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Correlation of In Vitro Time-Kill Curves and Kinetics of Bacterial Killing in Cerebrospinal Fluid During Ceftriaxone Therapy of Experimental *Escherichia coli* Meningitis

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Ceftriaxone was highly active in eliminating *Escherichia coli* from the cerebrospinal fluid of rabbits infected with experimental meningitis. However, concentrations equal to or >10 times the minimal bactericidal concentration had to be achieved to ensure optimal efficacy (rate of kill, 1.5 $\log_{10}$ CFU/ml per h). In contrast to other β-lactams studied in this model, ceftriaxone concentrations in cerebrospinal fluid progressively increased, whereas serum steady state was obtained by constant infusion. The percent penetration was 2.1% after 1 h of therapy, in contrast to 8.9% after 7 h ($P < 0.001$). In vitro time-kill curves done in cerebrospinal fluid or broth more closely predicted the drug concentrations required for a maximum cidal effect in vivo than that predicted by determinations of minimal inhibitory or bactericidal concentrations.

Antimicrobial therapy of gram-negative bacillary meningitis remains imperfect, and high rates of mortality and sequelae are still reported (2). It is hoped that the new cephalosporins with expanded antibacterial spectra and favorable pharmacokinetic properties will improve the prognosis of this condition (7). Ceftriaxone may be uniquely suited for therapy of meningitis, since it is active against most pathogens responsible for this disease (12), especially in neonates, and exhibits a very long half-life in serum (9, 10). In rabbits with experimental *Escherichia coli* and *Streptococcus pneumoniae* meningitis, half-lives in cerebrospinal fluid (CSF) of as long as 4.9 and 9.3 h, respectively, have been demonstrated (6, 8). The purposes of this study were (i) examine the kinetics of penetration of ceftriaxone into the CSF of animals with *E. coli* meningitis, (ii) compare activity of the drug in vitro and in vivo by studying the kinetics of bacterial killing in CSF in vivo under conditions of constant concentrations in CSF, and (iii) evaluate the predictive value of in vitro time-kill curves determined in CSF and in broth.

MATERIALS AND METHODS

Infecting organism. The *E. coli* strain used in this study was K1 antigen positive and was isolated from the CSF and blood of a 3-day-old premature infant at the University of Virginia, Charlottesville, in 1975. The strain was found to be resistant to killing by rabbit serum and CSF and infected rabbit CSF (filtered for sterility). The strain was stored on glass beads at −70°C, and a new bead was removed for each study.

Antibiotic. Ceftriaxone was provided by Hoffmann-La Roche, Inc., Nutley, N.J.

In vitro studies. Minimal inhibitory concentrations (MICs) were determined by a twofold dilution method in Mueller-Hinton broth and in a pool of sterile rabbit CSF. The MIC was defined as the lowest concentration of ceftriaxone that inhibited visible growth after 18 h of incubation at 35°C with an inoculum of 10^5 CFU/ml. Each tube without growth was quantitatively subcultured to tryptic soy agar to determine the minimal bactericidal concentration (MBC), which was defined as the lowest concentration of ceftriaxone that reduced viable colony counts to 0.1% that of the original inoculum.

Preparation of inocula. An overnight culture in tryptic soy broth was centrifuged, washed two times, resuspended in saline, and adjusted to a concentration of 10^5 to 10^6 CFU/0.2 ml.

Induction of meningitis. A total of 92 New Zealand white rabbits were prepared as described previously (4). In brief, anesthesia was induced by intravenous injection of 45 mg of pentobarbital (D. M. Pharmaceuticals, Inc., Rockville, Md.), and a dental acrylic helmet was attached to the skull of the animal, which immobilized the rabbit's head in a stereotaxic frame. A Quincke spinal needle (25 gauge, 3.5 inches [8.89 cm]) was introduced into the cisterna magna. A sample of 0.2 ml of CSF was withdrawn and replaced by an equivalent volume of the inoculum containing 10^5 to 10^6 CFU, and the animal was returned to its cage. At 18 h later, meningitis was present, as manifested by

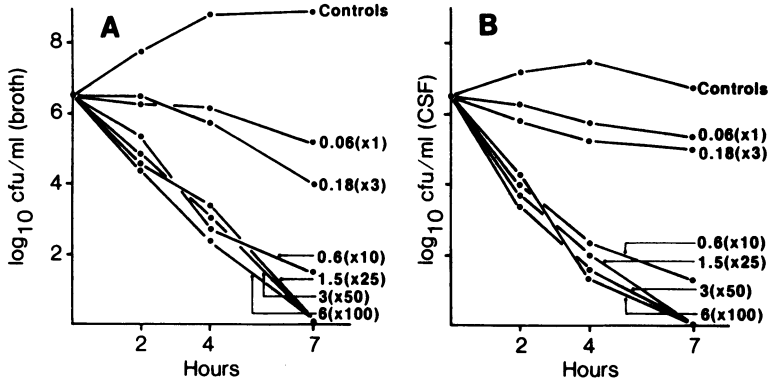

FIG. 1. Time-kill curves with *E. coli* determined in vitro in broth (A) and in a pool of rabbit CSF (B).

