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In Vivo Activity and Pharmacodynamics of Cefotaxime or Ceftriaxone in Combination with Fosfomycin in Fibrin Clots Infected with Highly Penicillin-Resistant *Streptococcus pneumoniae*

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Using a clinical pneumococcal strain for which MICs were 2, 0.5, 0.5, and 16 mg/liter for penicillin, cefotaxime, ceftriaxone, and fosfomycin, respectively, we studied the efficacies of these antibiotics alone and in combination in one or two doses or in continuous infusion over 6 h in the treatment of the prolonged (48-h) experimental fibrin clot infections in rabbits. Doses were chosen to obtain low antibiotic concentrations. We observed the highest bacterial reductions (change in $\log_{10}$ CFU per gram) with the following five regimens: combination of cefotaxime plus fosfomycin given in two divided doses 6 h apart (each at 50 mg/kg of body weight given intravenously (4.2 ± 0.7 CFU/g), ceftriaxone (8 mg/kg given once intravenously) along with one or two doses of fosfomycin (3.79 ± 0.6 and 3.95 ± 0.5 CFU/g), cefotaxime alone administered in two divided doses (3.6 ± 0.4 CFU/g), and a 6-h continuous infusion of cefotaxime (100 mg/kg) with fosfomycin (100 mg/kg) (3.5 ± 0.4 CFU/g). The bacterial reductions obtained with these five regimens were all higher than those obtained with the other regimens tested ($P < 0.05$). The time of bacterial regrowth was significantly delayed with the two doses of the cefotaxime-fosfomycin regimens (23.2 ± 11 h) compared with those with the other combinations ($P < 0.05$). The rate of bacterial regrowth with this regimen was even lower than that observed with cefotaxime alone given in two doses ($P < 0.05$). The critical concentration, below which bacterial regrowth was observed, was lower for antibiotics in the combinations; the most important decrease in this concentration was with fosfomycin: 27.3 ± 5.6 mg/liter for fosfomycin alone versus 14.7 ± 12 mg/liter for fosfomycin in the combinations ($P < 0.05$). By a multivariate analysis, the most important independent parameters for efficacy were the maximal concentrations of β-lactam antibiotics and the residual concentration of fosfomycin and, for the combinations, the log of the area under the concentration-time curve/MIC ratio for β-lactam antibiotics. From these findings, the combinations cefotaxime or ceftriaxone plus fosfomycin could be proposed for the treatment of infections caused by highly penicillin-resistant pneumococci.

Infections caused by *Streptococcus pneumoniae* continue to represent a significant cause of mortality and morbidity in humans (1). A dramatic increase in the incidence of pneumococcal strains with resistance to penicillin and a range of other antimicrobial agents has been observed over the past two decades (1, 6, 10, 12, 16, 17, 20, 21, 32), and subsequently, poor responses to therapy and increased mortality resulting from pneumococcal infections have been seen (7, 8, 11, 13, 14, 18, 20, 25, 30). The optimal therapeutic approach toward such cases is not known, but expanded-spectrum cephalosporins such as cefotaxime or ceftriaxone are the most commonly recommended. However, failures have been reported with monotherapy with these compounds (5, 15, 22, 27).

Fosfomycin is a unique bactericidal antibiotic which exhibits good in vitro activity against both penicillin-susceptible and penicillin-resistant pneumococcal strains (17, 26). It is also able to penetrate most infected tissues (24, 26, 29).

Several investigators have reported an in vitro synergy between β-lactam antibiotics and fosfomycin against penicillin-resistant pneumococcal strains (2, 3, 9, 26), and therefore, the study described here was undertaken to evaluate whether this documented synergy is also apparent in vivo.

By using a prolonged (48-h) fibrin clot model in rabbits, we tested the intrinsic therapeutic efficacies of cefotaxime and ceftriaxone alone and in combination with fosfomycin, and we voluntarily chose to investigate low antibiotic concentrations.

MATERIALS AND METHODS

Bacterial strain and susceptibility studies. The tested *S. pneumoniae* strain (strain 9093, serotype 9v; kindly provided by P. Geslin, Centre de Référence des Pneumocoques) was obtained from a culture of cerebrospinal fluid from a patient with meningitis. The MICs of the study drugs were determined by the standard tube dilution technique, in which an inoculum of 5×10^5 CFU and Mueller-Hinton broth supplemented with 5% horse blood, 0.25 ml of $MgCl_2$, and 0.5 ml $CaCl_2$ were used. The MIC was defined as the lowest concentration of drug that was required to inhibit visible growth.

Since we used the fibrin clot model in rabbits, we checked for the susceptibility of this pneumococcal strain in fibrin. The same dilution technique described above was used, but the broth was a 2.5% solution of sterile bovine fibrinogen in brain heart broth. MICs were the same by both methods and were 2, 0.5, 0.5, and 16 mg/liter for penicillin, cefotaxime, ceftriaxone, and fosfomycin, respectively. Moreover, we investigated the in vitro killing curves in this system since it allowed a significant inhibition of spontaneous autolysis; indeed, the rates of growth of this pneumococcal strain were the same over the first 6 h in both systems, but for the brain heart broth the lysis began after this time, leading to almost complete lysis at the 24th h (2.0 ± 1 log CFU/ml); on the contrary, for the fibrin clot system, the concentration at 24 h was 5.2 ± 2 log CFU/ml ($P = 0.02$). Thus, for this specific strain, this latter system allowed a better means of comparison of low concentrations of antibiotics. For these investigations, we used the

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same protocol as that used in the in vivo experiment (see below); the antibiotics were prepared according to the recommendations of the manufacturers. Briefly, fibrin clots containing the indicated antibiotic concentrations were sampled at time zero and at 3, 6, 12, and 24 h; the lysis of clots was obtained by adding trypsin, and 100 μl of an adequate dilution was plated onto blood agar. The results are the means of at least three sets of experiments performed in duplicate.

