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Pharmacokinetics and Antibacterial Efficacy of Cefpirome (HR 810) in Experimental *Escherichia coli* and *Haemophilus influenzae* Type b Meningitis

HAMID S. JAFARI, XAVIER SÁEZ-LLORENS, OCTAVIO RAMILO, SHARON L. SHELTON, AND GEORGE H. McCRACKEN, JR.*

Department of Pediatrics, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75235-9063

Received 9 August 1990/Accepted 15 November 1990

Cefpirome (HR 810) is a new cephalosporin related to cefotaxime that has potent bactericidal activity against a broad spectrum of gram-negative and gram-positive organisms. The pharmacokinetics and bacteriological efficacy of cefpirome administered as a single intravenous dose were assessed in rabbits with experimental *Haemophilus influenzae* type b and *Escherichia coli* K1 meningitis. The mean penetrations into the cerebrospinal fluid (CSF) in relation to the amount of drug in serum of animals infected with *H. influenzae* and *E. coli* were 25 and 54%, respectively. The median CSF bactericidal titers were 1:128 against both organisms at 1 h after the dose and 1:32 and 1:16 against *H. influenzae* and *E. coli*, respectively, at 8 h after the dose. In CSF of uninfected animals, the mean penetration was 4.5%. There was a significant reduction in the concentrations of bacteria in CSFs of both groups of animals treated with cefpirome compared with that in untreated groups. Mortality was also significantly lower in treated animals than it was in untreated animals. Intravenous administration of dexamethasone before the cefpirome dose did not compromise penetration, bactericidal titers, or antibacterial activity of cefpirome in CSF.

Cefpirome (HR 810) is a new cephalosporin related to cefotaxime that has potent bactericidal activity against a broad range of gram-negative and gram-positive organisms including *Pseudomonas aeruginosa* and methicillin-susceptible *Staphylococcus* spp. It is also highly active against *Haemophilus influenzae* type b and many members of the family *Enterobacteriaceae* (1, 2, 13). *Escherichia coli* K1 and *H. influenzae* type b are the two most important gram-negative pathogens involved in bacterial meningitis affecting infants and children.

The objectives of the present study were (i) to determine the concentrations of cefpirome, after a single intravenous (i.v.) dose, in the sera and cerebrospinal fluid (CSF) of rabbits with experimental *E. coli* K1 and *H. influenzae* type b meningitis; (ii) to evaluate the penetration and bacteriologic efficacy of this investigational cephalosporin in CSF of infected and uninfected animals; and (iii) to assess the effects of dexamethasone, administered i.v. before the antibiotic dose, on penetration, bacteriostatic and bactericidal titers, and antibacterial activity of cefpirome in CSF of infected animals.

MATERIALS AND METHODS

Six rabbits in each of the following groups of animals were used to study cefpirome pharmacokinetics: (i) *E. coli* meningitis, (ii) *H. influenzae* meningitis, (iii) uninfected controls, and (iv) *H. influenzae* meningitis with dexamethasone therapy. Because of the higher mortality in untreated animals, up to 18 animals had to be used in all groups in order to compare the antibacterial activities between the treated and untreated groups.

Experimental meningitis model. New Zealand White male rabbits (weight, 2 to 3 kg) were prepared as described previously (3). Briefly, a dental acrylic helmet was attached to the skull of each rabbit, and later the animals were anesthetized and placed in a stereotactic frame. A spinal needle was introduced into the cisterna magna and fixed in the frame. Bacteria were inoculated and CSF samples were taken via the intracisternal needle during the experiment.

Bacteriology. Volumes of 0.2 ml containing 10^5 to 10^6 CFU of a β-lactamase-negative strain of *H. influenzae* type b per ml and 10^6 to 10^7 CFU of *E. coli* K1 per ml were used. These inocula uniformly produced meningitis in the rabbits; larger inocula caused the deaths of 50% or more of the animals within 20 h of inoculation. The inoculum chosen resulted in CSF bacterial counts at the time of the experiments that were within the range of bacterial concentrations observed in clinical samples of CSF from infants and children with meningitis (4). The two test organisms were isolated from CSF samples of infants with bacterial meningitis and were characterized at our institution. Chocolate agar and blood agar plates were used for *H. influenzae* and *E. coli* cultures, respectively. Dilutions for quantitative cultures were done with pyrogen-free phosphate-buffered saline (pH 7.4).

