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Distribution of Amoxicillin and Clavulanic Acid in Infected Animals and Efficacy Against Experimental Infections

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The therapeutic effects produced by formulations of amoxicillin plus clavulanic acid (BRL 25000A and BRL 25000G) were compared with those of amoxicillin and clavulanic acid separately against a variety of infections produced by amoxicillin-susceptible and β-lactamase-producing (amoxicillin-resistant) bacteria. The infection models studied included intraperitoneal infections, a mouse pneumonia, experimental pyelonephritis, and local lesions caused by *Staphylococcus aureus* and *Bacteroides fragilis*. The distribution of amoxicillin and clavulanic acid in infected animals after the administration of amoxicillin-clavulanic acid was evaluated by measurement of the concentrations of the substances present in specimens collected at the sites of infection. The results showed that both amoxicillin and clavulanic acid were well distributed in the animal body after the administration of amoxicillin-clavulanic acid formulations, being present in significant concentrations at various sites of infection, e.g., peritoneal washings, pleural fluid, pus, and infected tissue homogenates. In a number of cases, the amoxicillin concentrations measured after the administration of BRL 25000 were higher than those found after treatment with amoxicillin alone, presumably as a result of inhibition of bacterial β-lactamases by clavulanic acid at the site of infection. The ability of clavulanic acid to protect amoxicillin in vivo was confirmed by the efficacy of amoxicillin-clavulanic acid formulations in the treatment of the infections studied, most of which were refractory to therapy with amoxicillin.

Clavulanic acid is a progressive inhibitor of β-lactamases produced by gram-negative and gram-positive bacteria (3, 15) and has been shown to be effective in protecting amoxicillin and other β-lactam antibiotics in antibacterial tests in vitro against β-lactamase-producing bacteria (1, 5, 8, 17). The compound is well absorbed by the oral route in animals and humans, and its pharmacokinetics are compatible with those of amoxicillin. In addition, clavulanic acid, like amoxicillin, has been shown to be well distributed throughout uninfected animals (5, 13). However, there is little evidence regarding the penetration of both compounds to infected sites, although in preliminary studies, amoxicillin-clavulanic acid formulations (BRL 25000A and BRL 25000G) were shown to be effective in the treatment of infections caused by amoxicillin-susceptible and amoxicillin-resistant bacteria (5, 9).

Accordingly, the studies reported here were designed to investigate the distribution of amoxicillin and clavulanic acid after the administration of BRL 25000 to animals infected with β-lactamase-producing bacteria. The sites of infection at which concentrations of the compounds were measured included peritoneal fluid from mice infected by the intraperitoneal route, pleural fluid from a mouse pneumonia, tissue homogenates from a localized staphylococcal infection, and pus from a localized *Bacteroides fragilis* lesion. As a rule, the specimens were collected when the course of infection was well advanced to ensure that significant numbers of bacteria were present. The penetration of the compounds to sites of infection was further assessed by evaluation of the efficacy of therapy of these infections with BRL 25000 compared with amoxicillin or other antibacterial agents.

MATERIALS AND METHODS

Compounds. The amoxicillin trihydrate and potassium clavulanate used in these studies were laboratory reference preparations (Beecham Pharmaceuticals, Worthing, England). Standard compounds tested for comparison included cefoxitin (Merck Sharp & Dohme Ltd., Hoddeson, England), clindamycin (Upjohn Ltd., Crawley, England), and metronidazole (May and Baker Ltd., Dagenham, England) and were generously provided by the manufacturers.

Two amoxicillin-clavulanic acid formulations were studied: (i) BRL 25000A, amoxicillin trihydrate and potassium clavulanate equivalent to two parts of

amoxicillin and one part of clavulanic acid as the pure free acids; and (ii) BRL 25000G, amoxicillin trihydrate and potassium clavulanate equivalent to four parts of amoxicillin and one part of clavulanic acid as the pure free acids.

Mice. Mice of the MFI-Olac strain (Oxford Laboratory Animal Colony, Oxford, England), with a weight range of 18 to 22 g, were used in all studies.

