ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1982, p. 312-315
0066-4804/82/080312-04$02.00/0

Vol. 22, No. 2

Comparative Efficacies of Ceftriaxone, Moxalactam, and Ampicillin in Experimental *Salmonella typhimurium* Infection

P. A. ANTON,[1] J. A. KEMP,[1] THOMAS BUTLER,[1]* AND M. R. JACOBS[1,2]

Departments of Medicine[1] and Pathology,[2] Case Western Reserve University and University Hospitals of Cleveland, Cleveland Ohio 44106

Received 2 November 1982/Accepted 14 May 1982

The activities of ceftriaxone, moxalactam, and ampicillin against *Salmonella typhimurium* LT-2 were compared in culture media at pH 5, 6, 7 and 8 and in mice inoculated intraperitoneally. The minimal inhibitory concentrations for strain LT-2 in Mueller-Hinton broth were 0.03 μg of ceftriaxone per ml, 0.08 μg of moxalactam per ml, and 0.4 μg of ampicillin per ml. A comparison of minimal inhibitory concentrations in buffered broth at pH 5 with those in media at higher pH values showed that ceftriaxone was more acid stable than the other antibiotics. Groups of CF-1 female mice inoculated intraperitoneally with 3×10^4 colony-forming units received saline or each drug in fourfold decremental doses by the subcutaneous route every 8 h for 3 days, beginning at 24 h after challenge. The mean $\log_{10}$ colony-forming units of *S. typhimurium* per spleen at the end of treatment and the mortality rates at 21 days after inoculation were measured for each treatment group. The mean $\log_{10}$ colony-forming units per spleen was significantly reduced from that of the saline control by dosages of ≥0.06 mg of ceftriaxone per kg, 64 mg of moxalactam per kg, or ≥16 mg of ampicillin per kg ($P < 0.05$). Mortality rates of infected mice were significantly reduced by dosages of ≥1 mg of ceftriaxone per kg or ≥64 mg of ampicillin per kg ($P < 0.05$), whereas moxalactam in dosages as high as 16 mg/kg did not significantly reduce mortality rate. These results demonstrate the superiority of ceftriaxone to the other tested antibiotics on a weight basis in this model of experimental *Salmonella* infection.

Moxalactam and ceftriaxone have in vitro activity against most clinical isolates of *Salmonella* spp. (1, 5, 6, 8, 9, 16, 17). These drugs are worthy of evaluation in experimental animal models of typhoid fever, an infection which requires new approaches for chemotherapy to overcome the problems of drug resistance and high relapse rates.

Salmonella typhimurium infection in mice results in bacteremia and, on occasion, in fatal accumulation of organisms in reticuloendothelial cells, which are similar to the pathogenesis of typhoid fever in humans. Because the intracellular environments in which *Salmonella* infection occurs may be more acidic than extracellular fluid (7, 15), we compared the activities of these antibiotics against *S. typhimurium* at pH values between 5 and 8. Using this experimental infection, we compared the in vivo activities of ceftriaxone and moxalactam with that of ampicillin, which is known to be effective against this infection (3, 4).

MATERIALS AND METHODS

***S. typhimurium* LT-2.** S. typhimurium LT-2 was used in both the in vitro and in vivo studies. It had been passaged in mice and was maintained on nutrient agar slants at 4°C. This strain is virulent for mice (3, 4), resembling in this respect other *Salmonella* strains that have been employed in experimental mouse typhoid infections. The 50% lethal dose of strain LT-2 for CF-1 mice was 3×10^3 colony-forming units (CFU) inoculated intraperitoneally.

In vitro testing of antibiotic susceptibility. Ampicillin trihydrate (Bristol Laboratories, Syracuse, N.Y.) was dissolved in 5% sodium carbonate and diluted with water. Moxalactam (Eli Lilly & Co., Indianapolis, Ind.) and ceftriaxone (Ro 13-9904; Hoffmann-La Roche, Inc., Nutley, N.J.) were dissolved in water. Diluted solutions of these agents were added to Mueller-Hinton broth. To determine the minimal inhibitory concentrations (MICs), tubes containing 1 ml of broth were inoculated with 5×10^5 bacteria and read for turbidity after 24 h of incubation at 35°C. The minimal bactericidal concentrations were determined by plating 0.01-ml samples from tubes without visible turbidity on blood agar and considering growth of less than five colonies to be bactericidal activity (99.9% killing).

