ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1994, p. 1501–1506
0066-4804/94/$04.00+0

Vol. 38, No. 7

Bay Y 3118, a New Quinolone Derivative, Rapidly Eradicates *Listeria monocytogenes* from Infected Mice and L929 Cells

THOMAS NICHTERLEIN,[1]* MARIANNE KRETSCHMAR,[1] CHRISTINA BUDEANU,[1] JENS BAUER,[1] WERNER LINSS,[2] AND HERBERT HOF[1]

Institute of Medical Microbiology and Hygiene, Mannheim, Faculty of Clinical Medicine, University of Heidelberg, 68167 Mannheim,[1] and Institute of Anatomy I, Friedrich Schiller University of Jena, 07743 Jena,[2] Germany

Received 4 August 1993/Returned for modification 10 December 1993/Accepted 11 April 1994

Bay Y 3118 is a new quinolone derivative with pronounced activity against gram-positive bacteria including the facultatively intracellular bacterium *Listeria monocytogenes*. Bay Y 3118 was tested in vitro and in animal models of listeriosis. All strains of *L. monocytogenes* and other *Listeria* spp. were highly susceptible in vitro; the MICs for these organisms ranged from 0.062 to 0.25 μg/ml. Bay Y 3118 was rapidly bactericidal in vitro, with a postantibiotic effect occurring for 3 h after removal of the antibiotic. *L. monocytogenes* was eliminated from infected L929 cells treated with Bay Y 3118, suggesting a bactericidal effect on the listeriae in these cells. Immunocompetent mice were rapidly cured by treatment with 4 mg every 12 h. Concomitantly, the levels of interleukin 6 and gamma interferon in mouse sera declined rapidly. In immunocompetent mice, treatment with 2 mg of Bay Y 3118 every 12 h resulted in a greater initial reduction in the listerial counts in the organs than treatment with 2 mg of ampicillin every 12 h. Bay Y 3118 completely eliminated *L. monocytogenes* from the livers and spleens of chronically infected nude mice. However, some of the bacteria reappeared after the cessation of therapy. In conclusion, Bay Y 3118 is an excellent candidate substance for the therapy of infections caused by facultatively intracellular gram-positive bacteria such as *L. monocytogenes*.

Bay Y 3118 (Fig. 1) is a novel fluoroquinolone with a broad spectrum of antibacterial activity (2–4, 12, 19). It is as active as ciprofloxacin against gram-negative bacteria. Moreover, its excellent activity against gram-positive aerobic and anaerobic bacteria distinguishes Bay Y 3118 from ciprofloxacin, ofloxacin, and fleroxacin. Bay Y 3118 has been shown to be effective in several animal models of infection with gram-positive and gram-negative bacteria (3a). In the work described here its in vitro activity against isolates of *L. monocytogenes* and other *Listeria* spp. was examined. Furthermore, a curative effect of Bay Y 3118 in cell cultures as well as in immunocompetent and immunocompromised mice infected with *L. monocytogenes* was demonstrated.

In mice *L. monocytogenes* resides and multiplies not only extracellularily but also in various host cells including macrophages (7). In this type of infection, the intracellular bacteria multiply in the cytoplasm (14). Immunity is, therefore, cell mediated (14) because the listeriae are inaccessible to humoral immune mediators such as antibodies. Immunocompromised hosts are likely to succumb to infection (18). Therapy of listeriosis in these subjects must rely on antibiotics that are bactericidal within the cytoplasm of eukaryotic cells and independent of the function of the cellular immune system (7). Bay Y 3118 seems to fulfil these requirements because it is able to eradicate *L. monocytogenes* from L929 cells and from immunocompetent and even immunocompromised mice.

MATERIALS AND METHODS

Drugs. Bay Y 3118 was a kind gift of A. Dalhoff (Bayer AG, Wuppertal, Germany). Ampicillin was purchased from Sigma (Deisenhofen, Germany).

* Corresponding author. Mailing address: Institute of Medical Microbiology and Hygiene, Mannheim, Faculty of Clinical Medicine, University of Heidelberg, 68167 Mannheim, Germany. Phone: (49) 621 383 2862. Fax: (49) 621 383 3816.

Bacteria. For determination of the MICs, strains of *L. monocytogenes* were taken from Seeliger's Listeria Culture Collection (SLCC) at Mannheim, Germany.

The following SLCC strains of *L. monocytogenes* were used: 2374, 2377, 2378, 2479, 5415, 5489, 5543, 6031, 6204, and 6793. In addition, five strains each of the listeriae *L. innocua*, *L. seeligeri*, *L. grayi*, and *L. ivanovii* and one strain of *L. welshimeri* were taken from the SLCC and tested. For all tissue culture and animal experiments *L. monocytogenes* EGD (SLCC 5835) was used. All bacteria were grown for 18 h in tryptose phosphate broth (Difco, Detroit, Mich.) at 37°C.