Experimental model. Infected fibrin clots were prepared as follows. A 2-ml sterile solution of 2.5% human fibrinogen was distributed into test tubes (13 by 100 mm) and infected with 0.1 ml of a 5-h culture of the strain (inoculum 10^5 to 10^6 CFU/ml). Human thrombin (Laboratoire Stago, Paris, France) was added to each tube, and then the tubes were incubated at 37°C for 1 h. The resulting clots were then gently removed and were immediately inserted subcutaneously into rabbits.

We used a modified technique of the subcutaneous fibrin clot model in rabbits (4) in order to obtain a prolonged period of observation (48 h). Male New Zealand White rabbits (weight, 2 to 3 kg) were anesthetized with ketamine and xylazine. Then, (i) a central silastic venous catheter was surgically placed into the rabbits by using a sterile operative technique as described previously (31) to provide for continued nontraumatic venous access, and (ii) one flank was shaved and swabbed with iodine and alcohol and then the skin was anesthetized with 2% lidocaine before making a 4-cm incision. On the day of the experiment, eight infected clots were placed into the subcutaneous pocket. Autoclips were applied to close the incision.

Antibiotic regimens. Antibiotics were injected into the rabbits immediately after the clots were inserted. Unless otherwise indicated, each antibiotic was infused as a 30-s bolus. Rabbits were randomized to receive no drug as a control; cefotaxime as one or two doses 6 h apart (the doses were each 50 mg/kg of body weight) or were given as a 6-h continuous infusion including a 25-mg/kg loading dose and then 75 mg/kg infused with an electric pump (total dose, 100 mg/kg); ceftriaxone as one dose of 8 mg/kg; fosfomycin as one or two doses given 6 h apart (the doses were each 50 mg/kg) or as a 6-h continuous infusion including a 25-mg/kg loading dose and then 75 mg/kg infused with an electric pump (total dose, 100 mg/kg); and the following combinations with the same doses given above: cefotaxime and fosfomycin at one dose each, cefotaxime and fosfomycin at two doses each, cefotaxime and fosfomycin as a continuous infusion, ceftriaxone and fosfomycin at one dose each, or ceftriaxone at one dose with fosfomycin as two divided doses.

Determination of drug concentration and pharmacokinetics. Each clot was weighed and homogenized in a 2.5% solution of trypsin (Laboratoire Eurobio, Les Ulis, France), the volume of which was equal in weight to the weight of the clot. Trypsin had no effect on antibiotic activity and did not influence the bacterial count. Dissolved clots and serum samples were assayed for antibiotic concentrations. Concentrations of fosfomycin were measured by an agar well diffusion microbioassay with *Serratia marcescens*; the detection limit was 0.625 mg/liter; the intraassay variability was <5% in the range of observed concentrations (from 1 to 4 mg/liter), and the interassay variability was <5%; in case of combination with cefotaxime or ceftriaxone, the β-lactam was hydrolyzed by the addition of cephalosporinase (600 U/ml) extracted from *Serratia liquefaciens* SL132. This procedure did not change the performance of the fosfomycin bioassays. The concentrations of ceftriaxone and cefotaxime were measured by high-performance liquid chromatography as described previously (19, 23); the detection limit was 0.082 mg/liter in serum and 0.095 mg/liter in trypsinized fibrin clots; intra- and interassay variations were <6%. Standards of serum samples were diluted in 100% normal rabbit serum, whereas standards of fibrin clots samples were diluted in trypsinized fibrin clots.

Serum samples were obtained at the following times: time zero; 5 min; 1, 3, and 6 h; 6 h and 5 min (if second injection); and 7, 12, 24, 36, and 48 h. Fibrin clots samples were obtained at time zero; 5 min; and 1, 3, 6, 7, 12, 24, 36, and 48 h. The area under the concentration-versus-time curve (AUC) was obtained by the method of successive trapezoidal approximation (4). The half-lives of elimination of the antibiotics in serum and clots were calculated by the least-squares method. The maximal concentration was considered as the concentration at the 5th min after the end of the infusion in serum and the highest observed concentration in clots. The time to reach the MIC and the time above the MIC in fibrin clots were calculated from each individual concentration-versus-time curve. The concentration at the time of regrowth was the concentration observed in clots at the time of bacterial regrowth (see below). This concentration defined the critical concentration below which the bacteria grew in this in vivo model.

In vivo efficacy. The efficacies of the various regimens were evaluated by analyzing the bacterial contents of the infected clots at each time interval by using appropriate dilutions of trypsinized clots, which were inoculated onto blood agar; this was followed by incubation at 37°C for 24 h (log CFU per gram of clot). The detection sensitivity of the method was ≥10 CFU/g. Thus, killing indices (versus time) were calculated (4).

Time of regrowth was defined for each individual experiment as that time when the rate of bacterial growth became positive. Also, the reduction of the bacterial content in fibrin clots between time zero and the time of regrowth ($\Delta \log_{10}$ CFU per gram at the time of regrowth) was calculated for each individual experiment; this parameter defined the best bacterial effect for each individual experiment. Following the bacterial curve after this latter point, the rate of regrowth was calculated and expressed as log CFU per gram per hour.

Statistical analysis. The required number of experiments was determined for a type α error of 5% and a type β error of 20% for a two-sided test and a difference of 1 log CFU at the 24th h. Therefore, results were expressed as the mean (± standard deviation) of at least five experiments for each antibiotic regimen. Statistics were performed by one-way analysis of variance, and the following tests were used for specific comparisons. The protected least significant difference test of Fisher was used to test the differences in the results between each pair of regimens, the contrast method was used to test the differences in the results between specific pairs or groups of regimens, and the least significant range with the Newman-Keuls test (with $\alpha = 0.05$) was used to compare treatments all together. Univariate coefficient ratio estimates were computed by exact methods. A forward stepwise multivariate linear regression was used in an attempt to correlate efficacy and pharmacodynamic criteria. In order to standardize the comparison of all the regimens, the best bacterial effect of each individual experiment ($\Delta \log_{10}$ CFU per gram) at the time of regrowth was considered. A result was considered significant if the P value was less than 0.05.

