Use of Ampicillin-Sulbactam for Treatment of Experimental Meningitis Caused by a β-Lactamase-Producing Strain of Escherichia coli K-1

LUIS GUERRA-ROMERO, STEPHEN L. KENNEDY, MICHAEL A. FOURNIER, JAY H. TUREEN, AND MARTIN G. TÄUBER*

The Medical Service, San Francisco General Hospital, and the Departments of Medicine and Pediatrics, School of Medicine, University of California, San Francisco, California 94110

Received 22 April 1991/Accepted 31 July 1991

We evaluated the pharmacokinetics and therapeutic efficacy of ampicillin combined with sulbactam in a rabbit model of meningitis due to a β-lactamase-producing strain of Escherichia coli K-1. Ceftriaxone was used as a comparison drug. The MIC and MBC were 32 and >64 μg/ml (ampicillin), >256 and >256 μg/ml (sulbactam), 2.0 and 4.0 μg/ml (ampicillin-sulbactam [2:1 ratio, ampicillin concentration]) and 0.125 and 0.25 μg/ml (ceftriaxone). All antibiotics were given by intravenous bolus injection in a number of dosing regimens. Ampicillin and sulbactam achieved high concentrations in cerebrospinal fluid (CSF) with higher dose regimens, but only moderate bactericidal activity compared with that of ceftriaxone was obtained. CSF bacterial titers were reduced by 0.6 ± 0.3 log10 CFU/ml/h with the highest ampicillin-sulbactam dose used (500 and 500 mg/kg of body weight, two doses). This was similar to the bactericidal activity achieved by low-dose ceftriaxone (10 mg/kg), while a higher ceftriaxone dose (100 mg/kg) produced a significant increase in bactericidal activity (1.1 ± 0.4 log10 CFU/ml/h). It appears that ampicillin-sulbactam, despite favorable CSF pharmacokinetics in animals with meningitis, may be of limited value in the treatment of difficult-to-treat β-lactamase-producing bacteria, against which the combination shows only moderate in vitro activity.

Bacterial meningitis remains a serious disease associated with significant morbidity and mortality despite antibiotic and supportive therapy (10, 15, 25, 28). The infecting organism is a major determinant of the prognosis, which is particularly poor in gram-negative-bacillus meningitis (1, 13, 23). The increasing rate of β-lactamase-producing gram-negative pathogens has prompted a search for effective agents to treat meningitis caused by these bacteria. Newer cephalosporins are generally highly effective in this regard (3, 4, 12, 19–21, 30). As an alternative approach, the combination of a penicillin-derivative drug and an agent that inhibits the β-lactamase elaborated by the pathogen has attracted interest. Recently, we have examined the combination of piperacillin and tazobactam, a β-lactamase inhibitor, in experimental meningitis due to a β-lactamase-producing Escherichia coli strain (9). We found favorable pharmacokinetic properties for both compounds and showed that the addition of the β-lactamase inhibitor protected the bactericidal activity of piperacillin in cerebrospinal fluid (CSF). However, because of the relatively weak in vitro activity of piperacillin against the test strain, maximal CSF bactericidal activity of the combination was achieved only with extremely high drug concentrations.

Sulbactam, a penicillanic acid sulfone, is an irreversible inhibitor of β-lactamase that extends the spectrum of ampicillin to include strains of Staphylococcus aureus, Haemophilus sp., E. coli, and other β-lactamase-producing gram-negative bacteria (8, 16). Ampicillin-sulbactam is extensively used in clinical practice (11). In the present study, we examined ampicillin-sulbactam in a model of meningitis due to a β-lactamase-producing, K-1-positive strain of E. coli. In addition to the CSF penetration of the two compounds after bolus intravenous (i.v.) administration, we were specifically interested in the bactericidal activity of ampicillin in this model. Ceftriaxone served as comparison drug, as it had in our previous study (9).

MATERIALS AND METHODS

Infecting organism. A K-1-positive, serum-resistant strain of E. coli, originally isolated from a neonate with meningitis, was used. The organism produced β-lactamase, as determined by the nitrocefin chromogenic assay, and was resistant to ampicillin with an MIC and MBC of 32 and >64 μg/ml. The organism was stored on glass beads at −70°C, grown for 5 to 6 h in tryptic soy broth, washed, and diluted in saline to the desired concentration.

Antimicrobial agents. Ampicillin and sulbactam were obtained from Roerig/Pfizer, New York, N.Y. Ceftriaxone was a commercial preparation (Roche Laboratories, Nutley, N.J.).