Determination of the MIC. For determination of the MIC, a standard agar dilution method described in the literature (17) was used. Bay Y 3118 was dissolved in isotonic saline immediately before use. Agar plates were prepared and used within 1 day after preparation. The inhibition of bacterial growth was also assessed in tissue culture medium (RPMI 1640 with L-glutamine and 10% fetal calf serum [Gibco, Eggenstein, Germany]) as described previously (10). In brief, 10^4 cells of *L. monocytogenes* EGD were incubated for 8 h in the presence of the antibiotic (37°C, 7.5% CO_2, pH 6.9 to 7.2). Thereafter, the number of bacteria was determined by plating in tryptose agar (Difco). The lowest concentration of the antibiotic that inhibited growth in this system was considered the minimal effective concentration in tissue culture medium.

Bactericidal effect and postantibiotic effect. The bactericidal effect of Bay Y 3118 was determined in Mueller-Hinton broth (Radiometer, Willich, Germany) with an inoculum of approximately 1,000 *L. monocytogenes* EGD per ml tested by standard methods (13) at 37°C. To avoid antibiotic carryover, the samples were appropriately diluted in isotonic saline and agar by a pour plate technique to reach concentrations of less than one-quarter the MIC. For determinations of the postantibiotic effect, the antibiotic was removed after 2 h by three washes with isotonic saline (three centrifugation steps at 15,000 × *g* for 5 min) and the bacteria were resuspended in fresh broth

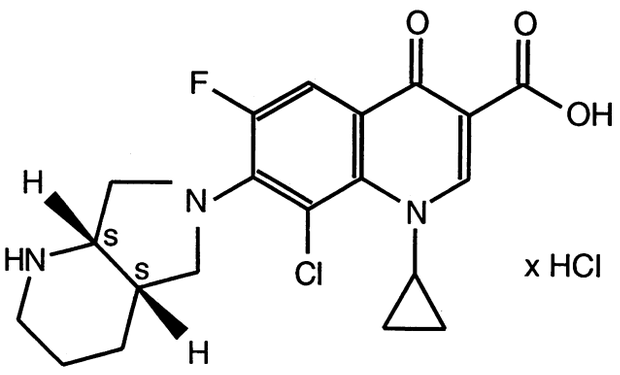

FIG. 1. Chemical structure of Bay Y 3118.

(37°C). The bacterial counts were determined by plating in tryptose agar (Difco).

Intracellular growth inhibition. L929 cells were purchased from ICN Biomedicals (Meckenheim, Germany). L929 cells were cultured in antibiotic-free medium as described previously (11). Confluent monolayers (2×10^5 cells per well) were infected for 2 h (37°C, 7.5% CO_2) with 2×10^4 cells of *L. monocytogenes* EGD in tissue culture medium (RPMI 1640 with L-glutamine and 10% fetal calf serum [Gibco]). After three washes with phosphate-buffered saline (PBS; Gibco), the extracellular bacteria were killed with 50 μg of gentamicin (Sigma) per ml in tissue culture medium (30 min, 37°C, 7.5% CO_2). In preliminary experiments, this treatment was shown to eliminate all of the inoculated listeriae from wells containing no cells or formalin-fixed L929 cells (data not shown). After killing of the extracellular listeriae, Bay Y 3118 was added in combination with gentamicin (50 μg/ml). Intracellular bacteria were enumerated after 8 h of incubation (37°C, 7.5% CO_2). After three washes with PBS, cells were lysed with 10 ml of sterile distilled water and appropriate dilutions were plated in tryptose agar (Difco) by a pour plate technique. Four wells were used for each dilution. Bacterial counts after killing with gentamicin (zero value) and after 8 h of incubation with gentamicin alone (growth control) served as controls. Intracellular growth was assumed when the bacterial counts after 8 h were greater than the zero value plus 1 standard deviation of the mean. Intracellular killing was assumed when bacterial counts were less than the zero value minus 1 standard deviation. In another experiment, the time course of listerial infection of L929 cells was determined. L929 cells were cultured and infected as described above. After the killing of extracellular bacteria for 30 min with gentamicin, eight times the minimal effective concentration of Bay Y 3118 in tissue culture medium (2 μg/ml) was added in combination with gentamicin (50 μg/ml). The intracellular bacteria were enumerated by plating at 1, 3, 5, and 8 h after the addition of Bay Y 3118.

Electron microscopy. L929 cells were grown on Transwell collagen-cellulose filters (pore size, 0.4 μm; Costar, Cambridge, Mass.). Cells were infected for 2 h with 10 to 100 *L. monocytogenes* EGD per L929 cell in tissue culture medium. The cells were washed with tissue culture medium, and gentamicin (50 μg/ml) or Bay Y 3118 (eight times the minimal effective concentration in tissue culture medium) was added. Samples were taken at 8 h after the addition of the antibiotics. Cells were fixed for 1 h at room temperature with 2.5% glutaraldehyde in cacodylate buffer (pH 7.2) and were rinsed in the same buffer. Postfixation was done for 1 h with 2% osmium tetroxide. Cells were dehydrated and embedded in resin (Durcupan; Fluka, Buchs, Switzerland). Ultrathin sections were collected and stained with uranyl acetate and lead citrate. Sections were observed with a JEM 100 B or Tesla BS 500 transmission electron microscope.