RESULTS

In vitro killing curves. As shown in Fig. 1, each antibiotic was tested at a fraction and a multiple of its MIC. No significant differences were noted between cefotaxime and ceftriaxone. The activities of cefotaxime, ceftriaxone, and fosfomycin increased as the antibiotic concentrations increased. The combinations of fosfomycin at half the MIC plus cefotaxime or ceftriaxone at the MIC were highly synergistic ($P < 0.01$). The same combinations but with β-lactam antibiotics at twice their MICs led to a higher bacterial reduction ($P < 0.01$).

Pharmacokinetic study. (i) Serum. Pharmacokinetic data for each drug was obtained from studies of serum from at least five animals (Table 1). The highest mean concentrations in serum were observed 5 min after injection of one bolus of cefotaxime, ceftriaxone, or fosfomycin. Following two injections 6 h apart, the concentrations of both cefotaxime and fosfomycin in serum were slightly increased 5 min after administration of the second bolus; the continuous infusions of cefotaxime and fosfomycin were associated with the lowest peak concentrations; the respective half-lives of cefotaxime and fosfomycin were not different, whatever the regimen used. Consequently, the AUCs were the lowest for the continuous infusions of cefotaxime and fosfomycin and the highest for the two injection regimens.

No interactions between β-lactam antibiotics and fosfomycin were observed.

(ii) Fibrin clots. Pharmacokinetic data for the drugs in fibrin clots are given in Table 1 and Fig. 2. The concentrations of cefotaxime and fosfomycin were highest following the two-injection regimens and the lowest following the one-dose regimens. The peak concentration of ceftriaxone was significantly lower than that of cefotaxime given as two divided doses.

The times of peak concentrations were obtained approximately 1 to 2 h after administration of the last bolus dose for both cefotaxime and fosfomycin. The times of peak concentrations of the continuous regimens were obtained approximately at the end of the 6-h infusion. The time of the peak ceftriaxone concentration was similar to that observed for the continuous infusion of cefotaxime.

The half-lives of elimination of cefotaxime and fosfomycin from clots were not different, whatever the modality of administration ($P > 0.6$) and were approximately 4 h and 3.5 h, respectively. For ceftriaxone, the value of this parameter was 10.3 ± 5 h.

Finally, the AUC of cefotaxime given as one dose was lower than that of cefotaxime given as two doses, and ceftriaxone exhibited a higher AUC than cefotaxime as a continuous infusion. The AUC of fosfomycin given as two doses was higher than that of fosfomycin given as a continuous infusion and was also higher than that of fosfomycin given as one dose.

No pharmacokinetic interactions were observed between the two β-lactam antibiotics and fosfomycin.

