ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1991, p. 1785–1790
0066-4804/91/091785-06$02.00/0

Vol. 35, No. 9

Failure of β-Lactam Antibiotics and Marked Efficacy of Fluoroquinolones in Treatment of Murine *Yersinia pseudotuberculosis* Infection

BERNARD C. LEMAITRE,[1,2] DANIEL A. MAZIGH,[1] AND MAURICE R. SCAVIZZI[2]*

Unite d'Ecologie Bactérienne, Institut Pasteur, 75724 Paris Cedex 15,[1] and Service de Bactério-virologie, Centre Hospitalier Universitaire Avicenne, Université Paris-Nord, 93009 Bobigny Cedex,[2] France

Received 5 September 1990/Accepted 19 June 1991

The treatment of yersiniosis by β-lactams is questionable considering the proven failure of newer β-lactams for treating murine *Yersinia enterocolitica* infection. Another modality of experimental treatment was performed with a virulent strain of *Y. pseudotuberculosis* (nonproducer of β-lactamase) highly susceptible (in terms of MICs) to amoxicillin, cefotaxime, ceftriaxone, imipenem, doxycycline, gentamicin, and ofloxacin. The in vivo comparative efficacy of these drugs was evaluated in a standardized and reproducible mouse model of systemic infection. Each single antibiotic was injected intravenously once, at 30 h after intravenous inoculation of the infective strain, and then repeatedly (at 30, 52, and 76 h postinfection). In vivo results were measured by counting the viable bacteria recovered from the whole spleens of mice sacrificed at selected times. Cefotaxime, even at high doses (250 mg/kg of body weight), was totally ineffective. Amoxicillin and imipenem at high doses (200 and 100 mg/kg, respectively) and ceftriaxone at usual doses (20 mg/kg) were active only in stopping bacterial proliferation to a more or less slight degree. Ceftriaxone was able to reduce viable counts in the spleen only at high doses (200 mg/kg), as were gentamicin (20 mg/kg) and doxycycline (125 mg/kg). Ofloxacin at the low dose of 5 mg/kg was demonstrated to be very effective by the very significant decrease observed in bacterial numbers from 10^6 to 10^3 CFU per spleen. The pharmacological parameters do not in themselves explain all the discrepancies between the in vitro and in vivo activities of β-lactams on yersiniae. No emergence of beta-lactam-resistant organisms, which could explain the failure of β-lactams, was detected. Thus, their use should be delayed in the therapy of human yersinosis until further investigations are carried out. The fluoroquinolone appeared more active and rapid than reference drugs in the treatment of murine yersinosis, which confirms initial clinical results.

The genus *Yersinia* includes three species which are pathogenic for humans. *Yersinia pestis* is the agent of the plague. *Y. enterocolitica* and *Y. pseudotuberculosis* cause intestinal and extraintestinal syndromes of various severities, ranging from mild gastroenteritis to mesenteric lymphadenitis and septicemia.

For yersiniosis, antibiotic treatment recommendations from the World Health Organization include tetracycline, chloramphenicol, gentamicin, and cotrimoxazole (43). Newer β-lactam antibiotics were suggested as alternatives in severe *Yersinia* infections (20, 21, 32, 37). However, an animal model showed that the treatment of mice infected by *Y. enterocolitica* failed with the newer β-lactams despite in vitro susceptibility of the strain (31). Therefore, we checked the in vivo efficacy of old and newer β-lactams and other in vitro active antibiotics (e.g., fluoroquinolones) against *Y. pseudotuberculosis* (a β-lactamase nonproducer), in a reproducible mouse model mimicking natural systemic infection (3, 35).

MATERIALS AND METHODS

Bacterial strain. *Y. pseudotuberculosis* IP 2637, serovar I, was used, harboring both a 47-MDa virulence-associated plasmid and a further cryptic one of 60 MDa (34). This strain was isolated at Necker Hospital, Paris, France. It was grown regularly on tryptocasein-soy-agar (TCS; Diagnostics Pasteur, Marnes-la-coquette, France) overnight at 25°C from stock cultures frozen at −70°C in 50% glycerol. The various phenotypic characteristics associated with the presence of the 47-MDa virulence plasmid were checked, i.e., Ca^{2+} dependence, autoagglutination, and released proteins and YOPs, visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by a method based on that of Heesemann et al. (19).

Infection of mice. We used Swiss pathogen-free female mice, aged 5 weeks and weighing 20 ± 2 g, purchased from IFFA CREDO, Lyon, France. The mice were injected intravenously (i.v.) with bacterial suspensions in sterile nonpyrogenic saline at two standardized doses, 10^6 and 10^3 CFU; both of these ensure a fatal systemic infection, but there is more exponential kinetic growth at 10^3 CFU.