Drug administration. Cefpirome sulfate (HR 810; provided by Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.) was administered 12 h (time zero) after bacterial inoculation in a single i.v. dose of 30 mg/kg of body weight to each rabbit as a 5-min infusion. The amount of cefpirome given was based on the achievable peak concentrations in serum in animals that were similar to those in adult humans. Dexamethasone was administered i.v. in a single 1-mg/kg dose 5 min before the antibiotic dose in a separate group of animals infected with *H. influenzae* type b.

Specimen processing. Serial blood from auricular arteries and CSF samples from the intracisternal needle were obtained before and at 0.25, 0.5, 1, 2, 4, and 8 h after the single i.v. dose of cefpirome; CSF samples obtained before and 8 and 24 h after the antibiotic dose were immediately cultured

* Corresponding author.

in serial 10-fold dilutions for quantitation of bacterial colonies. After centrifugation, serum and CSF samples were kept frozen at −70°C until the antibiotic concentrations and antibacterial titers were measured, usually within 2 weeks.

Antibiotic assay. Concentrations of antibiotic in serum and CSF were measured by an agar-disk diffusion microbioassay method using *E. coli* RO1346. Serum and CSF from untreated healthy and untreated infected rabbits did not inhibit the growth of the assay organism.

Susceptibility studies. The MICs and MBCs for the two test strains were determined by a microtiter technique with serial twofold dilutions in Mueller-Hinton broth for *E. coli* K1 and in Mueller-Hinton broth with supplement C (Difco Laboratories, Detroit, Mich.) for *H. influenzae*. The inoculum was approximately 10^5 CFU/ml. After 24 h of incubation at 37°C, the wells were inspected; the MIC was defined as the lowest concentration of antibiotic that inhibited visible growth. Each clear well was subcultured quantitatively onto blood agar plates for *E. coli* and chocolate agar plates for *H. influenzae*, and these plates were incubated for another 18 h. The MBC was defined as the lowest concentration of antibiotic that killed 99.9% or more of the original inoculum.

Bacteriostatic and bactericidal titers. Bacteriostatic and bactericidal titers in blood and CSF for the test strain that caused meningitis were determined by a microtiter technique similar to that used for MIC and MBC measurements, except that test serum and CSF were used instead of standard antibiotic solution. The diluent was Mueller-Hinton broth (for *E. coli*) or Mueller-Hinton broth plus supplement C (for *H. influenzae*).

Pharmacokinetic analysis. The postinfusion concentrations of cefpirome in sera and CSF of all groups of animals were fitted to nonlinear models by using PCNONLIN version 3.0 software (SCI Software, Lexington, Ky.). The concentration-time curves of the drug obtained in sera were fitted to a one-compartment model with bolus input and first-order output (model 1). The CSF concentration-time curves were fitted to a one-compartment model with first-order input, first-order output, and no lag time (model 3).

Statistical analysis. Student's *t* test was used to compare differences in the mean reduction in $\log_{10}$ bacterial CFU per milliliter of CSF between treated and untreated animals. The two-tailed Fisher exact test was used to compare the differences in mortality between treated and untreated animals. The Pearson correlation coefficient was applied to evaluate the correlation between logarithmic values of antibiotic concentrations approximated to the closest titer value and logarithmic values of inhibitory and bactericidal titers in CSF. A *P* value of less than 0.05 was considered significant.

RESULTS

Susceptibility studies. The cefpirome MIC and MBC for the test *E. coli* K1 strain were 0.0625 and 0.125 μg/ml, respectively, while the MIC and MBC for the test *H. influenzae* strain were both 0.0156 μg/ml.

Pharmacokinetics in serum and CSF. Comparable mean ± standard deviation peak concentrations in serum of 79.5 ± 3.6, 78 ± 17, and 75 ± 17 μg of cefpirome per ml were obtained at the first sampling time of 0.25 h in healthy, *E. coli*-infected, and *H. influenzae*-infected animals, respectively. The mean half-lives in serum were 0.47, 0.64, and 0.6 h, respectively, and the areas under the concentration-time curve (AUCs) for serum were 75.6, 93.1, and 81.3 μg · h/ml respectively, in these groups of animals. The mean ± standard deviation peak concentrations of antibiotic in CSF of healthy, *E. coli*-infected, and *H. influenzae*-infected groups reached 0.7 ± 0.3, 10.5 ± 2.3, and 5.3 ± 4.4 μg/ml, respectively, and were achieved 0.5 to 1 h after the i.v. dose. The mean half-lives in CSF were 0.69, 3.6, and 4 h; and the AUCs for CSF were 3.4, 50.7, and 20.2 μg · h/ml, respectively (Fig. 1).