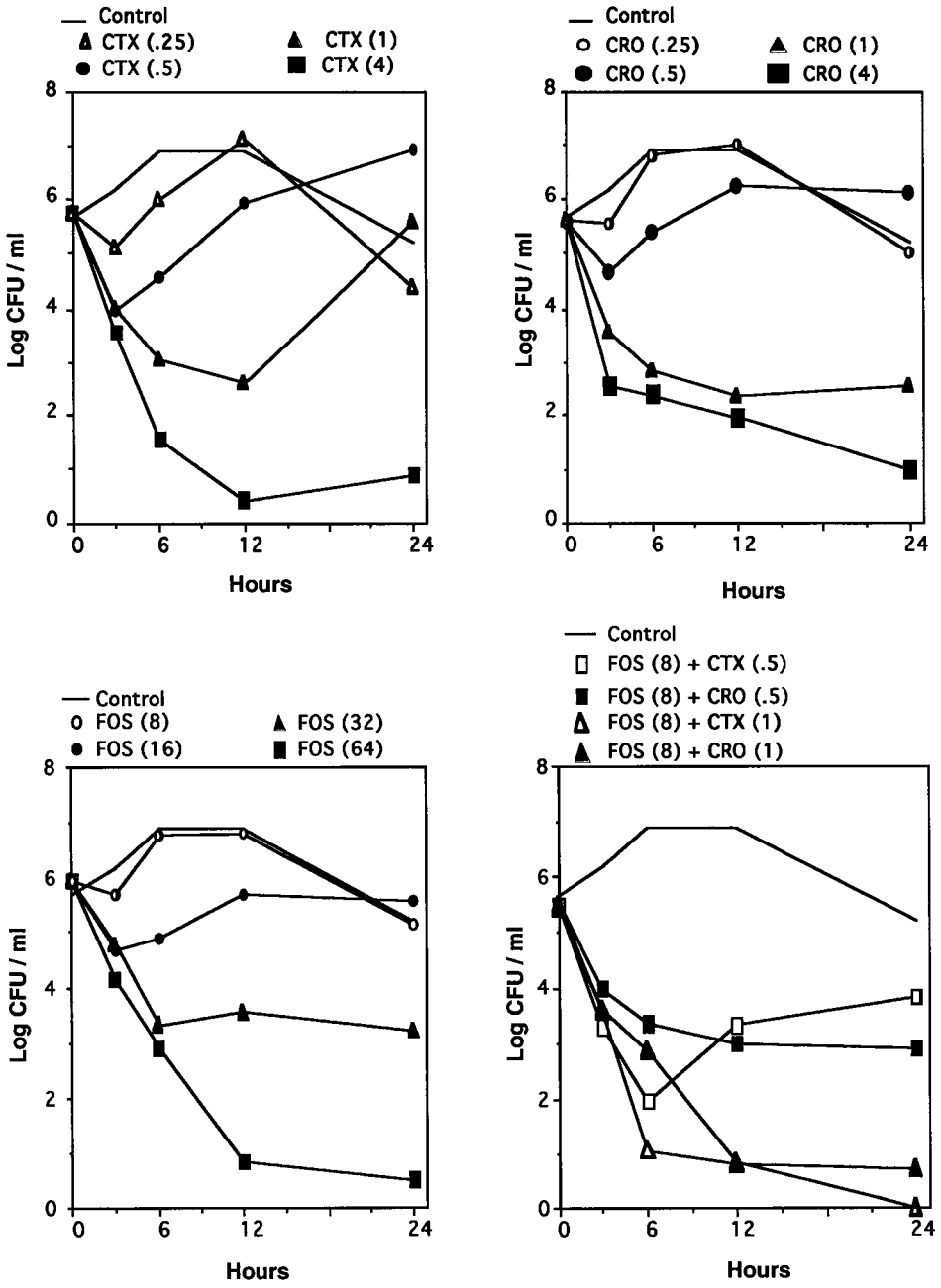

FIG. 1. In vitro kill-kinetic studies of strain 9093 for cefotaxime, ceftriaxone, and fosfomycin. CTX, cefotaxime; CRO, ceftriaxone; FOS, fosfomycin. Concentrations (in milligrams per liter) are given in parentheses.

The concentrations of cefotaxime in the fibrin clots rapidly reached the MIC if cefotaxime was administered in bolus form (Table 2); however, when it was infused in continuous form, this level was obtained at 60 ± 30 min versus 16 ± 6 and 13 ± 6 min for cefotaxime given as one and two doses, respectively ($P < 0.007$). The MIC was obtained at 36 ± 12 min for ceftriaxone, which was significantly sooner than that for cefotaxime given as a continuous infusion ($P = 0.01$). For fosfomycin, the time needed to reach the MIC in clots was also significantly delayed when continuous infusion was used (132 ± 28 min versus 39 ± 14 and 49 ± 20 min for fosfomycin given as one and two doses, respectively; $P < 0.001$).

The times during which the concentrations were greater than the MIC for this strain were 9.1 ± 1 h for cefotaxime given as one dose, 15.7 ± 5 h for cefotaxime given as a continuous infusion, 21.7 ± 4 h for cefotaxime given as two divided doses ($P = 0.008$ versus cefotaxime given as one dose), and 25.5 ± 10 h for ceftriaxone ($P < 0.004$ versus cefotaxime given as one dose and as a continuous infusion). The times that the concentrations were greater than the MICs were 6.4 ± 3, 8.4 ± 1.8, and 14.6 ± 4 h ($P = 0.001$ versus the two first regimens) for fosfomycin given as one and two doses and as a continuous infusion, respectively.

The critical concentration in vivo was that observed at the time of bacterial regrowth. These concentrations were 0.77 ± 0.4, 1.27 ± 0.9, 1.99 ± 0.9, and 1.64 ± 0.3 mg/liter for cefo-

TABLE 1. Pharmacokinetics of cefotaxime, ceftriaxone, and fosfomycin[a] in infected fibrin clots

Antibiotic	Regimen (dose [mg/kg])	Time (h), type of infusion	Abbreviation	C_{max} (mg/liter)	T_{max} (h)	Half-life (h)	AUC (mg · h/liter)
Cefotaxime	50	0, bolus	CTX_1	2 ± 0.7[b]	1.6 ± 1.7[c,d]	4.16 ± 1.3	13.1 ± 3.2[e,f]
	50	0 and 6, bolus	CTX_2	3.5 ± 0.9[b,c]	6.8 ± 3[c,d]	4.5 ± 1.4[c]	40.6 ± 11.5[a]
	100	0 to 6, continuous[h]	CTX_{cont}	2.3 ± 0.6[g]	5.7 ± 1.9	3.8 ± 1.6	24.3 ± 10.6[f]
Ceftriaxone	8	0, bolus	CRO	2.5 ± 1	5.1 ± 2.6	10.3 ± 5.3[c]	45 ± 38[f]
Fosfomycin	50	0, bolus	FOS_1	29.5 ± 9[f]	2.2 ± 1.6[c,i]	3.3 ± 1	225 ± 148[b,g]
	50	0 and 6, bolus	FOS_2	35.6 ± 5.5[f]	7.7 ± 1.8[c,j]	3.7 ± 1.5	520 ± 289[g]
	100	0 to 6, continuous	FOS_{cont}	30.4 ± 5.6	6.5 ± .5[i,j]	3.5 ± .9	322 ± 82[b]

[a] Values of pharmacokinetic parameters are means ± standard deviations. T_{max}, time to C_{max}. The abbreviations for the other pharmacokinetic parameters are given in the text.
[b] $P = 0.008$.
[c] $P < 0.001$.
[d] $P = 0.004$.
[e] $P = 0.003$.
[f] $P = 0.006$.
[g] $P = 0.001$.
[h] A 25-mg/kg loading dose plus 75 mg/kg over 6 h.
[i] $P = 0.04$.
[j] $P = 0.05$.

taxime given as one and two doses and as a continuous infusion and for ceftriaxone, respectively; the mean critical concentration of all of the β-lactam regimens was 1.5 ± 0.7 mg/liter. The critical concentrations were 23 ± 2, 26 ± 6, and 32.5 ± 3 mg/liter for fosfomycin given as one and two doses and as a continuous infusion, respectively; the mean critical concentration for all of the fosfomycin regimens was 27.3 ± 5.6 mg/liter. Concerning the antibiotic combinations, the critical concentrations of cefotaxime were 0.85 ± 0.2, 0.55 ± 0.6, and 1.2 ± 0.9 mg/liter for regimens including cefotaxime and fosfomycin given as one and two doses and as a continuous infusion, respectively; the mean of these concentrations was 0.83 ± 0.7 mg/liter, which was significantly lower than the mean of the critical concentrations for the cefotaxime regimens ($P < 0.04$). For ceftriaxone, the critical concentrations were 1.8 ± 1 and 1.7 ± 1 mg/liter when this antibiotic was combined with one and two doses of fosfomycin, respectively. The critical concentrations of fosfomycin in the combination regimens were 10 ± 7, 7.2 ± 8, and 12.3 ± 7 mg/liter with cefotaxime and fosfomycin given as one and two doses and as a continuous infusion, respectively, and 14.2 ± 11 and 22 ± 14 mg/liter for ceftriaxone with one and two doses of fosfomycin, respectively; all of these critical concentrations of fosfomycin were significantly lower than the critical concentration of fosfomycin given alone ($P < 0.01$). Furthermore, the mean critical concentration of fosfomycin in the combination regimens was 14.7 ± 12 mg/liter, which was significantly lower than the critical concentration of fosfomycin given alone ($P = 0.001$).

