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Comparative Efficacy of Cefotiam, Cefmenoxime, and Ceftriaxone in Experimental Endocarditis and Correlation with Pharmacokinetics and In Vitro Efficacy

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To determine the influence of in vitro activity, pharmacokinetic properties, and therapeutic regimen on the antibacterial effect in vivo, we compared three cephalosporins, cefotiam, cefmenoxime, and ceftriaxone, in a rabbit model of experimental *Escherichia coli* endocarditis after 4 days of treatment. The MBCs of cefotiam, cefmenoxime, and ceftriaxone for the *E. coli* strain were 0.5, 0.125, and 0.06 μg/ml, respectively. Killing curves at 10 times the MBC were similar for the three cephalosporins. In serum, the elimination half-life of ceftriaxone was twice as much as the elimination half-life of cefotiam or cefmenoxime (2.8 ± 0.45 versus 1.4 ± 0.25 or 1.3 ± 0.4 h, respectively). Ceftriaxone was much more effective than cefotiam. The bacterial titer in the vegetations (log_{10} CFU per gram of vegetation) was 7.56 ± 1 with cefotiam and 2.41 ± 2.6 with ceftriaxone, as their concentrations were 18 and 466 times higher, respectively, than their MBCs. Although ceftriaxone and cefmenoxime exhibited a similar rate of killing and percentage of protein binding, ceftriaxone was more effective than cefmenoxime at the same regimen of 15 mg/kg twice a day (3.08 ± 1.1 versus 4.82 ± 3.2 log_{10} CFU/g of vegetation). When antibiotic was given as a single daily injection of 30 mg/kg, the antibacterial effect persisted for ceftriaxone, but not for cefmenoxime. The longer elimination half-life and the higher local concentration/MBC ratio of ceftriaxone explained these results. The bacterial titer measured 24 h after the fourth injection of 30 mg of ceftriaxone per kg confirmed that this regimen prevented regrowth of bacteria. These results suggest that the local antibiotic level/MBC ratio roughly correlated with the antibacterial effect and could represent an adequate basis to explain the differences observed between the drugs in vivo. They also demonstrate that, provided that the dose is sufficient, a long-acting broad-spectrum cephalosporin may be effective in severe gram-negative infections, even when given at relatively long dosing intervals, in contrast with a rapidly cleared drug with the same intrinsic activity.

In severe infections such as endocarditis or meningitis, a bactericidal effect at the site of infection is essential for cure. Scheld and Sande (18), who investigated experimental pneumococcal meningitis in rabbits, demonstrated that only antimicrobial therapy with a bactericidal effect on the cerebrospinal fluid is associated with cure. In *Escherichia coli* meningitis, Decazes et al. (6) showed that ceftriaxone is effective when its level in the cerebrospinal fluid is at least 10 times the MBC. In addition, the rate at which antibiotics alone or in combination kill microorganisms in vitro is predictive of the rate at which they eradicate these organisms in vivo. Thus, a combination of penicillin and aminoglycoside is more effective than penicillin alone in streptococcal endocarditis (7), and vancomycin is more effective than penicillin in *Staphylococcus aureus* endocarditis (16); in each case this is because the former antibiotic kills bacteria faster than the latter. The antibacterial effect of a given drug in vivo also depends on its pharmacokinetic properties, which determine the rate of diffusion at the infected site and its local concentration. To study the relationship between the pharmacokinetic properties and the antibacterial efficacy of antibiotics, we chose three cephalosporins that differ in terms of in vitro activity and their pharmacokinetic properties; cefotiam (5), cefmenoxime (10), and ceftriaxone (15). We used an experimental model of *E. coli* endocarditis which, although an uncommon infection in humans, provides a rigorous test of antibiotic efficacy. It is a reliable and easily reproducible experimental model of severe infection for which cure requires a high in vivo antibacterial activity.

The purposes of this study were (i) to determine whether there is a relationship between the in vitro activity and pharmacokinetic properties of three different cephalosporins on the one hand and antibacterial effect in vivo on the other, and (ii) to evaluate the conditions under which a single daily administration of a long-acting antibiotic might prove effective.