Intraperitoneal infections. Mice were inoculated 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; Difco Laboratories, Detroit, Mich.) of the test organism standardized to give an infective inoculum of 10 to 100 50% lethal doses. In these tests, the animals were inoculated with mouse-virulent strains of a β-lactamase-producing (P^+) strain of *Staphylococcus aureus* MB9 and an amoxicillin-resistant strain of *Escherichia coli* JT39 (P^+) possessing a TEM-1 mediated β-lactamase. For therapy, the compounds (0.2 ml/20 g of mouse weight) were administered orally as acacia suspensions in phosphate-buffered saline with a stainless steel oral needle 1 and 5 h after infection. The substances were given at four or five dose levels for each test, and groups of 10 mice were treated at each dose level. The numbers of animals surviving for 4 days after infection were recorded, and the dose of compound required to produce 50% protection of infected animals was calculated (12).

The concentrations of amoxicillin and clavulanic acid present in peritoneal fluid after the administration of BRL 25000A formulation and the concentration of amoxicillin present after the administration of amoxicillin were measured in mice infected by the intraperitoneal route with *S. aureus* MB9 (P^+) and *E. coli* JT39 (P^+). BRL 25000A, amoxicillin, and clavulanic acid were administered orally to infected mice 4 h after infection. Groups of five mice were killed with carbon dioxide at intervals during the 6-h period after drug administration. The abdomen was swabbed with 75% alcohol, and 2 ml of phosphate buffer (pH 7.0) was forcefully injected into the peritoneal cavity. The abdomen was gently massaged to ensure adequate mixing, a small incision was made through skin and peritoneal wall, and a sample of washings was collected with a Pasteur pipette. The samples from mice given BRL 25000A were split into two factions; one was assayed for amoxicillin 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 amoxicillin content to prevent hydrolysis of amoxicillin during the in vitro procedures because previous experimentation had shown this procedure to be effective for this purpose. The addition of excess clavulanic acid did not affect the amoxicillin assay at the concentrations used. Peritoneal washings were diluted in 0.15 M phosphate buffer (pH 7.0) and assayed for amoxicillin by a large-plate agar diffusion assay with *Sarcina lutea* NCTC 8430 and for clavulanic acid by a β-lactamase-inhibition assay with *Klebsiella aerogenes* NCTC 11228 (15). The assay plates were incubated for 18 h at 30°C for *S. lutea* and at 37°C for *K. aerogenes*. Inhibition zones were measured, and the antibiotic concentrations were derived from standard lines prepared from standard solutions.

Mouse pneumonia. Mice were inoculated intranasally under light ether anesthesia with 50 μl of an overnight suspension of *K. pneumoniae* 62 diluted to give an inoculum of approximately 1,000 cells per mouse. Therapy by the oral route was started 18 h after inoculation and continued three times daily for 4 days. The efficacy of the compounds was studied during therapy and for 6 days after therapy had ceased.

Concentrations of amoxicillin and clavulanic acid present in pleural fluid after the administration of BRL 25000A were measured 36 h after the initiation of infection. At intervals during the 6-h period after drug administration, groups of five mice were killed with CO_2. The chest area was liberally swabbed with 75% alcohol, and the thorax was carefully dissected to expose the pleural cavity. Two sterile paper disks (6.5-mm diameter) were inserted into the cavity to absorb the pleural fluid; both disks were fully saturated with pleural fluid before removal (25 μl). The disks were dried in a hot-air stream to prevent further hydrolysis of amoxicillin by any β-lactamase present in the fluid. One disk from each of the five mice was assayed for amoxicillin, and the remaining disks were assayed for clavulanic acid as described above. Antibiotic concentrations in the pleural fluid were derived from standard lines prepared from 6.5-mm-diameter paper disks soaked in standard solutions.

Mouse pyelonephritis. Mice were inoculated intravenously with 0.2 ml of a suspension of an overnight culture of the test strains of *E. coli*, an inoculum of approximately 10^8 cells per mouse. The renal virulence of the bacteria was enhanced by intramuscular administration of iron sorbitol citrate (Jectofer; Astra Chemicals Ltd., Watford, England) at 30 mg/kg 16, 42, 66, and 90 h after inoculation (4). Oral treatment with the compounds started 17 h after inoculation and continued four times a day for 4 days. Groups of 10 mice at each dose level were killed 10 days after inoculation. Both kidneys were removed aseptically from all 10 mice in each group and examined for macroscopic abscesses. Bacterial counts were made from serial 10-fold dilutions in saline of homogenates prepared from the kidneys, which were pipetted onto drug-free agar. Colonies were counted after incubation for 18 h at 37°C.