Mouse infection models. Outbred female NMRI mice weighing 20 to 25 g were obtained in a specific-pathogen-free state from the Central Institute for Laboratory Animals (Hannover, Germany). Female NMRI nude mice were obtained from the same breeder. Immunocompetent NMRI mice were infected intravenously with a sublethal dose of *L. monocytogenes* EGD (10^4 bacteria per animal). Mice were treated intraperitoneally every 12 h with Bay Y 3118 (1, 2, or 4 mg per animal in 0.2 ml of PBS starting 6 h postinfection). Control animals received 0.2 ml of PBS only. At days 1, 3, and 6 postinfection, five mice from each group were killed by cervical dislocation and their spleens and livers were removed aseptically. The organs were homogenized in isotonic saline with Tenbroeck tissue grinders (Wheaton, Millville, N.J.) and were further diluted in isotonic saline. The bacterial counts per organ were determined by plating of appropriate dilutions of the homogenates in tryptose agar by a pour plate technique. In another experiment, Bay Y 3118 was compared with ampicillin. Mice were infected and treated as described above with 2 mg of Bay Y 3118 every 12 h or 2 mg of ampicillin every 12 h. Five mice from each group were killed at days 1, 2, 3, 4, and 7 postinfection.

Nude mice were infected intravenously with 10^5 *L. monocytogenes* EGD. Each animal was given 2 mg of Bay Y 3118 every 12 h intraperitoneally from days 6 to 12 of infection. Control animals received PBS only. At days 6, 12, and 20 of infection, five mice from each group were killed and the number of bacteria in their livers and spleens was determined. The organs were homogenized as described above, and the whole homogenate was poured into plates with agar in aliquots of 1 ml to reduce the detection limit to 1 CFU per organ.

IL determinations. Female NMRI mice weighing 20 to 25 g were used. The mice were infected with *L. monocytogenes* and then treatment was done as described above. Mice were treated with 2 mg of Bay Y 3118 every 12 h. Individual blood samples were obtained after cervical dislocation (five mice per group). The sera were removed and were stored frozen at −80°C until interleukin (IL) determinations were done. IL-6 and gamma interferon (IFN-γ) levels were determined by commercial tests (Endogen, Boston, Mass.) that were used as recommended by the producer.

Statistics. Student's *t* test was used to determine statistically significant differences ($P < 0.05$).

RESULTS

MICs. All strains were highly susceptible; the MICs of Bay Y 3118 for the strains ranged between 0.062 and 0.25 μg/ml. The MIC for *L. monocytogenes* EGD was 0.125 μg/ml. The minimal effective concentration of Bay Y 3118 in tissue culture medium was 0.25 μg/ml for *L. monocytogenes* EGD.

Bactericidal effect and postantibiotic effect. Bay Y 3118 was bactericidal at a concentration of eight times the MIC for *L. monocytogenes* EGD in Mueller-Hinton broth (Fig. 2) and tissue culture medium (data not shown). Bay Y 3118 caused a further growth inhibition for 3 h after removal of the antibiotic (Fig. 3).

Intracellular growth inhibition. By using eight times the minimal effective concentration in tissue culture medium (2 μg/ml), there was a reduction in the number of listeriae in the cells as early as 1 h after the addition of the antibiotic (Fig. 4). *L. monocytogenes* EGD was almost completely eliminated by

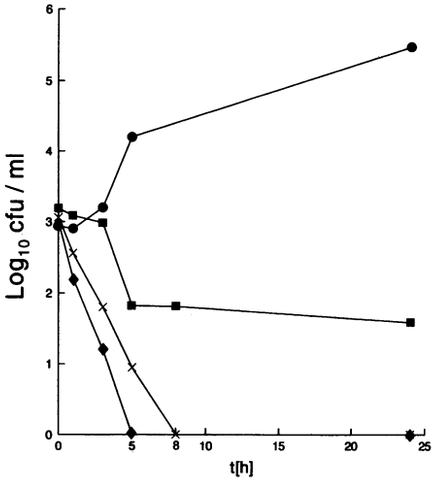

FIG. 2. Killing of *L. monocytogenes* EGD in Mueller-Hinton broth. A concentration of two times the MIC (■) reduced the inoculum to a steady state. Concentrations of eight times the MIC (×) and 32 times the MIC (◆) were bactericidal. ●, growth control.

