ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1986, p. 838–844
0066-4804/86/050838-07$02.00/0

Vol. 29, No. 5

Bactericidal Effects of Ticarcillin-Clavulanic Acid against β-Lactamase-Producing Bacteria In Vivo

RONALD J. BOON, ANGELA S. BEALE, AND ROBERT SUTHERLAND*

Beecham Pharmaceuticals Research Division, Brockham Park, Betchworth, Surrey RH3 7AJ, England

Received 13 August 1985/Accepted 8 February 1986

The comparative efficacies of ticarcillin and ticarcillin plus clavulanic acid have been determined in the mouse against experimental infections caused by ticarcillin-resistant bacteria. The infections studied comprised an intraperitoneal infection, local tissue infections, pyelonephritis, and pneumonia. Both ticarcillin and clavulanic acid penetrated readily to the sites of infection studied and at the doses employed were present at concentrations of the same order as those obtained in humans after the administration of ticarcillin-clavulanic acid formulations (Timentin; Beecham). At these concentrations, the ticarcillin-clavulanic acid combination caused significant bactericidal effects at the sites of infection against the ticarcillin-resistant strains of *Bacteroides fragilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* investigated. The efficacy of ticarcillin plus clavulanic acid against the infections resistant to therapy with ticarcillin demonstrated the β-lactamase-inhibitory activity of clavulanic acid in vivo.

Clavulanic acid is a potent inhibitor of a wide range of bacterial β-lactamases (9, 11), and its effectiveness in protecting β-lactam antibiotics from inactivation by β-lactamase-producing bacteria in vitro and in vivo has been reported (3, 6, 8, 16). Ticarcillin, a broad-spectrum penicillin of proven clinical efficacy, is a candidate as a constituent of a clavulanic acid-penicillin combination, because the compound is unstable to many of the β-lactamases prevalent among clinical isolates. Data from in vitro studies have demonstrated the enhanced antibacterial activity of ticarcillin in the presence of clavulanic acid against ticarcillin-resistant bacteria. These include strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Yersinia enterocolitica*, and the anaerobe *Bacteroides fragilis*. In addition, β-lactamase-producing isolates of *Haemophilus influenzae*, *Branhamella catarrhalis*, *Neisseria gonorrhoeae*, and *Staphylococcus aureus* are susceptible to ticarcillin-clavulanic acid (1, 4, 7, 10, 14).

The studies reported here were designed to measure the extent to which ticarcillin and clavulanic acid were capable of penetrating to the sites of infections caused by β-lactamase-producing bacteria in the mouse and to relate this to the effects of therapy of the ticarcillin-resistant infections with a combination of ticarcillin-clavulanic acid in comparison with that of ticarcillin alone.

MATERIALS AND METHODS

Compounds. Ticarcillin disodium and potassium clavulanate were laboratory reference materials (Beecham Pharmaceuticals, Worthing, England). The ticarcillin-clavulanic acid combination comprised ticarcillin disodium and potassium clavulanate equivalent to 30 parts of ticarcillin to 1 part of clavulanic acid, as the pure free acids.

MICs. Serial dilutions of ticarcillin were added to 18-ml volumes of molten Mueller-Hinton agar in petri dishes. The antibacterial activity of ticarcillin-clavulanic acid was determined as that of ticarcillin in the presence of 2 μg of clavulanic acid per ml (7). The plates were inoculated with 0.001 ml of an overnight broth culture of the test organism diluted to yield an inoculum of approximately 10^4 CFU per spot. The plates were incubated for 18 h at 37°C, and the MICs were determined as the lowest concentrations of antibiotic preventing visible growth.

Mice. Albino mice of the MF1-Olac strain (Oxford Laboratory Animal Colony, Bicester, England) ranging in weight from 18 to 22 g were used throughout.

Intraperitoneal infection. Mice were injected by the intraperitoneal route with 0.5 ml of a suspension in hog gastric mucin (American Laboratories Inc., Omaha, Nebr.) of a dilution of an overnight broth culture (veal infusion broth; Difco Laboratories, Detroit, Mich.) of *Pseudomonas aeruginosa* PU21(pMG19) standardized to an infective inoculum of 5.8 $\log_{10}$ cells per mouse. The bactericidal effects of ticarcillin-clavulanic acid and ticarcillin in the therapy of the infection produced by *P. aeruginosa* Pu21(pMG19) were studied by monitoring the growth of the bacteria in the blood and peritoneal cavity of infected mice after therapy with the antibiotics. Ticarcillin (375 mg/kg)–clavulanic acid (12.5 mg/kg) and ticarcillin (375 mg/kg) were administered subcutaneously in distilled water (0.2 ml/20 g of mouse weight) to the infected mice 1, 3, 5, and 7 h after infection. At these times, and also at 12, 24, 48, and 72 h after infection, groups of five mice were killed with carbon dioxide. Samples of blood were collected from the axillary region. The abdomen was swabbed with 75% alcohol, and 2 ml of citrate buffer (pH 6.5) was injected into the peritoneal cavity. The abdomen was gently massaged to ensure adequate mixing, a small incision was made through the skin and peritoneal wall, and a sample of washings was collected with a Pasteur pipette.

The samples were diluted in 0.2% yeast extract and plated in duplicate onto nutrient agar containing 13,000 U of penicillinase (Bacto-penase; Difco) per ml to inactivate any ticarcillin remaining in the sample. The plates were incubated for 18 h at 37°C. Colonies were counted, and the geometric mean and standard deviations were calculated.

