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Simulation of Human Serum Pharmacokinetics of Cefazolin, Piperacillin, and BRL 42715 in Rats and Efficacy against Experimental Intraperitoneal Infections

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Studies were performed to determine the effects of BRL 42715, a potent β-lactamase inhibitor, on the activity of cefazolin and piperacillin against experimental intraperitoneal infections caused by either *Escherichia coli* or *Serratia marcescens* in rats. Compounds were administered to rats as a continuous infusion of an exponentially diluted solution to simulate in rat plasma the concentration-versus-time curves obtained for humans following intravenous bolus administration. A simulated 1-g dose of cefazolin was ineffective in reducing the bacterial counts in blood and peritoneal fluid samples of animals infected with *S. marcescens* US20, which produced class Ia β-lactamase, and as a result, mortality was similar to that of infected controls. Similarly, a simulated 2-g dose of piperacillin was ineffective in reducing bacterial numbers and mortality in animals infected with *E. coli* 41548, producing a TEM-1 β-lactamase. However, when the antibiotics were coadministered with BRL 42715, bacterial numbers were reduced significantly and all animals survived at least 16 h after infection. These data demonstrate the ability of BRL 42715 to potentiate the activity of cefazolin and piperacillin against β-lactamase-producing bacteria that would otherwise be resistant to these antibiotics and illustrate the application of a model to simulate human serum concentrations in conscious rats.

BRL 42715 (Fig. 1) is a novel potent inhibitor of a wide range of bacterial β-lactamases, including chromosomally-mediated class I enzymes, and is effective in protecting β-lactam antibiotics from degradation by bacterial β-lactamases in vitro, thereby rendering many organisms that would otherwise have been resistant fully susceptible (1, 3).

The studies reported here, designed to measure the efficacy of BRL 42715 in vivo, were conducted using rats with an experimental intraperitoneal infection in which the infecting organisms were β-lactamase-producing bacteria resistant to cefazolin or piperacillin. However, in humans, the terminal half-life ($t_{1/2}$) of BRL 42715 (0.6 h [9]) is shorter than that of cefazolin (1.6 h [2]) and piperacillin (1.1 h [12]), and although this relationship is generally similar in the rat, the elimination of all three compounds is more rapid in this species ($t_{1/2}$ of 0.1, 0.51, and 0.33 h for BRL 42715, cefazolin, and piperacillin, respectively). Gerber et al. (6) have demonstrated that interspecies differences in elimination half-life can cause marked differences in the efficacy of antibiotics in animal models compared with clinical efficacy. Therefore, to compensate for species differences in the elimination of the compounds used in these studies, the elimination kinetics in the rat were modified by using an intravenous infusion of a continuously diluted solution of each compound. In the studies reported here, the efficacy of cefazolin and piperacillin was examined in the presence of concentrations in human serum following bolus administration of 1 g of cefazolin plus 100 mg of BRL 42715 or 2 g of piperacillin plus 100 mg of BRL 42715 simulated in conscious rats. The results were compared with those obtained after administration of cefazolin or piperacillin alone.

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MATERIALS AND METHODS

Bacteria. A cefazolin-resistant strain of *Serratia marcescens*, US20, producing a chromosomally mediated class Ia β-lactamase was used as the infecting organism in studies to examine the efficacy of cefazolin and cefazolin plus BRL 42715. A strain of piperacillin-resistant *Escherichia coli*, 41548, producing TEM-1 β-lactamase was used as the infecting organism in studies to examine the efficacy of piperacillin and piperacillin plus BRL 42715.

Compounds. Sodium cefazolin (Kefzol; Eli Lilly & Co. Ltd.) and sodium piperacillin (Pipril; Lederle Laboratories) were commercial preparations. BRL 42715B (sodium salt) was prepared by SmithKline Beecham Pharmaceuticals, Brockham Park, England. Compounds were used as the pure free acid equivalent.