Antibiotics. Seven antibiotics of known potency used in this study were kindly supplied by the manufacturers (or their subsidiary agents in France): amoxicillin (Beecham, Paris, France), gentamicin (Unilabo, Levallois-Perret, France), doxycycline (Pfizer, Paris, France), cefotaxime (Roussel, Romainville, France), ceftriaxone (Roche, Neuilly-sur-Seine, France), imipenem-cilastatin (Merck, Paris, France), and ofloxacin (Diamant, Puteaux, France).

In vitro susceptibility tests. The MICs and MBCs were determined by the broth microdilution method as previously described (20). The final antibiotic concentration ranged from 0.016 to 32 mg/liter. The inoculum was prepared from overnight Mueller-Hinton broth culture at 37°C and diluted so that final sizes of approximately 5×10^4, 5×10^5, and 5×10^6 CFU/ml were obtained to determine MICs and to look for a possible inoculum effect; 5×10^6 CFU/ml was used to

* Corresponding author.

determine MBCs. The microdilution trays were incubated for 24 h at 37°C. The MIC was defined as the lowest concentration of drug that prevented visible growth. The MBC was defined as the lowest concentration of drug that permitted at least 99.9% (10^3-fold) reduction in the number of CFU. At the same time, a strain of *Escherichia coli* (ATCC 25922) was used for quality control with each antibiotic in each experiment.

β-Lactamase production was tested by the iodometric spot method on sonicated bacteria, as previously described (20).

In vivo antibiotic experiments. In the first series of in vivo experiments, we used the same model as described previously with *Y. enterocolitica* (31). In fact, these experiments were performed as a screening of the in vivo antibiotic activity. In a second series of experiments, we used a lower inoculum and repeated antibiotic injections to follow up the activity of the drug during the infection. Thirty mice in each treatment group and each nontreated group (control) were inoculated, with five being sacrificed at each of the five time points and five observed at the end of each experiment.

Antibiotic solutions were prepared as recommended by the manufacturers. Their in vitro activity was immediately verified, and they were injected rapidly (bolus) i.v. into mice (7, 8, 25, 30). In the first series of experiments, the solutions were injected 30 h after i.v. injection of the 10^6 CFU bacterial inoculum; in the second series of experiments they were injected at 30, 52, and 76 h after i.v. injection of the 10^3 CFU inoculum. The times of injection of the drug were selected from the growth kinetics of the strain *Y. pseudotuberculosis* IP 2637 in control mice; 30 h corresponded approximately to the mid-exponential phase. The doses of drugs were as follows: doxycycline (13), 125 mg/kg of body weight (injected slowly to save the mice); gentamicin (28), 20 mg/kg; amoxicillin (1, 6), 200 mg/kg; cefotaxime (2, 23, 26), 20, 200, and 250 mg/kg; imipenem (23, 27), 100 mg/kg; ceftriaxone (4, 29), 20 and 200 mg/kg; and ofloxacin (33), 20 and 5 mg/kg.

These doses were selected first of all in accordance with previously reported data: in the above-mentioned studies, the doses were chosen to relate as closely as possible to the conventional doses used in humans. Other doses used here were modified by us; we increased the doses of ineffective antibiotics (β-lactams) and lowered those of the most effective one (ofloxacin). All these doses are expected to provide suitable concentrations of drug in mice because the previous works indicate the following: (i) a 200-mg/kg dose of amoxicillin injected subcutaneously (s.c.) produces both a peak level in serum of 80 mg/liter and a concentration in serum remaining above the MBC for our tested bacterial strain for at least 3 h (1, 6); (ii) with a 10-mg/kg dose of gentamicin injected s.c., these values are 9 mg/liter and >1.5 h, respectively (6, 28); (iii) with a 50-mg/kg dose of doxycycline injected i.v., the values are 50 mg/liter and >2.5 h, respectively (9); (iv) with a 180-mg/kg dose of cefotaxime injected s.c., the values are 80 mg/liter and 3 h, respectively (14); (v) with a 180-mg/kg dose of ceftriaxone injected s.c., the values are 150 mg/liter and 8.5 h, respectively (14); (vi) with a 70-mg/kg dose of imipenem injected s.c., the values are 19 mg/liter and >6 h, respectively (26a); (vii) no data are available for ofloxacin.

The doses will be named high, usual, or low below, in accordance with the efficacy of doses used in previous experiments. Each experiment was repeated once.

Bacterial counts and results. The spleens of mice were aseptically removed and homogenized. Viable bacteria recovered from spleen homogenates were counted as CFU on TCS incubated for 18 h at 25°C as described previously (35). Data were obtained for the whole spleens without regard to the size of this target organ. Serial 10-fold dilutions of each spleen homogenate were plated on agar. The times selected for in vivo bacterial growth measurement were based upon preliminary experiments that showed the growth kinetics of the strain IP 2637 in the control mice. In the two series of experiments, bacteria were counted at 3, 6, 30, 54, and 78 h postinfection, i.e., for the second series, 2 h after i.v. injections of drugs, which is three or four times the half-life of the antibiotics tested, except for ceftriaxone (twice) and doxycycline (once) (1, 9, 11a, 14, 22, 26a). The efficacy of each antibiotic was quantified from comparative bacterial counts in the spleens of five treated mice and five nontreated controls for each point. While the spleens were being removed, blood samples were collected by cardiac puncture and incubated on TCS in the same way.

