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Simulation of Human Serum Pharmacokinetics of Ticarcillin-Clavulanic Acid and Ceftazidime in Rabbits, and Efficacy against Experimental *Klebsiella pneumoniae* Meningitis

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The penetration into cerebrospinal fluid (CSF) and efficacy of ticarcillin-clavulanic acid, ticarcillin alone, and ceftazidime were compared in rabbits with experimentally induced *Klebsiella pneumoniae* meningitis. The compounds were administered to simulate in rabbit plasma the concentration-versus-time curves observed in humans after 30-min infusions of Timentin (3 g of ticarcillin plus 100 mg of clavulanic acid), ticarcillin (3 g), and ceftazidime (2 g). Single- and multiple-dosing schedules were used. The penetrations of clavulanic acid into CSF (expressed as [area under the concentration-time curve for CSF/area under the curve for plasma] × 100) after the two dosing schedules were 28 and 24.5%, similar to that for ceftazidime (21%; multiple-dosing only) and greater than those for ticarcillin (8.4 and 9.3%). Ticarcillin was ineffective in reducing viable counts in CSF but, in the presence of clavulanic acid, reduced bacterial numbers by approximately 99% at 4 h after a single dose and by 99.99% at 12 h after three doses given at 4-h intervals. Two doses of ceftazidime given 8 h apart were more effective than the three doses of ticarcillin-clavulanic acid, in keeping with the in vitro activities of these compounds against the infecting organism. These results illustrate the ability of clavulanic acid to penetrate the blood-CSF barrier such that concentrations of the inhibitor in CSF potentiate the activity of ticarcillin against the ticarcillin-resistant, β-lactamase-producing strain of *K. pneumoniae*.

In the presence of clavulanic acid the antibacterial spectrum of ticarcillin is extended to include ticarcillin-resistant, β-lactamase-producing strains of members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Bacteroides* spp., *Haemophilus influenzae*, and staphylococci (6, 8, 20). For clavulanic acid to inhibit β-lactamases produced in vivo and allow ticarcillin to exert its bactericidal effect, both compounds should display similar distribution characteristics. This has been demonstrated both in animals (4, 5, 24) and in humans (1, 3, 22). In the study reported here, the penetration into cerebrospinal fluid (CSF) and the efficacy of ticarcillin-clavulanic acid were investigated in a rabbit model of meningitis (18) in which the infecting organism was a strain of *Klebsiella pneumoniae*.

To compensate for the more rapid elimination of ticarcillin (elimination half-life, 0.4 to 0.6 h), clavulanic acid (0.3 to 0.6 h), and ceftazidime (0.75 to 0.88 h) from serum in the rabbit (16, 21, 23; unpublished data) than from serum in humans (half-life, 1.1 ± 0.5, 1.0 ± 0.06, and 1.8 ± 0.21, respectively) (7, 9), the antibiotics were administered to infected rabbits by using a procedure designed to simulate in rabbit plasma the concentration-versus-time curves observed in human serum after therapeutic doses. The efficacy of ticarcillin-clavulanic acid was compared with results for ticarcillin alone and with those for ceftazidime, a broad-spectrum cephalosporin considered useful for the treatment of gram-negative-bacilliary meningitis (2, 12).

MATERIALS AND METHODS

Test organism. A ticarcillin-resistant strain of *K. pneumoniae*, T767, producing the chromosomally mediated Richmond and Sykes class IV β-lactamase was used to induce meningitis in rabbits. The in vitro susceptibility of the infecting organism was determined by a microdilution technique. Serial two-fold dilutions were prepared in Iso-Sensitest broth (Oxoid Ltd.) for ceftazidime, ticarcillin, and ticarcillin in the presence of a range of clavulanic acid concentrations. The inoculum size was approximately 10^6 CFU/ml. After overnight incubation of the organism at 37°C, the MIC, which was interpreted as the lowest concentration of antibiotic that inhibited visible growth, was determined. The contents of each clear well were subcultured on nutrient agar (Oxoid Ltd.) and incubated overnight to determine the MBC, which was interpreted as the concentration that reduced the initial inoculum by more than 99.9%.

Time-kill curve studies in vitro. The inoculum of *K. pneumoniae* T767 was prepared from an overnight culture in Tryptone soya broth (Oxoid Ltd.). Solutions of the antibiotics were prepared in 10 ml of warmed nutrient broth (Oxoid Ltd.) to give final concentrations of 10 μg of ticarcillin per ml, 1.0 μg of clavulanic acid per ml, 10 μg of ceftazidime per ml, and 10 μg of ticarcillin per ml plus 1 μg of clavulanic acid per ml. The organism was added to give initial viable counts of 10^6 or 10^5 CFU/ml. Flasks were incubated at 37°C, and samples were taken for viable count estimation at 0, 1, 2, 4, 6, and 24 h.