The effect of acidity on the MICs was tested at pH values of 5 to 8. Buffers covering this range were prepared from 0.1 M citric acid and 0.2 M sodium diphosphate. The test system included 0.7 ml of one of these buffers at each pH, 0.1 ml of antibiotic solution in water, and 0.2 ml of Mueller-Hinton broth containing approximately 5×10^4 CFU of *S. typhimurium* LT-2. The addition of these volumes to Mueller-Hinton

broth (pH 7.4) and antibiotics in water resulted in pH changes of no more than 0.1 U. After 16 h of incubation at 35°C, tubes were read for turbidity.

Assessment of in vivo activity. Groups of CF-1 female mice (Carworth Farms, New City, N.Y.) approximately 8 weeks old and weighing 18 to 20 g were inoculated intraperitoneally with 3×10^4 CFU of *S. typhimurium* LT-2 (10 times the 50% lethal dose) suspended in 0.2 ml of 0.9% NaCl. Treatment with the various doses of antibiotics was started 24 h later. These doses in 0.1 ml of 0.9% NaCl or 0.1 ml of 0.9% NaCl alone for control mice were injected subcutaneously every 8 h for 3 days. In each dosage group of each experiment, 10 mice were used for observation of mortality, and 5 mice were used for enumeration of the numbers of bacteria in the spleen or blood.

Blood was obtained from the retroorbital plexus with a heparinized Pasteur pipette, and spleens were removed aseptically. The spleens were homogenized in 1 ml of 0.9% NaCl by using glass mortars fitted with Teflon pestles. Spleen homogenates and blood specimens were serially diluted 10-fold with 0.9% NaCl, and 0.1-ml aliquots were streaked onto tryptic soy agar plates (Difco Laboratories, Detroit, Mich.). Colony counts were made after 24 h of incubation at 35°C. The mean $\log_{10}$ CFU per spleen or per milliliter of blood at 8 h after the last antibiotic dose and the mortality rates during 21 days of observation were used as measurements of drug effect when compared with saline-treated control mice.

RESULTS

In vitro susceptibility testing. The geometric means of triplicate determinations of the MICs were 0.03 μg of ceftriaxone per ml, 0.08 μg of moxalactam per ml, and 0.4 μg of ampicillin per ml. The minimal bactericidal concentration of each compound, indicating ≥99.9% killing, was the same as the MIC.

When incubated in buffers at pH values less than 5, *S. typhimurium* LT-2 did not multiply, and MIC determinations could not be performed. All three agents were most active at pH values of 6 or 7 (Table 1). At pH 5, the MIC of ampicillin increased 8-fold, and the MIC of moxalactam increased 10-fold, whereas the MIC of ceftriaxone increased only 2.5-fold. At pH 8, the MIC of ampicillin increased fourfold, whereas the MICs of ceftriaxone and moxalactam showed little or no change from the values at pH 6 and 7.

Effects of antibiotic treatment in murine infections. The numbers of bacteria in the spleen and blood at the start of treatment and after 3 days of treatment are shown in Fig. 1. The number of bacteria in the spleen exceeded the number of bacteria per milliliter of blood by approximately 1,000-fold at both times. The excess number of bacteria in the spleen over that in the blood was still greater on a basis of tissue weight because the mouse spleens weighed less than 0.1 g each. Treatment with 64 mg of ampicillin per kg per dose for 3 days prevented the increases in bacterial numbers in both the spleen and the blood that occurred in saline-treated controls.

TABLE 1. Effects of pH of medium on MICs of antibiotics against *S. typhimurium* LT-2

Antibiotic	MIC (μg/ml)[a] at following pH:			
	5	6	7	8
Ampicillin	4.00	0.50	0.63	2.00
Ceftriaxone	0.10	0.04	0.05	0.06
Moxalactam	0.31	0.06	0.03	0.03

[a] Each value is the geometric mean of three determinations.