Staphylococcal thigh lesion. Mice were inoculated intramuscularly in the right hind leg with 0.2 ml of a suspension in veal infusion (Difco) containing about 10^8 cells of *S. aureus* MB9. The compounds were administered by the oral route to groups of 10 mice 1 and 5 h after inoculation. The thigh diameters were measured by means of calipers on day 5 after inoculation, and the effects of therapy were calculated as follows: Percent protection = {[(mean thigh enlargement of infected controls) − (mean thigh enlargement of treated animals)]/(mean thigh enlargement of infected controls)} × 100.

The concentrations present in tissue homogenates after administration of BRL 25000A or amoxicillin 3 days after infection with *S. aureus* were measured as follows. At intervals during the 6-h period after drug administration, groups of five mice were killed by dislocation of the neck. The thigh area was liberally swabbed with 75% alcohol, and the leg was carefully skinned. The entire infected area was removed, care being taken to avoid inclusion of healthy tissue, and homogenized in 2.0 ml of 0.1 M phosphate buffer (pH

7.0) for 1 min. In the case of specimens to be assayed for amoxicillin content, clavulanic acid (10 μg/ml) was added to inhibit hydrolysis in vitro. The specimens were assayed for amoxicillin with *S. lutea* NCTC 8340 and for clavulanic acid with *K. aerogenes* NCTC 11228.

***B. fragilis* localized lesion.** Mice were inoculated subcutaneously in the groin with 0.5 ml of a 22-h culture in semisolid agar (cooked meat broth + 0.25% agar no. 3; Oxoid Ltd., Basingstoke, England) of *B. fragilis* strain B3 or VPI 8908 (16). Therapy was started at the time of inoculation and was given four times a day for 4 days. Groups of 10 mice were given BRL 25000A, amoxicillin, clavulanic acid, metronidazole, and clindamycin by the oral route and cefoxitin by the subcutaneous route. The animals were killed 7 days after inoculation, 66 h after cessation of therapy, and the groin area and associated hind leg were skinned, amputated, and homogenized in a Colworth stomacher. Serial 10-fold dilutions of the homogenates were prepared in reduced thioglycolate medium (BBL Microbiology Systems, Cockeysville, Md.) and pipetted onto 10% blood agar. The plates were incubated for 48 h in 95% H_2 in a GasPak jar (BBL), and colonies were counted.

The drug concentrations in the pus of abscesses produced by subcutaneous inoculation with *B. fragilis* B3 after oral administration of BRL 25000A, amoxicillin, or clavulanic acid were determined 7 days after inoculation. Groups of five mice were killed by cervical dislocation at intervals during the 6-h period after drug administration. The groin area was swabbed with 75% alcohol, an incision was made in the abscess, and a measured volume of pus was removed by aspiration. The sample was thoroughly mixed with a known volume of 0.15 M phosphate buffer (pH 7.0), and in the case of the specimens to be assayed for amoxicillin content, clavulanic acid (10 μg/ml) was added to inhibit hydrolysis in vitro. The specimens were assayed for amoxicillin and clavulanic acid as described above.

RESULTS

Intraperitoneal infections. Figure 1 shows the distribution of amoxicillin and clavulanic acid in peritoneal washings from mice infected with either *S. aureus* MB9 (P^+) or *E. coli* JT39 (P^+) after oral administration of BRL 25000A. Both compounds were absorbed rapidly, with peak concentrations occurring at 45 min in mice infected with *S. aureus* MB9, falling to relatively low levels by 180 min. In mice infected with *E. coli* JT39, the peak levels for both compounds occurred at 60 min, and levels of 6 μg of amoxicillin per ml and 2 μg of clavulanic acid per ml were still detectable at 6 h. When amoxicillin was administered alone, none of the dose was detected in the peritoneal washings taken from mice infected with *S. aureus* MB9, presumably as a result of the β-lactamase produced in the peritoneal cavity ($P < 0.01$; Wilcoxon's signed rank test). In the case of the infection produced by *E. coli* JT39, amoxicillin was present in the peritoneal washings of mice treated