Detection of high concentrations of drugs in the spleen. The strain we inoculated into mice was seeded into TCS; then aliquots of spleen homogenate, removed from treated mice at time points 54 and 78 h, were poured into 0.1-ml wells bored into the agar; lastly, the plates were incubated for 24 h at 37°C.

Detection of antibiotic-resistant bacteria. Whole-spleen homogenates were plated on antibiotic concentration gradients (from 0 to 500 mg/liter) in agar (38) for each antibiotic treatment at the end of each experiment; the plates were incubated for 24 h at 37°C.

Statistical methods. Using the Student *t* test, we calculated the geometric mean and the confidence interval of the mean from the data obtained for each group of five mice at each time point. In vivo growth curves illustrate these results (Fig. 1 and 2). The significance of the difference between the results obtained from control and treated mice was evaluated at each time point.

RESULTS

In vitro susceptibility tests. Taking into account the MICs, all the drugs were active, and no inoculum effect was observed from 10^4 to 10^6 CFU/ml (Table 1). The difference between MBC and MIC expressed in $\log_2$ was equal to 0 or 1 for all drugs except the bacteriostatic drug doxycycline (6 $\log_2$). The iodometric spot test confirmed that the strain was not a β-lactamase producer.

Detection of antibiotic amounts in spleens. No bacteriostatic effect of spleen homogenates was observed on the plates, except with doxycycline. Moreover, numbers of CFU were multiples of 10 in each bacterial count except for doxycycline (drug effect on first plate). Therefore, we can conclude that no remaining antibiotic concentration in tissue had much influence on our results.

In vivo antibiotic activities. *Y. pseudotuberculosis* IP 2637 at 10^6 and 10^3 CFU was highly virulent for the Swiss mice. As illustrated in Fig. 1 and 2, the bacterial growth curves in the spleens of control mice showed an exponential phase, reaching a plateau at about 54 h and leading to the death of the mice between 80 and 96 h after a real septicemia. The kinetics of bacterial growth (<200 CFU/ml at 54 h) in the blood were judged unsuitable for demonstrating the effects of antibiotic therapy.

In the first series of experiments performed with a heavy inoculum (10^6 CFU), a single i.v. injection of a high dose of doxycycline (125 mg/kg) was effective in reducing viable counts in the spleen; a single normal dose of gentamicin (20