Susceptibility testing. The in vitro susceptibility of the infecting organisms was determined by a microdilution technique as described in the guidelines of the National Committee for Clinical Laboratory Standards (11). Serial twofold dilutions were prepared in Mueller-Hinton broth (Difco) for cefazolin and piperacillin either alone or in the presence of a range of BRL 42715 concentrations. The inoculum size was approximately 10^6 CFU/ml. After overnight incubation of the organism at 37°C, the MIC was determined as the lowest concentration of antibiotic that inhibited visible growth.

Infection. The bacterial inoculum for all studies was prepared from an overnight broth culture in tryptone soya broth diluted in sterile porcine gastric mucin (5% in 1% carboxymethylcellulose), and 2.5 ml (approximately 10^7 CFU per rat for studies with *E. coli* and approximately 10^8 CFU per rat for studies with *S. marcescens*) was injected intraperitoneally into each animal. These inocula were sufficient to cause 80 to 100% mortality in untreated animals between 10 and 14 h after infection.

Therapy. Male rats (250 to 300 g, Sprague-Dawley, Charles River) were surgically prepared before use. The external jugular vein was cannulated, and the cannula was

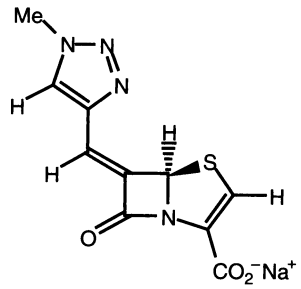

FIG. 1. Chemical structure of BRL 42715.

exteriorized dorsally, taken through a flexible metal sheath to the top of the cage, and attached to a free-moving swivel system.

The external carotid artery was cannulated in animals in which bioavailability of the compounds was to be measured. The carotid cannula was also exteriorized dorsally and taken through the flexible metal sheath. The cannula was not connected to a swivel system but was sealed and allowed to rotate freely between sampling times. Animals were housed individually and were allowed to recover for 2 days before infusion. The cannulae were kept patent by regular flushing of heparinized saline (0.3 ml of a solution containing 50 IU of heparin per ml).

Dosing commenced 1 h after infection, at which stage animals were divided into three groups. One group received a continuous infusion of saline only and served as infected untreated controls. The remaining infected groups were infused with an exponentially diluted solution of either antibiotic (cefazolin or piperacillin) or antibiotic plus BRL 42715. The infusion was designed to simulate in rat plasma the concentrations measured in human serum following intravenous bolus administration of either 1 g of cefazolin, 2 g of piperacillin, 1 g of cefazolin plus 100 mg of BRL 42715, or 2 g of piperacillin plus 100 mg of BRL 42715. In all therapy studies, a single intravenous bolus dose was simulated, animals were humanely killed 3 to 5 h after the end of infusion (16 to 18 h postinfection), and samples of blood and peritoneal fluid were taken, at this time or at death, if occurring earlier, for viable bacterial count estimation.

Simulation of human serum kinetics. The simulation of human serum concentration profiles in anesthetized rabbits has been described previously (13). Briefly, the terminal phase of the serum level curve was simulated by the infusion of phosphate-buffered saline (PBS; 0.1 M, pH 7.4) at a constant rate into a fixed-volume reservoir containing a solution of antibiotic or inhibitor. The rate of decline in concentration in the reservoir was equivalent to the human elimination rate constant (β) and was related to the infusion flow rate (F) and the reservoir volume (V_{res}) by using the equation $\beta = F/V_{res}$.

The exponential decline in concentrations in the reservoir was reflected in proportional changes in the concentration of the agent in the plasma of the rat. The initial phase of the serum level curves observed in humans was simulated by the administration of a bolus dose at the start of infusion equivalent to $C_0 \cdot V$, where C_0 was the theoretical concentration in human serum at 0 h and V was the volume of distribution in the rat.