Data from a representative evaluation of the effect of the three agents on splenic bacterial counts are shown in Fig. 2. Significant reductions in the number of bacteria occurred in mice treated with ceftriaxone in dosages of ≥0.06 mg/kg, moxalactam in a dosage of 64 mg/kg, or ampicillin in dosages of ≥16 mg/kg ($P < 0.05$ by Student's t test). The effects of the various dosages on mortality during 21 days of observation were recorded (Table 2). The mortality rate for saline-treated controls was 100%, and the median number of days after inoculation until death for those mice that died was 4.5. Dosages of ceftriaxone of ≥1 mg/kg or of ampicillin of ≥64 mg/kg reduced mortality significantly ($P < 0.05$ by chi-square test). The median number of days until death in these treatment groups also increased in comparison with the control groups. Moxalactam did not significantly de-

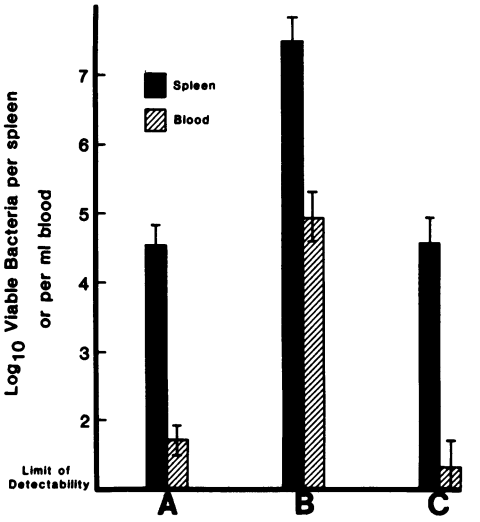

FIG. 1. The numbers of *S. typhimurium* in spleens and blood of mice. (A) At the time treatment was initiated (24 h after inoculation). (B) After 3 days in mice treated with saline (controls). (C) After 3 days in mice treated with 64 mg of ampicillin per kg per dose.

crease mortality or prolong the time of death in dosages up to 16 mg/kg.

DISCUSSION

Experimental infection of mice with *S. typhimurium* is an intracellular infection of reticuloendothelial cells in the spleen, liver, bone marrow, and other tissues. Fatal infection in this model is associated with a buildup of bacteria to concentrations in the vicinity of 10^7 bacteria per g of tissue. The intracellularity of this *Salmonella* infection was suggested in our experiments by the demonstration that the spleen contained greater than 1,000 times more bacteria than did the blood when CFUs per tissue weight were compared. For an antimicrobial drug to be effective in vivo, it must prevent bacterial multiplication in the intracellular environment. The drug first must penetrate into the tissues and then must be active at the site of bacterial multiplication. In regard to the intracellular location of *Salmonella* spp., the likely sites of multiplication include phagolysosomes of the mononuclear phagocytic cells, which are considerably more acidic than the extracellular fluid. Mouse peritoneal macrophages show intralysosomal pH values of about 4.8 (7), and the phagocytic vacuoles of human neutrophils and monocytes after phagocytosis of staphylococci show pH values as low as 6.0 (15).

These results with the new beta-lactam antibiotics ceftriaxone and moxalactam in murine *Salmonella* infection indicate that both drugs are effective in reducing the burden of bacteria in the spleen. On a weight basis, ceftriaxone was superior to both moxalactam and ampicillin. These differences in efficacy were greater than would be suggested by MICs alone. The MICs of ampicillin and moxalactam, respectively, against strain LT-2 were approximately 13 and 3 times greater than the MIC of ceftriaxone. Yet, doses of ampicillin and moxalactam required to reduce splenic bacterial populations significantly were, respectively, about 266 and 1,066 times that of ceftriaxone. A comparison of the efficacy of the drugs in reducing the mortality of infected mice showed that ceftriaxone was also superior on a weight basis to ampicillin and moxalactam. Dosages of ampicillin and moxalactam required to reduce mortality significantly were, respectively, 64 and at least 16 times greater than that of ceftriaxone.