The blood and peritoneal fluid of infected mice were studied for their content of ticarcillin and clavulanic acid. Ticarcillin (375 mg/kg) and ticarcillin (375 mg/kg)–clavulanic acid (12.5 mg/kg) were administered subcutaneously to the mice 1 h after infection. Groups of five mice were killed with

* Corresponding author.

carbon dioxide at intervals during the 4-h period after drug administration. The samples from mice given ticarcillin-clavulanic acid were split into two fractions; one was assayed for ticarcillin content, and the other was tested for clavulanic acid content. Excess clavulanic acid (10 μg/ml) was added to all samples to be assayed for ticarcillin content to prevent hydrolysis of ticarcillin during the in vitro procedures. The addition of excess clavulanic acid did not affect the ticarcillin assay at the concentrations used, because the MIC of clavulanic acid for the assay organisms was 128 μg/ml. Blood samples were diluted in horse blood, a diluent known not to differ significantly from mouse blood. Peritoneal washings were diluted in 0.1 M citrate buffer (pH 6.5) and assayed for ticarcillin by a large-plate agar diffusion assay with *P. aeruginosa* NCTC 10701 and for clavulanic acid by a β-lactamase inhibition assay with *K. pneumoniae* NCTC 11228 (11). The latter assay was unaffected by the ticarcillin concentrations in the samples. The assay plates were incubated for 18 h at 37°C. Inhibition zones were measured, and antibiotic concentrations were derived from standard lines prepared from standard solutions. Means and standard deviations were calculated.

Pyelonephritis. The method of infection and assessment of bacterial numbers in the kidneys 7 days after infection was as described previously (3, 5). In brief, mice were injected intravenously with 8.3 $\log_{10}$ cells of *P. aeruginosa* Pu21(RMS149) or *P. aeruginosa* Pu21(pMG19). Subcutaneous treatment with ticarcillin (900 mg/kg) or ticarcillin (900 mg/kg)–clavulanic acid (30 mg/kg) started 19 h after infection. Dosing was continued four times a day for 4 days, evenly spaced between 9:00 a.m. and 6:00 p.m. each day.

Staphylococcal thigh lesion. Mice were infected intramuscularly in the right hind leg with 0.2 ml of a suspension in veal infusion broth containing about 8.0 $\log_{10}$ cells of the β-lactamase-producing strain *S. aureus* MB9(P^+) or the isogenic β-lactamase-negative variant, *S. aureus* MB9(P^-). Ticarcillin (150 mg/kg) or ticarcillin (150 mg/kg)–clavulanic acid (5 mg/kg) was administered subcutaneously to groups of 10 mice at 1 h after infection and again at 5 h after infection. At the time of the first dose, 1 h after infection, groups of 10 untreated infected animals were killed. The infected limb was liberally swabbed with 75% alcohol and carefully skinned. The entire limb was amputated, weighed, and homogenized for 2 min in 0.2% yeast extract. The homogenates were serially diluted 10-fold in 0.2% yeast extract and plated onto nutrient agar, and bacterial colonies were counted after overnight incubation at 37°C. The procedure was repeated 2 h after the second dose, 7 h after infection, and also at 30, 53, and 72 h and at 168 h in the case of those animals allowed to survive until this time.

For evaluation of the pharmacokinetic profiles, animals were infected with both strains, one strain in the right hind leg and the other strain in the left hind leg. The concentrations of antibiotics present in blood and tissue homogenates after dosage 1 h after infection were measured by microbiological assay as previously described.

***B. fragilis* groin infection.** Mice were infected by subcutaneous injection in the groin with 0.5 ml of a 22-h culture of *B. fragilis* VPI 8908 (9.3 $\log_{10}$ cells) in semisolid agar (3, 15). Subcutaneous therapy with ticarcillin (900 mg/kg) or ticarcillin (900 mg/kg)–clavulanic acid (30 mg/kg) was started at the time of infection and continued three times a day for 4 days, evenly spaced between 9:00 a.m. and 6:00 p.m. each day. Groups of 10 mice were killed at intervals after infection, and the bacteria in homogenates prepared from groin tissue were counted (3). The drug concentrations in the pus of fully formed abscesses were also measured after subcutaneous administration of ticarcillin or ticarcillin-clavulanic acid (3).

TABLE 1. Activity of ticarcillin, ticarcillin-clavulanic acid, and clavulanic acid against infecting bacteria

Organism	β-Lactamase class[a]	MIC (μg/ml)		
		Ticarcillin	Ticarcillin-clavulanic acid	Clavulanic acid
K. pneumoniae 62	IV	128	4.0	32
P. aeruginosa Pu21(RMS149)	V (PSE-3)	>512	32	128
P. aeruginosa Pu21(pMG19)	V (PSE-4)	>512	256	128
B. fragilis VPI 8908	Ic	512	8.0	16
S. aureus MB9(P^+)	+[b]	64	1.0	16
S. aureus MB9(P^-)	−[c]	1.0	0.5	16

[a] Richmond and Sykes classification (12).
[b] β-Lactamase-producing strain.
[c] β-Lactamase-negative strain.