Bay Y 3118 from L929 cells after 5 h of incubation with eight times the effective concentration in tissue culture medium (Fig. 4). By using 8 h of incubation with various concentrations of Bay Y 3118, there was a reduction in the number of listeriae beyond the zero level at a concentration of two times the minimal effective concentration of Bay Y 3118 in tissue culture medium (0.5 μg/ml; Fig. 5). However, an inhibition of intracellular growth in comparison with the growth control was measurable already with 0.25 μg/ml (the minimal effective concentration in tissue culture medium). With electron microscopy and by using 2 μg of Bay Y 3118 per ml and an incubation time of 8 h, the bacteria that were found inside infected L929 cells were heavily damaged (Fig. 6B). On the contrary, in the gentamicin control (50 μg/ml), dividing bacteria were found in the cytoplasm (Fig. 6A). There were no signs of damage to the L929 cells caused by Bay Y 3118 found either by electron microscopy or by the neutral red assay for cell viability (Sigma) at doses of up to 100 μg of Bay Y 3118 per ml (data not shown).

Therapy of primary infection of mice with Bay Y 3118. *L.*

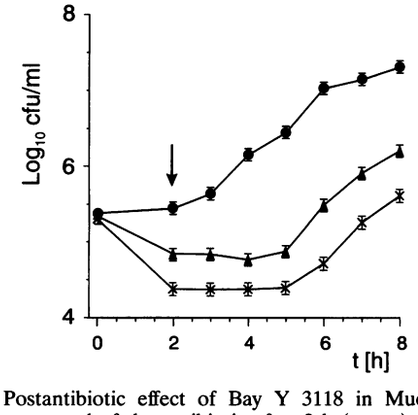

FIG. 3. Postantibiotic effect of Bay Y 3118 in Mueller-Hinton broth. After removal of the antibiotic after 2 h (arrow), there was a postantibiotic effect of 3 h. ▲, four times the MIC; ×, eight times the MIC; ●, growth control. Data are the means of five determinations.

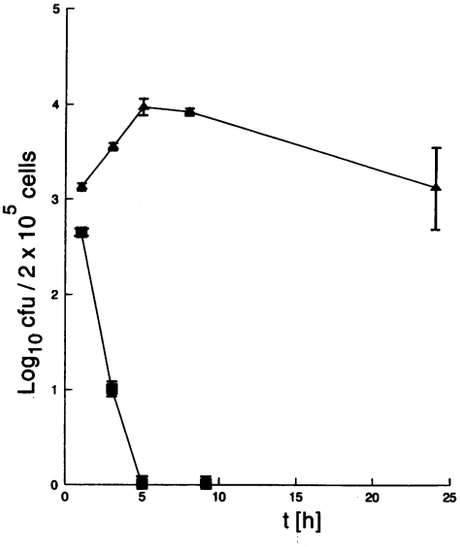

FIG. 4. Killing of *L. monocytogenes* in L929 cells with 2 μg of Bay Y 3118 per ml (▲, gentamicin control; ■, Bay Y 3118 plus gentamicin). Bay Y 3118 at 2 μg/ml led to a reduction in bacterial numbers as early as 1 h after the addition of the antibiotic, with virtual elimination after 5 h.

monocytogenes EGD was eradicated from the spleens of infected mice in a dose-dependent manner (Fig. 7). Similar results were obtained for the livers (data not shown). For animals given Bay Y 3118 at 4 mg every 12 h there was a complete eradication at day 3 of infection. Dosages of 2 mg every 12 h and 1 mg every 12 h inhibited the growth of the listeriae as early as at day 1 of infection and led to an accelerated elimination of the listeriae at day 6 of infection. Concomitantly, the levels of IL-6 and IFN-γ in the sera declined rapidly during therapy with 2 mg of Bay Y 3118 given every 12 h (Table 1). When given at the same dosage as

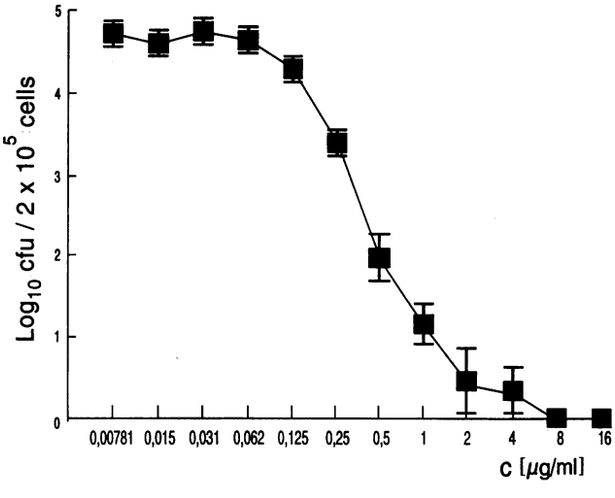

FIG. 5. Dose-dependent killing of *L. monocytogenes* in L929 cells by Bay Y 3118. C, Bay Y 3118 concentration. There was a reduction of the listerial numbers in L929 cells beyond that in the growth control (4.88 ± 0.05) with 0.25 μg of Bay Y 3118 per ml (the minimal effective concentration in tissue culture medium). A dose of 0.5 μg/ml reduced the listerial numbers beyond the zero level of 3.45 ± 0.05.