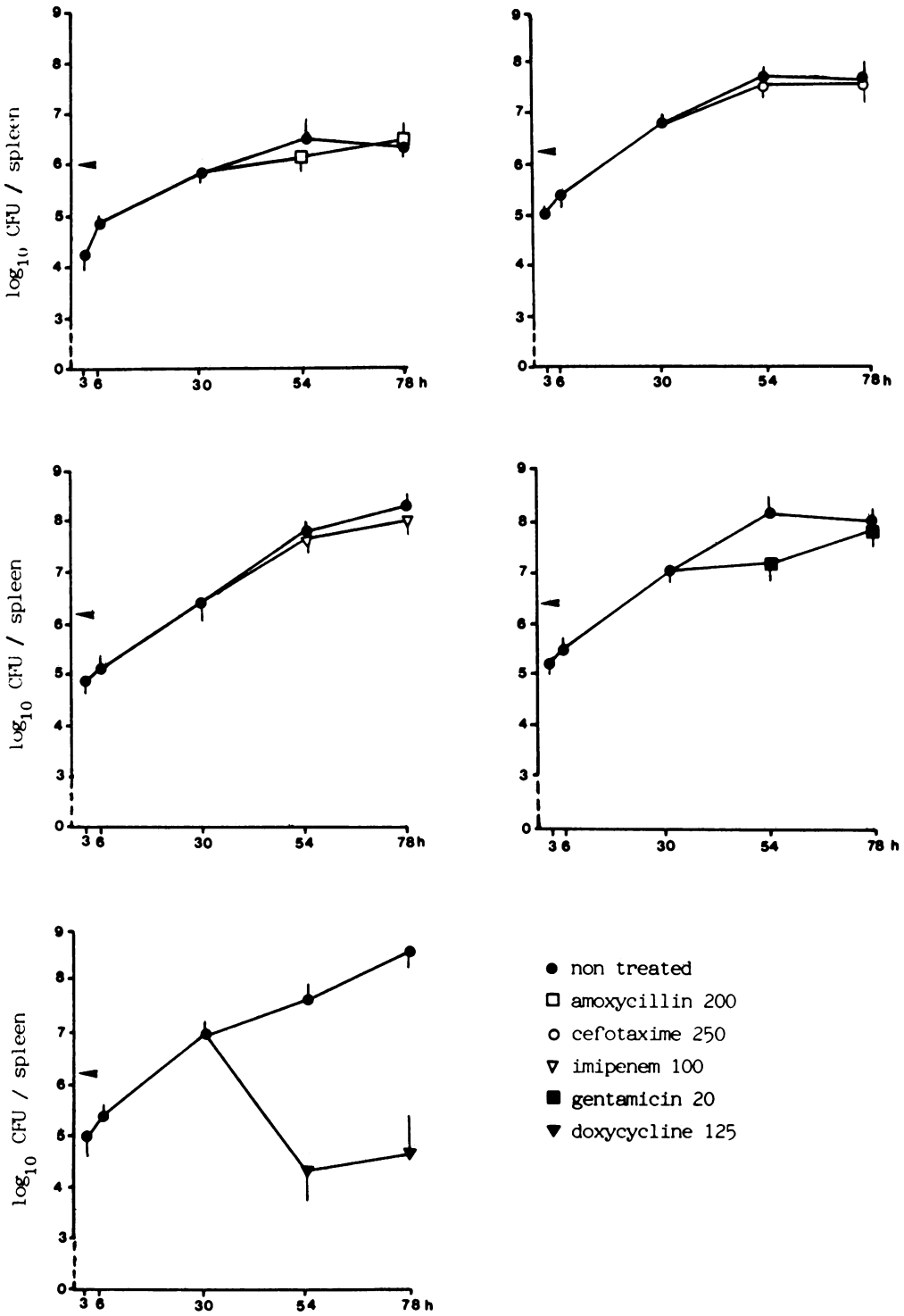

FIG. 1. Effect of one antibiotic injection (30 h) on *Y. pseudotuberculosis* infection. Kinetic growth survival of *Y. pseudotuberculosis* IP 2637 in the spleens of mice infected i.v. with a standardized inoculum (see the arrow on the vertical axis). Each point represents the geometric mean of bacterial enumeration, after spleen homogenization, from five mice either treated or not treated (control). The vertical bars indicate half of the confidence interval of the mean, *t* SEM (Student *t* test and standard error of the mean), with a 5% risk of error. The antibiotic doses are expressed in milligrams per kilogram.

