < 0.02$) also were alive 13 weeks after infection, as were 20% ($P = 0.1$) of the animals treated with chloramphenicol (100 mg/kg b.i.d.).

Long-term oral treatment and recovery of bacteria from livers of immunocompromised mice. Table 2 shows data on the number of bacteria per gram of liver during (day 17) and at the end of (day 28) therapy. Ciprofloxacin but not ampicillin or chloramphenicol reduced the number of bacteria below the level of detectability. A significant decrease in the number of bacteria per gram of liver as compared with bacterial numbers in untreated animals was also seen after treatment with chloramphenicol or ampicillin, but this was less than had been observed with ciprofloxacin. These results are correlated with the low liver weight in ciprofloxacin-treated animals, indicating a reduced inflammatory response (data not shown). No overt side effects were seen in the mice during long-term oral treatment with any of the chemotherapeutic agents.

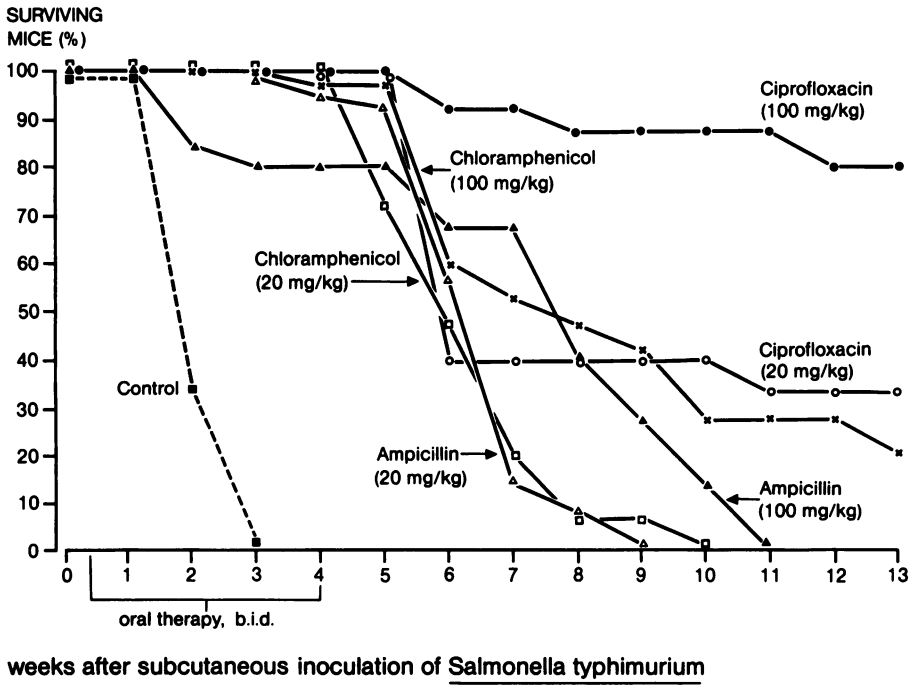

FIG. 6. Effect of long-term oral chemotherapy on the mortality of C57BL/6 (immunocompromised) mice after lethal infection (10^3 CFU per mouse) with *S. typhimurium* (30 mice per group).

TABLE 2. Effects of oral antibiotic treatments on recovery of bacteria from livers during experimental *S. typhimurium* infection of immunocompromised (C57BL/6, *Ity*^{s}) mice

Drug	Dosage (mg/kg)[a]	Log_{10} CFU (mean ± SD)/g of liver at indicated day after infection	
		17 (*P*)[b]	28
Ciprofloxacin	20	1.9 ± 0.3 (<0.001)	<1.7
Ciprofloxacin	100	<1.7[c] (<0.001)	<1.7
Ampicillin	20	3.8 ± 0.3 (<0.005)	4.1 ± 0.4
Ampicillin	100	4.2 ± 1.3 (<0.01)	3.1 ± 0.3
Chloramphenicol	20	5.1 ± 0.4 (<0.1)	4.9 ± 0.7
Chloramphenicol	100	3.3 ± 0.2 (<0.001)	3.5 ± 0.1
Control	0	5.8 ± 0.7	—[d]

[a] Dosages were b.i.d. from day 3 until day 28 after inoculation of the bacteria.
[b] Versus control (Student's *t* test [five mice per group]).
[c] Level of detectability.
[d] —, No untreated animals had survived until day 28.

Surviving animals were sacrificed 95 days after inocula tion. High-dose ciprofloxacin reduced the number of bacteria to undetectable levels (level of detectability: $10^{1.7}$ CFU/g), but it is obvious from the data of Fig. 6 that the bacteria had not been eliminated in all animals, because 20% of the mice died of the infection even after this high-dose, long-term therapy. There was no difference in liver weights compared with those of uninfected mice (data not shown).