Rat pharmacokinetic data for cefazolin, piperacillin, and BRL 42715 used to calculate the various dosing parameters were determined from antibiotic concentrations measured in uninfected animals after intravenous administration of a bolus dose of 100 mg/kg of body weight for cefazolin and piperacillin and 20 mg/kg for BRL 42715. Pharmacokinetic analysis (open one-compartment model) was based on lines of best fit analyzed with a program based on that described by Neilsen-Kudsk (8) installed on a Apple II+ microcomputer. Human pharmacokinetic data for cefazolin, piperacillin, and BRL 42715 were obtained from previous studies (2, 9, 12). The pharmacokinetic parameters, in rats and humans, used to calculate the dosing parameters are shown in Table 1, and the dosing parameters are also listed in Table 1.

TABLE 1. Pharmacokinetic and dosing parameters used for the simulation of human concentration-versus-time curves in rat plasma[a]

Parameter[b]	Results with:		
	Cefazolin	BRL 42715	Piperacillin
Pharmacokinetic			
Rat			
k_{el} (h^{-1})	1.4	2.3	1.9
V (liter/kg)	0.3	2.2	0.48
Human[c]			
β (h^{-1})	0.44	1.2	0.8
B (mg/liter)	108	0.9	53
C_0 (mg/liter)	300	8.0	184
Dosing			
V_{res} (ml)	1.5	0.48	0.75
C_{res} (mg/ml)	36.0	2.71	33.5
F (ml/h)	0.6	0.6	0.6
Bolus (mg/kg)	90.0	16.8	87.5

[a] Following bolus administration of 1 g of cefazolin, 2 g of piperacillin, 1 g of cefazolin plus 100 mg of BRL 42715, or 2 g of piperacillin plus 100 mg of BRL 42715.

[b] k_{el} and β, elimination rate constants; V, volume of distribution; B, time zero intercept for β phase; C_0, concentration at time zero in serum; V_{res}, volume of reservoir; C_{res}, initial concentration in reservoir; F, infusion flow rate.

[c] Human data were obtained from reference 9 for BRL 42715, from reference 2 for cefazolin, and from reference 12 for piperacillin.

Bactericidal studies. Animals were infected intraperitoneally with *E. coli* 41548 as in the therapy studies, and dosing, to simulate concentrations in human serum after a single intravenous bolus dose of piperacillin (2 g) or piperacillin (2 g) plus BRL 42715 (100 mg), was started 1 h postinfection. Two animals per group were humanely killed at the start of dosing and at 2, 4, 7, 10, and 13 h postinfection.

Samples of blood, by cardiac puncture, and peritoneal fluid were taken at death for microbiological assay and viable count determination.

Bacterial numbers in blood and peritoneal fluid samples for all studies were determined on nutrient agar with a spiral plater (Don Whitley Scientific Products) by a spread plate technique.

Bioavailability studies. Groups of two uninfected animals received cefazolin, piperacillin, or BRL 42715 by infusion to simulate concentrations of the compounds in human serum following intravenous bolus administration of 1 g of cefazolin, 2 g of piperacillin, or 100 mg of BRL 42715. Samples of blood were taken from the carotid artery at 5, 15, 30, 60, 90, 120, 240, and 360 min after the start of infusion.

Sample analysis. Plasma was separated and samples were stored at 4°C until assay within 4 h. Large plate agar diffusion was used to assay concentrations of the compounds

TABLE 2. Susceptibility of *E. coli* 41548 to piperacillin and piperacillin plus BRL 42715 and of *S. marcescens* US20 to cefazolin and cefazolin plus BRL 42715

Organism	Test agent	MIC (μg/ml) in the presence of BRL 42715 at a concn (μg/ml) of:					
		0.25	0.125	0.06	0.03	0.015	0
E. coli 41548	Piperacillin	0.125	0.25	0.5	128	≥128	1,024
S. marcescens US20	Cefazolin	8	8	8	8	≥128	≥512

in plasma and peritoneal fluid by using *Bacillus subtilis* ATCC 6633 and *Pseudomonas aeruginosa* ATCC 29366 as the assay organisms for cefazolin and piperacillin, respectively. BRL 42715 was assayed by using an agar diffusion β-lactamase inhibition assay with *Klebsiella pneumoniae* ATCC 29665 as the assay organism (7).