The ratios of the AUCs for CSF to those for serum were calculated for healthy, *E. coli*-infected, and *H. influenzae*-infected animals to express the percentages of drug penetration into CSF, which were 4.5, 54, and 25%, respectively.

Bacteriologic efficacy. The mean changes in bacterial colony counts in CSF (change in $\log_{10}$ CFU per milliliter) at 8 and 24 h after cefpirome administration were calculated to express the antibacterial activity of cefpirome. The initial bacterial concentrations at the time of antibiotic administration were from 1×10^5 to 1×10^8 CFU/ml (mean, 7×10^7 CFU/ml) and 1×10^4 to 1×10^7 CFU/ml (mean, 1×10^7 CFU/ml) in animals infected with *E. coli* and *H. influenzae*, respectively.

(i) *E. coli* K1 meningitis. In untreated animals with *E. coli* meningitis, the bacterial counts in CSF increased by a mean of 0.33 $\log_{10}$ CFU/ml in 8 h, and 83% of the rabbits were dead before 24 h. In the treated group of animals, the mean reductions in bacterial concentration were −2.5 and −3.4 $\log_{10}$ CFU/ml at 8 and 24 h after the antibiotic dose, respectively (Fig. 2). The difference in counts at 8 h for treated animals versus that for untreated animals was statistically significant ($P = 0.0001$). Because five of the six untreated animals died before 24 h, no comparisons could be made with treated animals at that time.

(ii) *H. influenzae* type b meningitis. In untreated animals with *H. influenzae* meningitis, the bacterial counts in CSF decreased by a mean of −0.41 $\log_{10}$ CFU/ml by 8 h, and two-thirds of the animals were dead before 24 h. In the treated animals the mean reductions in bacterial concentration were −2.3 and −5.3 $\log_{10}$ CFU/ml at 8 and 24 h after the antibiotic dose, respectively (Fig. 2). The reduction in counts at 8 h was significant ($P = 0.002$).

The combined mortality of untreated animals with *E. coli* and *H. influenzae* meningitis was significantly higher than that of the treated animals in both groups 8 to 24 h after the antibiotic dose. Only 3 of the 16 treated animals, compared with 13 of 18 untreated animals, died during the 24-h experimental period ($P = 0.0025$).

(iii) Titers in serum and CSF. A significant correlation was observed between cefpirome concentrations and inhibitory and bactericidal titers in CSF of infected animals. Peak median inhibitory and bactericidal titers in CSF of infected animals at 0.5 to 1 h after the i.v. antibiotic dose were 1:256 and 1:128, respectively. The median inhibitory and bactericidal titers in CSF obtained at 8 h were 1:32 and 1:16 and 1:64 and 1:32 in *E. coli*- and *H. influenzae*-infected animals, respectively. The peak median inhibitory and bactericidal titers achieved in CSF of uninfected control animals were 1:32, and the trough values were 1:16, when tested against the test *H. influenzae* type b strain used in the other experiments.

(iv) Rabbits with *H. influenzae* type b meningitis. Rabbits with *H. influenzae* type b meningitis that received dexamethasone before the antibiotic dose had higher antibiotic concentrations in serum and CSF; however, the penetration of antibiotic into CSF (29%) was comparable to that of *H. influenzae*-infected animals that did not receive dexamethasone (25%). The higher concentrations of antibiotic in CSF were reflected in higher median bactericidal titers in CSF of more than 1:256 at 1 and 8 h postinfusion. The mean