DISCUSSION

Typhoid fever is a disease which requires new approaches for chemotherapy to overcome the problems of drug resistance and high relapse rates with this illness in different parts of the world (3, 11, 21, 25, 43). Plasmid-mediated resistance to chloramphenicol, ampicillin, and other agents presently used to treat systemic salmonellosis is an ever-present problem. Increased use of the newer beta-lactam compounds is likely to lead to the development of resistance to them also. The successful treatment of *S. typhimurium* infection in normal and immunocompromised mice by oral administration of ciprofloxacin in short-term (12-day) and long-term (26-day) regimens is therefore described here in comparison with the effectiveness of ampicillin and chloramphenicol. These experiments extend previous studies by Easmon and Blowers (12), who used a 5-day parenteral (subcutaneous) regimen of ciprofloxacin.

The start of treatment in our studies was delayed until 72 h after inoculation to allow bacteria to become established in host tissue. This model closely resembles the clinical reality of therapy in patients with typhoid fever. Our observations are reported in a clinical context because it has been known for many decades that antibiotic therapy for infected patients with impaired host defense can present the physician with rather difficult problems (42). This is especially the case in infections caused by facultative intracellular bacteria, i.e., pathogens which are able to multiply within macrophages. In these infections, a specific stimulus by sensitized T cells of the immune system is required to activate the macrophages and enable them to kill the invading microorganisms (8, 16, 23, 34, 40). *S. typhi* is considered such an intracellular pathogen for humans, and *S. typhimurium* infection of mice resembles human typhoid fever caused by systemic salmonellosis in this and many other features.

S. typhimurium infection in mice results in bacteremia and, depending on the size of the inoculum, in fatal accumulation of organisms in reticuloendothelial cells of the spleen, liver, bone marrow, and other tissues which contain a large number of macrophages. Fatal *S. typhimurium* infection is associated with numbers of bacteria up to 10^7 CFU/g of tissue.

A most difficult task for an antibiotic is the cure of an infection caused by an intracellular bacterium in a host with impaired macrophage or T-cell function. C57BL/6 mice are susceptible to mouse typhoid. The animals succumb to an intraperitoneal or subcutaneous challenge of less than 10 CFU per mouse. Because of a genetic defect, the macrophages of these mice do not kill intracellular bacteria properly (22, 37). Thus, the cure of mouse typhoid in C57BL/6 mice is a remarkable challenge for an antibiotic.

Ciprofloxacin, a new chemotherapeutic agent with a broad spectrum, exhibits rather low MICs against gram-negative bacteria, and when given to volunteers, the drug is rapidly adsorbed after oral administration. Furthermore, Easmon and Crane (13, 14) demonstrated penetration of ciprofloxacin into granulocytes and macrophages of humans, indicating that the antibacterial agent could be suitable for treatment of intracellular infections. Easmon and Blowers (12) reported that 5 days of subcutaneous ciprofloxacin therapy with 10 mg/kg b.i.d. prevented lethal *S. typhimurium* disease in normal but not in immunocompromised (BALB/c) mice.

Data presented here indicate that ciprofloxacin given orally was superior to ampicillin and chloramphenicol in curing typhoid in immunocompromised mice. This was the case even though chloramphenicol, like ciprofloxacin, penetrates cells effectively (17).

Clinical studies have demonstrated the efficacy of ciprofloxacin in treating human typhoid fever, but comparative trials with other antimicrobial agents are lacking (20, 30). It must be emphasized that the results in favor of ciprofloxacin in our study were obtained in mice and on a weight basis. Maximal doses which can be used in patients differ for the three antimicrobial agents compared in the present study. Further clinical studies are needed for the evaluation of the efficacy of ciprofloxacin in comparison with those of other antibiotics for salmonella infections of normal and immunocompromised patients.

It is conceivable that host defense mechanisms contribute to the effects of the antimicrobial agents during long-term treatment, because the defect of host defense in C57BL/6 mice is apparent in the early stages (days 0 to 14) after *S. typhimurium* infection, whereas later in the disease, protective mechanisms can be detected (22).

Recently, Maskell and Hormaeche (24) reported on the efficacy of a rather high dosage of ampicillin (20 mg per mouse [i.e., approximately 1 g/kg of body weight] daily and intraperitoneally) in BALB/c mice, which have a defect in host defenses similar to that of C57BL/6 mice. Prolongation of therapy in those *Ity*^{s}-mice prevented the development of the fatal relapse seen after short-term therapy. These findings are confirmed by our experiments. On the other hand, the present experiments suggest that ciprofloxacin may provide an effective alternative treatment for typhoid, paratyphoid, and other systemic salmonelloses.

The exact role of antimicrobial chemotherapy in these salmonella infections, which are restricted to the gastrointestinal tract, also remains controversial, partly because of the self-limiting nature of the disease and the rapid emergence of resistant strains (4, 26). Ampicillin is used in the empirical treatment of acute diarrhea, whereas chloramphenicol is widely administered for enteric fever. Quinolones may also be an alternative in this respect (28).

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