MATERIALS AND METHODS

In vitro tests. (i) Organism. A strain of *E. coli* isolated from a patient with endocarditis was used for this study. This strain, which was resistant to rabbit serum, has been described previously (4).

(ii) Antibiotic susceptibility tests. MICs and MBCs were determined for each antibiotic by the macrotube dilution method in a mixture of 50% Mueller-Hinton broth–50% normal rabbit serum in a total volume of 2 ml, as suggested by Stratton et al. (19), for the in vivo study of highly bound antibiotics. Organisms in the log phase of growth were used, and their final concentration was 10^7 CFU/ml. The MIC was defined as the lowest concentration of drug that inhibited growth, i.e., visible turbidity after 24 h of incubation at 37°C. The MBC was determined by subculturing 0.01 ml from each

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clear tube on agar plates containing β-lactamase (penicillin amido-β-lactamhydrolase [EC 3.5.2.6] from *Bacillus cereus* 569/H9; kindly provided by Roche Laboratories, France) to avoid the effects of antibiotic carry-over. The activity of this β-lactamase against the three drugs tested was verified in vitro by the technique described by Lee and Komarmy (14). The MBC was defined as the lowest concentration that reduced the number of viable organisms by 99.9%.

Using concentrations of each cephalosporin equivalent either to 10 times the MBC or to the antibiotic levels measured in infected vegetations (Fig. 1), we determined the rate of killing of the *E. coli* strain in Mueller-Hinton broth. An inoculum of 10^7 CFU/ml in the log phase of growth, which was similar to bacterial titers in vegetations, was tested. At 1, 3, and 6 h after inoculation of bacteria in the antibiotic-containing broth, serial dilutions of 0.1-ml samples (to 10^{-4}) were subcultured on agar plates containing the β-lactamase and were incubated at 37°C for 24 h. Colonies were then counted. All determinations were performed at least twice.

(iii) Protein binding. Protein binding in rabbit serum was investigated by equilibrium dialysis for 4 h at 37°C in 0.10 M phosphate buffer (pH 7.4) by using a Dianorm system (Diachema AG, Rulickon, Switzerland) with 0.250 ml of cells and cellulose dialysis membranes. The stability of each antibiotic was verified after an incubation for 4 h at 37°C; 4 h of dialysis was sufficient to reach equilibrium. Antibiotic concentrations were measured on each side of the dialysis membranes. Protein binding was measured for each antibiotic four times at the following concentrations: 75 μg/ml for cefotiam, 100 μg/ml for cefmenoxime, and 100 and 150 μg/ml for ceftriaxone. Concentrations were above those in serum at the peak level following the highest dose.

In vivo tests. (i) Pharmacokinetic studies. Three groups of five rabbits, each (weight, 2.5 to 3.2 kg) were assigned for 4 days to one of the following regimens: seven intramuscular (i.m.) injections of 15 mg of cefotiam per kg twice daily (b.i.d.), seven i.m. injections of 15 mg of cefmenoxime per kg b.i.d., and 4 i.m. injections of 30 mg of ceftriaxone per kg once daily (o.d.). Blood samples were collected at 0.5, 1, 2, 4, 6, and 8 h after the last injection. Additional samples were collected at 12 and 24 h after ceftriaxone injection.

(ii) Experimental endocarditis. Prior to inoculation, *E. coli* isolates were cultured overnight in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), washed, and suspended in saline. All inocula were serially diluted in 0.9% NaCl and cultured to determine the number of injected CFU.

For the rabbit model, a modification of the method described by Perlman and Freedman was used to produce endocarditis in New Zealand rabbits (weight, 2.2 to 3.1 kg), as described previously (4). Briefly, a polyethylene catheter was inserted through the right carotid artery into the left ventricular cavity (day 1) and left in place throughout the experiment. Infection was produced 24 h later by injection into an ear vein of 1 ml of 10^7 CFU of *E. coli* per ml. This produced endocarditis in more than 95% of the rabbits that were inoculated, as established by positive blood cultures at day 7 and the presence of vegetations at killing.