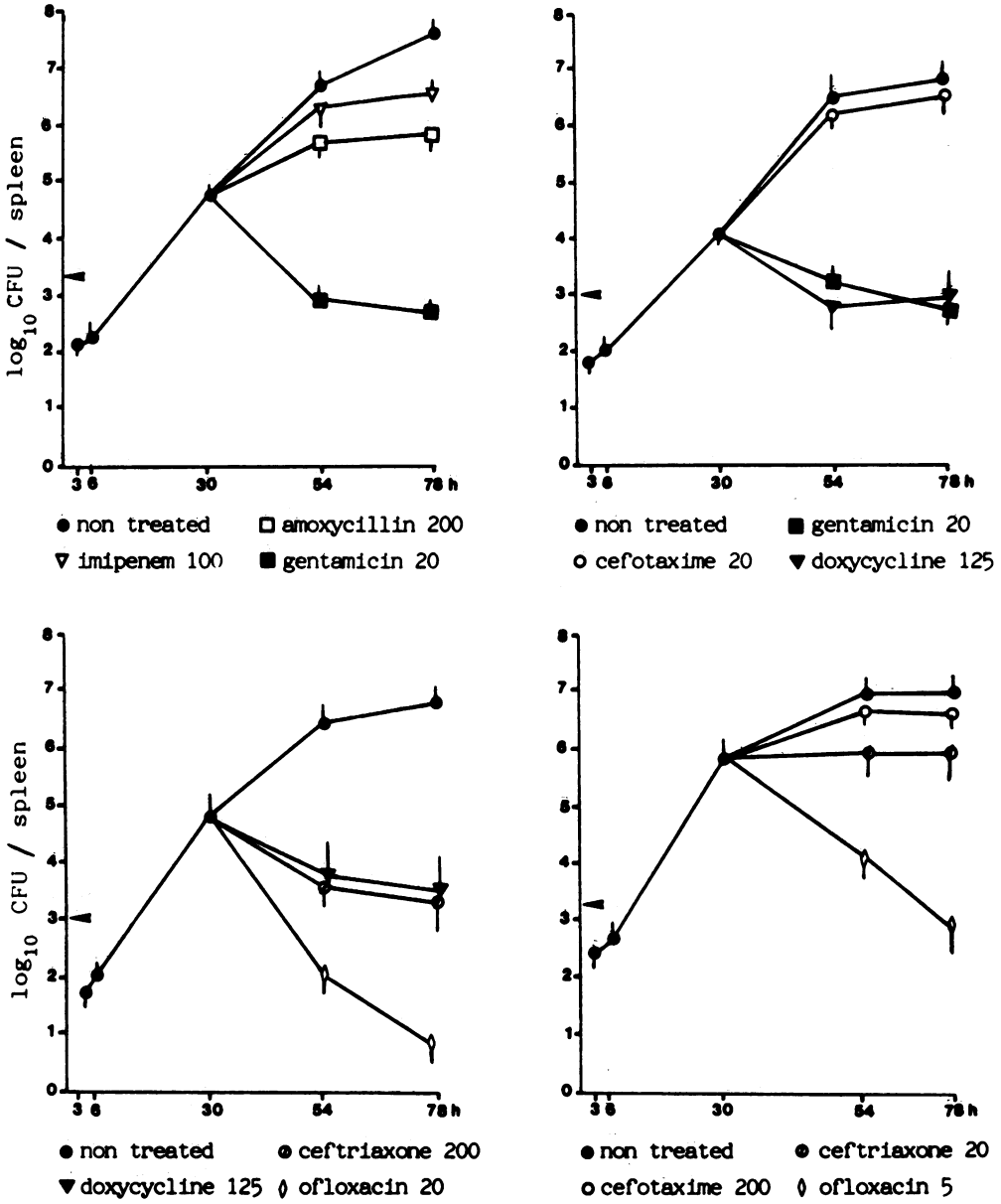

FIG. 2. Effect of repeated antibiotic injections (30, 52, and 76 h) on *Y. pseudotuberculosis* infection. Kinetic growth survival of *Y. pseudotuberculosis* IP 2637 in the spleens of mice infected i.v. with a standardized inoculum (see the arrow on the vertical axis). Each point represents the geometric mean of bacterial enumeration, after spleen homogenization, from five mice either treated or not treated (control). The vertical bars indicate half of the confidence interval of the mean, *t* SEM (Student *t* test and standard error of the mean), with a 5% risk of error. The antibiotic doses are expressed in milligrams per kilogram.

mg/kg) appeared temporarily effective, but high doses of amoxicillin (200 mg/kg), cefotaxime (250 mg/kg), and imipenem (100 mg/kg) were not effective. Therefore, with a high inoculum of a really virulent strain, none of the agents appeared to have a very potent antibacterial effect (Fig. 1). In contrast, with a low inoculum, some of the agents had a pronounced effect (Fig. 2).

In the second series of experiments carried out with an inoculum of 10^3 CFU and repeated antibiotic injections, gentamicin (20 mg/kg) and doxycycline (125 mg/kg) were effective in reducing viable bacterial counts ($P < 0.005$ for each point on the growth curve). Among the β-lactams we tested, amoxicillin (200 mg/kg) reduced bacterial develop-

TABLE 1. In vitro activity of seven antibiotics against *Y. pseudotuberculosis* IP 2637

Antibiotic	MIC[a]	MBC[a]
Amoxicillin	0.06	0.12
Doxycycline	0.12	8
Cefotaxime	0.015	0.03
Ceftriaxone	0.015	0.03
Imipenem	0.06	0.06
Ofloxacin	0.03	0.06
Gentamicin	0.12	0.25

[a] In milligrams per liter, with inoculum (in CFU per milliliter) of 10^4, 10^5, and 10^6 for MICs and 10^6 for MCBs, at 37°C.

ment in the spleen compared with its effect in the nontreated mice. Imipenem (100 mg/kg) also showed a significant difference between the treated and the nontreated mice, but only at 78 h. Cefotaxime was inactive even with a high dose of 200 mg/kg. Ceftriaxone growth curves were different from those for the nontreated mice: when we used high doses of 200 mg/kg, the results were comparable to those of doxycycline, but with usual doses of 20 mg/kg, there was only a slight effect. The greatest efficacy was observed with ofloxacin treatment (20 or 5 mg/kg, which is a low dose). The decrease in the bacterial count in the spleen obtained with the fluoroquinolone (from 10^5 to $\leq 10^1$ CFU per spleen) was always superior to the decrease obtained with other treatments.

The aim of this study was not to compare mortality rates; however, we noticed that our results for mortality rate were in agreement with bacterial counts. Remembering that experiments were repeated once and that five mice per group were observed after treatment, the results are as follows: (i) 10 of 10 mice died between 80 and 96 h with ineffective (or slightly active) drugs such as amoxicillin, imipenem, or cefotaxime at 20 mg/kg; (ii) no mice died with effective drugs such as ofloxacin, gentamicin, doxycycline, or ceftriaxone at 200 mg/kg; and (iii) the results with cefotaxime (200 mg/kg) and ceftriaxone (20 mg/kg) were mixed: 7 of 10 and 5 of 10 mice died, respectively.

Detection of antibiotic-resistant mutants. With unsuccessful treatments, no resistant mutant that might have been selected by therapy was detected on the antibiotic gradient concentration agar plate among bacteria recovered from total-spleen homogenates (38).

DISCUSSION

The *Y. pseudotuberculosis* experimental mouse infection model reproduced each lesional stage that is generally observed in human infection. Rapid bacterial contact with and penetration into phagocytes were made possible by the nonnatural i.v. injection. As previously described (3, 35), *Y. enterocolitica* and *Y. pseudotuberculosis* (facultative intracellular parasites) inoculated i.v. into mice rapidly colonized the spleen, multiplied with inflammatory granuloma formation, and then reached the blood, causing septicemia prior to death.

