

Comparative Efficacies of Amoxicillin-Clavulanic Acid and Ampicillin-Sulbactam against Experimental *Bacteroides fragilis*-*Escherichia coli* Mixed Infections

JOHN GISBY AND ANGELA S. BEALE*

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

Received 29 April 1988/Accepted 8 September 1988

Amoxicillin-clavulanic acid was compared with ampicillin-sulbactam in preventing the development of mixed infections produced in mice by subcutaneous inoculation of amoxicillin-resistant strains of *Bacteroides fragilis* and *Escherichia coli*. At doses designed to produce concentrations in mouse plasma similar to those obtained in humans, both amoxicillin-clavulanic acid and ampicillin-sulbactam were effective in preventing an infection caused by *B. fragilis* VPI 8908 mixed with *E. coli* E96, both strains being susceptible in vitro to each combination. However, ampicillin-sulbactam failed to arrest the progression of infections involving a more potent β -lactamase-producing strain, *E. coli* 41548, even when a comparatively low inoculum was tested. In contrast, amoxicillin-clavulanic acid therapy effectively reduced the bacterial numbers at the site of infection. These data illustrate the need to treat polymicrobial infections with agents effective against the responsible aerobic as well as anaerobic bacteria.

Clavulanic acid and sulbactam are potent inhibitors of a wide range of bacterial β -lactamases, and combinations containing amoxicillin plus clavulanic acid and ampicillin plus sulbactam have been shown to be active against many β -lactamase-producing bacteria resistant to the aminopenicillins (14, 18). Both antibiotic combinations have been reported to be effective against acute and discriminative experimental infections (3, 5, 18) and in the clinic (12, 13, 15).

The majority of infections arising from lower bowel and gynecological surgery are polymicrobial, and the organisms most frequently isolated from such infections include the anaerobe *Bacteroides fragilis*, with *Escherichia coli* as the most commonly isolated gram-negative aerobe (4, 17). Many of these organisms are β -lactamase-producing strains, and the antibacterial spectra of amoxicillin-clavulanic acid and ampicillin-sulbactam are therefore suited for the prevention and treatment of such infections. The studies reported here were designed to assess the efficacies of amoxicillin-clavulanic acid and ampicillin-sulbactam in preventing the development of mixed *B. fragilis*-*E. coli* infections in the mouse, using a model described previously (2).

MATERIALS AND METHODS

Experimental animals. Female MF1 mice weighing 18 to 22 g were used (Oxford Laboratory Animal Colony, Bicester, England).

Test organisms. Two mixed infections were established, both involving the β -lactamase-producing strain *B. fragilis* VPI 8908 plus a different strain of *E. coli* possessing a TEM-mediated β -lactamase: (i) *E. coli* E96, a strain typical of the majority of clinical isolates of *E. coli*, which are susceptible to both amoxicillin-clavulanic acid and ampicillin-sulbactam (Table 1); (ii) *E. coli* 41548, exemplifying the proportion of clinical isolates of *E. coli* which are more potent β -lactamase producers. *E. coli* 41548 was tested at two inoculum levels.

Preparation of inocula. Overnight broth cultures of *E. coli* were grown in veal infusion broth (Difco Laboratories, Detroit, Mich.). *B. fragilis* was grown in chopped-meat broth (Difco). The cultures were diluted and mixed so that, when 0.5 ml of the mixed culture was injected subcutaneously into the groin area, the inocula per mouse were as follows: study 1, 5.8 log₁₀ CFU of *E. coli* E96 plus 8.3 log₁₀ CFU of *B. fragilis* VPI 8908; study 2, 6.3 log₁₀ CFU of *E. coli* 41548 plus 8.3 log₁₀ CFU of *B. fragilis* VPI 8908; study 3, 4.7 log₁₀ CFU of *E. coli* 41548 plus 8.4 log₁₀ CFU of *B. fragilis* VPI 8908.

Antibiotics. All antibiotics used were dry-powder preparations: amoxicillin sodium and ampicillin sodium were commercial preparations (Amoxil and Penbritin; Beecham Research, Brentford, England); potassium clavulanate was supplied by Beecham Pharmaceuticals, Worthing, England; and sulbactam was prepared in the laboratories of Beecham Pharmaceuticals Research Division, Betchworth, England. Amoxicillin, ampicillin, and sulbactam were dissolved in phosphate-buffered saline, pH 7.2, while potassium clavulanate was dissolved in 0.1 M citrate buffer, pH 6.5. The mixture of ampicillin-sulbactam consisted of 2 parts ampicillin to 1 part sulbactam, while the amoxicillin-clavulanic acid combination consisted of 10 parts amoxicillin to 1 part clavulanic acid. Solutions were mixed immediately prior to dosing, having been adjusted to contain the pure free acid equivalents.