Bacteriologic effect. (i) Monotherapies. Following a single injection of each antibiotic alone, the reductions in the bacterial contents were similar ($P > 0.15$) and very brief (Fig. 3); the bacterial reductions were 1.6 ± 0.7 and 1 ± 1 log CFU/g for cefotaxime and ceftriaxone, respectively; the bacterial reduction was 1.65 ± 1.3 log CFU/g for fosfomycin (Table 2). The times of bacterial regrowth were 6 ± 2.8, 6.6 ± 1.2, and 2.6 ± 0.8 h for the three drugs, respectively; these differences did not reach statistical significance (Table 2; $P > 0.2$). The rates of regrowth were similar (0.23 ± 0.2, 0.25 ± 0.2, and 0.25 ± 0.1 log CFU/h for cefotaxime, ceftriaxone, and fosfomycin respectively; Table 2).

Following the continuous infusions of cefotaxime and fosfomycin, the bacterial reductions were not higher than those for

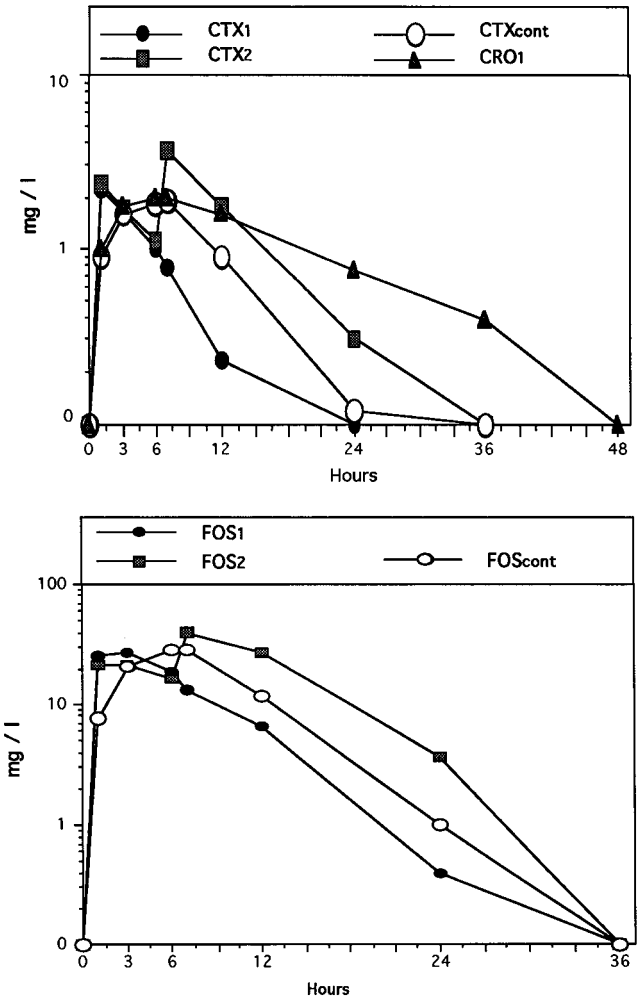

FIG. 2. Fibrin clot concentrations of cefotaxime and ceftriaxone and fosfomycin (means of at least five experiments in rabbits). CTX_1, cefotaxime, 50 mg/kg at hour 0; CTX_2, cefotaxime, 50 mg/kg at hours 0 and 6; CTX_{cont}, cefotaxime, 25-mg/kg loading dose plus 75 mg/kg as a continuous infusion over 6 h; CRO_1, ceftriaxone, 8 mg/kg; FOS_1, fosfomycin, 50 mg/kg at hour 0: FOS_2, fosfomycin, 50 mg/kg at hours 0 and 6; FOS_{cont}, fosfomycin, 25-mg/kg loading dose plus 75 mg/kg as a continuous infusion over 6 h.

TABLE 2. Pharmacodynamic parameters of cefotaxime, ceftriaxone, or fosfomycin and their combinations in infected fibrin clots

Regimen[b]	Time to reach MIC (min)[c]	Time above MIC (h)[d]	Time of regrowth (h)[e]	Concentration at regrowth (mg/liter)[f]	$\Delta\log_{10}$ CFU at time of regrowth[g]	Rate of regrowth log CFU/h[h]
CTX_1	16 ± 6	9.1 ± 1	6 ± 2.8	0.77 ± 0.4	−1.6 ± 0.7	0.23 ± 0.2
CTX_2	13 ± 6	21.7 ± 4	12 ± 0	1.27 ± 0.9	−3.6 ± 0.4	0.29 ± 0.08
CTX_{cont}	60 ± 30	15.7 ± 5	7.5 ± 1.8	1.99 ± 0.9	−2 ± 0.9	0.15 ± 0.08
CRO_1	36 ± 12	25.5 ± 10	6.6 ± 1.2	1.64 ± 0.3	−1 ± 1	0.25 ± 0.2
FOS_1	39 ± 14	6.4 ± 3.7	2.6 ± 0.8	23 ± 2	−1.65 ± 1.3	0.25 ± 0.1
FOS_2	49 ± 20	14.6 ± 4.6	6 ± 2	26 ± 6	−0.94 ± 0.6	0.15 ± 0.05
FOS_{cont}	132 ± 28	8.4 ± 1.8	12.5 ± 9	32.5 ± 3	−2.45 ± 1.5	0.12 ± 0.09
CTX_1-FOS_1			6 ± 2	0.85 ± 0.2 (CTX) 10 ± 7 (FOS)	−2.4 ± 0.9	0.2 ± 0.1
CTX_2-FOS_2			23.2 ± 11	0.55 ± 0.6 (CTX) 7.2 ± 8 (FOS)	−4.2 ± 0.7	0.08 ± 0.1
CTX_{cont}-FOS_{cont}			11 ± 2.4	1.2 ± 0.9 (CTX) 12.3 ± 7 (FOS)	−3.5 ± 0.4	0.27 ± 0.1
CRO_1-FOS_1			16.5 ± 11	1.82 ± 1.4 (CRO) 14 ± 11 (FOS)	−3.79 ± 0.6	0.11 ± 0.08
CRO_1-FOS_2			13.1 ± 6	1.79 ± 1.2 (CRO) 22.9 ± 14 (FOS)	−3.95 ± 0.5	0.13 ± 0.09