Two types of experiments are presented here: one used a 10^6 CFU inoculum and the other used an inoculum 10^3-fold lower, both leading to the same bacterial burden of approximately 10^7 to 10^8 CFU per spleen. In the experiments with a heavy inoculum, no antimicrobial agent, except doxycycline, produced a clear-cut result. One can argue that this was due to an inoculum effect. However, this cannot in itself explain the failure of the drug; there are many reasons why the effect of a drug on a highly complex process of infection might be much more evident at a low than a high inoculum (11, 36).

Here, the β-lactam treatments administered at usual doses (20 mg/kg) (2, 4, 26, 27, 29) demonstrated less efficacy than classic treatments such as gentamicin or doxycycline. Therefore, a treatment can be considered really successful when administration of the drug leads to a decrease in the growth kinetics curves and not merely to a significant difference between treated and nontreated groups of mice.

As discussed in a previous paper (31) for *Y. enterocolitica*, the failure of β-lactams in the murine systemic infection model cannot be explained by the selection of β-lactam-resistant mutants during the treatment or, at least for the moment, the influence of local host factors.

Pathogenic yersiniae are mostly intracellular parasites; the success of the treatment could depend on the intracellular penetration of the drug, so the activity of doxycycline, even though it is a bacteriostatic antibiotic, might be due to its cellular penetration. Furthermore, fluoroquinolones have a good intracellular diffusion (12, 39, 41, 42): ofloxacin was the most effective antibiotic in this study even at low doses of 5 mg/kg.

After all, β-lactams are largely excluded from cells (15–18, 39) and may be ineffective against intracellular bacterial cells; even imipenem, which binds rapidly with leukocytes, may be ineffective because this cell-associated drug declines progressively with time (15–17); what is more, the molecule is unstable. Therefore, the result observed in the present work makes it clear that the pharmacological parameters of antibiotics affect their in vivo efficacy.

However, imipenem, cefotaxime, and other β-lactams are active at their usual doses on murine systemic infections due to other bacterial species which replicate intracellularly (23, 26, 27); gentamicin and other aminoglycosides are accumulated slowly into cells in an active form (10, 39, 40); and ceftriaxone penetrates cells reasonably well (5, 24), but despite its excellent in vitro activity, 10 times more ceftriaxone (200 mg/kg) than gentamicin is needed to obtain the same in vivo efficacy. Therefore, the pharmacological parameters do not in themselves explain all the discrepancies between the in vitro and in vivo activities of β-lactams on yersiniae. Thus, phenotypic variations of pathogenic yersiniae, expressed mainly in vivo (19), could be involved in this failure of β-lactams.

Finally, we have studied only a single isolate of *Y. pseudotuberculosis*. Even though the IP 2637 strain has a phenotype representative of prominent human isolates, we cannot determine that all strains of *Y. pseudotuberculosis* are resistant in vivo or slightly susceptible to chemotherapy with beta-lactams (amoxicillin, cefotaxime, ceftriaxone, and imipenem-cilastatin). However, on the basis of this study, it would be wise to delay the use of beta-lactam antibiotics in human yersinosis until further investigations are carried out.

However, the success of ofloxacin for murine yersinosis, due to *Y. pseudotuberculosis*, confirms preliminary clinical results obtained with fluoroquinolones (14a).

ACKNOWLEDGMENTS

We thank J. M. Alonso for stimulating discussion; C. Mouché for typing the manuscript, composing the table, and drawing the figures; and J. C. Barber for supervising the translation.

This study was supported in part by grants from the Syndicat National des Médecins des Hôpitaux (SNAM–Fonds de Recherche) and the Direction de la Recherche et des Etudes Doctorales (DRED).

REFERENCES

1. **Acred, P., P. A. Hunter, L. Mizen, and G. N. Rolinson.** 1971. α-Amino-*p*-hydroxybenzylpenicillin (BRL 2333), a new broad-spectrum semisynthetic penicillin: in vivo evaluation, p. 416–422. Antimicrob. Agents Chemother. 1970.
2. **Actor, P., and S. F. Grappel.** 1982. The evaluation of cephalosporins in animal models of infection, p. 137–146. *In* H. C. Neu (ed.), New β-lactam antibiotics: a review from chemistry to clinical efficacy of the new cephalosporins. Proceedings of the Cephalosporins Symposium of the American College of Clinical Pharmacology. Francis Clark Wood Institute for the History of Medicine, Philadelphia.
3. **Alonso, J.-M., D. Mazigh, H. Bercovier, and H. H. Mollaret.** 1978. Infection experimentale de la souris par *Yersinia enterocolitica* (souche du chimiotype 4, du sérogroupe O:3, du lysotype VIII): devenir de l'inoculum chez des souris athymiques ou traitées par le cyclophosphamide. Ann. Microbiol. (Paris). **129B:**27–36.