Meningitis. Meningitis was induced in New Zealand White male rabbits (2 to 3 kg; Regal Rabbits, Great Bookham, England) by introducing the infecting organism directly into the cisterna magna (18). *K. pneumoniae* T767 was passaged intracisternally in the rabbits three times and maintained on nutrient agar slopes. Prior to infection of the rabbits, the organism was grown in Tryptone soya broth for 4 to 5 h at 37°C with shaking. The culture was then centrifuged at 2,000 × *g* for 10 min at 5°C and washed twice in pyrogen-free physiological saline, suspended, and diluted in saline to give an inoculum size of 10^4 CFU per rabbit. Under anesthesia induced by alphaxolone-alphadolone (Saffan; Glaxovet Ltd.,

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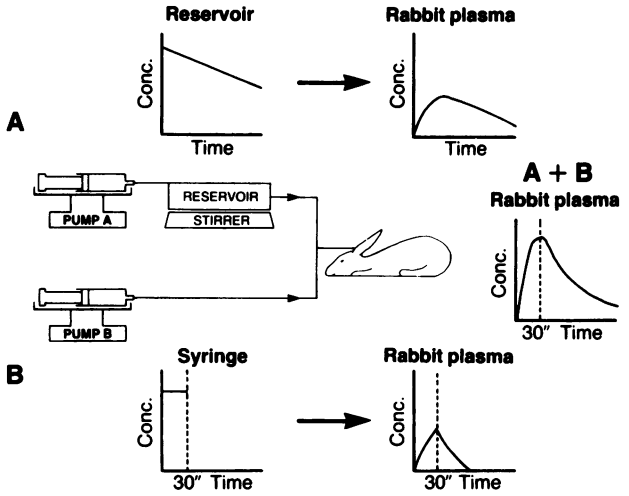

FIG. 1. Procedure for simulating in rabbit plasma the antibiotic concentration-time curve seen in human serum after administration of 30-min intravenous infusion.

Uxbridge, England) at a dose of approximately 10 mg/kg, 0.25 ml of CSF was removed from the cisterna magna, and 0.25 ml of the inoculum was introduced slowly through the same needle. The rabbits were allowed to recover and were returned to their cages until 12 h after infection, at which time the animals were febrile (body temperatures >40°C) and lethargic. Bacterial titers in CSF were between 10^4 and 10^8 CFU/ml, and pleocytosis was evident, with leukocyte counts of 10^5 to 10^6 cells per ml, of which >90% were polymorphoneutrophils.

Antibiotic administration. Disodium ticarcillin and potassium clavulanate were obtained from Beecham Pharmaceuticals, Worthing, England. Ceftazidime was a commercial preparation (Fortum; Glaxo Pharmaceuticals, Ltd., Greenford, England). Doses were adjusted to contain the pure free-acid equivalents.

Before being dosed at 12 h after infection, the animals were anesthetized with 32 mg of sodium pentobarbitol per kg (Sagatal; May & Baker, Dagenham, England), and the external jugular vein was cannulated for the intermittent administration of doses of Sagatal to maintain light anesthesia throughout the study. The femoral artery and the femoral vein were cannulated for sampling of blood and administration of the compounds, respectively.

The procedure for simulating in rabbit plasma the concentration-versus-time curves approximating those in human serum for a 30-min intravenous infusion of a 3.1-g dose of Timentin (3 g of ticarcillin plus 100 mg of clavulanic acid) and a 30-min intravenous infusion of a 2-g dose of ceftazidime is illustrated in Fig. 1. The animals received two infusions (A and B) simultaneously for the first 30 min; thereafter they received only infusion A. Infusion A was used to stimulate human elimination-phase concentrations of antibiotic in rabbit plasma, and infusion B was used to produce the peak concentration observed in human serum at 30 min.

The simulation of human elimination-phase kinetics in rabbit plasma has been described previously (23). In brief, phosphate-buffered saline (0.1 M, pH 7.4) was infused at a constant rate by pump A into a fixed-volume reservoir (V^{res}) containing an antibiotic solution (Fig. 1). This resulted in a progressive dilution of the solution as it was infused into the rabbit. The rate of decline in the concentrations was equivalent to the human elimination phase rate constant (β). The infusion flow rate (F) for infusion A and the initial concentration required in the reservoir (C^{res}) were calculated by using the following two equations: $F = \beta V^{res}$ and $C^{res} = Vk^rB/F$, where k^r is the elimination rate constant in the rabbit, V is the volume of distribution in the rabbit, and B is the intercept of the back-extrapolated β phase in humans with the ordinate.

TABLE 1. Pharmacokinetic parameters for ticarcillin, clavulanic acid, and ceftazidime in rabbits and humans, as used for the calculation of infusion rates to stimulate in rabbit plasma the antibiotic concentration-versus-time curves seen in human serum

Parameter[a]	Ticarcillin	Clavulanic acid	Ceftazidime
Rabbits			
k_{el} (h^{-1})	1.84	1.4	0.78
V (liter/kg)	0.20	0.19	0.17
Humans			
β (h^{-1})	0.76	0.81	0.4
B (μg/ml)	215	2.9	97
30-min concn in serum (μg/ml)	240	6.7	160

[a] k_{el}, Elimination rate constant; V, volume of distribution; β, elimination rate constant; B, zero time intercept for β phase.

The infusion rate for infusion B was calculated as follows. The concentration in plasma 30 min (C_x) after infusion B would be dependent on the infusion rate and clearance in the rabbit: $C_x = X_0(1 - e^{-k'30})/CL$, where X_0 is the infusion rate (milligrams per kilogram per hour) and CL is clearance in the rabbit ($k'V$). Adjustment of this equation is required to allow for the concentrations present as a result of infusion A. The concentration 30 min after infusion A (C_i) is defined as $C_i = (X_0e^{-\beta 30}/CL)(1 - e^{-k'30})$. The concentration required 30 min after infusion B will therefore be $C_h - C_i$, where C_h is the observed value in humans. By substitution and rearrangement, the infusion rate for B can be calculated as follows: $X_0 = [(C_h - C_i)CL]/(1 - e^{-k'30})$.