lethargy, temperature of >39.5°C, CSF pleocytosis marked by >500 leukocytes per mm^3 with 90% polymorphonuclear leukocytes, and 10^4 to 10^7 CFU of *E. coli* ml. Anesthesia was reinduced by intravenous infusion of 1.75 g of urethane (Sigma Chemical Co., St. Louis, Mo.) per kg of body weight over 20 min, and the animal was kept in the frame for the duration of treatment (7 h). Untreated, the disease was uniformly fatal within 36 to 48 h.

Rabbits were treated in groups of four. Each group received the same number of organisms, and the interval between inoculation and initiation of therapy was the same. A treatment regimen was assigned to each group before inoculation but was not randomized.

Ceftriaxone administration. Four animals received no drug and were used as controls. In initial studies, ceftriaxone was given to 32 animals as a constant infusion of 0.1, 0.5, 0.75, 1.0, 5.0, or 10.0 mg/kg per h, starting immediately after an initial bolus of 20% of the total 7 h dose, to produce constant drug concentrations in serum. However, this procedure did not result in constant CSF concentrations. A total of 56 animals then received the initial bolus, followed 4 h later with a constant infusion of 0.1, 0.5, 1.0, 5.0, or 10.0 mg/kg per h. This method successfully achieved constant drug concentrations in CSF (steady state).

Sampling. CSF samples were obtained every hour through the spinal needle, which remained in place in the cisterna magna. CSF was quantitatively cultured on tryptic soy agar, and 0.1 ml of each CSF sample was stored at −70°C until assayed (within 1 week). Serum samples were obtained hourly through a femoral arterial catheter (Intramedic PE-90; Clay Adams, Parsippany, N.J.) and stored at −70°C until assayed. Animals that did not live for the 7 h of the experiment or from which all expected samples could not be obtained (48 in all) were excluded from the study.

Ceftriaxone assay. Concentrations of ceftriaxone were determined by an agar-well diffusion technique, with *E. coli* ATCC 10536 used as the test strain. Standards for assay in serum and CSF were prepared in a pool of rabbit serum and saline, respectively. The lowest ceftriaxone concentration that was accurately measurable was 0.02 μg/ml.

Analysis of Data. Statistical analysis on paired observations was done by Student's *t* test.

RESULTS

Antibacterial activity of ceftriaxone in vitro. The MIC and MBC of ceftriaxone for the infecting organisms in broth were both 0.06 μg/ml. Results determined in CSF were identical. When the dynamics of bacterial killing were studied in vitro with time-kill curves, organisms did not grow as well in CSF as in broth. The final number of organisms in control tubes was greater in broth than in CSF (Fig. 1). The bactericidal effect of ceftriaxone was influenced by both the drug concentration and the medium used. Drug concentrations equal to and three times greater than the MBC produced a better bactericidal effect in broth than in CSF (−2.5 versus −1.5 $\log_{10}$ CFU/ml per 7 h), whereas drug concentrations 10 times greater than the MBC were rapidly bactericidal in both media and resulted in a maximal bactericidal effect. Increasing the concentration above this level did not achieve a more rapid decline in bacterial titers.

Penetration of ceftriaxone into CSF. The percent penetration into CSF was used to compare drug levels in CSF with simultaneous serum levels and was defined as follows: percent penetration = (concentration in CSF/concentration in serum) ×100.

In animals receiving the drug as a constant infusion after the initial bolus, concentrations in serum were relatively constant during the 7 h of therapy (Fig. 2). However, a large variability of mean concentrations in serum was observed between animals receiving the same dosage. In contrast, concentrations in CSF of the same animals tended to increase throughout the 7 h of therapy (Fig. 2). The percent penetration after 1 h differed significantly from that after 7 h of therapy. At 1 h, penetration (mean ± standard deviation) was 2.1 ± 2.9%, whereas at 7 h, penetration was 8.9 ± 4.5% ($P < 0.001$). In animals receiving the drug by a constant infusion starting at 4 h after the initial bolus, ceftriaxone