Standards were prepared in the appropriate dilution of rat plasma for the assay of plasma samples and in PBS for the assay of peritoneal fluid samples. Samples were assayed in duplicate against standards over the concentration range of 1.25 to 0.01 μg/ml for BRL 42715 and 50 to 0.78 μg/ml for cefazolin and piperacillin. The lowest concentration was taken as the limit of detection for the assay. The correlation coefficients for the regression lines of the standard solutions were not less than 0.997. The within-day coefficients of variation were 5% for BRL 42715, 7% for cefazolin, and 7% for piperacillin. Coefficients of variation were measured at 0.6 and 0.03 μg/ml for BRL 42715 and at 25 and 3.13 μg/ml for cefazolin and piperacillin.

RESULTS

MIC determinations. The MICs of cefazolin alone and of cefazolin in the presence of twofold dilutions of BRL 42715 against *S. marcescens* US20 are shown in Table 2. Also shown in Table 2 are the MICs of piperacillin alone and of piperacillin in the presence of twofold dilutions of BRL 42715 against *E. coli* 41548. The MIC of cefazolin against *S. marcescens* US20 was ≥512 μg/ml, which was reduced to 8 μg/ml in the presence of concentrations of BRL 42715 ranging from 0.03 to 0.25 μg/ml. The MIC of BRL 42715 alone against *S. marcescens* US20 was ≥64 μg/ml. The MIC of piperacillin against *E. coli* 41548 was 1,024 μg/ml, which was reduced to 0.5 μg/ml in the presence of 0.06 μg of inhibitor per ml. This was reduced further, to 0.125 μg/ml in the presence of 0.25 μg of BRL 42715 per ml. The MIC of BRL 42715 alone against this organism was 16 μg/ml.

The susceptibility of both organisms before dosing was similar to that measured for organisms isolated from blood or peritoneal fluid after dosing.

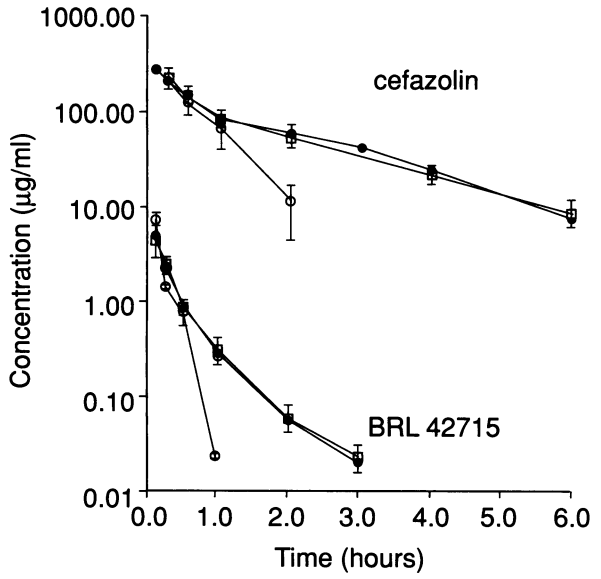

FIG. 2. Concentrations of cefazolin and BRL 42715 in rat plasma after either an intravenous bolus dose (100 mg of cefazolin per kg or 20 mg of BRL 42715 per kg [○]) or an infusion (□) to simulate the concentration-versus-time curves seen for human serum (●) (2, 9) after an intravenous bolus dose of 1 g of cefazolin plus 100 mg of BRL 42715. Each point is a mean and range for two animals.