These results showing efficacy of ceftriaxone in murine typhoid infection confirm the results of Beskid et al. (2), who showed that a single dose of 0.07 mg/kg protected mice against lethal infection with *Salmonella schottmuelleri*. These workers administered the antibiotic immediately after inoculation of bacteria. In our experimental model, treatment was delayed until 24 h after inoculation, allowing bacteria to become established intracellularly, and this model more closely resembles the clinical reality of treating patients with typhoid fever.

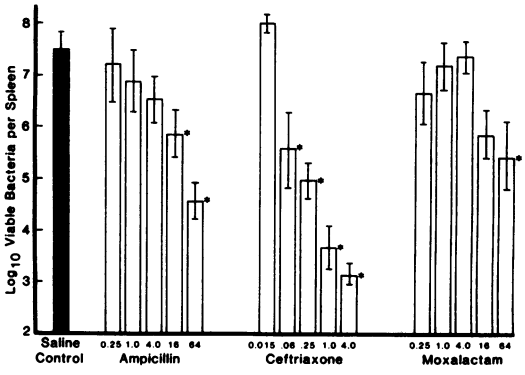

FIG. 2. The numbers of *S. typhimurium* in the spleens of mice 8 h after completion of treatment with various doses of ampicillin, ceftriaxone, and moxalactam. Each bar shows the mean ± standard error of the mean of bacteria in five spleens of mice that received each dosage. *, Dosage resulting in mean value that was significantly lower than the mean of the saline controls ($P < 0.05$ by Student's t test).

The superiority of ceftriaxone over the other drugs may be explained in part by the known longer serum half-life of ceftriaxone in humans and animals. The reported serum half-life of ceftriaxone in humans is 8.8 h, whereas that reported for moxalactam ranges from 1.8 to 3.5 h, and that of ampicillin is about 1.3 h (10, 13,

TABLE 2. Effect of antibiotic treatment on mortality rate and time of death

Treatment	Dose (mg/kg)	% Mortality	Median days after inoculation until death of mice that died
Saline		100	4.5
Ampicillin	64	40[a]	8
	16	78	7
	4	90	5
	1	90	5
Ceftriaxone	4	40[a]	10.5
	1	40[a]	12
	0.25	80	7
	0.06	80	6
Moxalactam	16	80	5
	4	100	5
	1	90	4
	0.25	100	5

[a] $P < 0.05$ when compared with saline controls by the Fisher exact test.

14). Schaad et al. found that in experimental meningitis in rabbits, the serum half-life of ceftriaxone is 2.1 h, compared with 0.7 h for moxalactam (12). More sustained serum concentrations of ceftriaxone than of other tested antibiotics have also been detected in rats (11) and mice (2) after intravenous injection.

Another factor that may have contributed to the superiority of ceftriaxone is its better stability at low pH. Our examination of the MICs of these antibiotics in buffers containing Mueller-Hinton broth and *S. typhimurium* indicated that at pH 5 the MIC of ceftriaxone increased to only two times that obtained at pH 7, whereas the MICs of ampicillin and moxalactam, respectively, increased to 6.3 and 10 times that obtained at pH 7. This greater relative activity of ceftriaxone in an acid environment may be an advantage in providing anti-*Salmonella* activity in the phagocytic vacuoles inside reticuloendothelial cells. Differences in the localizations of these antimicrobial agents in tissue in general and in the spleen in particular merit more attention than has been accorded here.