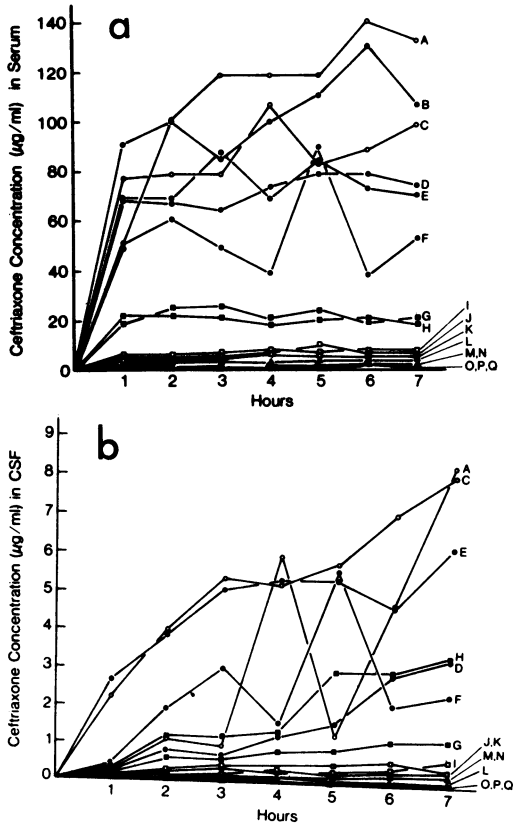

FIG. 2. Concentrations of ceftriaxone in serum (a) and CSF (b) of animals receiving various doses (0.1 to 10.0 mg/kg per h) by constant infusion after initial bolus. Letter designations indicate datum points from each animal on both graphs. Each line represents data from one or more animals, as indicated. Ceftriaxone was administered as described in the text. Doses (in milligrams per kilogram of body weight) given by continuous infusion after initial bolus are indicated as follows: ●, 10; ○, 5; ■, 1; □, 0.75; ▲, 0.5; △, 0.1. Ceftriaxone concentrations in CSF of rabbit B exceeded the scale on the graph; therefore, it was omitted from the graph and is given as follows (in micrograms per milliliter) at each time: 1 h, 9.9; 2 h, 12.4; 3 h, 11.3; 4 h, 16.9; 5 h, 17.7; 6 h, 16.9; 7 h, 17.7.

concentrations in CSF were relatively constant (Fig. 3). These animals were then used to determine the relationship between drug concentrations in CSF and rate of bacterial killing in CSF in vivo.

Activity of ceftriaxone in vivo. The activity of ceftriaxone in vivo was determined by performing quantitative CSF cultures hourly. For each animal, the rate of bacterial killing was calculated and expressed as follows: rate of kill = mean $\Delta\log_{10}$ CFU/ml per h = $[(\log_{10}$ CFU/ml$_0$ h$)$ $-(\log_{10}$ CFU/ml$_x$ h$)]/x$ h, where x = the time necessary to achieve CSF sterility. Ceftriaxone concentrations lower than or equal to the MBC failed to reduce bacterial titers in CSF in vivo (Fig. 4), and even in 10 animals with mean concentrations of 1 to 10 times the MBC, bacterial counts were minimally and inconsistently reduced (mean reduction, −0.7 CFU/ml per h). Animals with drug concentrations in CSF of >10 times the MBC had a mean rate of kill of −1.5 $\log_{10}$ CFU/ml per h, and the difference between both groups was statistically significant ($P < 0.05$). These studies demonstrate that concentrations of ceftriaxone in CSF of up to 10 times the MBC fail to produce maximal killing in CSF in vivo, and only when constant drug concentrations greater than this were achieved was a maximal therapeutic effect achieved.

DISCUSSION

The primary purpose of this study was to evaluate the penetration of ceftriaxone into the CSF of animals with gram-negative bacillary meningitis. Other investigators have reported drug concentrations in CSF to be 3 to 9% those in serum (5, 7). Although our results were in this range, we were unable to determine the specific ratio because the steady state in CSF was not achieved with a steady state in serum. We empirically found that giving the infusion 4 h after the bolus would produce constant concentrations in CSF, but, consequently, concentrations in serum will decline. Thus, when drug concentrations were constant in serum, the ceftriaxone concentration in CSF gradually increased; this tendency seems to be unique to ceftriaxone and has not been observed with any