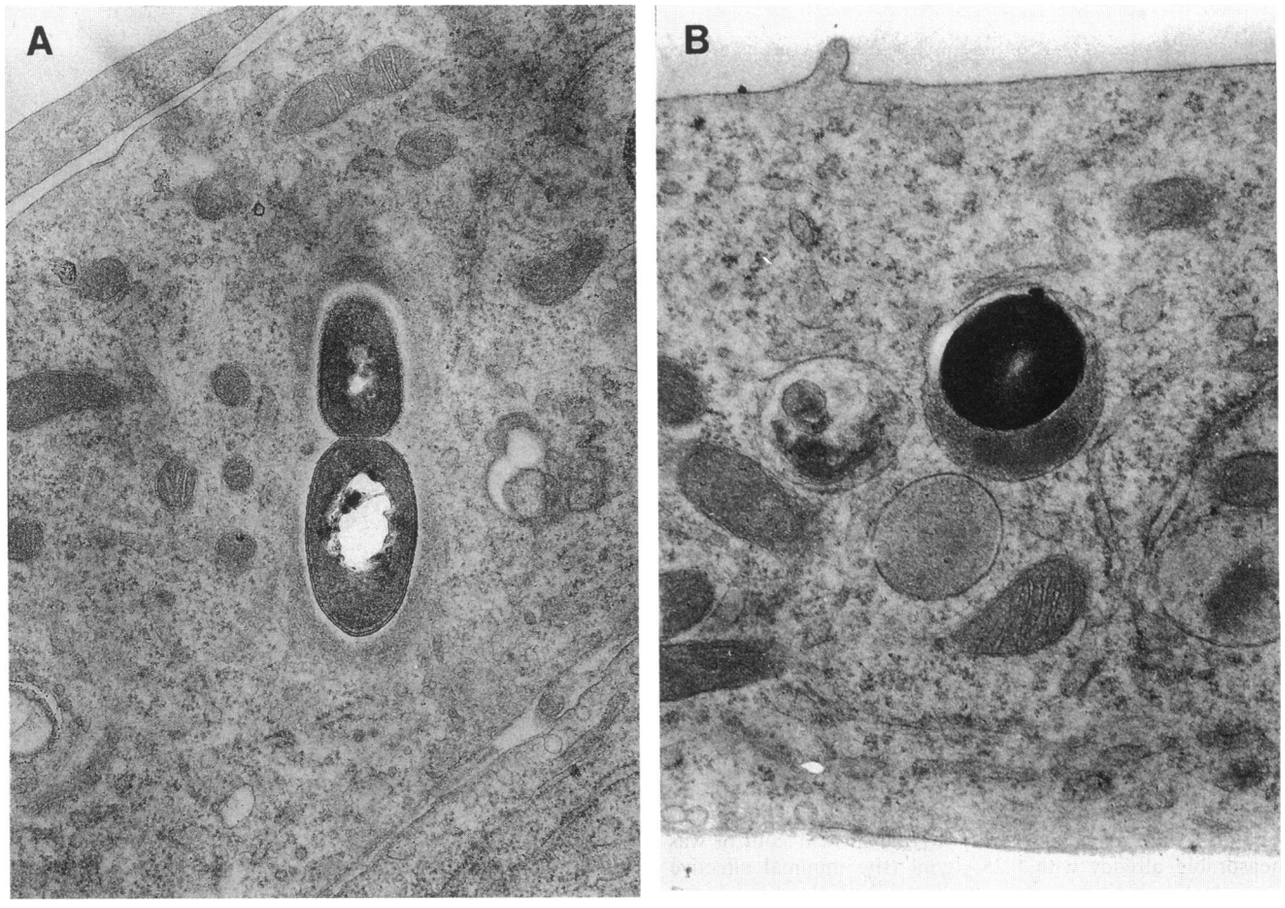

FIG. 6. Intracellular localization of listeriae after 8 h of incubation with 50 μg of gentamicin per ml (growth control) (A) and 2 μg of Bay Y 3118 per ml (B). Note that in the growth control, bacteria multiply in the cytoplasm, whereas with Bay Y 3118, there are damaged bacteria enclosed within membranes.

ampicillin, Bay Y 3118 treatment resulted in a greater initial reduction in the listerial numbers in the livers and spleens of mice as early as 1 day after infection, but there were similar declines in listerial numbers between days 1 and 7 (Fig. 8).

Therapy of chronic infection of nude mice. Bay Y 3118 given at 2 mg every 12 h completely eliminated the listeriae from the livers (data not shown) and spleens (Fig. 9; detection limit, 1 CFU) of infected *nu/nu* NMRI mice. However, some bacteria reappeared after 6 days after the cessation of therapy.

DISCUSSION

At present, the recommended therapy for listeriosis is ampicillin or amoxicillin, which is eventually combined with an aminoglycoside (1). This regimen is, however, not satisfactory since about 30% of the patients treated in this manner die (6, 8). Furthermore, recurrent listeriosis after inadequate therapy has been described (9). Therefore, it is urgent that there be a search for new and better drugs.