[a] Values are means ± standard deviations. Rankings are based on the Newman Keuls's test (level of significance, $P < 0.05$) (≈, not different).
[b] CTX_1, cefotaxime, 50 mg/kg at hour 0; CTX_2, cefotaxime at 50 mg/kg at hours 0 and 6; CTX_{cont}, cefotaxime, 25-mg/kg loading dose plus 75 mg/kg as a continuous infusion over 6 h; FOS_1, fosfomycin, 50 mg/kg at hour 0; FOS_2, fosfomycin, 50 mg/kg at hours 0 and 6; FOS_{cont}, fosfomycin, 25-mg/kg loading dose plus 75 mg/kg as a continuous infusion over 6 h; CTX_1-FOS_1, combination of one dose of each antibiotic; CTX_2-FOS_2, combination of two doses of each antibiotic 6 h apart; CTX_{cont}-FOS_{cont}, combination of CTX_{cont} plus FOS_{cont}; CRO_1, ceftriaxone, 8 mg/kg; CRO_1-FOS_1, ceftriaxone, 8 mg/kg, and fosfomycin, 50 mg/kg, at hour 0; CRO_1-FOS_2, ceftriaxone, 8 mg/kg, and fosfomycin, 50 mg/kg, at hours 0 and 6.
[c] $CTX_1 \approx CTX_2 \approx CRO_1 < CTX_{cont}$ and $FOS_1 \approx FOS_2 < FOS_{cont}$.
[d] $CTX_1 < CTX_{cont} \approx CTX_2 \approx CRO_1$ and $FOS_1 < FOS_{cont} < FOS_2$.
[e] $FOS_1 \approx FOS_2 \approx CTX_1$-$FOS_1 \approx CTX_1 \approx CRO_1 \approx CTX_{cont} < CTX_{cont}$-$FOS_{cont} \approx CTX_2 \approx FOS_{cont} < CRO_1$-$FOS_2 \approx CRO_1$-$FOS_1 < CTX_1$-$FOS_2$.
[f] The concentrations of FOS in each of the combination were lower than those in the FOS regimens.
[g] CTX_2-$FOS_2 \approx CRO_1$-$FOS_2 \approx CRO_1$-$FOS_1 \approx CTX_2 \approx CTX_{cont}$-$FOS_{cont} > FOS_{cont} \approx CTX_1$-$FOS_1 \approx CTX_{cont} \approx CTX_1 \approx FOS_1 \approx CRO_1 \approx FOS_2$.
[h] The only difference was between CTX_2-FOS_2 and CTX_2. By using the contrast method: $CRO_1 < CRO_1$-$FOS_1 \approx CRO_1$-FOS_2.

each antibiotic given in bolus form but were higher than that for ceftriaxone ($P < 0.01$). The times of regrowth were 7.5 ± 1.8 h for cefotaxime and 12.5 ± 9 h for fosfomycin ($P = 0.18$). This latter time was significantly more prolonged than that for fosfomycin given as one bolus ($P = 0.01$). The rates of regrowth were similar for both antibiotics; these rates were not different from the rate of regrowth for ceftriaxone (Table 2).

Following two doses given 6 h apart, the bacterial reductions were 3.6 ± 0.4 log CFU/g for cefotaxime and 0.94 ± 0.6 log CFU/g for fosfomycin ($P < 0.001$). This bacterial effect of cefotaxime given as two divided doses was better than those of cefotaxime given once ($P = 0.003$), cefotaxime given as a continuous infusion ($P = 0.003$), or ceftriaxone ($P < 0.001$). In contrast, the bacterial reductions for fosfomycin were not different, whatever the modality of administration ($P > 0.2$). By using the two-injection regimen given 6 h apart, the mean time of regrowth occurred at the 12th h for cefotaxime and at the 6th h for fosfomycin (Table 2). The rate of regrowth was 0.29 ± 0.08 log CFU/h for cefotaxime and 0.15 ± 0.05 log CFU/h for fosfomycin.

(ii) Combination therapies. By using the single bolus dose, whatever the combination of β-lactam and fosfomycin used, the result was a significantly higher reduction in the bacterial load (Table 2; Fig. 3). For cefotaxime and fosfomycin given once, the bacterial reduction was 2.4 ± 1.5 log CFU/g, which was higher than that for each monotherapy, but these differences did not reach statistical significance ($P = 0.15$). The times and rates of bacterial regrowth were similar for these three regimens. The addition of one dose of fosfomycin to ceftriaxone led to a greater antibacterial effect (3.79 ± 0.6 log CFU/g, $P < 0.001$); this combination was better than the combination of cefotaxime and fosfomycin given once ($P = 0.009$). The time of bacterial regrowth for ceftriaxone and fosfomycin given as one dose was 16.5 ± 11 h, which was significantly longer than that for cefotaxime and fosfomycin given once in bolus form ($P = 0.01$). In addition, the rate of regrowth with this regimen was lower (0.11 ± 0.08 log CFU/h) than that with fosfomycin given once ($P = 0.0004$).