Susceptibility tests. MICs and MBCs were measured in Mueller-Hinton broth by the standard 0.5-mm diameter dilution technique, against an inoculum of 5 × 105 CFU/ml. The MIC was the lowest concentration that prevented visible growth. The MBC, defined as the concentration that killed ≥99.9% of the original inoculum, was determined after subculturing 0.1 ml from each clear tube onto a blood agar plate and overnight incubation at 35°C.

Rabbit model. New Zealand White rabbits weighing 1.8 to 2.6 kg each were anesthetized by intramuscular injection of acepromazine (3 mg/kg of body weight), ketamine (30 mg/kg), and xylazine (15 mg/kg). The cisterna magna was punctured with a 25-gauge butterfly needle, and the inoculum of 2 × 107 to 1 × 108 CFU of E. coli in 0.3 ml of saline was administered intracisternally. At 12 to 14 h later, the animals were lethargic and febrile. At that time, the animals

* Corresponding author.
were given an i.v. infusion of urethane (1.75 g/kg) as a long-acting anesthetic and the cisterna magna was punctured again. Mean CSF bacterial titers ranged from 4.3 log_{10}/ml to 5.6 log_{10}/ml in the different experimental groups at this time, with no significant difference between the experimental groups. Following the second puncture, antibiotic administration was started.

**Drug administration.** All antibiotics were administered through a peripheral ear vein as a bolus injection in saline. Pharmacokinetics were studied in healthy rabbits with ampicillin doses of 100 and 200 mg/kg and sulbactam doses of 50 and 100 mg/kg. The following antibiotic regimens were evaluated in infected rabbits: (i) 100 mg of ampicillin per kg plus 50 mg of sulbactam per kg at 0 h; (ii) 100 mg of ampicillin per kg plus 50 mg of sulbactam per kg at 0 and 2.5 h; (iii) 50 mg of ampicillin per kg plus 250 mg of sulbactam per kg at 0 and 2.5 h; (iv) 500 mg of ampicillin per kg plus 500 mg of sulbactam per kg at 0 and 2.5 h; (v) 10 mg of ceftriaxone per kg at 0 h; (vi) 10 mg of ceftriaxone per kg at 0 h.

**Specimen collection and processing.** Serial blood samples drawn from an indwelling femoral artery catheter and CSF samples from the cisterna magna were obtained at 1 and 5 h after the beginning of antimicrobial therapy. CSF bacterial titers were determined by quantitative cultures on blood agar plates after inactivation of the β-lactam compound by adding a broad-spectrum β-lactamase that was incompletely inhibited by sulbactam (broad-spectrum β-lactamases 1 and II; Oxoid, Hampshire, England). The CSF and the serum samples were stored at −70°C until drug assays were performed within a month after collection, for which time all drugs were stable.

**Antibiotic assays.** Drug concentrations in serum and CSF were determined by agar well diffusion bioassays. *Bacteroides subtilis* (Difco; Detroit, Mich.) was used as the test strain for ampicillin, *Pasteurella hemolytica* ATCC 43823 was used as the test strain for sulbactam, and *E. coli* ATCC 10536 was used as the test strain for ceftriaxone. Sulbactam concentrations were determined by use of a β-lactamase inhibition assay. This assay is based on the production of a β-lactamase by the test strain (*P. hemolytica*). The zone of inhibition produced by an excess amount of ampicillin added to the test samples is directly proportional to the amount of β-lactamase inhibitor (sulbactam) present in the sample. The lower limit of detection by bioassay was 0.3 µg/ml for all drugs.

**Statistical analysis.** All results are given as the mean ± standard deviation. Differences between comparable groups were examined by one-way analysis of variance, followed by Student's *t*-test, corrected for multiple comparisons.

**RESULTS**

**In vitro susceptibility.** The MICs and MBCs of the study drugs were as follows: ampicillin as a single agent, 32 and >64 µg/ml; sulbactam as a single agent, >256 and >256 µg/ml; and ceftriaxone, 0.125 and 0.25 µg/ml. The MIC and MBC of the combination ampicillin-sulbactam were 2.0 and 4.0 µg/ml (ampicillin concentration) when tested in a 2:1 ratio.

**Pharmacokinetics.** In uninfected rabbits, both ampicillin and sulbactam reached high concentrations in serum of 55.1 ± 16.2 and 50.1 ± 14.8 µg/ml, respectively, 30 min after a bolus i.v. injection of 100 mg–50 mg. This was followed by rapid elimination with a calculated half-life at β phase of 30 min for ampicillin and 25 min for sulbactam. Concentrations of both drugs in CSF were low in healthy animals (0.0 ± 0.6 and 1.4 ± 0.5 µg/ml, respectively, 1 h after drug administration), but elimination from CSF appeared slower than that from serum.