4. **Angehrn, P., P. Probst, R. Reiner, and R. L. Then.** 1980. Ro 13-9904, a long-acting broad-spectrum cephalosporin: in vitro and in vivo studies. Antimicrob. Agents Chemother. **18**:913–921.
5. **Auvergnat, J. C., J. Lemozy, F. Lokiec, and M. Pecking.** 1986. Diffusion lymphatique et ganglionnaire de la ceftriaxone. Incidence dans le traitement des fièvres thyphoides. Pathol. Biol. **34**:508–509.
6. **Beale, A. S., J. Gisby, and R. Sutherland.** 1988. Efficacy of amoxycillin/clavulanic acid in experimental *Bacteroides fragilis*/*Escherichia coli* mixed infections. J. Antimicrob. Chemother. **21**:451–459.
7. **Bergan, T.** 1981. Pharmacokinetics of tissue penetration of antibiotics. Rev. Infect. Dis. **3**:45–66.
8. **Bergeron, M. G., D. Beauchamp, A. Poirier, and A. Bastille.** 1981. Continuous vs. intermittent administration of antimicrobial agents: tissue penetration and efficacy in vivo. Rev. Infect. Dis. **3**:84–97.
9. **Böcker, R., and C.-J. Estler.** 1981. Comparison of distribution of doxycycline in mice after oral and intravenous application measured by a high-performance liquid chromatographic method. Drug Res. **31 (II)**:2116–2117.
10. **Bonventre, P. F., and J. G. Imhoff.** 1970. Uptake of ^{3}H-dihydrostreptomycin by macrophages in culture. Infect. Immun. **2**:89–95.
11. **Brook, I.** 1989. Inoculum effect. Rev. Infect. Dis. **11**:361–368.
11a. **Diamant Laboratories.** Unpublished data.
12. **Easmon, C. S. F., J. P. Crane, and A. Blowers.** 1986. Effect of ciprofloxacin on intracellular organisms: in vitro and in vivo studies. J. Antimicrob. Chemother. **18**(Suppl. D):43–48.
13. **English, A. R., and J. E. Lynch.** 1967. α-6-Deoxyoxytetracycline. II. Activity in chemotherapeutic studies in the mouse. Proc. Soc. Exp. Biol. Med. **124**:586–591.
14. **Frimodt-Møller, N., M. W. Bentzon, and V. F. Thomsen.** 1986. Experimental infection with *Streptococcus pneumoniae* in mice: correlation of in vitro activity and pharmacokinetic parameters with in vivo effect for 14 cephalosporins. J. Infect. Dis. **154**:511–517.
14a. **Gayraud, M., and M. Scavizzi.** Unpublished data.
15. **Hand, W. L., and N. L. King-Thompson.** 1989. The entry of antibiotics into human monocytes. J. Antimicrob. Chemother. **23**:681–689.
16. **Hand, W. L., and N. L. King-Thompson.** 1990. Uptake of antibiotics by human polymorphonuclear leukocyte cytoplasts. Antimicrob. Agents Chemother. **34**:1189–1193.
17. **Hand, W. L., N. King-Thompson, and J. W. Holman.** 1987. Entry of roxithromycin (RU 965), imipenem, cefotaxime, trimethoprim, and metronidazole into human polymorphonuclear leukocytes. Antimicrob. Agents Chemother. **31**:1553–1557.
18. **Hand, W. L., N. L. King-Thompson, and T. H. Steinberg.** 1983. Interactions of antibiotics and phagocytes. J. Antimicrob. Chemother. **12**(Suppl. C):1–11.
19. **Heesemann, J., U. Gross, N. Schmidt, and R. Laufs.** 1986. Immunochemical analysis of plasmid-encoded proteins released by enteropathogenic *Yersinia* sp. grown in calcium-deficient media. Infect. Immun. **54**:561–567.
20. **Hornstein, M. J., A. M. Jupeau, M. R. Scavizzi, A. M. Philippon, and P. A. D. Grimont.** 1985. In vitro susceptibilities of 126 clinical isolates of *Yersinia enterocolitica* to 21 β-lactam antibiotics. Antimicrob. Agents Chemother. **27**:806–811.
21. **Jupeau, A., M. Hornstein, A. Philippon, and M. Scavizzi.** 1982. Sensibilité in vitro de *Yersinia* aux antibiotiques. Activité des béta-lactamines sur *Yersinia enterocolitica*. Med. Mal. Infect. **12**:675–681.
22. **Kallay, Z., T. Trnovec, M. Keitner, T. Macicková, J. Navarová, and M. Greguskovä.** 1982. Kinetics of gentamicin accumulation in subcellular structures of the mouse kidney. J. Pharm. Pharmacol. **34**:276–277.
23. **Kropp, H., J. G. Sundelof, J. S. Kahan, F. M. Kahan, and J. Birnbaum.** 1980. MK 0787 (*N*-formimidoyl thienamicin): evaluation of in vitro and in vivo activities. Antimicrob. Agents Chemother. **17**:993–1000.
24. **Kuhn, H., P. Angehrn, and L. Havas.** 1986. Autoradiographic evidence for penetration of ^{3}H-ceftriaxone (Rocephin*) into cells of spleen, liver and kidney of mice. Chemotherapy **32**:102–112.