Following continuous infusion of the combination of cefotaxime and fosfomycin over 6 h, the bacterial reduction was 3.5 ± 0.4 log CFU/g, which was significantly better than that from the continuous infusion of cefotaxime ($P = 0.002$) or fosfomycin ($P = 0.03$) alone. The time of regrowth and the rate of regrowth of this regimen were 11 ± 2.4 h and 0.27 ± 0.1 log CFU/h, respectively, which were not different from the times

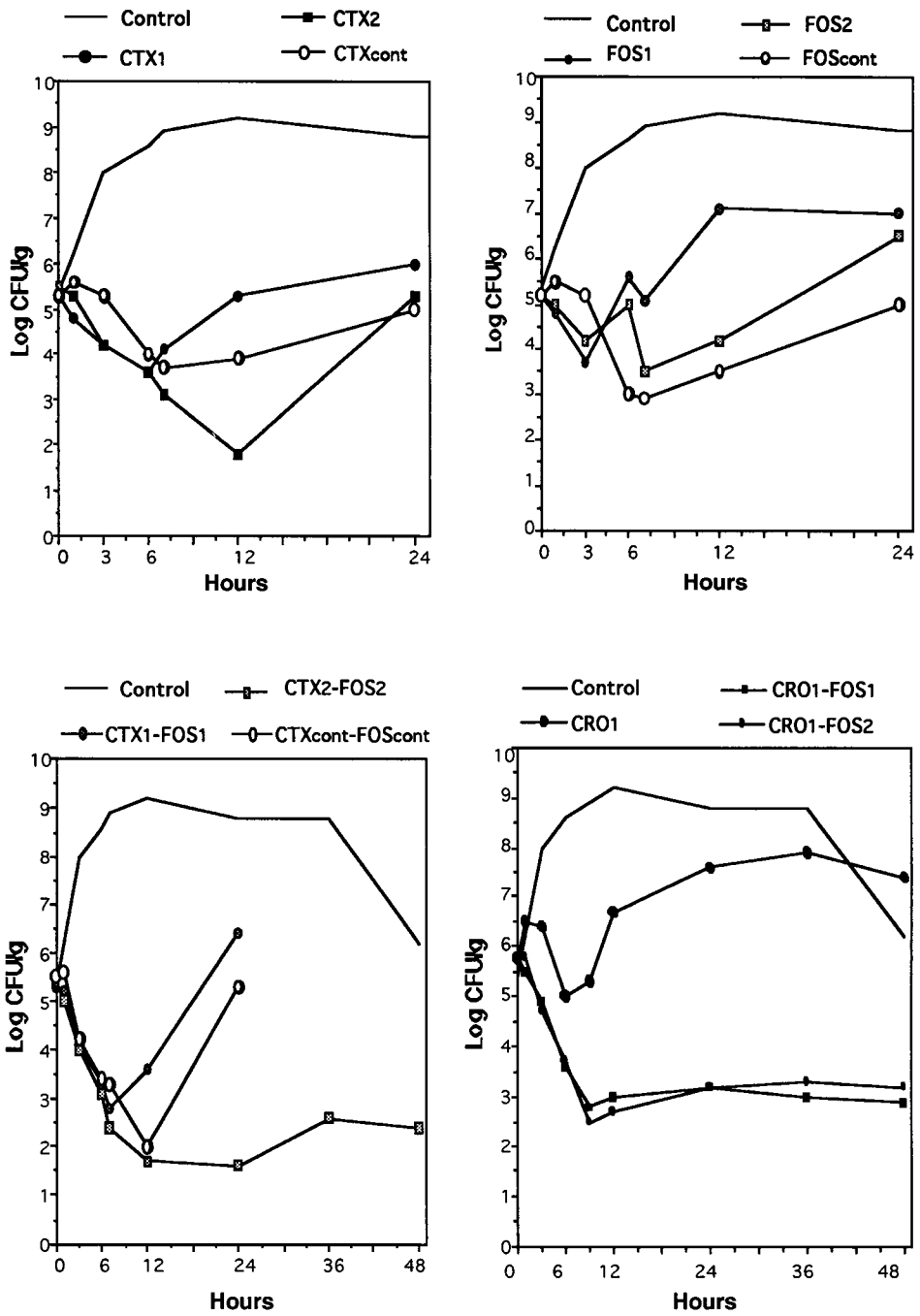

FIG. 3. Bacterial concentrations in fibrin clots (means of at least five experiments in rabbits). CTX_1, cefotaxime, 50 mg/kg at hour 0; CTX_2, cefotaxime, 50 mg/kg at hours 0 and 6; CTX_{cont}, cefotaxime, 25-mg/kg loading dose plus 75 mg/kg as a continuous infusion over 6 h; FOS_1, fosfomycin, 50 mg/kg at hour 0; FOS_2, fosfomycin, 50 mg/kg at hours 0 and 6; FOS_{cont}, fosfomycin, 25-mg/kg loading dose plus 75 mg/kg as a continuous infusion over 6 h; CTX_1-FOS_1, combination of one dose of each antibiotic; CTX_2-FOS_2 combination of two doses of each antibiotic given 6 h apart; CRO_1, ceftriaxone, 8 mg/kg; CRO_1-FOS_1, ceftriaxone, 8 mg/kg, and fosfomycin, 50 mg/kg, at hour 0; CRO_1-FOS_2, ceftriaxone, 8 mg/kg, and fosfomycin, 50 mg/kg, at hours 0 and 6.

and rates of regrowth observed for each antibiotic alone or in combination given once in bolus form.