The experimental design was as follows. Treatment was begun 72 h after injection (day 5). Animals were assigned to one of the following regimens for the next 72 h: seven i.m. injections of cefotiam (15 mg/kg b.i.d), seven i.m. injections of cefmenoxime (15 mg/kg b.i.d), four i.m. injections of cefmenoxime (30 mg/kg o.d.), four i.m. injections of ceftriaxone (15 mg/kg o.d.), four i.m. injections of ceftriaxone (30 mg/kg o.d.), seven i.m. injections of ceftriaxone (15 mg/kg b.i.d.). A control group of untreated animals was also studied. Rabbits were killed by chloroform inhalation on day 8, 1 h after the last injection of cefotiam or cefmenoxime and 2 h after the last injection of ceftriaxone. This was when the level of antibiotic in the serum was approximately at its peak. Serum samples for antibiotic assays were collected at the time of sacrifice. To evaluate the residual effect of the antibiotic, three other groups of rabbits were also studied. In one group, each rabbit was given eight i.m. injections of cefmenoxime (15 mg/kg b.i.d.) and was killed 12 h after the last injection, while in the two other groups each rabbit was given four i.m. injections of ceftriaxone (30 mg/kg o.d.) and killed either 12 or 24 h after the last injection.

The effect of therapy was determined by measuring the antibiotic levels and the reduction of bacterial titers ($\log_{10}$ CFU per gram) in infected vegetations. In each case the heart was removed at the time of sacrifice, and vegetations were excised aseptically and rinsed in sterile saline to avoid contamination by blood. A part of the vegetations was used for bacterial counts, and the remainder was stored at −20°C for determination of antibiotic concentrations. The absence of hemoglobin in the supernatant, as measured by spectrophotometry, was taken to indicate that the vegetations were not contaminated by blood.

To determine bacterial titers in vegetations, the vegetations were immediately homogenized, diluted, and cultured on agar plates containing β-lactamase, which was done to inactivate the antibiotics, and then incubated for 24 h at 37°C. Bacterial titers are expressed as $\log_{10}$ CFU per gram of vegetation. If bacterial titers were below 2 $\log_{10}$ CFU/g, the vegetations were considered sterile. The corresponding value was considered as zero in calculating the means.

For antibiotic assays, blood samples were allowed to clot and were centrifuged at 3,500 rpm for 5 min. Serum and vegetations were stored at −20°C until assay. Antibiotic concentrations were measured by the agar diffusion method. For concentrations of 5 to 40 μg/ml, samples were tested with *E. coli* ATCC 25922; and for concentrations of less than 5 μg/ml, samples were tested with *E. coli* 1346 (kindly provided by Roche Laboratories). Standards for the serum sample assay were prepared in normal rabbit serum.

Antibiotic levels in vegetations were determined by assaying the supernatant of a vegetation sample, which was homogenized in 0.3 ml of sterile phosphate buffer, with *E. coli* 1346, as described previously (3). The lower limits of detectability were 0.16, 0.03 and 0.02 μg/ml, which were equivalent to 4.9, 0.9, and 0.6 μg/g of vegetation for cefotiam, cefmenoxime, and ceftriaxone, respectively. Standards for the assay of the homogenized vegetation supernatants were prepared in a 0.10 M phosphate buffer (pH 7.4). Each sample was studied in duplicate. Samples were diluted when necessary.

Pharmacokinetic analysis. Blood concentration-time data were plotted on a semilogarithmic scale, and the final points were selected by eye for the determination of the terminal half-life ($t_{1/2\beta}$). The latter was calculated by linear regression. Areas under the curves were calculated by the trapezoidal rule method.

Statistical evaluation. Statistical evaluation was determined by the unpaired Student t test for comparison of two means; $P < 0.05$ was considered significant. For comparison of more than two means, we used an analysis of variance and the Bonferroni correction (9), in which $P < 0.01$ was considered significant. Statistical differences in the sterilization of vegetations were analyzed by the chi-square test.