The reasons for the high failure rate are manifold (7). (i) Listeriae are very rarely resistant to antibiotics, but most of the common antibiotics are only bacteriostatic against these bacteria. This holds especially true for ampicillin, since most isolates are tolerant because of a particular cell wall architecture. (ii) Listeriosis often occurs in compromised hosts, so that it is necessary that an antibiotic have bactericidal activity. (iii)

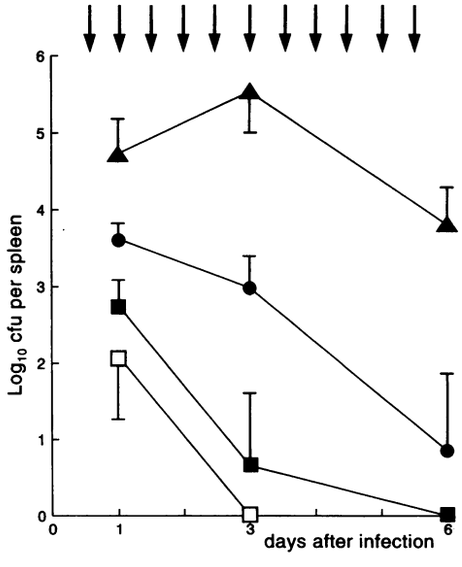

FIG. 7. Therapy of primary infection of immunocompetent mice with Bay Y 3118 (↓). At a dosage of 4 mg given every 12 h (□), there was an eradication from the spleens at day 3. At a dosage of 2 mg given every 12 h (■) and 1 mg given every 12 h (●), listerial numbers in the spleens were reduced significantly at days 1, 3, and 6 in comparison with those in the spleens of the untreated controls (▲).

TABLE 1. Levels of IL-6 and IFN-γ in the sera of mice during primary infection with *L. monocytogenes* EGD

Mouse group[a]	Day postinfection	Concn (pg/ml)[b]			
		IL-6		IFN-γ	
		$\bar{x}$	s	$\bar{x}$	s
Control	1	170	80	1,220	200
	3	750	130	12,000	1,500
	6	110	45	2,700	500
Therapy	1	40	40	100	70
	3	32	40	0	
	6	22	30	0	

[a] Female NMRI mice were infected with 10^4 *L. monocytogenes* EGD. At 6 h after infection, treatment with 2 mg of Bay Y 3118 was started. Each mouse was treated with 2 mg of Bay Y 3118 every 12 h throughout the experiment.
[b] $\bar{x}$, mean of five determinations; s, standard deviation of the mean.

Pathogenic *L. monocytogenes* are able to reside and multiply in phagocytic as well as parenchymal cells, but it is difficult for many common antibiotics to reach these sites because of poor intracellular penetration.

Quinolones represent a forthcoming group of antimicrobial agents (15). They are effective against intracellular bacteria (5, 6). Yet, most of the previously developed members of this group of agents are poorly active against gram-positive bacteria (15). Ciprofloxacin is almost ineffective against infections with *L. monocytogenes* in immunocompromised hosts (20), whereas CI 934 is definitely more active (5). Bay Y 3118 is a new quinolone which is very effective against several gram-positive organisms (2, 3, 12, 19). This may also be demonstrated for *L. monocytogenes*. All strains of *L. monocytogenes* and other *Listeria* spp. were highly susceptible to Bay Y 3118 in vitro. It could be shown that this drug has a marked postantibiotic effect (Fig. 3) against *L. monocytogenes*. Bay Y 3118 is bactericidal for *L. monocytogenes* (Fig. 2). Thus, the in vitro activity of Bay Y 3118 on *L. monocytogenes* is outstanding in comparison with those of most other antimicrobial agents.

Furthermore, the number of *L. monocytogenes* EGD in infected L929 mouse fibroblast-like cells was reduced (Fig. 4 and 5), with Bay Y 3118 having a measurable effect on bacterial multiplication at the minimal effective concentration in tissue culture medium, reducing the listerial numbers beyond the zero level at two times the minimal effective concentration in tissue culture medium and eliminating the bacteria with eight times the minimal effective concentration as early as 5 h after

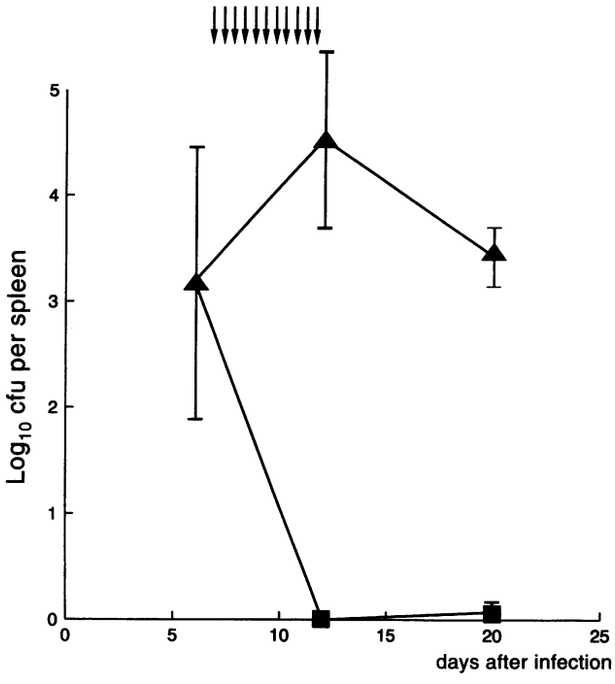

FIG. 9. Therapy of nude mice with Bay Y 3118 (2 mg every 12 h [↓]). The listeriae were rapidly eliminated from the spleens (■). Control mice (▲) were not able to eliminate the bacteria from their spleens.