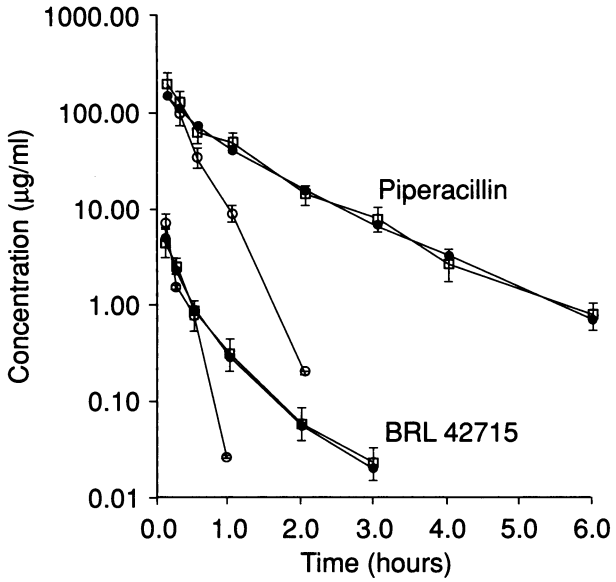

FIG. 3. Concentrations of piperacillin and BRL 42715 in rat plasma after either an intravenous bolus dose (100 mg of piperacillin per kg or 20 mg of BRL 42715 per kg [○]) or an infusion (□) to simulate the concentration-versus-time curves seen in human serum (●) (9, 12) after an intravenous bolus dose of 2 g of piperacillin plus 100 mg of BRL 42715. Each point is a mean and range of values for two animals.

TABLE 3. Efficacy of cefazolin and cefazolin plus BRL 42715 against an intraperitoneal infection caused by *S. marcescens* US20 in rats[a]

Treatment group	No. surviving/ no. tested	Median time to death (h)[b]	Mean bacterial count (CFU/ml)[c]	
			Blood	Peritoneal fluid
Infected controls	1/10	12	7.4×10^7	$\geq 10^{10}$
Cefazolin alone	3/10	13	7.3×10^7	$\geq 10^{10}$
Cefazolin + BRL 42715	10/10		3.2×10^3	2.1×10^5[d]

[a] Following infusion to simulate concentrations obtained in human serum after bolus administration of 1 g of cefazolin or 1 g of cefazolin plus 100 mg of BRL 42715.

[b] Time after infection.

[c] Samples taken at death or at 16 h after infection.

[d] Counts significantly different from those of infected controls and cefazolin-alone group ($P < 0.001$).

TABLE 4. Efficacy of piperacillin and piperacillin plus BRL 42715 against an intraperitoneal infection caused by *S. marcescens* US20 in rats[a]

Treatment group	No. surviving/ no. tested	Median time to death (h)[b]	Mean bacterial count (CFU/ml)[c]	
			Blood	Peritoneal fluid
Infected controls	1/6	11	1.8×10^7	$\geq 10^{10}$
Piperacillin alone	2/6	12	1.2×10^7	$\geq 10^{10}$
Piperacillin + BRL 42715	6/6		$\leq 10^{2d}$	4.5×10^{2d}

[a] Following infusion to simulate concentrations obtained in human serum after bolus administration of 2 g of piperacillin or 2 g of piperacillin plus 100 mg of BRL 42715.
[b] Time after infection.
[c] Samples taken at death or at 16 h after infection.
[d] Counts significantly different from those of infected controls and piperacillin-alone group ($P < 0.001$).

Bioavailability studies. Concentrations of cefazolin, piperacillin, and BRL 42715 in rat plasma following infusion are shown in Fig. 2 and 3, and values are compared with those measured in the sera of humans following administration of 1 g of cefazolin plus 100 mg of BRL 42715 and 2 g of piperacillin plus 100 mg of BRL 42715 (also shown in Fig. 2 and 3) (2, 9, 12). The results for rat plasma are within the ranges reported for humans but differ greatly from the concentrations in rat plasma after an intravenous bolus dose of each compound (Fig. 2 and 3).