Following two injections of cefotaxime and fosfomycin 6 h apart, the bacterial reduction was 4.2 ± 0.7 log CFU/g, which was significantly higher than that for fosfomycin given as two doses ($P < 0.001$). The addition of two doses of fosfomycin 6 h apart to ceftriaxone led to a bacterial reduction of 3.95 ± 0.6 CFU/g, which was not different from the bacterial effect of ceftriaxone given with only one dose of fosfomycin or the combination of cefotaxime and fosfomycin given as two doses ($P > 0.2$). The time of regrowth of this latter combination was 23.2 ± 11 h, which was significantly later than that obtained with cefotaxime and fosfomycin given as one dose ($P < 0.0001$) or as a continuous infusion ($P = 0.0005$), the combination of ceftriaxone plus fosfomycin given as one dose ($P = 0.02$), and the combination of ceftriaxone plus two doses of fosfomycin

(13.1 ± 6 h; $P = 0.0003$). The rates of regrowth of all of the combinations including cefotaxime or ceftriaxone plus fosfomycin as either one or two doses were similar ($P > 0.3$). However, the rate of bacterial regrowth for cefotaxime given as two doses was significantly higher than that for cefotaxime plus fosfomycin given as two doses ($P = 0.01$); similarly, the rates for ceftriaxone plus fosfomycin given as one and two doses were significantly lower than that for ceftriaxone given alone ($P = 0.03$ and $P = 0.05$, respectively).

Correlation of pharmacokinetic parameters with therapeutic efficacy. For this analysis, in an attempt to compare all of the regimens, the $\Delta\log_{10}$ CFU per gram at the time of bacterial regrowth was used (Table 2). The maximal and residual concentrations, the time to reach the MIC, the time above the MIC, and the log AUC/MIC ratio were entered into this analysis as independent parameters.

For cefotaxime or ceftriaxone, the only parameter that significantly correlated with efficacy was the maximum concentration in serum (C_{max}) ($P = 0.005$, coefficient = 0.91, $R^2 = 0.28$). The efficacy of fosfomycin correlated significantly with the residual concentration ($P = 0.004$, coefficient = 0.06, $R^2 = 0.56$) and C_{max} ($P = 0.05$, coefficient = 0.07, $R^2 = 0.31$); a multivariate analysis showed that the most important parameter was the residual concentration ($P = 0.0047$). The efficacy of the combinations correlated with the log AUC/MIC ratios for cefotaxime and ceftriaxone ($P < 0.001$, coefficient = 0.81, $R^2 = 0.34$), the log AUC/MIC ratio for fosfomycin ($P = 0.0006$, coefficient = 0.25, $R^2 = 0.25$), the C_{max}s of cefotaxime and ceftriaxone ($P = 0.001$, coefficient = 0.31, $R^2 = 0.21$), the C_{max} of fosfomycin ($P = 0.01$, coefficient = 0.01, $R^2 = 0.12$), the time above the MIC for cefotaxime and ceftriaxone ($P = 0.0005$, coefficient = 0.03, $R^2 = 0.26$), and the time above the MIC for fosfomycin ($P = 0.004$, coefficient = 0.06, $R^2 = 0.18$). By using the multivariate analysis, the most important parameter was the log AUC/MIC ratios for β-lactam antibiotics ($P < 0.001$).

DISCUSSION

The main findings of the experiments involving an infection caused by a highly penicillin-resistant pneumococcal strain was that cefotaxime or ceftriaxone combined with fosfomycin led to either a greater bacterial reduction, a more prolonged antibacterial effect, or a lower regrowth rate than those observed with the other regimens tested.

All of the combinations with the exception of the combination of cefotaxime and fosfomycin given once led to higher bacterial reductions than did either monotherapy. This latter observation could partly be explained by the fact that after the 6th h, the concentrations of both antibiotics were too low to give any antibacterial effect (Fig. 2). Nevertheless, it was observed that the critical concentration of fosfomycin in the combinations was significantly decreased to approximately reach the MIC (Table 2). Furthermore, there was also a significant decrease in the critical concentrations of cefotaxime in the combinations versus those in the monotherapies. These in vivo observations were in accordance with the in vitro findings. Thus, in vivo, the presence of fosfomycin decreased the critical concentration of cefotaxime and vice versa.

Combinations of cefotaxime or ceftriaxone with fosfomycin were associated with a prolonged antibacterial effect. Indeed, except for the combination of cefotaxime and fosfomycin given once, all of the combinations led to a delay in bacterial regrowth compared with relative monotherapy. The best example was observed with cefotaxime and fosfomycin administered as two divided doses. With this latter regimen, the time of regrowth occurred significantly later than with monotherapy. Similarly, the ceftriaxone-fosfomycin combinations significantly delayed regrowth compared with each relative monotherapy. However, the fact that a second dose of fosfomycin did not increase the delay in bacterial regrowth with this combination could partly be explained by the fact that this antibiotic is poorly active against slowly growing bacteria.

Moreover, the intensity of the regrowth of this pneumococcal strain seemed to be reduced when both fosfomycin and β-lactam antibiotics were administered concomitantly to rabbits. By using the contrast method, the rate of regrowth after administration of cefotaxime alone at two doses was higher than that after administration of the combination cefotaxime plus fosfomycin at two doses ($P = 0.01$; Table 2); similarly, the rate of bacterial regrowth for ceftriaxone was higher than that for ceftriaxone plus fosfomycin ($P < 0.05$).

The efficacies of the cefotaxime or the ceftriaxone regimens and the fosfomycin regimens were found to depend on their respective concentrations, which is in complete accordance with previous data (2, 26, 28). Finally, the antipneumococcal effect of the combination of β-lactam antibiotic and fosfomycin in this model seemed to be both time and concentration dependent. With these regimens, the most important independent parameter was the AUC of the β-lactam antibiotic; this finding was also in complete accordance with the fact that both cefotaxime and ceftriaxone decreased by half the critical concentration of fosfomycin, providing conditions for a greater antibacterial effect.

Conclusion. Using an in vivo model infection caused by a highly penicillin-resistant pneumococcal strain, we demonstrated that combinations that included cefotaxime or ceftriaxone plus fosfomycin had very large antibacterial effects; these findings corroborated the in vitro data. Finally, the β-lactam–fosfomycin regimens tested in the experiments increased the bacterial reduction, delayed the time of regrowth, or decreased the rate of regrowth of this pneumococcal infection. Consequently, these combinations could be proposed for use in the treatment of pneumococcal infections.

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