Rabbit pharmacokinetic data for ceftazidime, ticarcillin, and clavulanic acid used in the above calculations were determined from antibiotic concentrations measured in the plasma of anesthetized, uninfected animals after intravenous administration of a bolus dose of 100 mg/kg for ticarcillin and ceftazidime and 50 mg/kg for clavulanic acid. Pharmacokinetic analysis was based on lines of best fit for data conforming to an open one-compartment model (ticarcillin and clavulanic acid), and a curve-stripping procedure was used for analysis of data conforming to an open two-compartment model (ceftazidime). Lines were fitted and analyzed with an Apple II+ microcomputer. The program was a modification of that described by Neilsen-Kudsk (13). Human pharmacokinetic data for ticarcillin-clavulanic acid and ceftazidime were obtained from the literature (7, 9). The pharmacokinetic parameters in rabbits and humans that were used for calculating dosing parameters are given in Table 1, and the dosing parameters are listed in Table 2.

Two series of studies were undertaken. In the first, animals received a single dose of ticarcillin-clavulanic acid or ticarcillin alone. In the second series, three doses of ticarcillin-clavulanic acid were administered at 4-h intervals and two doses of ceftazidime were administered 8 h apart. In each study a group of rabbits was infused with saline only and served as infected controls.

Sampling and analysis. In the single- and multiple-dose studies, blood samples were taken at intervals after dosing, and plasma was separated for microbiological assay of

TABLE 2. Dosing parameters used to simulate in rabbit plasma the antibiotic concentration-versus-time curves seen in humans after 30-min infusions of ticarcillin, clavulanic acid, and ceftazidime at doses of 3 g, 100 mg, and 2 g, respectively

Parameter	Ticarcillin	Clavulanic acid	Ceftazidime
Infusion A			
Initial infusion rate (mg/kg per h)	80	0.75	12.3
Flow rate (ml/h)	3	3	1.5
Reservoir volume (ml)	4	4	4
Infusion B			
Initial infusion rate (mg/kg per h)	15	2	26.7
Flow rate (ml/h)	3	3	3

antibiotic concentrations. Samples of CSF were removed from the cisterna magna for microbiological assays and viable count determinations. The CSF samples were removed just before dosing and at intervals up to 6 h in the single-dose studies and up to 12 h in the multiple-dose studies.

Portions of the CSF samples were immediately diluted in 0.9% saline in a series of 10-fold dilutions, and the appropriate dilutions were dropped onto nutrient agar (0.02 ml per drop; four drops per dilution). Colonies were counted after overnight incubation at 37°C. The remaining portions of the samples were stored at 4°C for microbiological assay of antibiotic concentrations within 4 h of sample collection. Statistical comparison of the viable counts for the different treatment groups was done with the unpaired Student *t* test.

For the microbiological assay of antibiotic concentrations, the large-plate agar diffusion assay was used. Concentrations of ticarcillin were assayed with *P. aeruginosa* NCTC 10701. The enzyme inhibition assay with *K. pneumoniae* NCTC 11228 as the test organism (10) was used for clavulanic acid. The assay organism for ceftazidime was *Proteus mirabilis* C977. Samples were assayed in duplicate against standard solutions over the concentration ranges of 1.56 to 50 μg/ml for ticarcillin, 0.08 to 5 μg/ml for clavulanic acid, and 0.39 to 25 μg/ml for ceftazidime. The lowest concentrations were taken as the limits of detection. The correlation coefficients for the regression lines of the standard solutions were not less than 0.997. The coefficients of variation for the assays were below 10% for all three compounds. Plasma samples were assayed against standard solutions prepared in rabbit plasma, and any necessary dilutions were made in phosphate-buffered saline and assayed against standards prepared in the appropriate dilution of plasma. CSF samples were assayed against standards prepared in phosphate-buffered saline; previous studies had indicated that phosphate-buffered saline was a suitable diluent and that presence of the infection did not influence the assay results.

Pharmacokinetic analysis of distribution. In the single-dose studies, the area under the concentration-time curve (AUC) for CSF and plasma was determined by using a program for the Apple II+ microcomputer that was designed to estimate (i) the AUC up to the last concentration-time point for the terminal elimination phase by the trapezoidal rule and (ii) the remaining area to infinity by dividing this point by the elimination rate constant. In the multiple-dose studies, the AUC values for CSF and plasma were determined from 0 to 12 h by the trapezoidal rule. The extent of penetration into CSF was defined by expressing the AUC in CSF as a percentage of the AUC in plasma.

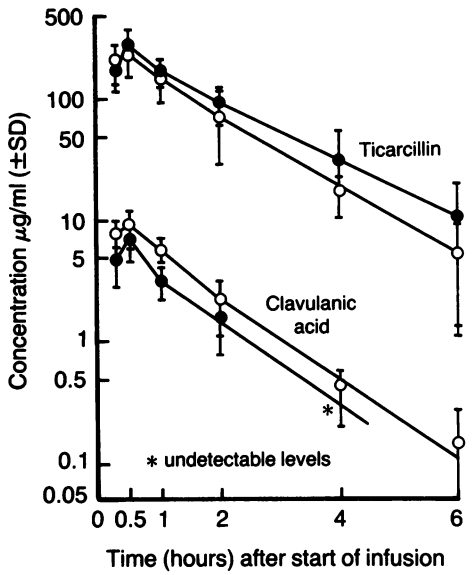

FIG. 2. Ticarcillin and clavulanic acid concentrations in the plasma of rabbits (○) with *K. pneumoniae* meningitis after an infusion to simulate the antibiotic concentration-time curve seen in human serum after a 30-min intravenous infusion of 3 g of ticarcillin plus 100 mg of clavulanic acid. The actual concentrations observed in humans (●) (9) are included for comparison. Each datum point represents the mean ± standard deviation for a group of four rabbits.