Therapy. Treatment was initiated 1 h after infection and continued twice daily for 4 days. All combinations were given subcutaneously, as follows: amoxicillin-clavulanic acid, 200/20 mg/kg (all studies); ampicillin-sulbactam, 200/100 (all studies) and 400/200 (study 3 only) mg/kg. The dose of amoxicillin-clavulanic acid, and the lower dose of ampicillin-sulbactam, produced peak levels in plasma in the mouse of the same order as those attainable in humans following intravenous administration (Table 2). Previous studies have indicated that amoxicillin, ampicillin, clavulanic acid, and sulbactam were ineffective when given alone against infections caused by β -lactamase-producing bacteria, including *E. coli* and *B. fragilis* (3, 18).

* Corresponding author.

TABLE 1. Antimicrobial susceptibility of infecting bacteria

Organism	MIC ($\mu\text{g/ml}$) ^a					
	Amoxicillin	Amoxicillin-clavulanic acid (2:1)	Ampicillin	Ampicillin-sulbactam (2:1)	Clavulanic acid	Sulbactam
<i>E. coli</i> E96	>512	8/4	>512	16/8	16	16
<i>E. coli</i> 41548	>512	16/8	>512	128/64	16	64
<i>B. fragilis</i> VPI 8908	512	2/1	>512	4/2	16	16

^a Serial dilution in Wilkins-Chalgren agar. The MIC for *E. coli* was read after 18 h of aerobic incubation at 37°C; that for *B. fragilis* was read after 24 h of anaerobic incubation at 37°C.

Assessment. At intervals after infection, groups of five mice from each of the treatment regimens were sacrificed. The groin and associated hind legs were swabbed with 75% (vol/vol) ethanol before being skinned, amputated, and weighed. The tissue was homogenized in 4 ml of Wilkins-Chalgren broth (Oxoid Ltd., London, England) by a Colworth stomacher (A. J. Seward & Co. Ltd., London, England). Supernatants were serially diluted in Wilkins-Chalgren broth, and 20- μl volumes were plated in triplicate onto agar to determine the numbers of viable bacteria in the tissue. To enumerate *E. coli*, the dilutions were plated onto nutrient agar and incubated aerobically at 37°C.

Viable counts of *B. fragilis* were performed on Wilkins-Chalgren agar (Oxoid) supplemented with 50 μg of gentamicin per ml to prevent the growth of *E. coli* and incubated in an atmosphere of H₂-N₂-CO₂ (10:80:10) at 35°C in an anaerobic work station (Don Whitley Scientific Ltd., Shipley, England). Both isolation media were supplemented with 0.5% (vol/vol) β -lactamase (Penase; Difco).

Distribution. Concentrations of the agents tested were measured 1 h after infection, in plasma and in the fluid accumulating at the site of infection. Infected animals were given a single subcutaneous injection of ampicillin-sulbactam (200/100 mg/kg) or amoxicillin-clavulanic acid (200/20 mg/kg). At intervals after dosing, groups of five mice per treatment were sacrificed and samples of blood were collected from the axillary vein into heparinized tubes (5,000 U/ml) (Multiparin; Weddel Pharmaceuticals, Wrexham, Wales). The blood samples were centrifuged at 15,000 $\times g$ for 2 min to obtain a plasma fraction. Site-of-infection fluid was sampled by inserting two sterile 6.5-mm filter paper disks into an incision made into the infected groin area to absorb the fluid. Samples contaminated with blood were discarded. The samples were assayed by large-plate agar diffusion assay: amoxicillin and ampicillin against *Bacillus subtilis* ATCC 6633 (with clavulanic acid at 5 $\mu\text{g/ml}$ to

prevent further hydrolysis in vitro), and sulbactam and clavulanic acid by β -lactamase inhibition assay with *Klebsiella pneumoniae* NCTC 11228 (10). For sulbactam, the sensitivity of the assay was enhanced by seeding a smaller volume (200 ml) of agar to produce a layer thinner than that used for the clavulanic acid assay (400 ml), and by increasing the benzylpenicillin concentration threefold to 180 $\mu\text{g/ml}$ of agar. The plates were incubated for 18 h at 37°C, and antibiotic concentrations were derived from standard lines prepared from standard solutions in pooled mouse plasma. Concentrations of ampicillin and sulbactam in infected mice following a dose of 400/200 mg/kg were not measured.