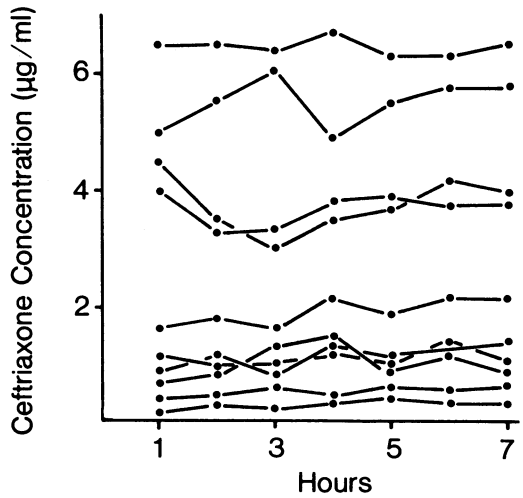

FIG. 3. Concentrations of ceftriaxone in CSF of animals receiving various doses (from 0.1 to 10.0 mg/kg per h) by constant infusion started 4 h after the initial bolus.

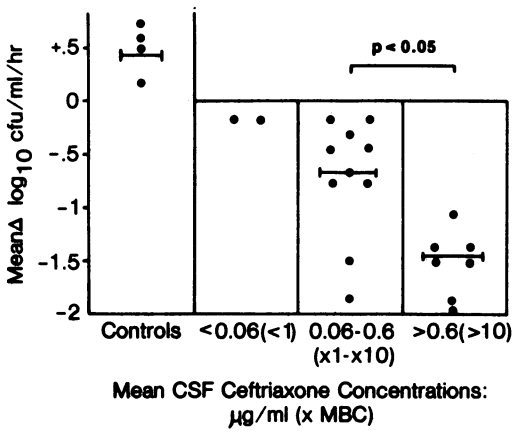

FIG. 4. Correlation between mean ceftriaxone concentrations in CSF and rate of bacterial killing in CSF in vivo. Each datum point represents one animal.

of the 27 β-lactams or any antibiotic studied to date in this model (J. M. Decazes, submitted for publication). The explanation for this characteristic is unclear. High protein binding has been found to be partially responsible for the exceptionally long serum half-life in humans (11), and binding to the proteins of infected CSF may restrain elimination of the drug from the CSF. In addition, progressive increase in CSF protein during the course of treatment might allow a highly protein-bound drug to accumulate in CSF. Studies in humans have also failed to demonstrate substantial active secretion of ceftriaxone by the kidney tubules (10). Since this mechanism appears to correlate with the active secretion of β-lactams from CSF back into the bloodstream, it suggests that ceftriaxone is not affected by the activity of the exit pump. Future studies in which probenecid is used should help clarify this point. Evidence was found in the current study that accumulation of the drug in CSF under conditions of constant infusion is consistent with extremely long CSF half-lives determined in other studies in experimental meningitis after one single injection (4.9 and 9.3 h in rabbits infected with *E. coli* and *S. pneumoniae*, respectively [6, 8]). This unique property may provide an important therapeutic advantage for ceftriaxone over other new antimicrobial agents in the treatment of meningitis and may be especially critical in countries where only intermittent intramuscular injections may be possible. The ability of ceftriaxone to cure meningitis in humans with a single injection has already been reported (1).

In vivo findings demonstrate that ceftriaxone was highly active in eliminating viable, susceptible organisms from the CSF of the infected animals. However, maximal rate of kill was obtained only when concentrations of >10 times the MBC were achieved in CSF in vivo. This observation is consistent with those of Doroshow et al. (C. Doroshow, T. A. Drake, and M. A. Sande, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, Ill., abstr. no. 456, 1981), who found that ceftriaxone and moxalactam concentrations required to obtain maximal killing of *S. pneumoniae* in vivo were 10 to 20 times those required to obtain a maximal rate of kill in broth in vitro, but were similar to those required to produce a maximal rate of kill in CSF in vitro. In the current study, our in vitro studies demonstrated that the ceftriaxone MIC and MBC for the infective strains were not different when determined in Mueller-Hinton broth or in a pool of rabbit CSF. In vitro time-kill curves were better in predicting efficacy in vivo, regardless of whether the various concentrations were incubated in CSF or in broth. Thus, traditional methods of in vitro testing of susceptibility of a strain to antimicrobial drugs may be misleading or bear little resemblance to in vivo efficacy at the site of infection. In 1967, it was suggested by Chabert (3) that bactericidal activity in the CSF was necessary for treatment of bacterial meningitis. In a recent study by Scheld and Sande (9), the importance of bactericidal activity in cure of experimental pneumococcal meningitis was demonstrated. The current study suggests that drug concentrations in CSF that are appropriate for the treatment of bacterial meningitis should not only reach the MBC for the infecting organism but exceed it by 10 to 20 times to ensure maximum efficacy. Future studies in humans with ceftriaxone should determine whether such concentrations can be safely reached in CSF.

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