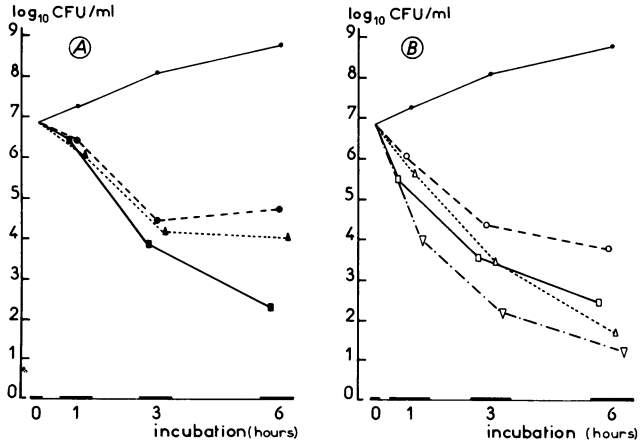

FIG. 1. (A) In vitro killing of *E. coli* incubated with each cephalosporin at a concentration 10 times greater than the MBC. Symbols: ●—●, control; ▲, ceftriaxone (0.6 μg/ml); ■, cefotiam (5 μg/ml); ●---●, cefmenoxime (1.25 μg/ml). (B) In vitro killing of *E. coli* incubated with each cephalosporin at concentrations equivalent to those measured in infected vegetations. Symbols: ●, control; △, ceftriaxone (8 μg/ml); ▽, ceftriaxone (28 μg/ml); □, cefotiam (9 μg/ml); ○, cefmenoxime (7 μg/ml).

RESULTS

In vitro antibiotic susceptibility. Both the MICs and MBCs of cefotiam, cefmenoxime, and ceftriaxone for *E. coli* were 0.5, 0.125, and 0.06 μg/ml, respectively.

Killing curves. After 3 and 6 h of drug-bacteria contact, there was no major difference in the killing rate of cefotiam, cefmenoxime, or ceftriaxone at concentrations equal to 10 times the MBC; there was a decrease between 2.5 and 4.5 orders of magnitude at 6 h (Fig. 1A). At concentrations equivalent to those obtained in the vegetations when the level in serum was at its peak on day 4 of treatment, ceftriaxone killed the most bacteria, i.e., a decrease of 5 orders of magnitude at 6 h (Fig. 1B). However, when the concentration of ceftriaxone was raised from 8 to 28 μg/ml (142 to 466 times the MBC, which was equivalent to the value obtained in the vegetations with 15 and 30 mg of ceftriaxone and per kg o.d., respectively), the decline of bacterial titers in vitro was not accelerated. Concentrations in vegetations were 9 μg/ml (18 times the MBC) and 7 μg/ml (58 times the MBC) for cefotiam and cefmenoxime, respectively. At these concentrations the effect of cefotiam was similar. With cefmenoxime, although there was no greater killing than with 10 times the MBC, the numbers of bacteria indicated in Fig. 1A rose between 3 and 6 h, and those indicated in Fig. 1B fell over this interval.

Protein binding study. The percentages of cefotiam, cefmenoxime, and ceftriaxone (100 μg/ml) and ceftriaxone (150 μg/ml) bound to proteins in serum were 51 ± 7, 93 ± 2, 90 ± 0.8, and 91 ± 1, respectively.

Pharmacokinetics. Levels of cefotiam and cefmenoxime in serum reached their peak at 30 min for cefotiam (15 mg/kg) and cefmenoxime (15 mg/kg) (10.5 ± 2.5 and 14.4 ± 5.7 μg/ml, respectively). With ceftriaxone (30 mg/kg) the peak value (72 ± 8 μg/ml) was reached at 1 h. The $t_{1/2\beta}$ values of cefotiam, cefmenoxime, and ceftriaxone in serum were 1.4 ± 0.25, 1.3 ± 0.4, and 2.8 ± 0.45 h, respectively. The areas under the curve of cefotiam, cefmenoxime, and ceftriaxone in serum were 17.5 ± 4.4, 22 ± 9.9, and 326 ± 91.4 μg h/ml, respectively.