Pneumonia. Mice were inoculated intranasally under light ether anesthesia with *K. pneumoniae* 62 at an inoculum of 4.0 $\log_{10}$ cells per mouse. Therapy with ticarcillin (900 mg/kg) or ticarcillin (900 mg/kg)–clavulanic acid (30 mg/kg) was started 18 h after infection and continued three times a day for 4 days between 9:00 a.m. and 6:00 p.m. On the first day of treatment 10 mice were sacrificed 1 h before the first dose. Samples of blood were collected from the axillary region. The lungs were carefully excised and washed in sterile phosphate-buffered saline to remove any blood. The tissues were homogenized in 0.2% yeast extract, and bacterial counts were performed on serially diluted blood and lung homogenates. This procedure was repeated on groups of 10 mice from each treatment regime 1 h after cessation of the first day of therapy and was continued likewise (i.e., samples taken 1 h before commencement of and 1 h after cessation of therapy) for 4 days after infection. In a separate group of mice, the infection was allowed to progress for 36 h before concentrations of ticarcillin and clavulanic acid in pleural fluid after the administration of ticarcillin or ticarcillin-clavulanic acid were measured. At intervals during the 3-h period after drug administration, groups of five mice were sampled as described previously (3).

RESULTS

Antibacterial activity in vitro. Table 1 shows the MICs of ticarcillin, clavulanic acid, and of ticarcillin in the presence of 2 μg of clavulanic acid per ml against the mouse-virulent strains of bacteria. The activity of ticarcillin against the β-lactamase-producing strains of bacteria was notably enhanced in the presence of clavulanic acid as a result of the β-lactamase-inhibitory properties of the latter. The susceptibility of the β-lactamase negative strain, *S. aureus* MB9(P^-), to ticarcillin was not influenced by clavulanic acid.

Intraperitoneal infection. Concentrations of ticarcillin and of clavulanic acid in the peritoneal fluid and blood after the administration of ticarcillin-clavulanic acid and of ticarcillin alone in mice infected with the β-lactamase-producing strain *P. aeruginosa* Pu21(pMG19) are shown in Fig. 1. In perito-

neal fluid, a peak level of 227 ± 25 μg of ticarcillin per ml was measured 10 min after dosage with ticarcillin alone, whereas when combined with clavulanic acid, the peak level of ticarcillin was higher, 293 ± 64 μg/ml at 10 min. The difference in antibiotic concentrations was reflected in the areas under the curves (AUC), which were 277 μg · h/ml for ticarcillin alone and 345 μg · h/ml for ticarcillin in the presence of clavulanic acid. However, this difference was not statistically significant. A peak concentration of 19.6 ± 3.6 μg of clavulanic acid per ml was measured in the peritoneal fluid at 10 min (AUC, 21.0 μg · h/ml). In the blood of the infected animals there was also a difference in the levels of ticarcillin attained in the presence and absence of clavulanic acid; the peak concentration of ticarcillin after administration of the combination was 197 ± 44 μg/ml (AUC, 124 μg · h/ml) compared with 112 ± 19 μg/ml for ticarcillin alone. A peak concentration of 10.6 ± 4.4 μg of clavulanic acid per ml of blood was measured at 10 min (AUC, 6.1 μg · h/ml).

Figure 2 shows the growth of *P. aeruginosa* Pu21(pMG19) in the blood and peritoneal fluid of infected and treated mice. In the untreated control mice, the bacterial numbers in the peritoneal cavity increased steadily from the starting inoculum of 5.8 $\log_{10}$ cells per ml to 8.04 ± 0.60 $\log_{10}$ cells per ml at 7 h after infection; between 7 and 12 h there was a decline in cell numbers to 6.41 ± 1.80 $\log_{10}$ cells per ml, but by 24 h all of the control mice had died. In the mice treated with ticarcillin, the bacterial growth pattern was very similar to that in the untreated control mice; by 12 h the bacterial count had reached 8.74 ± 1.03 $\log_{10}$ cells per ml, and none of the animals were alive 24 h after the initiation of the infection. In contrast, therapy with ticarcillin-clavulanic acid resulted in a decline in the bacterial numbers from the commencement of dosing, and at 12 h the mean count was 1.35 ± 0.79 $\log_{10}$ cells per ml, which was significantly different ($P < 0.05$) from the counts in the untreated and ticarcillin-treated mice. There was some regrowth between 12 and 72 h after infection (the numbers fluctuated between 1.00 and 3.00 $\log_{10}$ cells per ml), but no deaths occurred in the mice treated with ticarcillin-clavulanic acid.

In the blood of the infected mice, 4.59 ± 0.21 $\log_{10}$ cells per ml were present 1 h after infection; in the untreated control animals, the numbers remained at approximately this level for 12 h, after which all of the mice died (Fig. 2). Therapy with either ticarcillin or the combination had little apparent effect on the growth of the organisms in the blood up to 7 h after infection, the cell numbers approximating

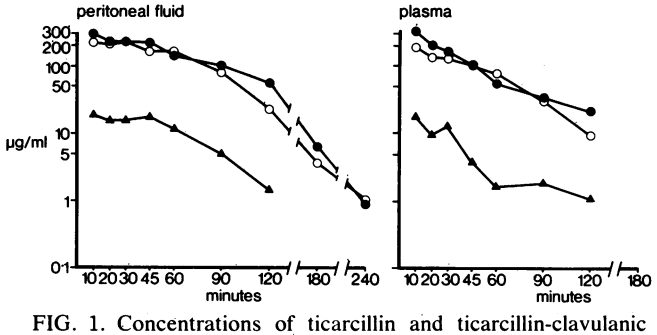

FIG. 1. Concentrations of ticarcillin and ticarcillin-clavulanic acid in the peritoneal fluid and blood of mice infected intraperitoneally with *P. aeruginosa* Pu21(pMG19). Symbols: ○, ticarcillin (375 mg/kg); ●, ticarcillin (375 mg/kg)–clavulanic acid (12.5 mg/kg); ▲, clavulanic acid (12.5 mg/kg).