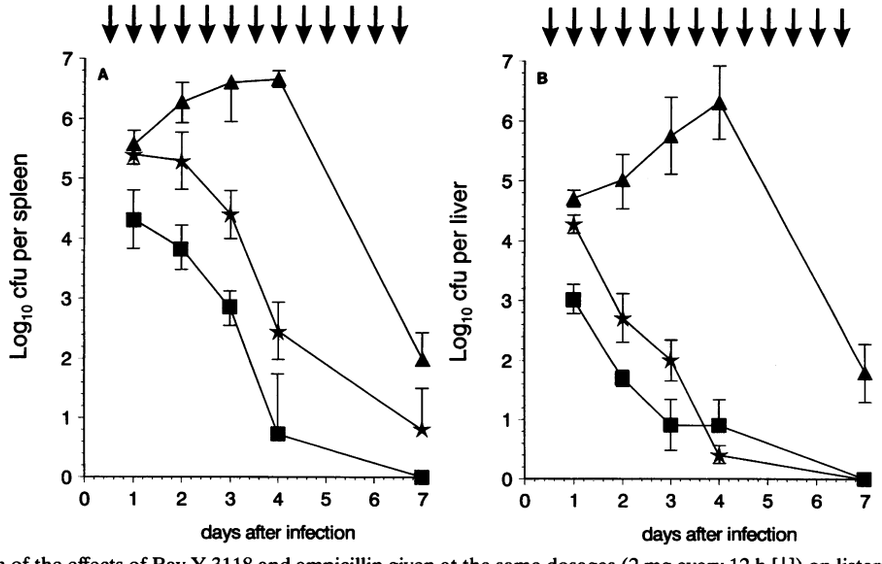

FIG. 8. Comparison of the effects of Bay Y 3118 and ampicillin given at the same dosages (2 mg every 12 h [↓]) on listerial counts in the spleens (A) and livers (B) of immunocompetent mice during primary infection. ■, Bay Y 3118; ★, ampicillin; ▲, untreated controls.

the addition of the antibiotic. This reduction was not due to an effect of Bay Y 3118 or gentamicin on the listeriae in dead cells because there was no detectable toxic effect of Bay Y 3118 on L929 cells, as measured by a commercial neutral red assay (data not shown). In addition, by electron microscopy, the L929 cells appeared morphologically intact, although heavily damaged membrane-enclosed listeriae were found (Fig. 6B). It may be concluded, therefore, that Bay Y 3118 is bactericidal against *L. monocytogenes* EGD in infected L929 cells. We were, however, not able to demonstrate an accumulation of the effective antibiotic within the cytoplasms of L929 cells because at least the effective concentration in tissue culture medium was necessary to reduce the numbers of listeriae in comparison with the numbers of the growth control after 8 h of incubation (Fig. 5). This is comparable to the findings of Rudin et al. (16) for other fluoroquinolones.

When immunocompetent mice infected with *L. monocytogenes* EGD were treated with Bay Y 3118, the bacteria were rapidly eliminated at a dosage of 4 mg every 12 h (Fig. 7). Lower doses significantly reduced the bacterial load of the organs at days 1, 3, and 6 of infection. The effect of Bay Y 3118 at a dosage of 4 mg every 12 h is comparable to the action of the gyrase inhibitor coumermycin, the hitherto most effective substance in the treatment of murine listeriosis (6, 7). When given at the same dosages, Bay Y 3118 was more effective than ampicillin (Fig. 8), which is widely used in the therapy of human listeriosis. At day 1 of infection, treatment with Bay Y 3118 resulted in a significantly greater reduction in the bacterial load than treatment with ampicillin did. Later on, however, there were similar declines in listerial numbers in the organs. One possible explanation for this may be that Bay Y 3118 is more potent in initially eradicating the listeriae from professional phagocytes that take up and kill the listeriae than from nonprofessional phagocytes (e.g., hepatocytes), where the listeriae reside later in infection. This assumption must be proven in appropriate models of infection.

The rapid eradication of the bacteria from the organs of infected mice by Bay Y 3118 agrees well with the rapid decline in the levels of IL-6 and IFN-γ in the sera of mice treated during primary infection (Table 1).

In chronically infected athymic nude mice, a 6-day course of therapy with Bay Y 3118 (2 mg every 12 h) completely eliminated the listeriae from the livers and spleens of the animals (Fig. 9), indicating that no functional T cells need to be present for Bay Y 3118 to be effective. However, small numbers of listeriae reappeared 6 days after the end of therapy. Possibly, the bacteria persist at other sites of the body than in the livers or spleens in spite of therapy. It is also possible that the mice were reinfected from external sources, because the listeriae are excreted in the feces after intravenous infection (unpublished data).