Therapy studies. Results of therapy studies in which rats, infected intraperitoneally with *S. marcescens* US20, were treated with a single bolus simulation of either cefazolin or cefazolin plus BRL 42715 are shown in Table 3. Nine of 10 animals receiving saline died approximately 13 h postinfection (10^7 CFU/ml in blood samples and $\geq 10^{10}$ CFU/ml in peritoneal fluid samples). In the group treated with cefazolin alone, 7 of 10 animals died by 14 h after infection, and these animals also had high numbers of bacteria in both blood and peritoneal fluid. All of the animals in the group treated with cefazolin plus BRL 42715 survived to 16 h postinfection, and bacterial counts in the blood (10^3 CFU/ml) and peritoneal fluid (10^5 CFU/ml) samples of these animals were significantly lower ($P < 0.001$) than those in untreated controls or cefazolin-treated animals. Table 4 shows the results obtained from animals infected intraperitoneally with *E. coli* 41548 following a single bolus simulation of either piperacillin or piperacillin plus BRL 42715. Five of six animals receiving saline only died approximately 11 h postinfection (10^7 CFU/ml in blood samples and $\geq 10^{10}$ CFU/ml in peritoneal fluid samples). In the piperacillin-alone group, four of six animals died by 12 h after infection, and these animals had bacterial counts similar to those of infected controls for both blood and peritoneal fluid samples. All of the animals in the piperacillin plus BRL 42715-treated group survived to 16 h postinfection, and bacterial counts in the blood ($\leq 10^2$ CFU/ml) and peritoneal fluid (10^2 to 10^3 CFU/ml) samples of these animals were low.

Bactericidal studies. Bacterial counts in peritoneal fluid and blood samples of rats infected with *E. coli* 41548 are shown in Fig. 4a and b, respectively.

Bacterial counts in the peritoneal fluid samples of control animals increased steadily during the first 4 h postinfection and remained at $\geq 10^{10}$ CFU/ml for the remainder of the study. The numbers of viable bacteria in peritoneal fluid samples of piperacillin-treated animals were relatively static during the first 7 h postinfection, approximately 10^5 to 10^6 CFU/ml, but after this time, numbers increased and were similar to those for control animals ($\geq 10^{10}$ CFU/ml). In