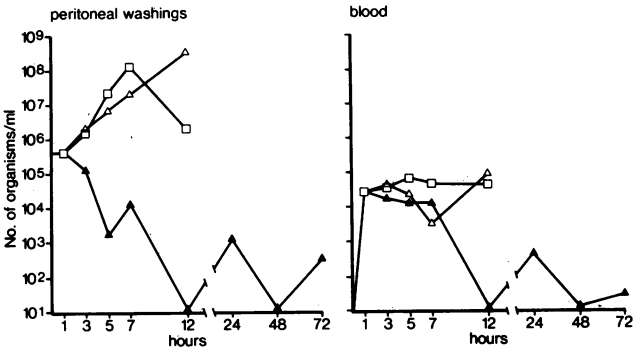

FIG. 2. Effects of therapy with ticarcillin and ticarcillin-clavulanic acid on the growth of *P. aeruginosa* Pu21(pMG19) in peritoneal fluid and blood of mice infected intraperitoneally. Symbols: □, untreated controls; △, ticarcillin (375 mg/kg); ▲, ticarcillin (375 mg/kg)–clavulanic acid (12.5 mg/kg).

those in the untreated mice. In the ticarcillin-treated mice, the cell numbers remained at 5.00 ± 0.18 $\log_{10}$ cells per ml at 12 h, after which the animals became moribund and died. However, from 7 h onward in the animals treated with ticarcillin-clavulanic acid there was a rapid fall in bacterial numbers, and at 12 h the counts in the blood were significantly different ($P < 0.05$) from those in the ticarcillin-treated and untreated mice, being below the detectable limit (1.23 $\log_{10}$ cells per ml). The bacterial numbers in the blood of the ticarcillin-clavulanic acid-treated mice did not exceed 3.00 $\log_{10}$ cells per ml in the 12- to 72-h period.

Pyelonephritis. The results of therapy of kidney infections due to the β-lactamase-producing, ticarcillin-resistant strains *P. aeruginosa* Pu21(pMG19) and *P. aeruginosa* Pu21 (RMS149) are shown in Table 2. In each of these infections, macroscopic multiple focal suppurative lesions were observed in 70 to 80% of the untreated animals, and mean bacterial counts of 6.34 and 7.57 $\log_{10}$ cells per g of kidney tissue, respectively, were measured at the end of the test.

Treatment with ticarcillin or clavulanic acid was relatively ineffective against both organisms; 70 to 80% of the animals had visible kidney lesions and bacterial counts of the same order as the untreated controls. In contrast, none of the animals treated with ticarcillin-clavulanic acid showed visible kidney lesions, and this was reflected in the bacterial counts of the kidney homogenates. No bacteria (<1.70 $\log_{10}$ cells per g of tissue) were recovered from the kidneys of mice infected with *P. aeruginosa* Pu21(RMS149); in the mice infected with *P. aeruginosa* Pu21(pMG19), bacteria were detected in only 4 of 10 animals (mean count, 3.14 ± 1.71 $\log_{10}$ cells per g).

Staphylococcal thigh lesion. The comparative activities of ticarcillin-clavulanic acid and of ticarcillin were determined against the localized thigh muscle infections caused by the β-lactamase-producing strain *S. aureus* MB9(P^+) and the isogenic β-lactamase-negative variant, *S. aureus* MB9(P^-) (Fig. 3). At the start of therapy, the mean bacterial counts in infected thigh tissue were 7.52 ± 0.95 $\log_{10}$ (P^+) and 7.77 ± 0.27 $\log_{10}$ (P^-) cells per g. In untreated control animals the tissue counts were 7.62 ± 0.54 $\log_{10}$ (P^+) and 8.08 ± 0.70 $\log_{10}$ (P^-) cells per g at 72 h after infection, when the mice were killed before extensive tissue damage occurred. Treatment of both infections with ticarcillin-clavulanic acid resulted in a continuing reduction of the bacterial numbers in the infected thighs during and after therapy; at the end of the

TABLE 2. Activity of ticarcillin and ticarcillin-clavulanic acid in the treatment of mouse pyelonephritis infections due to ticarcillin-resistant strains of *P. aeruginosa*

Organism	Therapy	No. with visible lesions/total	No. with bacteria in kidneys/total	Bacterial count (log_{10} CFU/g of kidney)[a]
P. aeruginosa Pu21(RMS149)	Ticarcillin (900 mg/kg)–clavulanic acid (30 mg/kg)	0/10	0/10	<1.70[b]
	Ticarcillin (900 mg/kg)	8/10	7/10	7.78 ± 0.86
	Clavulanic acid (30 mg/kg)	8/10	8/10	7.78 ± 0.30
	Nontreated controls	8/10	8/10	7.57 ± 1.04
P. aeruginosa Pu21(pMG19)	Ticarcillin (900 mg/kg)–clavulanic acid (30 mg/kg)	0/10	4/10	3.14 ± 1.71[b]
	Ticarcillin (900 mg/kg)	7/10	7/10	8.27 ± 0.32
	Clavulanic acid (30 mg/kg)	8/10	8/10	7.57 ± 1.91
	Nontreated controls	7/10	9/10	6.34 ± 2.33

[a] Geometric mean bacterial count of mice with recoverable bacteria in kidneys ± standard deviation.
[b] Significantly more effective than ticarcillin ($P < 0.05$).