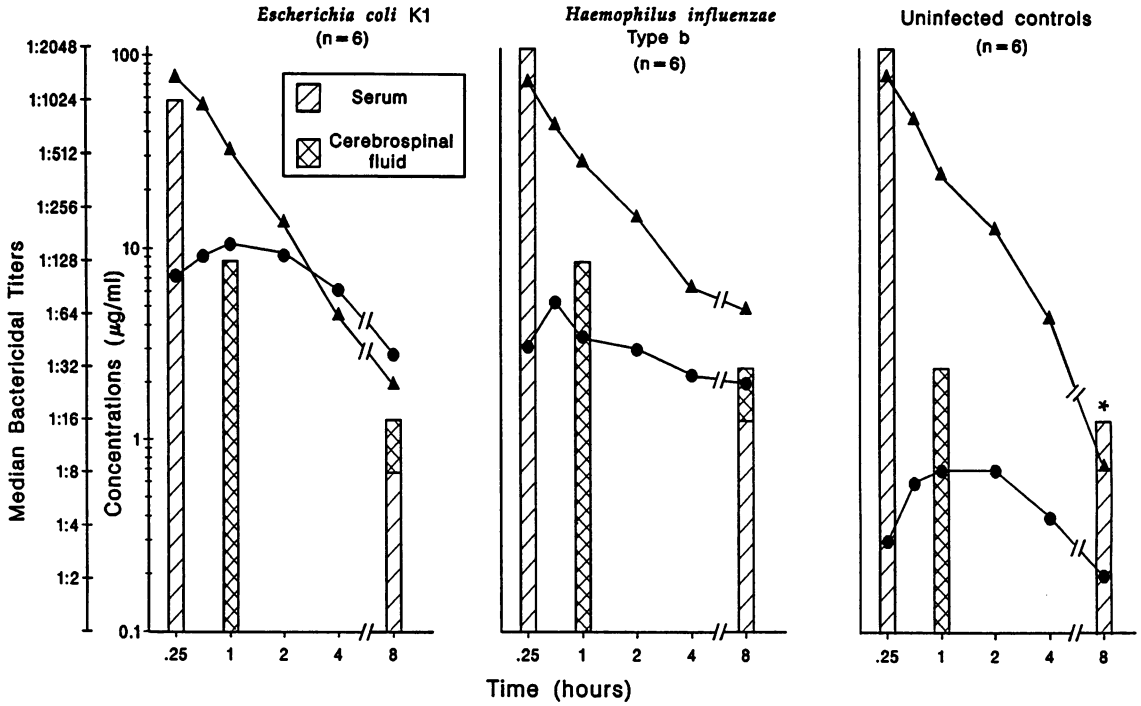

FIG. 1. Concentrations of cefpirome in paired serum (▲) and CSF (●) specimens in rabbits with experimental meningitis in relation to bactericidal titers (vertical bars). The correlation between antibiotic concentrations versus inhibitory and bactericidal titers in CSF was ≥0.952 in each group. Bactericidal titers in uninfected animals were determined against the test *H. influenzae* strain used in the other experiments. The asterisk corresponds to both serum and CSF titers.

reductions in bacterial concentration of −2.0 and −6.0 $\log_{10}$ CFU/ml during 8 and 24 h, respectively, after the antibiotic dose were similar to those in *H. influenzae*-infected animals that received antibiotic alone.

DISCUSSION

Cefpirome is highly active against the three most common meningeal pathogens that are encountered in the pediatric age group: *H. influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis* (5, 14). *E. coli* K1 and *H. influenzae* type b are the two most important gram-negative pathogens isolated from infants and children with bacterial meningitis. Cefpirome has excellent antibacterial activity against these two organisms, with reported MICs for 90% of organisms tested of 0.06 and 0.015 μg/ml, respectively (1, 2, 13).

In the present study, relative penetration of cefpirome into CSF in the presence of meningeal inflammation ranged from 25 to 54%, and high inhibitory and bactericidal titers against *E. coli* K1 and *H. influenzae* type b were achieved 1 h after the dose. Bactericidal activity against both pathogens was also present in CSF at 8 h. This remarkably high penetration into CSF is similar to that reported by other investigators who used a similar animal model of experimental pneumococcal meningitis (14). The antibacterial activity of cefpirome, which was assessed by the reduction in bacterial concentrations over time, was comparable to that in previous reports of other highly active cephalosporins such as ceftazidime and ceftriaxone against both these organisms. By using the same animal model and similar experimental conditions and concentrations of bacterial inocula, there was a mean reduction of −2.7 $\log_{10}$ CFU of *E. coli* per ml in CSF 5 h after a single dose of ceftazidime. The mean reduction in *H. influenzae* concentration in CSF was −4.4 $\log_{10}$ CFU/ml after 6 h and −2.4 $\log_{10}$ CFU/ml after 5 h of single doses of ceftriaxone and ceftazidime, respectively (8, 11).