In conclusion, Bay Y 3118 is bactericidal against *L. monocytogenes* in vivo and in vitro. It is a candidate substance for the treatment of infections with facultatively intracellular bacteria in immunocompromised patients.

ACKNOWLEDGMENTS

We thank Irena Kotyllo for typing the manuscript and Wolfgang Meister for drawing the figures.

REFERENCES

1. **Armstrong, D.** 1990. *Listeria monocytogenes*, p. 1587–1593. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill Livingstone, New York.
2. **Bongaerts, G. P. A., and J. A. A. Hoogkamp-Korstanje.** 1993. In vitro activities of Bay Y 3118, ciprofloxacin, ofloxacin, and fleroxacin against gram-positive and gram-negative pathogens from respiratory tract and soft tissue infections. Antimicrob. Agents Chemother. **37**:2017–2019.
3. **Bremm, K. D., U. Petersen, K. G. Metzger, and R. Endermann.** 1992. In vitro evaluation of Bay Y 3118, a new full-spectrum fluoroquinolone. Chemotherapy **38**:376–387.

3a. **Brunner, H., K. D. Bremm, R. Endermann, and K. G. Metzger.** 1992. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 646 and 648.

4. **Fass, R. J.** 1993. In vitro activity of Bay 3118, a new quinolone. Antimicrob. Agents Chemother. **37**:2348–2357.
5. **Hof, H.** 1990. Treatment of experimental listeriosis by CI 934, a new quinolone. J. Antimicrob. Chemother. **25**:121–126.
6. **Hof, H.** 1991. Therapeutic activities of antibiotics in listeriosis. Infection **19**(Suppl. 4):229–233.
7. **Hof, H., and G. Waldenmeier.** 1988. Therapy of experimental listeriosis—an evaluation of different antibiotics. Infection **16**(Suppl. 2):171–174.
8. **Marget, W., and H. P. R. Seeliger.** 1988. *Listeria monocytogenes* infections. Therapeutic possibilities and problems. Infection **16**(Suppl. 2):175–177.
9. **McLauchlin, J., A. Audurier, and A. G. Taylor.** 1991. Treatment failure and recurrent human listeriosis. J. Antimicrob. Chemother. **27**:851–857.
10. **Nichterlein, T., J. Bauer, and H. Hof.** 1992. Clarithromycin is superior to erythromycin in a tissue culture model of infection with *Listeria monocytogenes*. Med. Microbiol. Lett. **1**:85–90.
11. **Nichterlein, T., and H. Hof.** 1991. Effect of various antibiotics on *Listeria monocytogenes* multiplying in L 929 cells. Infection **19**(Suppl. 4):234–238.
12. **Nord, C. E., A. Lindmark, and I. Persson.** 1993. In vitro activity of the new quinolone Bay Y 3118 against anaerobic bacteria. Eur. J. Clin. Microbiol. Infect. Dis. **12**:640–642.
13. **Peterson, L. R., and C. J. Shanholtzer.** 1992. Tests for bactericidal effects of antimicrobial agents: technical performance and clinical relevance. Clin. Microbiol. Rev. **5**:420–432.
14. **Portnoy, D. A.** 1992. Innate immunity to a facultative intracellular bacterial pathogen. Curr. Opin. Immunol. **4**:20–24.
15. **Rubinstein, E., and C. Carbon.** 1992. The fluoroquinolones—reappraisal. Int. J. Antimicrob. Agents **1**:147–152.
16. **Rudin, D. E., P. X. Gao, C. X. Cao, H. C. Neu, and S. C. Silverstein.** 1992. Gemfibrozil enhances the listeriacidal effects of fluoroquinolone antibiotics in J774 macrophages. J. Exp. Med. **176**:1439–1447.
17. **Sahm, D. F., and J. A. Washington.** 1991. Antibacterial susceptibility tests: dilution methods, p. 1105–1116. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.). Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
18. **Skogberg, K., J. Syrjänen, M. Jahkola, O. V. Renkonen, J. Paavonen, J. Ahonen, S. Kontiainen, P. Ruutu, and V. Valtonen.** 1992. Clinical presentation and outcome of listeriosis in patients with and without immunosuppressive therapy. Clin. Infect. Dis. **14**:815–821.
19. **Wexler, H. M., E. Molitoris, and S. M. Finegold.** 1993. In vitro activity of Bay Y 3118 against anaerobic bacteria. Antimicrob. Agents Chemother. **37**:2509–2513.
20. **Van Ogtrop, M. L., H. Mattie, B. Razab Sekh, E. van Strijen, and R. van Furth.** 1992. Comparison of the antibacterial efficacies of ampicillin and ciprofloxacin against experimental infections with *Listeria monocytogenes* in hydrocortisone-treated mice. Antimicrob. Agents Chemother. **36**:2375–2380.