test (168 h), the mean counts were 3.73 ± 1.70 log_{10} (P^-) and 3.70 ± 1.23 log_{10} (P^+) cells per g, a significant reduction in numbers ($P < 0.05$) from the tissue counts in the untreated mice at 72 h. None of the ticarcillin-clavulanic-acid treated mice showed visible evidence of infection. Against the infection caused by the penicillin-sensitive strain *S. aureus* MB9(P^-), ticarcillin therapy reduced the bacterial numbers to a similar extent by 168 h (3.58 ± 0.90 log_{10} cells per g). On the other hand, in mice infected with the β-lactamase-producing strain *S. aureus* MB9(P^+), ticarcillin therapy had no appreciable effect on the viability of the staphylococci, and the tissue counts in these animals at 72 h (7.66 ± 0.92 log_{10} cells per g) were similar to those in untreated animals. These animals also developed thigh swellings of a similar severity to the control animals and were therefore killed at 72 h before extensive tissue damage occurred.

The concentrations of ticarcillin and ticarcillin-clavulanic acid in the blood and thigh tissue of mice infected with both staphylococcal strains are presented in Table 3. Ticarcillin tissue concentrations after administration of the drug alone were considerably lower and less prolonged in the limbs infected with the β-lactamase-producing (P^+) strain than in those infected with the β-lactamase-negative variant (P^-); by comparing individual AUC values, this difference was very nearly significant at the 5% level ($P = 0.065$). However, after administration of ticarcillin-clavulanic acid, the ticarcillin tissue concentrations were similar in limbs infected with either the β-lactamase-producing strain (mean AUC, 6.1 μg · h/g) or the β-lactamase-negative strain (mean AUC, 6.5 μg · h/g). The inhibition of the staphylococcal β-lactamase at the site of infection was achieved with low and transient concentrations of clavulanic acid, 0.36 ± 0.10 μg/g of tissue being measured at 20 min, falling to undetectable levels (<0.06 μg/g) at 60 min.

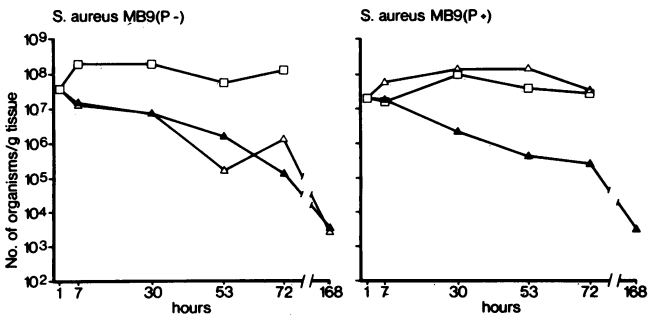

FIG. 3. Effects of therapy with ticarcillin and ticarcillin-clavulanic acid on the growth of *S. aureus* MB9(P^-) and *S. aureus* MB9(P^+) in mouse thigh muscle. Symbols: □, untreated controls; △, ticarcillin (150 mg/kg); ▲, ticarcillin (150 mg/kg)–clavulanic acid (5 mg/kg).

***B. fragilis* groin infection.** Figure 4 shows the concentrations of ticarcillin and clavulanic acid in the pus of abscesses produced in mice 7 days after injection of *B. fragilis* VPI 8908. When ticarcillin alone was administered, a peak concentration of 114.2 ± 60.8 μg/ml was attained in pus at 30 min. In the presence of clavulanic acid, the peak level of ticarcillin (90.9 ± 51.9 μg/ml) was reached later, at 60 min. From 60 min onward, the ticarcillin pus concentrations remained higher in the mice dosed with ticarcillin-clavulanic acid; this was reflected in the AUC, which were 151 μg · h/ml for ticarcillin alone and 219 μg · h/ml for ticarcillin in the presence of clavulanic acid. A peak concentration of 4.56 ± 2.37 μg of clavulanic acid per ml was attained at 60 min (AUC, 7.0 μg · h/ml).

The β-lactamase-inhibitory activity of clavulanic acid during the course of the *B. fragilis* infection was determined by measurement of the bacterial numbers in infected tissue over a period of 7 days in animals treated with ticarcillin-clavulanic acid or ticarcillin (Fig. 5). In untreated mice, the bacterial numbers fell from the starting inoculum of 9.30 log_{10} cells per g of tissue to 6.65 ± 0.51 log_{10} cells per g during the first 24 h, but rose to 7.35 ± 0.69 log_{10} cells per g by 32 h. The counts remained at this level, or higher, for the duration of the 7-day test period. Ticarcillin therapy was ineffective; the bacterial numbers in groin tissue of ticarcillin-treated mice were similar to those in the untreated mice at 32 h (7.47 ± 0.93 log_{10} cells per g). However, therapy with the ticarcillin-clavulanic acid combination caused a marked reduction in the viability of *B. fragilis* so that the bacterial counts fell steadily to 3.04 ± 0.88 log_{10} cells per g by 96 h; a significant reduction in cell numbers ($P < 0.05$) was achieved in comparison with the control and ticarcillin-treated groups. After cessation of therapy at 96 h, the count rose to 4.39 ± 2.51 log_{10} cells per g and remained at this level until the end of the study.