25. **Lavoie, G. Y., and M. G. Bergeron.** 1985. Influence of four modes of administration on the penetration of aztreonam, cefuroxime, and ampicillin into interstitial fluid and fibrin clots and on in vivo efficacy against *Haemophilus influenzae*. Antimicrob. Agents Chemother. **28**:404–412.
26. **Marre, R., and K. Sack.** 1979. Animal experiments on therapeutic efficacy in relation to pharmacokinetics and on renal tolerance of cefamandole, cefuroxime, cefoxitin and HR 756. Drugs Exp. Clin. Res. **5**:31–35.
26a. **Merck Laboratories.** Unpublished data.
27. **Mitsuhashi, S.** 1983. In vitro and in vivo antibacterial activity of imipenem against clinical isolates of bacteria. J. Antimicrob. Chemother. **12**(Suppl. D):53–64.
28. **Nicas, T. I., and L. E. Bryan.** 1978. Relationship between gentamicin susceptibility criteria and therapeutic serum levels for *Pseudomonas aeruginosa* in a mouse infection model. Antimicrob. Agents Chemother. **13**:796–801.
29. **Reiner, R., U. Weiss, U. Brombacher, P. Lanz, M. Montavon, A. Furlenmeier, P. Angehrn, and P. J. Probst.** 1980. Ro 13-9904/001, a novel potent and long-acting parenteral cephalosporin. J. Antibiot. **33**:783–786.
30. **Renneberg, J., and M. Walder.** 1988. A mouse model for simultaneous pharmacokinetic and efficacy studies of antibiotics at sites of infection. J. Antimicrob. Chemother. **22**:51–60.
31. **Scavizzi, M. R., J. M. Alonso, A. M. Philippon, A. M. Jupeau-Vessieres, and A. Guiyoule.** 1987. Failure of newer beta-lactam for murine *Yersinia enterocolitica* infection. Antimicrob. Agents Chemother. **31**:523–526.
32. **Scribner, R. K., M. I. Marks, A. Weber, and C. H. Pai.** 1982. *Yersinia enterocolitica*: comparative in vitro activities of seven new β-lactam antibiotics. Antimicrob. Agents Chemother. **22**:140–141.
33. **Sesnie, J. C., P. W. Fritsch, T. J. Griffin, C. L. Heifetz, E. T. Leopold, T. E. Malta, M. A. Shapiro, and P. W. Vincent.** 1989. Comparative chemotherapeutic activity of new fluorinated 4-quinolones and standard agents against a variety of bacteria in a mouse infection model. J. Antimicrob. Chemother. **23**:729–736.
34. **Simonet, M., P. Berche, and D. Mazigh.** 1983. Une septicémie due à une souche hautement virulente de *Yersinia pseudotuberculosis*. Med. Mal. Infect. **13**:767–771.
35. **Simonet, M., D. Mazigh, and P. Berche.** 1984. Growth of *Yersinia pseudotuberculosis* in mouse spleen despite loss of a virulence plasmid of mol. wt. 47×10^6. J. Med. Microbiol. **18**:371–375.
36. **Soriano, F., C. Ponte, M. Santamaria, and M. Jimenez-Arriero.** 1990. Relevance of the inoculum effect of antibiotics in the outcome of experimental infections caused by *Escherichia coli*. J. Antimicrob. Chemother. **25**:621–627.
37. **Soriano, F., and J. Vega.** 1982. The susceptibility of *Yersinia* to eleven antimicrobials. J. Antimicrob. Chemother. **10**:543–547.
38. **Szybalski, W., and V. Bryson.** 1952. Genetic studies on microbial cross-resistance to toxic agents. I. Cross-resistance of *Escherichia coli* to 15 antibiotics. J. Bacteriol. **64**:489–499.
39. **Tulkens, P. M.** 1990. Intracellular pharmacokinetics and activity of antibiotics, p. 243–259. *In* H. C. Neu (ed.), Frontiers of infectious diseases: new antibacterial strategies. Churchill Livingstone/Longman Group, Edinburgh.
40. **Tulkens, P. M., and A. Trouet.** 1978. The uptake and intracellular accumulation of aminoglycoside antibiotics in lysosomes of cultured rat fibroblasts. Biochem. Pharmacol. **27**:415–424.
41. **Une, T., and Y. Osada.** 1988. Penetrability of ofloxacin into cultured epithelial cells and macrophages. Drug. Res. **38(II)**:1265–1267.
42. **Van der Auwera, P., T. Matsumoto, and M. Husson.** 1988. Intraphagocytic penetration of antibiotics. J. Antimicrob. Chemother. **22**:185–192.
43. **World Health Organization.** 1983. Yersiniosis. Euroreports and studies no. 60. Report of a workshop, 1982. Regional Board of Europe, Copenhagen. World Health Organization, Geneva.