In infected animals, both compounds examined in this study reached remarkably high concentrations in CSF 1 h after an i.v. bolus injection compared with the simultaneously determined concentrations in serum (Table 1). While there were large variations from animal to animal, some experimental groups actually had mean drug concentrations that were higher in the CSF than in serum at 1 h (Table 1). This finding is likely explained by the pharmacokinetic variability observed in this model and by the fact that after i.v. bolus administration, the time to peak is shorter and the drug elimination is more rapid in serum than in CSF.

As a result of the short half-life, the concentrations of both drugs in serum were below detectability at 5 h at the lowest dose, and most animals also had concentrations in CSF below detectability at this time (Table 1). For this reason, other dosing regimens were evaluated, with two doses given during the 5-h treatment period. With this schedule, concentrations in CSF tended to be relatively similar at the 1- and 5-h time points, while concentrations in serum were generally lower by 5 h (Table 1), again reflecting the short half-life in serum. The two injections of high-dose ampicillin-sulbactam produced concentrations in serum and CSF well above...
the MBC of the infecting organism for most of the 5-h treatment period.

The mean concentrations of ceftriaxone in CSF for the two regimens (10 and 100 mg/kg as i.v. bolus) at 1 and 5 h are given in Table 1. Because of the long half-life of this drug (5 h in the CSF [20]), only one injection was administered for the 5-h treatment period and high concentrations in CSF relative to the MBC were achieved, particularly with the higher dose.

**Bactericidal activity.** Mean CSF bacterial titers remained stable over the 5-h treatment period in control animals (Table 2). Administration of ampicillin-sulbactam, 100 and 50 mg/kg one time, the regimen that resulted in low concentrations in CSF during most of the treatment period, resulted in only a slight decrease of bacterial titers in the CSF that was not significant compared with titers in the control group (Table 2). In contrast, the same dose of ampicillin-sulbactam given twice as well as the higher dose of 500 and 250 mg/kg given twice resulted in a significant decrease in CSF bacterial titers ($P < 0.05$) (Table 2). In order to examine whether a further increase of the sulbactam dose would improve the bactericidal activity of ampicillin, we also examined ampicillin-sulbactam at 500 and 500 mg/kg given twice. Despite a slight further increase in the bactericidal rate, this regimen was not significantly different from the regimen of 500 and 250 mg/kg given twice. (Table 2). Treatment with the lower dose of ceftriaxone achieved bactericidal rates in CSF similar to those achieved with the highest dose of ampicillin-sulbactam (Table 2). In contrast, the higher dose of ceftriaxone produced a bactericidal rate that was significantly higher than those of all of the other regimens (Table 2; $P < 0.01$).

**DISCUSSION**

The production of β-lactamase by pathogenic bacteria has compromised the clinical utility of ampicillin and some other β-lactam antibiotics. These enzymes, however, can be blocked by different β-lactamase antagonists. Among them, sulbactam is a potent and irreversible β-lactamase inhibitor that restores and extends the spectrum of ampicillin against resistant strains of many pathogenic bacteria (5, 8, 16). In the present study, we examined the pharmacokinetics and the dose-dependent bactericidal activity of ampicillin-sulbactam in rabbits with meningitis caused by a β-lactamase-producing strain of *E. coli*.

Both ampicillin and sulbactam achieved excellent levels in CSF in animals with meningitis, and within the first hour after i.v. bolus administration, mean concentrations in CSF were similar to or even exceeded simultaneous concentrations in serum. This is most likely due to differences in drug kinetics between CSF and serum. The peak concentration in serum after i.v. bolus injection is reached within minutes, whereas the peak concentration in CSF occurs later. Because the half-life in serum is approximately 0.5 h for both compounds, the concentration in serum should be approximately 25% of the peak by 1 h. In contrast, the peak concentration in CSF is reached later (approximately 30 min after i.v. administration; unpublished observations) and the CSF elimination half-life for both compounds is about 1 h, resulting in a 1-h concentration in CSF that reflects the true peak concentration in CSF. Thus, even though a formal calculation of the CSF penetration based on comparison of areas under the curve was not attempted because we had obtained insufficient time points to do this reliably, it is likely that the actual peak in CSF was considerably lower than the peak in serum, as can be expected from previous antibiotic studies (7, 9, 14, 24, 26). Pharmacokinetic studies of ampicillin and sulbactam in humans, in agreement with the present findings, have shown that ampicillin and sulbactam have very similar pharmacokinetic properties, produce high concentrations in serum, and are well distributed into extravascular fluid and tissues (6–8, 24). Concentrations of sulbactam in CSF of up to 12 μg/ml have been detected between 1 and 4 h after administration to patients with bacterial meningitis, with higher levels present in patients with the most severe meningeal inflammation (24).