Mouse pneumonia. The penetration of ticarcillin and clavulanic acid into the pleural cavity of mice infected with *K. pneumoniae* 62 is shown in Fig. 6. Pleural exudate concentrations of ticarcillin, whether dosed alone or with clavulanic acid, were generally similar, falling from 213.5 ± 82.5 and

TABLE 3. Concentrations of ticarcillin and clavulanic acid in plasma and thigh tissue of *S. aureus*-infected mice[a]

S. aureus strain	Therapy	Time (min)	Ticarcillin concn in:		Clavulanic acid concn in:		AUC for:	
			Blood (μg/ml)	Tissue (μg/g)	Blood (μg/ml)	Tissue (μg/g)	Blood (μg·h/ml)	Tissue (μg·h/g)
MB9(P$^+$)	Ticarcillin (150 mg/kg)	20	38.7	3.2			18.9	1.6
		60	7.3	<2				
		90	3.7	<2				
		105	2.8	<2				
		120	<1	<2				
	Ticarcillin (150 mg/kg)–clavulanic acid (5 mg/kg)	20	38.4	6.3	1.4	0.36	18.7	6.1
		60	7.3	2.5	0.2	0		
		90	3.6	NS[b]	0	NS		
		105	2.0	2.3	0	0		
		120	<1	<2				
MB9(P$^-$)	Ticarcillin (150 mg/kg)	20		9.3				7.1
		60		2.9				
		90		2.4				
		105		<2				
		120		<2				
	Ticarcillin (150 mg/kg)–clavulanic acid (5 mg/kg)	20		6.6		0.26		6.1
		60		2.8		0		
		90		NS		NS		
		105		2.5		0		
		120		<2				

[a] Mice were infected with MB9(P$^+$), a β-lactamase-producing strain, or MB9(P$^-$), a β-lactamase-negative variant. Results are mean values from five animals.
[b] NS, No sample.

187.5 ± 44.4 μg/ml, respectively, at 15 min to 3.82 ± 1.18 and 2.96 ± 0.86 μg/ml at 180 min (AUCs, 136 and 162 μg · h/ml, respectively). A peak concentration of clavulanic acid of 8.93 ± 2.95 μg/ml was attained at 15 min, falling to 0.2 ± 0.1 μg/ml by 120 min (AUC, 5.1 μg · h/ml).

Intranasal instillation of *K. pneumoniae* 62 produced a respiratory tract infection in mice, causing consolidation of the lungs between 16 and 48 h; untreated mice died 48 to 112 h after infection, with the majority (80%) succumbing to the infection by 88 h. This was reflected in the lung counts of the untreated animals (Fig. 7), in which the numbers of bacteria rose to 7.23 ± 1.79 $\log_{10}$ cells per lung by 48 h. Therapy with ticarcillin was ineffective, the bacterial lung counts being similar to those of the untreated animals at 48 h (7.32 ± 1.44 $\log_{10}$ cells per lung). By 168 h there were no survivors in the untreated group or in the group treated with ticarcillin. In contrast, ticarcillin-clavulanic acid treatment produced a marked decrease in bacterial numbers in the lungs. By 88 h the count had fallen to 2.18 ± 0.48 $\log_{10}$ cells per lung, a significant reduction compared with the untreated and ticarcillin-treated mice ($P < 0.05$). After therapy ended, the count increased to 3.64 ± 0.78 $\log_{10}$ cells per lung at 112 h. By 168 h, when the experiment was terminated, 90% of the ticarcillin-clavulanic acid-treated mice were still alive.

A very similar pattern was observed with the bacterial counts in the blood of the mice infected with *K. pneumoniae* 62. (Fig. 7). That is, there was a rapid increase in the counts after infection, and they remained high during the test both in the untreated group and in that treated with ticarcillin. Therapy with ticarcillin-clavulanic acid resulted in a drop in

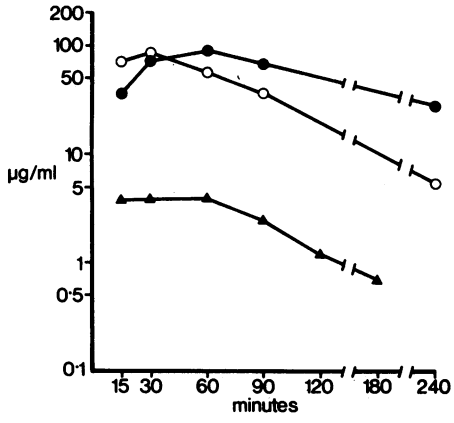

FIG. 4. Concentrations of ticarcillin and ticarcillin-clavulanic acid in pus aspirated from abscesses in mice 7 days after subcutaneous infection with *B. fragilis* VPI 8908. Symbols: ○, ticarcillin (900 mg/kg); ●, ticarcillin (900 mg/kg)–clavulanic acid (30 mg/kg); ▲, clavulanic acid (30 mg/kg).

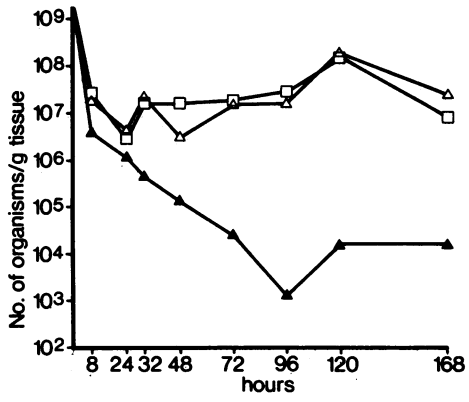

FIG. 5. Effects of therapy with ticarcillin and ticarcillin-clavulanic acid on the growth of *B. fragilis* VPI 8908 in the groin of mice infected subcutaneously. Symbols: □, untreated controls; △, ticarcillin (900 mg/kg); ▲, ticarcillin (900 mg/kg)–clavulanic acid (30 mg/kg).