Experimental endocarditis. In control animals that were all given the same inoculum (10^7 CFU of the *E. coli* strain), there was no correlation ($P > 0.20$) between weight (2.2 to 2.8 kg) and mean bacterial titer in vegetations. Thus rabbits were inoculated with 10^7 CFU of *E. coli* regardless of their weight. Comparison of cefotiam, cefmenoxime, and ceftriaxone efficacy at the same daily dose (Table 1) showed that the mean vegetation titers were significantly more reduced by cefmenoxime (15 mg/kg b.i.d.) than by cefotiam (15 mg/kg b.i.d.). Ceftriaxone (30 mg/kg o.d.) exhibited the best antibacterial effect, but ceftriaxone (15 mg/kg o.d.) did not reduce bacterial titers in vegetations compared with the control group. Cefmenoxime (30 mg/kg o.d.) exhibited less of an antibacterial effect than ceftriaxone (30 mg/kg o.d.). The differences obtained according to the time of sacrifice are shown in Table 2. When animals that received 30 mg of ceftriaxone per kg o.d. were sacrificed at 12 or 24 h after the last injection, the antibacterial effect of ceftriaxone persisted, although local antibiotic levels gradually declined. After the fourth injection, the mean vegetation titer was slightly less at 12 than at 2 h and was less at 24 than at 12 h, but these differences were not significant. Two of the nine rabbits that were killed at 2 h, 5 of the 10 rabbits that were killed at 12 h, and 6 of the 9 rabbits that were killed at 24 h had sterile vegetations; but again the differences were not significant. With cefmenoxime, the mean bacterial titer increased slightly from 4.82 ± 3.2 to 6.63 ± 1.3 $\log_{10}$ CFU/g in the animals killed 12 h after the last injection (not significant). Whatever the time of sacrifice, mean bacterial titers were lower in animals treated with 30 mg of ceftriaxone per kg o.d. than in those treated with 15 mg of cefmenoxime per kg b.i.d. The difference between the two treatments became significant ($P < 0.01$) when animals were sacrificed at the end of the interval between injections, which was 24 h for ceftriaxone and 12 h for cefmenoxime. The levels of both antibiotics in vegetations decreased significantly during the interval between injections. Trough levels of cefmenoxime and ceftriaxone in vegetations were roughly similar for cefmenoxime and ceftriaxone (19 versus 13 times the MBC, respectively).

DISCUSSION

Experimental endocarditis provides an adequate in vivo model for studying the efficacy of antimicrobial agents. In this study we compared the therapeutic efficacy of cefotiam, cefmenoxime, and ceftriaxone and attempted to correlate the results with the in vitro activity, $t_{1/2\beta}$, local concentrations, and dosing regimens of these antibiotics. Measurement of MBCs demonstrated that cefotiam is less active than cefmenoxime and ceftriaxone. In the in vitro time-kill experiments, if cefotiam was best for the results shown in Fig. 1A, ceftriaxone (at a high dose; > 142 times MBC) was best for the results shown Fig. 1B; the differences, however, appeared slight in each case. This bactericidal effect of ceftriaxone obtained in vitro, which was concentration and dose dependent, was not observed with cefotiam or cefmenoxime. In fact, no increase in cidal effect was measured when these two drugs were tested in vitro at 10 times the MBC or at the levels obtained in vegetations with the dosage regimens used. It must be stressed, however, that the concentrations of cefotiam and cefmenoxime in the vegetations did not exceed 60 times the MBC, when these antibiotics were administered at the same dose as ceftriaxone.

TABLE 1. Comparative efficacy of cefotiam, cefmenoxime, and ceftriaxone administered at the same daily dose[a]

Treatment	No. of rabbits	Antibiotic regimen	$\log_{10}$ CFU/g of vegetation (mean ± SD)	Antibiotic in vegetations		Antibiotic level in serum (μg/ml; mean ± SD)[b]
				μg/g (mean ± SD)	Ratio concn/MBC	
Control	9		7.41 ± 0.9			
Cefotiam	8	15 mg/kg b.i.d.	7.56 ± 1	8.9 ± 4.6	18	8.3 ± 5.5
Cefmenoxime	7	30 mg/kg o.d.	7.64 ± 0.3	13 ± 4.7	104	12.4 ± 4.3
Cefmenoxime	11	15 mg/kg b.i.d.	4.82 ± 3.2[c]	7.2 ± 2.6	58	8.1 ± 2.6
Ceftriaxone	9	30 mg/kg o.d.	2.41 ± 2.6[d]	28 ± 16	466	59 ± 16
Ceftriaxone	6	15 mg/kg o.d.	7.17 ± 0.5	8.5 ± 3.8	142	47 ± 8
Ceftriaxone	6	15 mg/kg b.i.d.	3.08 ± 1.1[d]			