The modest bactericidal activity of the drug combination found in this study is most likely a reflection of the relatively low in vitro activity of ampicillin against our particular strain of *E. coli* and is unrelated to β-lactamase production by the test strain. With the highest ampicillin concentration examined, the ratio between the CSF drug concentration and the MBC for the test strain was only slightly greater than 10. On the basis of our previous experiences, this is often insufficient for a β-lactam antibiotic to achieve maximal bactericidal activity in CSF (27, 29). The concentration in CSF/MBC ratio of the low-dose ceftriaxone was similar (12), and both regimens achieved a similar decrease in CSF bacterial titers of about 0.5 log$_{10}$–ml$^{-1}$. In contrast, the ratio was over 70 for the high-dose ceftriaxone at 1 h, and this regimen produced the highest bactericidal activity of more than 1 log$_{10}$–ml$^{-1}$, which is similar to the maximal bactericidal rates found in previous comparable studies (3, 9, 27). The results with ampicillin-sulbactam are similar to the results in our previous study with piperacillin-tazobactam, in which we found only moderate bactericidal activity with clinically achievable drug levels (9). We were, however, surprised in the present study by the apparent lack of increased bactericidal activity between the regimens containing 100 and 500 mg of ampicillin. This is in contrast to our previous studies and those of other authors, in which a strong dose-response relationship between achieved CSF drug concentrations and bactericidal activity in CSF was found (9, 18, 22, 26, 27). We do not have an obvious explanation for the lack of such an association in the present study.

Despite the inherent problems of extrapolating from animal studies to the situation in humans, observations made in the rabbit model of meningitis have previously been shown to have reasonable predictive value for the clinical situation (29). On the basis of the present results, it appears therefore that ampicillin-sulbactam may be of limited value in the

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**Table 2. Bacteriologic efficacy of ampicillin-sulbactam and ceftriaxone in experimental meningitis due to *E. coli***

<table>
<thead>
<tr>
<th>Drug (mg/kg), no. of doses</th>
<th>No. of rabbits</th>
<th>Initial titer (log$_{10}$/ml)</th>
<th>Change in titer (log$_{10}$/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>5.5 ± 1.1</td>
<td>+0.01 ± 0.1</td>
</tr>
<tr>
<td>Ampicillin (100)–sulbactam (50), 1</td>
<td>5</td>
<td>4.3 ± 1.4</td>
<td>−0.2 ± 0.6</td>
</tr>
<tr>
<td>Ampicillin (100)–sulbactam (50), 2</td>
<td>9</td>
<td>5.6 ± 2.1</td>
<td>−0.4 ± 0.3*</td>
</tr>
<tr>
<td>Ampicillin (500)–sulbactam (250), 2</td>
<td>20</td>
<td>5.2 ± 1.0</td>
<td>−0.4 ± 0.3*</td>
</tr>
<tr>
<td>Ampicillin (500)–sulbactam (500), 2</td>
<td>18</td>
<td>5.3 ± 1.0</td>
<td>−0.6 ± 0.3*</td>
</tr>
<tr>
<td>Ceftriaxone (10), 1</td>
<td>8</td>
<td>4.3 ± 0.9</td>
<td>−0.6 ± 0.2*</td>
</tr>
<tr>
<td>Ceftriaxone (100), 1</td>
<td>13</td>
<td>5.4 ± 1.3</td>
<td>−1.1 ± 0.4**</td>
</tr>
</tbody>
</table>

* * $P < 0.05$ versus controls; ** $P < 0.01$ versus all other groups.

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treatment of difficult-to-treat β-lactamase-positive organisms, while the favorable CSF pharmacokinetics appear to make this drug combination potentially effective against organisms with relatively low in vitro MICs and MBCs. For example, ampicillin-sulbactam was very effective in an infant rat model of meningitis caused by an ampicillin-resistant strain of *Haemophilus influenzae* (5). Furthermore, Rodríguez et al. (17) have demonstrated that ampicillin-sulbactam was as effective as chloramphenicol-ampicillin in the treatment of meningitis in infants and children. However, the findings in our study may identify the limitations of this combination in the treatment of bacterial meningitis. It appears prudent to routinely perform MIC and MBC determinations against the infecting pathogen when ampicillin-sulbactam is chosen to treat meningitis caused by gram-negative bacilli, and in cases in which high MIC and MBC values make it unlikely that CSF drug concentrations will exceed these values by at least 30-fold, alternative therapies should be considered.

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REFERENCES


27. Täuber, M. G., C. A. Doroshow, C. J. Hackbart, M. G.