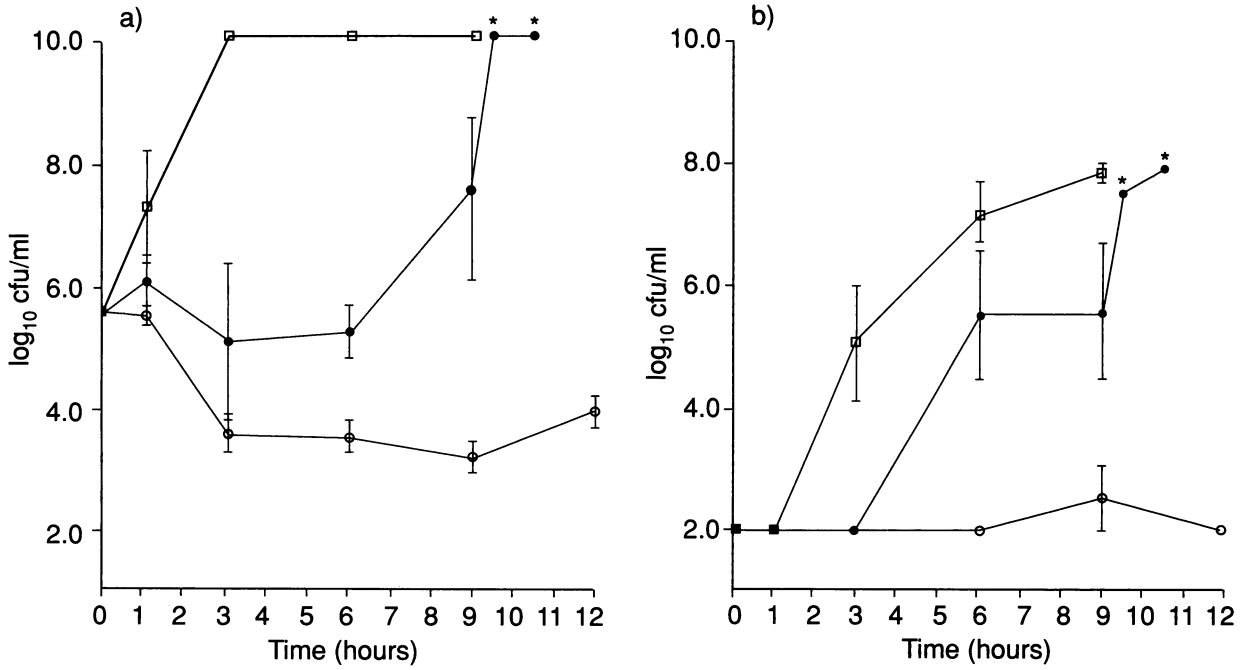

FIG. 4. Antibacterial activity of piperacillin and piperacillin plus BRL 42715 in peritoneal fluid (a) and blood (b) of rats infected intraperitoneally with *E. coli* 41548 after infusion to simulate the concentration-versus-time curve seen in human serum following administration of either 2 g of piperacillin (●) or 2 g piperacillin plus 100 mg of BRL 42715 (○). Data for infected controls (□) are included. Each point is a mean and range of values for two animals, except those marked with an *, which are values for only one animal.

contrast, the number of bacteria in the peritoneal fluid samples of animals treated with piperacillin plus BRL 42715 decreased during the first 3 h of infusion (4 h postinfection) to approximately 10^3 CFU/ml and remained at this level until 13 h postinfection, at which time there was a small increase in the numbers of viable bacteria in peritoneal fluid samples (Fig. 4a). Bacterial counts in the blood samples (Fig. 4b) generally reflected those in peritoneal fluid samples, with numbers in control animals and in piperacillin-treated animals increasing to approximately 10^8 CFU/ml while the number of bacteria in the blood samples of animals treated with piperacillin plus BRL 42715 were undetectable for the majority of the experimental period. However, counts in the blood samples of control animals were undetectable until 2 h postinfection, and this lag period was extended to approximately 4 h after infection in animals treated with piperacillin.

Concentrations of piperacillin and BRL 42715 in plasma and peritoneal fluid were generally similar throughout the experimental period.

DISCUSSION

In these studies of rats, the antibacterial activity of cefazolin and piperacillin was determined in the presence of antibiotic concentrations in rat plasma that simulated those in human serum following intravenous bolus administration. The importance of considering interspecies differences in pharmacokinetics has been demonstrated in several different experimental infection models (5, 6, 10, 13). The results presented here have shown that the infusion method described by Woodnutt et al. (13) for anesthetized rabbits can be adopted to produce human serum concentration profiles in conscious rats. The simulation of human serum concentrations of the β-lactamase inhibitor BRL 42715 in combination with either cefazolin or piperacillin adjusted for the species differences seen in the elimination kinetics and allowed the assessment of the β-lactamase inhibitory activity of BRL 42715 in vivo at clinically relevant concentrations. This method also allowed the evaluation of the effect that the more rapid elimination of the inhibitor compared with that of the antibiotics may have had on antibacterial activity in the rat intraperitoneal infection model.

The combinations of BRL 42715 with either cefazolin or piperacillin were highly effective in reducing the intraperitoneal fluid and blood viable counts of *S. marcescens* US20 and *E. coli* 41548, respectively, with subsequent survival of the rats treated. The concentration of BRL 42715 required to significantly reduce the MIC of either piperacillin or cefazolin against *E. coli* 41548 and *S. marcescens*, respectively, was 0.03 to 0.06 μg/ml. Concentrations of BRL 42715 in plasma were below this range approximately 2 to 3 h after the start of dosing, at which time the concentrations of the antibacterial agents would be subinhibitory. Previous studies using an intraperitoneal infection in mice (4) have indicated that a reduction in the viable count in peritoneal fluid lasting for approximately 4 to 6 h after the administration of an effective agent was sufficient to prevent development of a systemic, lethal infection. The course of infection in rats was not monitored for the intraperitoneal infection with *S. marcescens*, but the results for rats infected with *E. coli* 41548 support this observation. Piperacillin alone demonstrated only a transitory bacteriostatic effect in peritoneal fluid which did not prevent spread of the bacteria into the blood stream between 3 and 6 h after infection. In contrast, a bactericidal effect was observed within the first 3 h after administering piperacillin plus BRL 42715, and numbers were maintained at a low level in peritoneal fluid thereafter, with only low numbers appearing in blood samples at 12 h after infection.