[a] Daily dose of 30 mg/kg. Animals were sacrificed 1 h after the last injection of cefotiam and cefmenoxime and 2 h after the last injection of ceftriaxone.
[b] Antibiotic level was determined at the time of sacrifice.
[c] Significantly different from control values; $P < 0.05$.
[d] Significantly different from control values; $P < 0.01$.

Antibiotics with different MBCs can be compared by calculating the ratio of antibiotic concentration in the vegetation to the MBC. The different effects obtained with cefotiam and ceftriaxone provide an example. With cefotiam (15 mg/kg b.i.d.) the bacterial titer in the vegetations was 7.56 ± 1 $\log_{10}$ CFU/g of vegetation; and the concentration/MBC ratio was 18, while with ceftriaxone (30 mg/kg o.d.) the titer was 2.41 ± 2.6 and the concentration/MBC ratio was 466. Our results also show, however, that in its intermediate range of values, the concentration/MBC ratio alone cannot predict therapeutic efficacy. The latter depends on the half-life of the drug and on the interval between injections, both of which influence the duration of bacterial exposure to high antibiotic concentrations. Thus, cefmenoxime, which has the shortest half-life of the three drugs tested here, was more effective when given at 15 mg/kg b.i.d. than in a single large injection of 30 mg/kg o.d., although the peak value of the concentration/MBC ratio obtained with 15 mg of cefmenoxime per kg b.i.d. was only 58, which is considerably less than the peak of 104 obtained with 30 mg/kg o.d. The effect of the mode of administration on the extravascular penetration of antibiotic has been studied previously (1, 13), but simultaneous evaluation of therapeutic efficacy has rarely been investigated (2). Lavoie and Bergeron (13) have shown that in infected fibrin clots, the antibacterial effect of cefuroxime 24 h after administration is more marked with four injections of 100 mg/kg given once every 6 h than with a single large injection, because at 12 h, as drug levels decreased below the MIC, bacteria grew again in the clots. These results are similar to those we obtained with the two different regimens of cefmenoxime; thus, the failure of a single injection of 30 mg/kg o.d. to exert a significant antibacterial effect might be similarly explained by the regrowth of bacteria at the end of the interval between the injections. On the other hand, Kapusnik and Sande (11) have shown for experimental pneumonia in a guinea pig that the same daily dose of tobramycin is more effective if administered every 24 h in a single injection than if given every 6 h, except in neutropenic animals (12), in which regrowth of bacteria occurs when the antibiotic is administered in a single large injection. The model of bacterial endocarditis described here represents an invasive infection in which local cellular defenses are absent, which is perhaps why the results obtained in neutropenic guinea pigs are similar to those that we observed with cefmenoxime. This antibiotic must be administered frequently, because its half-life is short. In both types of infection, sustained bactericidal levels are necessary to effect cure.

The same antibacterial effect was obtained with ceftriaxone, whether this antibiotic was administered at 15 mg/kg b.i.d. or as a single daily injection (30 mg/kg o.d.). Although ceftriaxone and cefmenoxime showed the same intrinsic activity and protein binding, ceftriaxone was more effective than cefmenoxime at the same regimen of 15 mg/kg b.i.d. The long elimination half-life of ceftriaxone accounts for these results; but the dose adminstered must be sufficient to obtain antibacterial efficacy: ceftriaxone at 15 mg/kg o.d. was not as potent as ceftriaxone at 30 mg/kg o.d.