RESULTS

Distribution. (i) Single-dose study with ticarcillin-clavulanic acid and ticarcillin. The concentrations of ticarcillin and clavulanic acid in the plasma of rabbits with *K. pneumoniae* meningitis after a single dose infused to stimulate concentration-versus-time curves in humans for a 30-min intravenous infusion of ticarcillin (3 g) and clavulanic acid (100 mg) are shown in Fig. 2, together with results for human serum. There was generally good agreement between the results for rabbit and human serum. In rabbits, results for ticarcillin administered in the absence of clavulanic acid (data not shown) were similar to those for administration of ticarcillin-clavulanic acid.

After administration of ticarcillin-clavulanic acid, maximum concentrations in CSF were observed at 1 h for clavulanic acid and at 2 h for ticarcillin (Fig. 3). Concentrations of both compounds declined more slowly in CSF than in plasma, but differences were more marked for clavulanic acid. The mean elimination half-lives of ticarcillin in CSF and plasma were 1.2 and 1.0 h, respectively. For clavulanic acid the mean half-lives in plasma and CSF were 0.89 and 1.7 h. Concentrations of ticarcillin in CSF of infected rabbits receiving ticarcillin alone were initially similar to those in rabbits administered ticarcillin-clavulanic acid but were lower 2 to 6 h after dosing (Fig. 3).

The mean values for percent penetration into CSF are shown in Fig. 3. In rabbits receiving ticarcillin-clavulanic acid, the penetration of ticarcillin was less than that of clavulanic acid. The penetration of ticarcillin in rabbits receiving the penicillin alone was lower than that observed after coadministration of the inhibitor, but there was overlap of the individual results obtained for the two groups. The better penetration of clavulanic acid was reflected in the concentration ratios of ticarcillin to clavulanic acid in plasma and CSF. In the plasma samples obtained over 0.25 to 6 h,

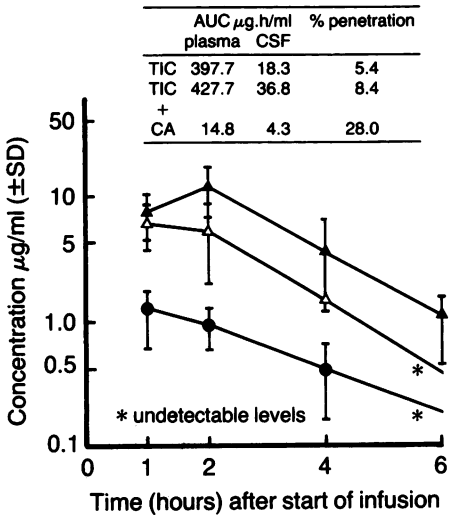

FIG. 3. Pharmacokinetic values and concentrations of ticarcillin (TIC) and clavulanic acid (CA) in the CSF of rabbits with *K. pneumoniae* meningitis after administration of ticarcillin alone (△) or ticarcillin (▲) with clavulanic acid (●). Compounds were infused to simulate in rabbit plasma the antibiotic concentration-time curve seen in human serum after a 30-min intravenous infusion of 3 g of ticarcillin or 3 g of ticarcillin plus 100 mg of clavulanic acid.

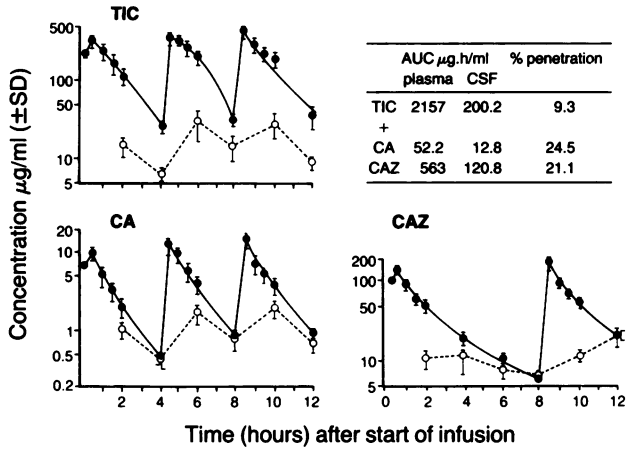

FIG. 5. Pharmacokinetic values and concentrations of ticarcillin (TIC), clavulanic acid (CA), and ceftazidime (CAZ) in the plasma (●) and CSF (○) of rabbits with *K. pneumoniae* meningitis after an infusion to simulate in rabbit plasma the antibiotic concentration-time curve seen in human serum after a 30-min intravenous infusion of 3 g of ticarcillin plus 100 mg of clavulanic acid or 2 g of ceftazidime. The doses of ticarcillin plus clavulanic acid were administered every 4 h, and the two doses of ceftazidime were given 8 h apart. Each datum point represents the mean ± standard deviation for a group of four rabbits.

these ratios ranged from 22:1 to 41:1, whereas in CSF the ratios ranged from 6.2:1 to 12:1.