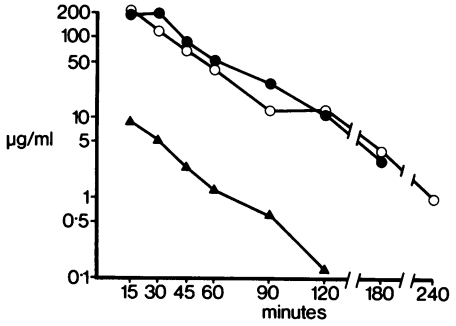

FIG. 6. Concentrations of ticarcillin and ticarcillin-clavulanic acid in pleural exudate of mice infected intranasally with *K. pneumoniae* 62. Symbols: ○, ticarcillin (900 mg/kg); ●, ticarcillin (900 mg/kg)–clavulanic acid (30 mg/kg); ▲, clavulanic acid (30 mg/kg).

the bacterial blood count from 4.77 ± 1.14 $\log_{10}$ cells per ml at the start of therapy at 24 h to numbers which were generally below the limit of detection (16 cells per ml) or did not exceed 100 cells per ml throughout the experiment.

DISCUSSION

The data reported here illustrate the penetration of ticarcillin and clavulanic acid into various sites of infection and describe the activity in vivo of ticarcillin-clavulanic acid and of ticarcillin alone, in terms of the dynamics of bacterial killing during and after therapy.

In these studies, there was little significant difference in the pharmacokinetic parameters of ticarcillin (C_{max}, $t_{1/2}$, and AUC) in the presence or absence of clavulanic acid, in keeping with studies in uninfected animals (L. Mizen and G. Woodnutt, Program Abstr. 22nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 294, 1982). Likewise, studies in human volunteers have confirmed the compatability of the penicillin and the β-lactamase inhibitor and have demonstrated rapid tissue penetration as exemplified by blister fluid concentrations (2, 13).

In the case of the infections caused by the β-lactamase-producing strains of gram-negative bacteria, there was little evidence of inactivation of ticarcillin at sites of infection, which may not be unexpected since the β-lactamases produced by these organisms are intracellular and hydrolysis of β-lactam substrate occurs within the bacterial cell. In the staphylococcal localized infection, the fact that the ticarcillin tissue concentrations were markedly lower at the sites infected with the β-lactamase-producing strain is consistent with the extracellular location of staphylococcal β-lactamases. In the presence of clavulanic acid, the ticarcillin tissue concentrations were similar whether the limb was infected with the β-lactamase-producing strain or the β-lactamase-negative strain, as a result of inhibition of the staphylococcal β-lactamase.

Despite the fact that overt destruction of ticarcillin could not readily be detected in some of the models studied, the role of bacterial β-lactamases in the failure of ticarcillin therapy and the efficiency of inhibition of bacterial β-lactamases by clavulanic acid in vivo were demonstrated by the superior bactericidal activity of the combination compared with that of ticarcillin alone. The latter was ineffective in preventing the growth of the infecting bacteria and hence the development of an intraperitoneal infection, pyelonephritis infections caused by *P. aeruginosa*, localized tissue infections produced by either *S. aureus* or *B. fragilis*, and a bacterial pneumonia initiated by *K. pneumoniae*. In contrast, ticarcillin-clavulanic acid exerted significant bactericidal action against the organisms during therapy, bringing about sufficient reduction in the numbers of viable bacteria to enable the host defense mechanisms to control the infection.

The dosage schedules used in these studies produced peak concentrations of ticarcillin and clavulanic acid in blood and at sites of infection of the same order as those measured in humans after infusion of ticarcillin-clavulanic acid formulations (2, 13). However, the elimination of the compounds from the infected mice was much more rapid than is the case in humans; nonetheless, therapy with ticarcillin-clavulanic acid of the ticarcillin-resistant infections was highly effective despite the relatively low and transient concentrations of clavulanic acid. For instance, in the staphylococcal tissue infection, the elimination of the β-lactamase-producing strain of *S. aureus* was achieved as rapidly with ticarcillin-clavulanic acid as was the ticarcillin-susceptible strain. This was the case also in the *B. fragilis* infection and in the pneumonia caused by *K. pneumoniae*, which responded readily to ticarcillin in the presence of low concentrations of clavulanic acid measured during therapy.

Unexpectedly good bactericidal effects were produced against the infections caused by *P. aeruginosa* Pu21(pMG19). This strain was classed as resistant to ticarcillin-clavulanic acid in vitro (MIC, 256 μg of ticarcillin per ml in the presence of 2 μg of clavulanic acid per ml). However, in the case of the intraperitoneal infection, the organism was exposed to relatively high concentrations of ticarcillin and, particularly, of clavulanic acid, and it may be that the susceptibility of *P. aeruginosa* Pu21(pMG19) to ticarcillin-clavulanic acid in vitro may have been underestimated. In the case of the pyelonephritis, it is likely that the kidney tissue levels of ticarcillin and clavulanic acid would be concentrated, which may again account for the efficacy of the combination.

Some discrepancies were noted with regard to the antibiotic concentrations measured in the different infection models. In particular, the concentrations of ticarcillin and clavulanic acid in blood of mice from the localized staphylococcal thigh infection were much lower than those measured in the animals infected intraperitoneally with *P. aeruginosa*. The explanation for these differences, which were observed in repeat studies, is not yet known.