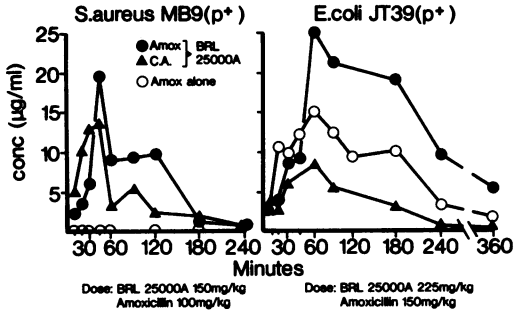

FIG. 1. Concentrations of amoxicillin and clavulanic acid in peritoneal fluid after an oral dose of BRL 25000A or amoxicillin to mice infected intraperitoneally with *S. aureus* (P^+) and *E. coli* (P^+). Amox, Amoxicillin; C.A., clavulanic acid.

with amoxicillin alone, but the levels were significantly lower after 30 min ($P < 0.05$–0.1; Wilcoxon's signed rank test) than those in mice treated with amoxicillin plus clavulanic acid (BRL 25000A).

These effects were reflected in the results of therapy in which BRL 25000A was effective in protecting mice against infections with *S. aureus* MB9 or *E. coli* JT39, presumably as a result of the β-lactamase-inhibitory effects of clavulanic acid, whereas treatment with amoxicillin was ineffective (Table 1).

Staphylococcal thigh lesion. The penetration of amoxicillin and clavulanic acid into the infected limb after oral administration of BRL 25000A was assessed by measurement of the concentrations present in thigh homogenates prepared from animals infected with *S. aureus* MB9 (P^+). Figure 2 shows that both amoxicillin and clavulanic acid were present for up to 6 h after infection. The peak concentrations of both compounds were measured at 30 min, and these fell to relatively low levels by 6 h. Over the first 60 min, the amoxicillin levels in recipients of this drug plus clavulanic acid were higher than in recipients of amoxicillin alone; after 60 min, there was no difference in amoxicillin levels in animals on either regimen. This was possibly due to the fact that after 60 min the clavulanic acid levels were so low that protection of amoxicillin was not provided, and the levels measured after this time period are indicative of unprotected amoxicillin concentrations.

Table 2 shows the results of therapy of thigh lesions produced by *S. aureus* MB9 (P^+) and by the β-lactamase-negative variant of equal mouse virulence, *S. aureus* MB9 (P^-) derived from it. In this study, the relative efficacies of BRL 25000A and BRL 25000G were compared. Amoxicillin, administered alone, was effective in protecting the animals infected with the β-lactamase-negative variant, but had little or no

TABLE 1. Comparative efficacies of BRL 25000 A, amoxicillin, and clavulanic acid in the treatment of mouse intraperitoneal infections caused by amoxicillin-resistant bacteria

Organism	Study no.	PD_{50}[a] (mg/kg)		
		BRL 25000A	Amoxicillin	Clavulanic acid
S. aureus MB9 (P^+)	1	25 (10– 63)	>800	>200
	2	46 (18– 82)	>1,000	>200
	3	40 (17– 92)	>800	>200
E. coli JT39 (P^+)	4	32 (15– 67)	>1,000	>200
	5	43 (22– 86)	>1,000	>200
	6	114 (90–169)	>1,000	>200

[a] PD_{50}, 50% protective dose, administered orally 1 and 5 h after infection. Numbers in parentheses are 95% confidence limits (12).

effect against infection caused by the β-lactamase-producing strain. In contrast, both infections were almost equally susceptible to the amoxicillin-clavulanic acid formulations, illustrating the β-lactamase-inhibitory effects of clavulanic acid in vivo. Both formulations showed similar orders of activities, the protective effects increasing with increasing doses of antibiotic and inhibitor.

Mouse pneumonia. Figure 3 shows the concentrations of amoxicillin and clavulanic acid measured in the pleural fluid of mice infected with *K. pneumoniae* 62 after a single oral dose of BRL 25000A and of amoxicillin alone. The peak concentrations of clavulanic acid were reached at 45 min, and these fell to quite low levels by 3 to 4 h after drug administration. Clavulanic acid was not detected in the 6-h specimens. Peak concentrations of amoxicillin administered alone and in the presence of clavulanic acid were similar, were attained more slowly (90 min), and persisted longer than levels of clavulanic acid, so that 2 to 5 μg/ml was still present 6 h after drug administration.