LITERATURE CITED

1. **Angehrn, P., P. J. Probst, R. Reiner, and R. Then.** 1980. Ro 13-9904, a long-acting broad-spectrum cephalosporin: in vitro and in vivo studies. Antimicrob. Agents Chemother. **18**:913–921.
2. **Beskid, G., J. G. Christenson, R. Cleeland, W. DeLorenzo, and P.W. Trown.** 1981. In vivo activity of ceftriaxone (Ro 13-9904), a new broad-spectrum semisynthetic cephalosporin. Antimicrob. Agents Chemother. **20**:159–167.
3. **Butler, T., C. W. Shuster, and P. Dixon.** 1981. Treatment of experimental *Salmonella typhimurium* infection with mecillinam and ampicillin. Antimicrob. Agents Chemother. **19**:328–331.
4. **Butler, T., C. W. Shuster, and A. Franco.** 1979. Effect of a *Salmonella* group H1 R factor on virulence and response of infections to antimicrobial therapy. Antimicrob. Agents Chemother. **15**:478–480.
5. **Chau, P. Y., W. S. Ng, J. Ling, and K. Arnold.** 1981. In vitro susceptibility of *Salmonella* to various antimicrobial agents, including a new cephalosporin, Ro 13-9904. Antimicrob. Agents Chemother. **19**:8–11.
6. **Clarke, A. M., and S. J. V. Zemcov.** 1981. Ro 13-9904 and GR 20263, two new cephalosporins with broad-spectrum activity: an *in vitro* comparison with other β-lactam antibiotics. J. Antimicrob. Chemother. **7**:515–520.
7. **de Duve, C., S. Ohkuma, B. Poole, and P. Tulkens.** 1978. The intralysosomal pH, p. 371–380. *In* P. A. Srere and R. W. Estabrook (ed.), Microenvironments and metabolic compartmentation. Academic Press, Inc., New York.
8. **Neu, H. C., N. Aswapokee, K. P. Fu, and P. Aswapokee.** 1979. Antibacterial activity of a new 1-oxa cephalosporin compared with that of other β-lactam compounds. Antimicrob. Agents Chemother. **16**:141–149.
9. **Neu, H. C., N. J. Meropol, and K. P. Fu.** 1981. Antibacterial activity of ceftriaxone (Ro 13-9904), a β-lactamase-stable cephalosporin. Antimicrob. Agents Chemother. **19**:414–423.
10. **Parsons, J. N., J. M. Romano, and M. E. Levison.** 1980. Pharmacology of a new 1-oxa-β-lactam (LY127935) in normal volunteers. Antimicrob. Agents Chemother. **17**:226–228.
11. **Reiner, R., U. Weiss, U. Brombucher, P. Lanz, M. Montavon, A. Furlenmeier, P. Angehrn, and P. J. Probst.** 1980. Ro 13-9904/001, a novel and long-acting parenteral cephalosporin. J. Antibiot. **33**:783–786.
12. **Schaad, U. B., G. H. McCracken, C. A. Loock, and M. L. Thomas.** 1981. Pharmakokinetics and bacteriologic efficacy of moxalactam, cefotaxine, cefoperazone and rocephin in experimental bacterial meningitis. J. Infect. Dis. **143**:156–163.
13. **Scheld, W. M., D. A. Spyker, G. R. Donowitz, W. K. Bolton, and M. A. Sande.** 1981. Moxalactam and cefazolin: comparative pharmacokinetics in normal subjects. Antimicrob. Agents Chemother. **19**:613–619.
14. **Seddon, M., R. Wise, A. P. Gillett, and R. Livingston.** 1980. Pharmacokinetics of Ro 13-9904, a broad-spectrum cephalosporin. Antimicrob. Agents Chemother. **18**:240–242.
15. **Segal, A. W., M. Geisow, R. Garcia, A. Harper, and R. Miller.** 1981. The respiratory burst of phagocytic cells is associated with a rise in vacuolar pH. Nature (London) **290**:406–409.
16. **Shelton, S., J. D. Nelson, and G. H. McCracken, Jr.** 1980. In vitro susceptibility of gram-negative bacilli from pediatric patients to moxalactam, cefotaxime, Ro 13-9904, and other cephalosporins. Antimicrob. Agents Chemother. **18**:476–479.
17. **Wise, R., J. M. Andrews, and K. A. Bedford.** 1979. LY127935, a novel oxa-β-lactam: an in vitro comparison with other β-lactam antibiotics. Antimicrob. Agents Chemother. **16**:341–345.