In a previous study (4), we have shown that the elimination half-life of moxalactam and gentamicin in the vegetations parallels that in serum. Because the elimination of ceftriaxone from the vegetations is slow, local bactericidal levels of this drug are maintained longer than those of a rapidly cleared drug, such as cefmenoxime, preventing regrowth of bacteria between injections. Lavoie and Bergeron (13) have reported that, unlike cefuroxime, aztreonam is more effective in infected fibrin clots when

TABLE 2. Comparison of cefmenoxime and ceftriaxone efficacy on the day 4 of therapy, according to the time of sacrifice

Treatment	No. of rabbits	Antibiotic regimen	Time (h) of sacrifice	$\log_{10}$ CFU/g of vegetation (mean ± SD)	Antibiotic in vegetations		No. of sterile vegetations/no. of sampled vegetations
					μg/g (mean ± SD)	Ratio concn/MBC	
Control	9			7.41 ± 0.9			
Cefmenoxime	11	15 mg/kg b.i.d.	1	4.88 ± 3.2	7.2 ± 6.2	58	0/11
Cefmenoxime	7	15 mg/kg b.i.d.	12	6.63 ± 1.3	2.4 ± 2.8	19	0/7
Ceftriaxone	9	30 mg/kg o.d.	2	2.41 ± 2.6[a]	28 ± 16	466	2/9
Ceftriaxone	10	30 mg/kg o.d.	12	1.96 ± 1.9	1.7 ± 1.2	28	5/10
Ceftriaxone	9	30 mg/kg o.d.	24	1.42 ± 2.3[b]	0.8 ± 0.5	13	6/9

[a] Not significantly different from the value obtained in rabbits receiving cefmenoxime (15 mg/kg b.i.d.) and killed 1 h after the last injection.
[b] Significantly different ($P < 0.01$) from the value obtained in rabbits receiving cefmenoxime (15 mg/kg b.i.d.) and killed 12 h after the last injection.

administered as a single large injection than as intermittent injections, because in fibrin clots, the mean half-lives of aztreonam and cefuroxime were 5.5 and 2.9 h, respectively. Schaad et al. (17) also have shown that ceftriaxone is not rapidly cleared from cerebrospinal fluid and is more effective than cefotaxime and cefoperazone. The efficacy of ceftriaxone is probably due to a high peak concentration in the vegetations and to the persistence of a high local concentration/MBC ratio. A similar phenomenon was observed in this study in animals sacrificed 12 h after the last injection, when trough levels were similar for ceftriaxone and cefmenoxime. We did not investigate a potential postantibiotic effect of the three cephalosporins in vitro. This highly doubtful effect could not be involved in our study because the trough concentration of ceftriaxone and cefmenoxime remained over the MIC in the vegetations. Because cefotiam was ineffective 1 h after the last injection, we did not investigate this drug further.

In a recent study, Gerber et al. (8) have shown that the results obtained with β-lactams cannot be extrapolated to other antibiotics. Despite a short half-life, aminoglycosides are effective when administered in a bolus injection. The postantibiotic effect and the rapid in vitro killing time of aminoglycosides may explain their good efficacy in vivo when given as a single large daily dose, in contrast with cephalosporins. The association of ceftriaxone with netilmicin delivered as a single daily injection was proved synergistic in vivo in experimental *E. coli* endocarditis, despite the short half-life of netilmicin (B. Fantin, B. Pangon, G. Potel, J. M. Vallois, and C. Carbon. Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 293, 1986).

Among the three cephalosporins studied here ceftriaxone seems to exhibit the strongest antibacterial effect in the rabbit model *E. coli* experimental endocarditis. High levels of ceftriaxone in vegetations were obtained, even though a high percentage of this antibiotic is bound to proteins. Because the MBC of ceftriaxone was low, the local concentration/MBC ratio was high. Gerber et al. (8) have stated that results obtained in small animals with cephalosporins are an underestimate of the effects of these drugs in humans. The extrapolation to humans of our results, which show the efficacy of a single daily injection in a severe infection, must be made cautiously. A possibility is to determine the interval between doses as a multiple of $t_{1/2\beta}$. In this study the antibiotics were administered roughly every 8 times the $t_{1/2\beta}$, whereas the usual therapeutic regimen of ceftriaxone in humans is a dose every 3 times the $t_{1/2\beta}$, i.e., one dose every 24 h. The difference between regimens in animal and human probably reflects a large security margin of the single daily dose in the treatment of infections in humans.

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