RESULTS

Study 1: *B. fragilis* VPI 8908 plus *E. coli* E96. The data in Fig. 1 show that both the *E. coli* E96 and *B. fragilis* VPI 8908 components of the mixed infection grew rapidly and persisted well in the groin tissue of nontreated animals. In this study, 4 of 15 of the control animals died between 9 and 48 h after infection due to *E. coli* bacteremia, while all surviving mice developed lesions in the groin area. Treatment with amoxicillin-clavulanic acid and ampicillin-sulbactam produced similar responses; numbers of both organisms were rapidly reduced during the therapy period. By 7 days (168 h), no bacteria were recovered from the mice treated with amoxicillin-clavulanic acid (limit of detection, 100 CFU/g of tissue), and no anaerobes were detected in the tissue homogenates of ampicillin-sulbactam-treated mice. Two of five ampicillin-sulbactam-dosed animals, however, had *E. coli*

TABLE 2. Peak concentrations in plasma obtained in infected mice, with peak concentrations in human serum for comparison

Compound	Mice		Humans ^a	
	Dose (mg/kg)	$\mu\text{g/ml}$ ^b	Dose (g)	$\mu\text{g/ml}$
Amoxicillin	200	90.4 \pm 26.0	1	50-100
+	+		+	
Clavulanic acid	20	11.0 \pm 2.4	0.2	5-15
Ampicillin	200	143.5 \pm 26.0	2	120
+	+		+	
Sulbactam	100	87.1 \pm 14.8	1	60

^a Reference 7; J. M. Andrews, R. Wise, I. A. Donovan, and J. Drumm, Proc. 13th Int. Congr. Chemother., p. 31-34, 1983.

^b Mean peak concentrations, with standard deviations, measured in five animals 15 min after the dose.

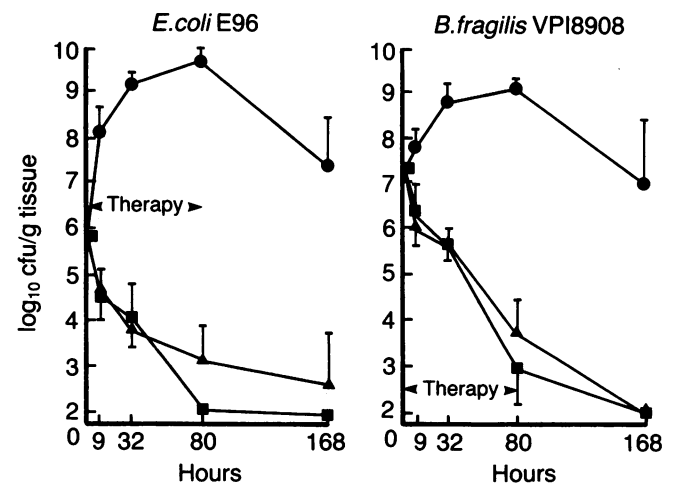


FIG. 1. Efficacies of amoxicillin-clavulanic acid (■; 200/20 mg/kg) and ampicillin-sulbactam (▲; 200/100 mg/kg) in preventing the development of a *B. fragilis* VPI 8908-*E. coli* E96 infection in the mouse. Symbol: ●, Controls.

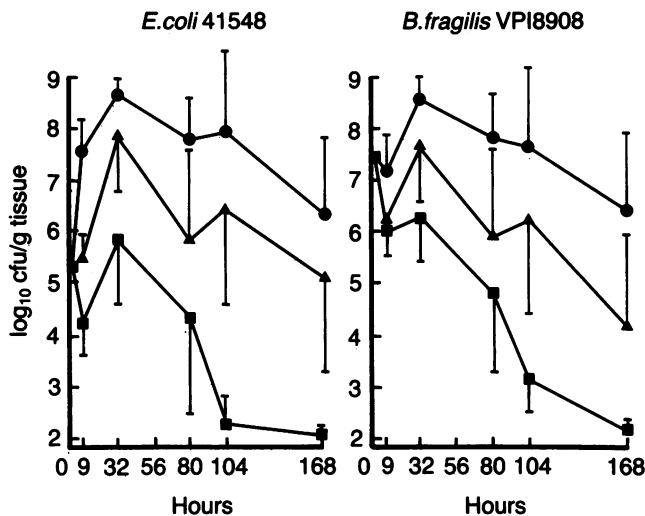


FIG. 2. Efficacies of amoxicillin-clavulanic acid (■; 200/20 mg/kg) and ampicillin-sulbactam (▲; 200/100 mg/kg) in preventing the development of a *B. fragilis* VPI 8908-*E. coli* 41548 infection in which the *E. coli* inoculum was 6.3 log₁₀ CFU per mouse. Symbol: ●, Controls.

counts of 3 to 4 log₁₀ CFU/g at 168 h, the remaining samples yielding no aerobes. Abscess development was prevented by both treatments.