The results of the studies reported here suggest that clavulanic acid exerts a profound inhibitory effect against

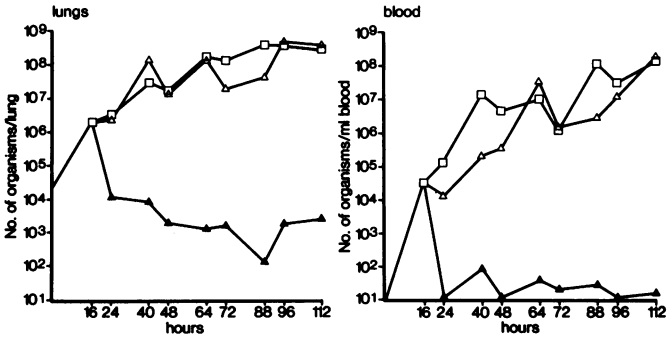

FIG. 7. Effects of therapy with ticarcillin and ticarcillin-clavulanic acid on the growth of *K. pneumoniae* 62 in the lungs and blood of mice infected intranasally. Symbols: □, untreated controls; △, ticarcillin (900 mg/kg); ▲, ticarcillin (900 mg/kg)–clavulanic acid (30 mg/kg).

bacterial β-lactamases in vivo and that the inhibitor might only need to be present at a site of infection for short periods of time, as was postulated by Bennett et al. (2) on the basis of the data from tissue penetration studies in human volunteers and clinical studies (2, 17). The efficacy of ticarcillin-clavulanic acid against experimental infections caused by ticarcillin-resistant bacteria illustrates the potential of the combination in clinical therapy.

ACKNOWLEDGMENTS

We thank Nigel Eaton and John Gisby for skilled technical assistance, David Merrikin for advice on the statistical analyses, and Liz Clarke for typing the manuscript.

LITERATURE CITED

1. **Barry, A. L., L. W. Ayers, T. L. Gavan, E. H. Gerlach, and R. N. Jones.** 1984. *In vitro* activity of ticarcillin plus clavulanic acid against bacteria isolated in three centers. Eur. J. Clin. Microbiol. **3**:203–206.
2. **Bennett, 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.
3. **Boon, R. J., A. S. Beale, K. R. Comber, C. V. Pierce, and R. Sutherland.** 1982. Distribution of amoxicillin and clavulanic acid in infected animals and efficacy against experimental infections. Antimicrob. Agents Chemother. **22**:369–375.
4. **Clarke, A. M., and S. J. V. Zemcov.** 1984. Clavulanic acid in combination with ticarcillin: an *in vitro* comparison with other beta-lactams. J. Antimicrob. Chemother. **13**:121–128.
5. **Comber, K. R.** 1976. Pathogenesis of an experimental pyelonephritis model in the mouse and its use in the evaluation of antibiotics, p. 311–316. *In* J. D. Williams and A. M. Geddes (ed.), Chemotherapy, vol. 2. Plenum Publishing Corp., New York.
6. **Dumon, L., P. Adriaens, J. Anne, and H. Eyssen.** 1979. Effect of clavulanic acid on the minimum inhibitory concentration of benzylpenicillin, ampicillin, carbenicillin, or cephalothin against clinical isolates resistant to beta-lactam antibiotics. Antimicrob. Agents Chemother. **15**:315–317.
7. **Fuchs, P. C., A. L. Barry, C. Thornsberry, and R. N. Jones.** 1984. In vitro activity of ticarcillin plus clavulanic acid against 632 clinical isolates. Antimicrob. Agents Chemother. **25**:392–394.
8. **Hunter, P. A., K. Coleman, J. Fisher, and D. Taylor.** 1980. *In vitro* synergistic properties of clavulanic acid with ampicillin, amoxicillin and ticarcillin. J. Antimicrob. Chemother. **6**:455–470.
9. **Neu, H. C., and K. P. Fu.** 1978. Clavulanic acid, a novel inhibitor of β-lactamases. Antimicrob. Agents Chemother. **14**:650–655.
10. **Paisley, J. W., and J. A. Washington.** 1978. Combined activity of clavulanic acid and ticarcillin against ticarcillin-resistant, gram-negative bacilli. Antimicrob. Agents Chemother. **14**:224–227.
11. **Reading, C., and M. Cole.** 1977. Clavulanic acid: a beta-lactamase-inhibiting beta-lactam from *Streptomyces clavuligerus*. Antimicrob. Agents Chemother. **11**:852–857.
12. **Richmond, M. H., and R. B. Sykes.** 1973. The beta-lactamases of Gram-negative bacteria and their possible physiological role. Adv. Microb. Physiol. **9**:31–88.
13. **Scully, B. E., E. Steinman, N. X. Chin, and H. C. Neu.** 1984. The pharmacology of clavulanic acid and ticarcillin combined. Chemioterapia **3**:385–389.
14. **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 clavulanate potassium. Am. J. Med. **79**(Suppl. 5B):13–24.
15. **Walker, C. B., and T. D. Wilkins.** 1976. Use of semisolid agar for initiation of pure *Bacteroides fragilis* infection in mice. Infect. Immun. **14**:721–725.
16. **Wise, R., J. M. Andrews, and K. A. Bedford.** 1978. In vitro study of clavulanic acid in combination with penicillin, amoxycillin and carbenicillin. Antimicrob. Agents Chemother. **13**:389–393.
17. **Wise, R., A. Donovan, J. Drumm, J. M. Andrews, and P. Stephenson.** 1983. The penetration of amoxycillin/clavulanic acid into peritoneal fluid. J. Antimicrob. Chemother. **11**:57–60.