In conclusion, these studies, in which concentrations in human serum were simulated in rat plasma, demonstrate that BRL 42715 displays potent inhibitory activity against β-lactamases in vivo and that at clinically attainable concentrations, the inhibitor was able to protect the β-lactam antibiotics, cefazolin and piperacillin, from inactivation by β-lactamase-producing bacteria. The data also illustrate the potential of the infusion method described in the assessment of the likely clinical utility of novel antibacterial agents.

REFERENCES

1. **Bennett, I. S., G. Brooks, N. J. P. Broom, K. Coleman, S. Coulton, R. A. Edmundson, D. R. J. Griffin, J. B. Harbridge, N. F. Osborne, I. Stirling-Francois, and G. Walker.** 1988. BRL 42715: a new potent, broad spectrum β-lactamase inhibitor. Synthesis and structure-activity relationships of C6-substituted methylene penems. Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 118.
2. **Bergan, T.** 1977. Comparative pharmacokinetics of cefazolin, cephalothin, cephacetril, and cephapirine after intravenous administration. Chemotherapy **23**:389–404.
3. **Coleman, K., D. R. J. Griffin, J. W. Page, and P. A. Upshon.** 1989. In vitro evaluation of BRL 42715, a novel β-lactamase inhibitor. Antimicrob. Agents Chemother. **33**:1580–1587.
4. **Comber, K. R., C. D. Osborne, and R. Sutherland.** 1975. Comparative effects of amoxycillin and ampicillin in the treatment of experimental mouse infections. Antimicrob. Agents Chemother. **7**:179–185.
5. **Gerber, A. U.** 1991. Impact of the antibiotic dosage schedule on efficacy in experimental soft tissue infections. Scand J. Infect. Dis. Suppl. **74**:147–154.
6. **Gerber, A. U., H. P. Brugger, C. Feller, T. Stritzko, and B. Stalder.** 1986. Antibiotic therapy of infections due to *Pseudomonas aeruginosa* in normal and granulocytopenic mice: comparison of murine and human pharmacokinetics. J. Infect. Dis. **153**:90–97.
7. **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. Croyden and M. F. Michel (ed.), Augmentin, clavulanate potentiated amoxycillin. Proceedings of the European Symposium 1982, Scheveningen, The Netherlands. Excerpta Medica, Amsterdam.
8. **Nielsen-Kudsk, F.** 1981. Pharmacokinetic analysis and calculations using a program for the minicalculator TI-59. Int. J. Biomed. Comput. **12**:83–96.
9. **Prince, W. T., J. C. Thow, B. E. Davies, R. Sutherland, and R. Horton.** 1990. Effect of BRL 42715 on the pharmacokinetics of piperacillin following intravenous administration. Program Abstr. 30th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 823.
10. **Roosendaal, R., and I. A. J. M. Bakker-Woudenberg.** 1991. Impact of the antibiotic dosage schedule on efficacy in experimental lung infections. Scand. J. Infect. Dis. Suppl. **74**:155–162.
11. **Thornsberry, C., J. Anhalt, A. L. Barry, J. L. Cotton, E. H. Gerlach, R. N. Jones, R. C. Moellering, and R. A. Norton.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
12. **Tjandramaga, T. B., A. Mullie, R. Verbesselt, P. J. De Schepper, and L. Verbist.** 1978. Piperacillin: human pharmacokinetics after intravenous and intramuscular administration. Antimicrob. Agents Chemother. **14**:829–837.
13. **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.