The results of therapy of the infection with BRL 25000A and BRL 25000G, amoxicillin alone, or clavulanic acid are illustrated in Fig. 4. Intranasal inhalation of *K. pneumoniae* 62 produced a severe infection, causing consolidation of both lungs within 48 h, and most of the untreated mice died 48 to 96 h after infection. Mice treated with amoxicillin or clavulanic acid alone showed little prolongation of life expectancy compared with untreated animals. In contrast, BRL 25000A and BRL 25000G were effective in protecting 100% of animals for the duration of therapy. Thereafter, the BRL 25000G-treated mice (the formulation with the higher amoxicillin content) continued to survive until day 7; 20% of the animals died between days 7 and 8, and no more animals died after day 8, resulting in an overall protection of 80%. In comparison, BRL 25000A afforded protection to 60% of the animals, and generally the mice died earlier than those treated with BRL 25000G. Cephalexin was less effective than the formulations in protecting the animals once therapy had ceased, and only 10% of the cephalexin-treated animals survived at day 10.

***B. fragilis* lesion.** Figure 5 shows the concentrations of amoxicillin and clavulanic acid in blood and in pus aspirated from groin abscesses of mice inoculated with *B. fragilis* B3 and given BRL 25000A or amoxicillin alone orally. Both

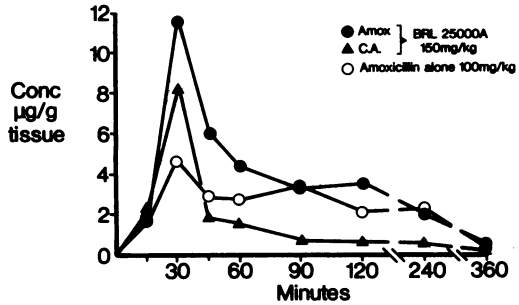

FIG. 2. Concentrations of amoxicillin and clavulanic acid in thigh homogenates after a single oral dose of BRL 25000A or amoxicillin to mice infected intramuscularly with *S. aureus* MB9 (P^+). Amox, Amoxicillin; C.A., clavulanic acid.

TABLE 2. Efficacy of BRL 25000 against mouse intramuscular infections due to *S. aureus* MB9 (P^+) and (P^-)

Compound	Dose (mg/kg)	% Protection	
		P^-	P^+
BRL 25000A	150	86	79
	75	83	75
BRL 25000G	250	90	83
	125	81	66
Amoxicillin	200	89	19
	100	78	0
Clavulanic acid	50	1.0	11
	25	3.0	7.0

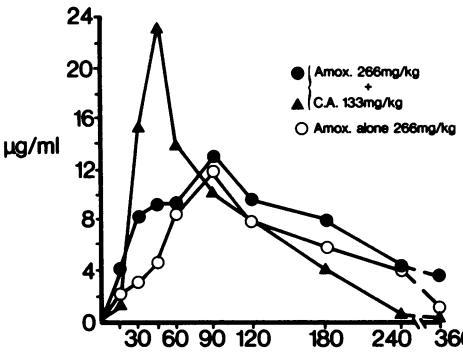

FIG. 3. Concentrations of amoxicillin and clavulanic acid in pleural fluid after a single oral dose of BRL 25000A to mice infected intranasally with *K. pneumoniae* 62. Amox, Amoxicillin; C.A., clavulanic acid.

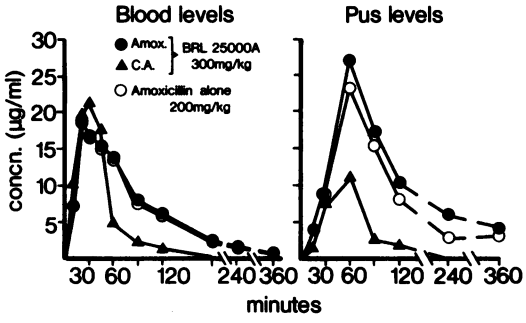

FIG. 5. Concentrations of amoxicillin and clavulanic acid in blood and pus after a single oral dose of BRL 25000A or amoxicillin to mice infected with *B. fragilis* B3. Amox, Amoxicillin; C.A., clavulanic acid.

amoxicillin and clavulanic acid were rapidly absorbed in the infected animals, reaching peak concentrations in the blood at 20 to 30 min (Fig. 5). Both amoxicillin and clavulanic acid penetrated the abscesses. The levels of amoxicillin in pus were of the same order as those found in blood, whereas the levels of clavulanic acid were approximately half those found in blood.