Study 2: *B. fragilis* VPI 8908 plus *E. coli* 41548. Figure 2 shows the efficacy of amoxicillin-clavulanic acid and ampicillin-sulbactam against a mixed infection caused by *B. fragilis* VPI 8908 and *E. coli* 41548. In this study, the aerobic inoculum was similar (6.3 log₁₀ CFU per mouse) to that used in study 1. Therapy with ampicillin-sulbactam proved ineffective, with numbers of *E. coli* increasing throughout therapy to 7.90 ± 1.11 log₁₀ CFU/g by 32 h, 5.15 ± 1.99 log₁₀ CFU/g being present at the termination of the study. The numbers of *B. fragilis* mirrored those of *E. coli*, and 70% of the mice developed abscesses in the groin area. In contrast, amoxicillin-clavulanic acid reduced the numbers of *B. fragilis* during therapy to 4.84 ± 1.43 log₁₀ CFU/g by 80 h and to the limit of detection by 168 h. Against *E. coli* 41548, amoxicillin-clavulanic acid suppressed growth during the therapy period (1 to 80 h), after which the *E. coli* 41548 numbers fell to the detectable limit at 168 h, and 75% of the mice were free of abscesses.

Study 3: *B. fragilis* VPI 8908 plus *E. coli* 41548 (lower aerobic inoculum). In this study, the inoculum of *E. coli* 41548 was approximately 10-fold lower than that used in study 2. High numbers of both organisms were still recovered from the groin tissue of nontreated control animals as the infection developed (Fig. 3). Against this lower inoculum, ampicillin-sulbactam (200/100 mg/kg) produced a transient fall in the *E. coli* 41548 count, but failed to prevent a steady increase thereafter. At 168 h, 6.38 ± 0.99 log₁₀ CFU/g were recovered from the groin tissue of mice in this treatment group. Reduction in *B. fragilis* numbers to 5.85 ± 0.35 log₁₀ CFU/g was observed over the initial treatment period, but the anaerobe count remained at this level for the rest of the study. Only when the ampicillin-sulbactam dose was increased to 400/200 mg/kg was activity comparable to that of amoxicillin-clavulanic acid seen, i.e., a rapid reduction in the numbers of both aerobes and anaerobes throughout therapy, with prevention of abscess development. Thus, amoxicillin-clavulanic acid showed activity against this infection similar

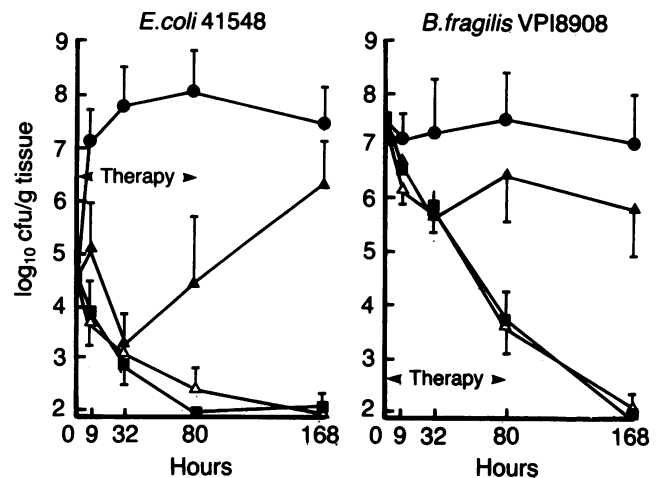


FIG. 3. Efficacies of amoxicillin-clavulanic acid (■; 200/20 mg/kg) and ampicillin-sulbactam (▲, 200/100 mg/kg; △, 400/200 mg/kg) in preventing the development of a *B. fragilis* VPI 8908-*E. coli* 41548 infection in which the *E. coli* inoculum was 4.7 log₁₀ CFU per mouse. Symbol: ●, Controls.

to that seen against the *B. fragilis* VPI 8908 + *E. coli* E96 infection (study 1).

Concentrations in plasma and site-of-infection fluid. Levels in plasma and site-of-infection fluid following single subcutaneous administration of the agents 1 h after infection with *E. coli* E96 and *B. fragilis* VPI 8908, at the doses used in the therapy studies, are illustrated in Fig. 4. In brief, at the time of commencement of therapy, concentrations of the agents at the site of infection generally reflected those measured in plasma, and the peak concentrations were of the same order as those achievable in humans. Similar concentrations of the agents were achieved in plasma and site-of-infection fluid after infection with *E. coli* 41548-*B. fragilis* VPI 8908 (not illustrated).