(ii) Multiple-dose studies with ticarcillin-clavulanic acid and ceftazidime. Simulated human serum concentration-versus-time curves for ceftazidime in rabbit plasma agreed closely with results for human serum, and values for rabbits after the first dose of ceftazidime are shown in Fig. 4 together with results for humans after a 30-min intravenous infusion of a 2-g dose. The concentrations of ceftazidime in rabbit serum and CSF after two doses are shown in Fig. 5 together with those of ticarcillin and clavulanic acid after repeated dosing.

The mean values for the penetration of ticarcillin and clavulanic acid into CSF over 0 to 12 h (Fig. 5) approximated those observed in the single-dose studies. The penetration of ceftazidime over 12 h (21.1%) was similar to that observed for clavulanic acid (24.5%).

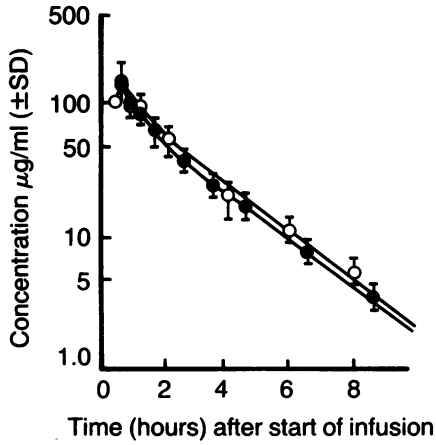

FIG. 4. Ceftazidime concentrations in the plasma of rabbits (○) with *K. pneumoniae* meningitis after an infusion to simulate the antibiotic concentration-time curve seen in human serum after a 30-min intravenous infusion of 2 g (7). The actual concentrations observed in humans are included for comparison (●). Each datum point represents the mean ± standard deviation for a group of four rabbits.

MIC and MBC determinations. The MICs and MBCs of ticarcillin-clavulanic acid and ceftazidime are shown in Table 3. In the presence of 0.5 μg of clavulanic acid per ml, the MBC of ticarcillin (16.0 μg/ml) was twofold higher than the MIC, but at higher concentrations of clavulanic acid, the MICs and MBCs of ticarcillin were the same. The MIC and MBC of ceftazidime were the same (0.5 μg/ml) and were lower than for ticarcillin in the presence of clavulanic acid.

Time-kill curve studies in vitro. The concentrations of ticarcillin (10 μg/ml), clavulanic acid (1.0 μg/ml), and ceftazidime (10 μg/ml) used in these studies were selected to approximate the midpoint concentrations of these agents observed in infected CSF. Neither ticarcillin nor clavulanic acid alone reduced the viable counts of cultures initially containing 10^5 CFU/ml (Fig. 6). In contrast, in the presence of clavulanic acid, ticarcillin was markedly bactericidal, with no bacteria being detected at 4 and 6 h. Ceftazidime exerted a similar effect. At 24 h bacteria were not detected in the ceftazidime-containing flask but a count of 1.5×10^5 CFU/ml was measured in the presence of ticarcillin and clavulanic acid. An inoculum effect was observed in these studies, and both ticarcillin-clavulanic acid and ceftazidime were less effective in reducing bacterial numbers when the initial count

TABLE 3. Susceptibility of *K. pneumoniae* T767 to ticarcillin alone, ticarcillin with clavulanic acid, and ceftazidime

Parameter	Ticarcillin (μg/ml) in presence of clavulanic acid at:				Ceftazidime (μg/ml)
	0 μg/ml	0.5 μg/ml	1.0 μg/ml	2.0 μg/ml	
MIC	500	8	8	4	0.5
MBC	ND[a]	16	8	4	0.5

[a] ND, Not done.

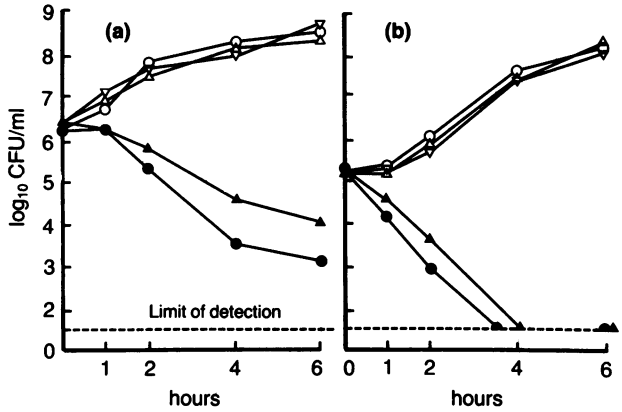

FIG. 6. Bactericidal activity of 10 μg of ticarcillin per ml plus 1 μg of clavulanic acid per ml (▲), 10 μg of ceftazidime per ml (●), 10 μg of ticarcillin per ml (△), and 1 μg of clavulanic acid per ml (▽) against an inoculum of 6 $\log_{10}$ CFU/ml (a) or 5 $\log_{10}$ CFU/ml (b) of *K. pneumoniae* T767. Values for untreated controls (○) are also represented.

was 10^6 CFU/ml. Ceftazidime reduced bacterial numbers by 99.9%, and ticarcillin-clavulanic acid reduced bacterial numbers by 99%. At 24 h there was regrowth of the culture in the presence of ticarcillin-clavulanic acid (3.9×10^6 CFU/ml) but not in the presence of ceftazidime.