Figure 6 compares the activities of a variety of therapeutic agents against infections due to *B. fragilis* B3, a strain moderately susceptible to amoxicillin (minimal inhibitory concentration [MIC], 25 μg/ml) and clavulanic acid (MIC, 50 μg/ml) and susceptible to BRL 25000A (MIC, 2.5 μg/ml). BRL 25000A and metronidazole were the most active of the compounds in reducing the bacterial groin counts in infected animals ($P < 0.05$, Kruskale Wallis test) as compared with control animals, whereas amoxicillin or clavulanic acid alone had little effect. The standard compounds cefoxitin and clindamycin were less effective than BRL 25000A in reducing the groin counts ($P = 0.05$ to 0.1) but were more effective than amoxicillin or clavulanic acid alone. The formulation of amoxicillin and clavulanic acid (BRL 25000A) was much more effective than metronidazole in reducing the incidence of palpable abscesses. However, there appeared to be little correlation between the presence of abscesses and the bacterial count in metronidazole-treated animals, since metronidazole was as effective as BRL 25000A in reducing the groin tissue bacterial counts.

The results of therapy of an infection produced by *B. fragilis* VPI 8908, a strain highly resistant to amoxicillin (MIC, >500 μg/ml) and resistant to clavulanic acid (MIC, 50 μg/ml), but susceptible to BRL 25000A (MIC, 5 μg/ml), are shown in Fig. 6. BRL 25000A was the most effective of the compounds tested in reducing the number of palpable abscesses and was distinctly more effective than amoxicillin, clavulanic acid, and cefoxitin in this respect. Similarly,

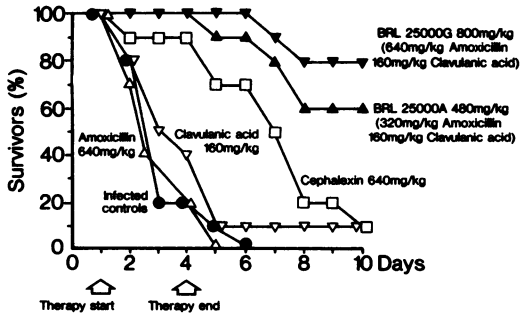

FIG. 4. Relative efficacies of BRL 25000A, BRL 25000G, amoxicillin, clavulanic acid, and cephalexin administered orally against a mouse pneumonia caused by *K. pneumoniae* 62.

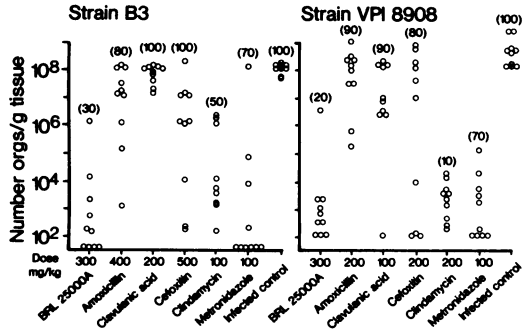

FIG. 6. Effects of oral therapy with BRL 25000A, amoxicillin, clavulanic acid, clindamycin, and metronidazole and parenteral cefoxitin therapy against *B. fragilis* groin infections in mice. The numbers in parenthesis are the percentages of palpable abscesses. Each circle represents the groin count from one mouse.

the formulation was significantly more effective than amoxicillin, clavulanic acid, and cefoxitin and as effective as clindamycin and metronidazole in reducing bacterial counts in groin tissue. Bacterial groin counts were significantly different in animals treated with these three compounds from those of control animals ($P < 0.05$; Kruskal and Wallis test).

Mouse pyelonephritis. The results of therapy with BRL 25000A, BRL 25000G, amoxicillin, and clavulanic acid of kidney infections due to an amoxicillin-resistant strain of *E. coli*, JT39 (P^+), and to an amoxicillin-susceptible variant, *E. coli* JT39 (P^-), derived from the parent strain, are shown in Fig. 7. Amoxicillin and BRL 25000A were equally effective ($P < 0.05$; Kruskal and Wallis test) against infection caused by *E. coli* JT39 (P^-) (Fig. 7), reducing the incidence of visible kidney abscesses and the viable counts to very low levels. Clavulanic acid had little effect at the dose tested; there was no significant difference in abscess formation and bacterial counts in the treated animals compared with infected untreated controls ($P < 0.05$; Kruskal and Wallis test). BRL 25000A and BRL 25000G were also similarly efficacious ($P < 0.05$) against infection caused by *E. coli* JT39 (P^+) and were in fact as effective against this infection as against that caused by the amoxicillin-susceptible strain. In contrast, amoxicillin had little effect on the course of the infection, even at the relatively high dose tested, and the high incidence of abscess production (100%) and the bacterial counts (10^7 to 10^8/ml of kidney homogenate) were not significantly different from those of the untreated control animals ($P < 0.05$).