DISCUSSION

Clavulanic acid and sulbactam are both effective inhibitors of many bacterial β-lactamases. However, there are significant differences between the compounds in the inhibitory effects exerted against certain β-lactamases. For instance, sulbactam is much less effective against class III plasmid-mediated TEM β-lactamases than is clavulanic acid (6, 16), and this is reflected in the antibacterial activities of the ampicillin-sulbactam and amoxicillin-clavulanic acid combinations against TEM-producing isolates of the family *Enterobacteriaceae*. Thus, in comparison with amoxicillin-clavulanic acid, ampicillin-sulbactam is relatively ineffective *in vitro* against such isolates (1, 8, 9, 11, 19).

The results of the *in vivo* studies reported here demonstrate that ampicillin-sulbactam was efficacious against the mixed *B. fragilis*-*E. coli* infection in which the aerobic, *E. coli* E96, produced moderate quantities of β-lactamase and was susceptible to ampicillin-sulbactam *in vitro*. On the other hand, the combination was ineffective against the mixed infections caused by *B. fragilis* and *E. coli* 41548, a more potent β-lactamase-producing strain than *E. coli* E96. In contrast, amoxicillin-clavulanic acid was active against the infections involving both strains of *E. coli*, as might be predicted from *in vitro* data. In these infection studies, the dose of sulbactam, chosen to simulate peak concentrations in human serum, was five times higher than that of clavulanic

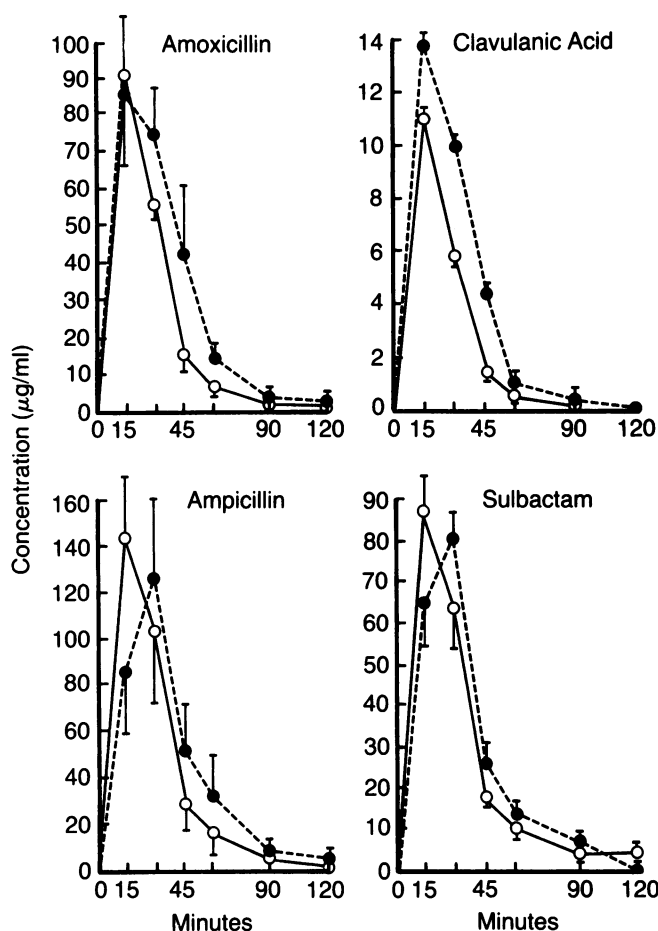


FIG. 4. Concentrations of amoxicillin-clavulanic acid (200/20 mg/kg) and ampicillin-sulbactam (200/100 mg/kg) in the plasma (○) and site-of-infection fluid (●) of mice infected with *B. fragilis* VPI 8908-*E. coli* E96.

acid, further illustrating the differences in β -lactamase-inhibitory activities of the compounds against TEM-mediated β -lactamases.

These data suggest the potential of amoxicillin-clavulanic acid in prophylaxis or in the treatment of polymicrobial infections. However, the efficacy of ampicillin-sulbactam may be limited by the relatively low inhibitory activity of the combination against potent plasmid-mediated β -lactamase-producing strains of *E. coli* likely to be encountered in such infections.

ACKNOWLEDGMENT

We thank Helen Thomas for typing the manuscript.

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