Antibacterial effects in vivo. (i) Single-dose study with ticarcillin-clavulanic acid and ticarcillin. Ticarcillin was ineffective in reducing the viable count of *K. pneumoniae* T767 in CSF, and as was the case in untreated animals, bacterial numbers increased over 6 h (Fig. 7). The mean change in the viable counts was +1.2 $\log_{10}$ CFU/ml in ticarcillin-treated rabbits and +0.9 $\log_{10}$ CFU/ml in infected controls. In contrast, ticarcillin-clavulanic acid markedly reduced the viable counts in all rabbits by 2 h, such that bacterial numbers in the ticarcillin-clavulanic acid-treated groups were significantly lower than in the rabbits receiving ticarcillin alone and in the untreated controls ($P = 0.02$ and 0.006, respectively). Regrowth had occurred after 4 h in one rabbit given ticarcillin-clavulanic acid, but bacterial numbers overall were still significantly lower than in either the ticarcillin-treated groups ($P = 0.001$) or the infected controls ($P = 0.003$). At 6 h, regrowth had occurred in all the ticarcillin-clavulanic acid-treated rabbits but bacterial numbers remained significantly lower ($P = 0.05$) than in the other two groups. The regrowth was presumably a result of the concentrations in CSF declining to subinhibitory levels. Concentrations of clavulanic acid at 4 and 6 h ranged from <0.15 to 0.8 μg/ml, and the concomitant concentrations of ticarcillin were below the MBC observed in the presence of clavulanic acid concentrations of 0.5 and 1.0 μg/ml (Table 3).

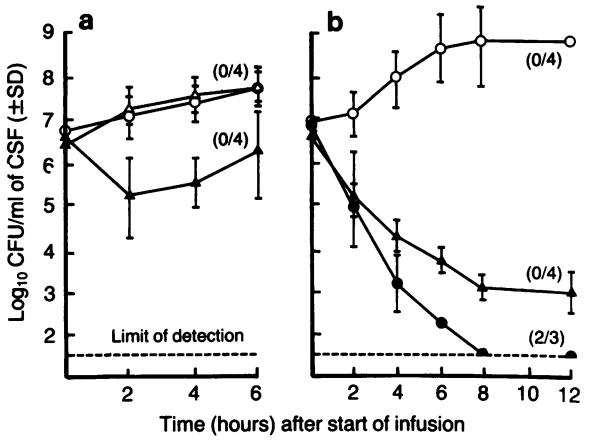

FIG. 7. Antibacterial activity in CSF of ticarcillin (△), ticarcillin plus clavulanic acid (▲), and ceftazidime (●) in rabbits with *K. pneumoniae* meningitis after single doses (a) of ticarcillin plus clavulanic acid or ticarcillin alone and after multiple doses (b) of ticarcillin plus clavulanic acid (three doses at 4-h intervals) and ceftazidime (two doses at 8-h intervals). Results for infected, untreated rabbits (○) are included. Compounds were administered to simulate in rabbit plasma the antibiotic concentration-time curves seen in human plasma after 30-min intravenous infusions of 3 g of ticarcillin, 3 g of ticarcillin plus 100 mg of clavulanic acid, and 2 g of ceftazidime. Numbers in parentheses give the number of rabbits with sterile CSF (<2.0 $\log_{10}$ CFU/ml).

Results for two rabbits administered ticarcillin-clavulanic acid were not included in the mean data reported in Fig. 7 because the initial viable counts in CSF (4.7 and 4.8 $\log_{10}$ CFU/ml) were lower than the values for the group receiving ticarcillin and for the infected controls (5.41 to 7.5 $\log_{10}$ CFU/ml). It is of interest, however, that bacterial numbers in these two rabbits were reduced to undetectable levels in one animal by 4 h and in both by 6 h. This observation is in keeping with the results of the in vitro kill-curve studies, in which an inoculum effect was also demonstrated (Fig. 6).

(ii) Multiple-dose study with ticarcillin-clavulanic acid and ceftazidime. Bacterial numbers increased in the untreated rabbits. At 8 h the overall increase was 1.6 $\log_{10}$ CFU/ml. At 12 h it was possible to sample the CSF in only one of the four rabbits; the viable count was 8.6 $\log_{10}$ CFU/ml. After repeated administration of ticarcillin-clavulanic acid, viable counts in CSF declined gradually, with an overall reduction ranging from 99.9 to 99.99% at 12 h. Ceftazidime displayed similar bactericidal activity up to 4 h, although at this time point, bacterial numbers in CSF were significantly lower ($P = 0.03$) than in rabbits administered ticarcillin-clavulanic acid. Bacterial numbers in ceftazidime-treated rabbits continued to decline, and no bacteria were detected in three of the four animals by 8 h; the viable count in the rabbit with detectable bacteria in CSF was 2.4 $\log_{10}$ CFU/ml. At 12 h, bacteria were not detected in two of three rabbits in the ceftazidime group. It was not possible to obtain a CSF sample from one rabbit at this time. The viable count in the rabbit with detectable bacteria at 12 h was 3.0 $\log_{10}$ CFU/ml.