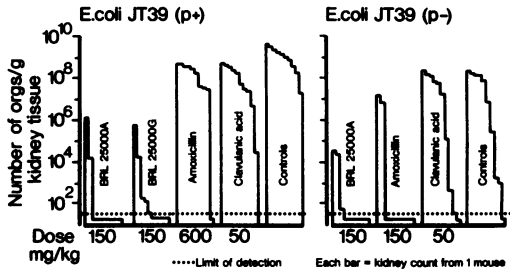

FIG. 7. Effects of oral therapy with BRL 25000A, BRL 25000G, amoxicillin, and clavulanic acid against *E. coli* pyelonephritis infections in mice caused by *E. coli* JT39 (P^+) and *E. coli* JT39 (P^-).

DISCUSSION

The results reported here demonstrate that both amoxicillin and clavulanic acid were distributed in a similar fashion in infected animals in the various infection models studied and were present together at the different sites of infection investigated. Relatively high concentrations of both compounds were measured in the peritoneal washings from mice infected by the intraperitoneal route, in pleural fluid from murine pneumonia, in homogenates prepared from staphylococcal lesions, and in pus obtained from abscesses caused by anaerobic bacilli. In general, the penetration studies were performed when the infection had progressed to a severe form to ensure that both amoxicillin and clavulanic acid did indeed have the ability to reach the site of infection and to penetrate well-advanced lesions, such as the *Bacteroides* groin abscess and staphylococcal thigh lesion. Previous studies from this laboratory have shown that the pharmacokinetics of amoxicillin and clavulanic acid are compatible and that both compounds are well distributed throughout uninfected animals after administration of BRL 25000 (5, 13). The data from this study show that this is also true for infected animals. Moreover, the data show that in certain of the infection models, amoxicillin was protected by clavulanic acid from inactivation by the bacterial β-lactamases present at the sites of infection; the amoxicillin levels measured after the administration of BRL 25000 were higher than those seen when the animals were treated with amoxicillin alone.

Confirmation of the presence of amoxicillin and clavulanic acid at effective concentrations at sites of infection is evidenced by the results of the therapy studies. Amoxicillin has been shown previously to be effective in the treatment of experimental infections (6), and, as expected, BRL 25000 showed activity equivalent to that of amoxicillin against infections due to amoxicillin-susceptible bacteria. In addition, BRL 25000 was also very effective in the treatment of infections caused by β-lactamase-producing (amoxicillin-resistant) strains of bacteria that failed to respond to therapy with amoxicillin.

A further proof of the efficiency of inhibition of bacterial β-lactamases by clavulanic acid in vivo is given by the efficacy of BRL 25000 in the treatment of the infections produced by the isogenic β-lactamase-negative and β-lactamase-producing strains of *S. aureus* and *E. coli*. These isogenic strains were of comparable mouse virulence, and consequently the resistance to amoxicillin of the infections caused by the β-lactamase-producing strains was almost certainly due solely to β-lactamase activity. The fact that BRL 25000 was as effective in the treatment of the infections due to the amoxicillin-resistant strains of *S. aureus* and *E. coli* as it was against those caused by the amoxicillin-susceptible strains illustrates the protection of amoxicillin by clavulanic acid in these particular experimental infections.

The data reported here, showing that amoxicillin and clavulanic acid were present in thera-

peutic concentrations at sites of infection after administration of BRL 25000 to infected animals and that, as a consequence, amoxicillin-clavulanic acid formulations are effective in the treatment of experimental infections due to amoxicillin-susceptible and amoxicillin-resistant bacteria, are in keeping with published studies reporting the efficacy of amoxicillin plus clavulanic acid in clinical infection (1, 2, 7, 10, 11, 14).

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