Dexamethasone significantly modulates the meningeal inflammatory response in experimental meningitis in rabbits and in infants and children with bacterial meningitis (9, 10). This modulation is reflected in lower leukocyte, protein, and lactate values and higher glucose concentrations in CSF of animals and patients and in fewer neurological sequelae in infants and children given dexamethasone compared with those in infants and children given placebo (6, 7). Because dexamethasone therapy results in less of a disruption of the blood-brain barrier, it is possible that penetration of antibiotics into CSF might be adversely affected. When dexamethasone was given to *H. influenzae*-infected rabbits 5 min before cefpirome administration, CSF penetration was comparable to that in non-steroid-treated animals. The bactericidal titers in CSF corresponded to the concentration of the drug. Additionally, the mean reduction of bacterial concentration in the CSF of dexamethasone-treated animals was similar to that in the CSF of non-steroid-treated animals.

Studies of antibiotic treatment of experimental meningitis in rabbits have shown this model to be a reliable predictor of CSF penetration and of the antibacterial activity of the drugs in infants and children with bacterial meningitis (8, 11, 12). It is difficult, however, to extrapolate results from the animal model to therapeutic efficacy in patients. Nonetheless, the results of this study indicate a possible role of cefpirome for the treatment of bacterial meningitis caused by *E. coli* K1 and *H. influenzae* type b in infants and children. Additional studies of the safety and efficacy of cefpirome in infants and children are necessary before any firm recommendations can be made for its use in the treatment of this disease.

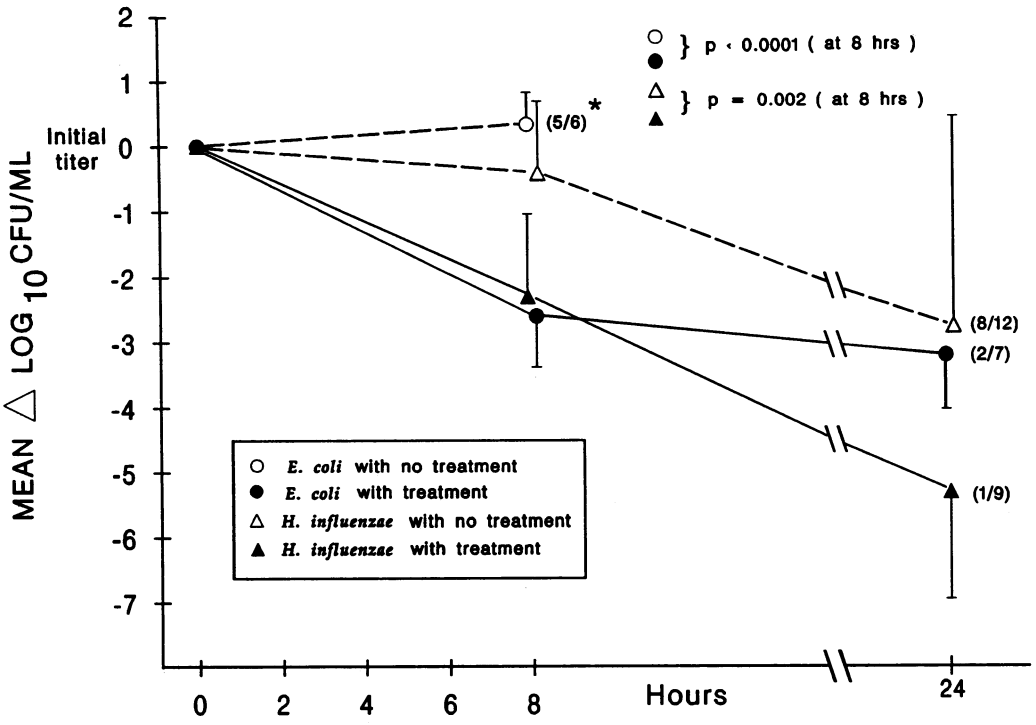

FIG. 2. Mean ± standard deviation change in $\log_{10}$ CFU of *H. influenzae* and *E. coli* per milliliter of CSF in rabbits given no therapy and those given cefpirome by i.v. bolus injection. There was a significant reduction in bacterial concentrations from 0 to 8 h in treated animals compared with that in untreated animals. At 24 h, mortality in untreated groups precluded such calculations. Numbers in parentheses indicate mortality (number of rabbits that died/total number of rabbits in group).

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