DISCUSSION

The usual approach to therapy in the rabbit model of meningitis involves the infusion of compounds to steady state or bolus intravenous dosing, and the doses are calculated to produce concentrations in serum that are within the range of values observed in human serum (18, 21). For the studies reported here, it was decided to simulate more precisely the concentration-versus-time curves observed in adult humans after administration of therapeutic doses of ticarcillin-clavulanic acid, ticarcillin, and ceftazidime, thereby compensating for the species differences in pharmacokinetics. The method involves the use of pharmacokinetic parameters derived from studies of healthy human volunteers and uninfected rabbits to calculate the infusion rates, and concentrations of ticarcillin and clavulanic acid (G. Woodnutt, I. Kernutt, and L. Mizen, Program Abstr. 6th Mediterr. Congr. Chemother., abstr. no. 300, 1988) and ceftazidime (unpublished data) observed in human serum after therapeutic doses have been successfully simulated in

uninfected rabbits. The presence of infection in the rabbits could lead to changes in the pharmacokinetics of antibacterial agents (19). However, after the described infusion method was used for ticarcillin, clavulanic acid, and ceftazidime in rabbits with experimental *K. pneumoniae* meningitis, antibiotic concentrations in plasma and elimination half-lives were in accordance with results for humans. It appears, therefore, that the use of infusion rates derived from pharmacokinetic parameters for infected rabbits was not essential in these studies for the simulation in rabbits of the antibiotic concentration-versus-time curves observed for humans.

The penetration of β-lactam antibiotics into CSF is limited as a result of the nature of the blood-CSF barrier (14), but in the presence of bacterial meningitis, permeability is increased. Concentrations of ticarcillin, clavulanic acid, and ceftazidime were detectable in CSF of rabbits with *K. pneumoniae* meningitis, and the percent penetration of clavulanic acid was similar to that for ceftazidime and greater than that for ticarcillin. The percent penetration values were comparable to those reported by others for ceftazidime, ticarcillin, and clavulanic acid in this experimental infection model (15, 16, 21). It has been shown that results for penetration in the rabbit model of meningitis give a good indication of the relative penetration of antibacterial agents into human CSF (11, 18), and the following categories have been suggested (17) for assessing the extent of penetration into rabbit CSF in the presence of meningitis: low (<6%), moderate (6 to 15%), and high (>15%). The penetration of ticarcillin was therefore moderate, whereas clavulanic acid and ceftazidime penetrated CSF relatively well.

In the single-dose studies the concentrations of clavulanic acid attained in CSF contributed to the activity of ticarcillin against the infecting strain of *K. pneumoniae*, although bacterial numbers were reduced to undetectable levels only when the viable counts in CSF at the start of ticarcillin-clavulanic acid therapy were between 10^4 and 10^5 CFU/ml. The results for the single-dose studies are in keeping with those of Syrogiannopoulos et al. (21) in their studies of *H. influenzae* type b and *Escherichia coli* K-1 meningitis in rabbits, in which treatment over a short period (6 h) with either a bolus dose or a continuous infusion to steady state also demonstrated the contribution of clavulanic acid to the antibacterial efficacy of ticarcillin.

The repeated dosing of ticarcillin-clavulanic acid over a relatively prolonged period (12 h) resulted in a more extensive reduction in the viable counts of *K. pneumoniae* T767 than in the single-dose studies, but in contrast to the results for ceftazidime, there were no rabbits with sterile CSF at the end of the study. The comparative bactericidal activities of ticarcillin-clavulanic acid and ceftazidime in CSF after multiple dosing seem consistent with results for the in vitro time-kill curve studies and for the susceptibility of the infecting organism to the antibacterial agents. In CSF the concentrations of ceftazidime exceeded MBCs over the 2 to 12-h period, and maximum concentrations were 15 to 50 times greater than the MBC, whereas concentrations of ticarcillin and clavulanic acid fell below or were similar to MBC values prior to administration of subsequent doses, and the highest ticarcillin concentrations in CSF for which concomitant clavulanic acid concentrations ranged from 1.0 to 2.6 μg/ml were 2 to 10 times higher than the MBCs for ticarcillin in the presence of 1 or 2 μg of clavulanic acid per ml.

In conclusion, in the presence of concentrations in rabbit plasma that closely simulate those observed in human serum after administration of recommended therapeutic doses, ticarcillin-clavulanic acid showed good activity against experimental *K. pneumoniae* meningitis, whereas ticarcillin was ineffective. These results demonstrate that the concentrations of the β-lactamase inhibitor were sufficient to potentiate the activity of ticarcillin against this particularly severe experimental infection and confirm the compatibility of the distribution characteristics of ticarcillin and clavulanic acid.

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LITERATURE CITED

1. **Adam, D., H.-D. Heilmann, and K. Weismeier.** 1987. Concentrations of ticarcillin and clavulanic acid in human bone after prophylactic administration of 5.2 g of Timentin. Antimicrob. Agents Chemother. **31**:935–939.
2. **Alstig, K., L. Olaison, and M. Rylander.** 1985. Ceftazidime for pseudomonas meningitis. Lancet **i**:161–162.
3. **Bennet, S., R. Wise, D. Weston, and J. Dent.** 1983. Pharmacokinetics and tissue penetration of ticarcillin combined with clavulanic acid. Antimicrob. Agents Chemother. **23**:831–834.
4. **Boon, R., A. S. Beale, and R. Sutherland.** 1986. Bactericidal effects of ticarcillin-clavulanic acid against β-lactamase-producing bacteria in vivo. Antimicrob. Agents Chemother. **23**: 831–834.
5. **Catherall, E. J., and L. Mizen.** 1984. Protection of amoxycillin and ticarcillin with clavulanic acid from inactivation by β-lactamases in an experimental localized infection. Drugs Exp. Clin. Res. **10**:697–702.
6. **Chattopadhyay, B., and I. Hall.** 1984. In vitro activity of ticarcillin and clavulanic acid (BRL 28500, Timentin) against ticarcillin resistant gram-negative rods. Curr. Med. Res. Opin. **9**:157–160.
7. **Drusano, G. L., H. C. Standiford, B. Fitzpatrick, J. Leslie, P. Tangtatsawasdi, P. Ryan, B. Tatem, M. R. Moody, and S. C. Schimpff.** 1984. Comparison of the pharmacokinetics of ceftazidime and moxalactam and their microbiological correlates in volunteers. Antimicrob. Agents Chemother. **26**:388–393.
8. **Hunter, P. A., K. Coleman, J. Fisher, and D. Taylor.** 1980. In vitro synergistic properties of clavulanic acid with ampicillin, amoxycillin and ticarcillin. J. Antimicrob. Chemother. **6**:455–470.
9. **Jackson, D., A. Cockburn, D. L. Cooper, P. F. Langley, T. C. G. Tasker, and D. J. White.** 1985. Clinical pharmacology and safety evaluation of Timentin. Am. J. Med. **79**(Suppl. 5B):44–55.
10. **Jackson, D., D. L. Cooper, R. Horton, P. F. Langley, D. S. Staniforth, and A. J. Sutton.** 1983. Absorption, pharmacokinetic and metabolic studies with Augmentin, p. 83–101. *In* E. A. P. Croydon and M. F. Michel (ed.), Augmentin, clavulanate potentiated amoxycillin. Proceedings of the European Symposium, Scheveningen. Excerpta Medica, Amsterdam.
11. **McCracken, G. H., Jr.** 1983. Pharmacokinetic and bacteriological correlations between antimicrobial therapy of experimental meningitis in rabbits and meningitis in humans: a review. J. Antimicrob. Chemother. **12**(Suppl. D):97–108.
12. **Modai, J.** 1986. Role of third-generation cephalosporins in the treatment of bacterial meningitis. Chemotherapia **5**:313–318.
13. **Neilsen-Kudsk, F.** 1981. Pharmacokinetic analysis and calculations using a program for the minicalculator T159. Int. J. Bio-Med. Comput. **12**:83–96.
14. **Norrby, R.** 1978. A review of penetration of antibiotics into CSF and its clinical significance. Scand. J. Infect. Dis. Suppl. **14**: 296–309.
15. **Sakata, Y., A. Boccazzi, and G. H. McCracken, Jr.** 1983. Pharmacokinetics and bacteriological effect of ceftazidime in experimental *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Escherichia coli* meningitis. Antimicrob. Agents Chemother. **23**:213–217.
16. **Sakata, Y., G. H. McCracken, Jr., M. L. Thomas, and K. D.**

Olsen. 1984. Pharmacokinetics and therapeutic efficacy of imipenem, ceftazidime, and ceftriaxone in experimental meningitis due to an ampicillin- and chloramphenicol-resistant strain of *Haemophilus influenzae* type b. Antimicrob. Agents Chemother. **25**:29–32.

17. **Scheld, W. M.** 1984. Rationale for optimal dosing of beta-lactam antibiotics in therapy for meningitis. Eur. J. Clin. Microbiol. **3**:579–591.
18. **Scheld, W. M.** 1986. Experimental animal models of bacterial meningitis, p. 139–186. *In* O. Zak and M. A. Sande (ed.), Experimental models in antimicrobial chemotherapy, vol. 1. Academic Press, Inc. (London), Ltd., London.
19. **Sears, M. R., J. M. O'Donoghue, H. K. Fisher, and H. N. Beaty.** 1974. Effect of experimental pneumococcal meningitis on respiration and circulation in the rabbit. J. Clin. Invest. **54**:18–23.
20. **Sutherland, R., A. S. Beale, R. J. Boon, K. E. Griffin, B. Slocombe, D. H. Stokes, and A. R. White.** 1985. Antibacterial activity of ticarcillin in the presence of clavulanic acid. Am. J. Med. **76**:(Suppl. 5B):13–24.
21. **Syrogiannopoulos, G. A., A. Al-Sabbagh, K. D. Olsen, and G. H. McCracken, Jr.** 1987. Pharmacokinetics and bacteriological efficacy of ticarcillin-clavulanic acid (Timentin) in experimental *Escherichia coli* K-1 and *Haemophilus influenzae* type b meningitis. Antimicrob. Agents Chemother. **31**:1296–1300.
22. **Walstad, R. A., K. B. Hellum, E. Thurmann-Nielsen, and L. G. Dale.** 1986. Pharmacokinetics and tissue penetration of Timentin: a simultaneous study of serum, urine, lymph, suction blister and subcutaneous thread fluid. J. Antimicrob. Chemother. **17**(Suppl. C):71–80.
23. **Woodnutt, G., E. J. Catherall, I. Kernutt, and L. Mizen.** 1988. Temocillin efficacy in experimental *Klebsiella pneumoniae* meningitis after infusion into rabbit plasma to simulate antibiotic concentrations in human serum. Antimicrob. Agents Chemother. **32**:1705–1709.
24. **Woodnutt, G., I. Kernutt, and L. Mizen.** 1987. Pharmacokinetics and distribution of ticarcillin-clavulanic acid (Timentin) in experimental animals. Antimicrob. Agents Chemother. **31**: 1826–1830.