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Comparison of Cefoperazone with Penicillin, Ampicillin, Gentamicin, and Chloramphenicol in the Therapy of Experimental Meningitis

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Cefoperazone was compared with penicillin against *Streptococcus pneumoniae*, gentamicin against *Escherichia coli*, and ampicillin and chloramphenicol against *Haemophilus influenzae* in the therapy of experimental meningitis in rabbits. Meningitis was produced by intracisternal inoculation into cerebrospinal fluid, and all antibiotics were administered intravenously over 8 h in dosages that would achieve serum levels comparable to those found in humans. The mean percent penetration into purulent cerebrospinal fluid, expressed as (cerebrospinal fluid concentration/serum concentration) × 100%, was 2.6% for penicillin, 22.0% for gentamicin, 12.1% for ampicillin, 23.8% for chloramphenicol, and 6.4% for cefoperazone. The mean cerebrospinal fluid antibiotic concentrations exceeded the minimum bactericidal concentration for the test strain in each experimental model, except for ampicillin in experimental meningitis due to the β-lactamase-producing *H. influenzae*. Cefoperazone produced a significantly faster bactericidal effect after 4 h of treatment when compared with penicillin ($P = 0.037$) and ampicillin ($P = 0.01$) in meningitis caused by *S. pneumoniae* and *H. influenzae* (ampicillin susceptible), respectively. In meningitis caused by *E. coli*, cefoperazone was significantly ($P = 0.006$) more rapidly bactericidal after 8 h of treatment when compared to gentamicin. In addition, cefoperazone was significantly more rapidly bactericidal than either ampicillin or chloramphenicol in experimental meningitis due to β-lactamase-producing *H. influenzae*. Cefoperazone deserves further evaluation in the therapy of bacterial meningitis in humans.

The recent emergence of penicillin- or chloramphenicol-resistant pneumococci (13), ampicillin- or chloramphenicol-resistant *H. influenzae* (7, 22), and the poor therapeutic results with aminoglycosides in gram-negative bacillary meningitis (17, 18) make it important to develop new antimicrobial agents in the therapy of these infections. Cefoperazone, with its high activity against the above organisms, including β-lactamase-positive *H. influenzae* and aminoglycoside-resistant Enterobacteriaceae (3, 8, 10, 12, 14, 15, 16, 19), and its capacity to cross the inflamed meninges (20, 23) is a relatively new agent that was deemed deserving of such evaluation. This study compares cefoperazone with accepted antibiotics in the therapy of experimental pneumococcal, *E. coli*, and *H. influenzae* meningitis in rabbits.

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MATERIALS AND METHODS

Test organisms. All strains, except the ampicillin-resistant *H. influenzae* type b ("Wylie," kindly provided by J. Nelson, University of Texas Southwestern Medical School, Dallas) were clinical isolates from cerebrospinal fluid (CSF) of patients at the University of Virginia hospital, as described previously (9, 29).

Preparation of inocula. These procedures have been described in detail previously (9, 29). The final inocula (in colony-forming units (CFU), final volume, 0.15 to 0.3 ml) were as follows: pneumococci ≃ 10^7, *E. coli* ≃ $10^{5.5}$, *H. influenzae* ≃ $10^{8.5}$.

Induction of meningitis. Two-kilogram New Zealand white rabbits were prepared with modifications as described previously (9, 25). A dental acrylic helmet was attached to the animals' skulls, which allowed rigid immobilization in a stereotaxic frame. A Quincke spinal needle (25 gauge by 3.5 inches [ca. 9 cm]) was introduced into the cisterna magna without trauma by a geared electrode introducer. These needles were used for both initial bacterial inoculation and for CSF sampling later during the course of treatment.

TABLE 1. Mean serum and CSF concentrations and percent penetration into the CSF in experimental bacterial meningitis

Drug	Dose (mg/kg per h)	No. of animals	Concn (μg/ml) in:[a]		Mean % penetration[b]
			Serum	CSF	
Penicillin	30	11	17.0 ± 4.3	0.41 ± 0.23	2.6
Ampicillin	30	12	31.9 ± 16.5	3.6 ± 2.4	12.1
Gentamicin	2.5	11	11.5 ± 5.5	2.6 ± 1.6	22.0
Chloramphenicol	30	12	27.2 ± 6.1	6.2 ± 2.0	23.8
Cefoperazone	60	58	147.9 ± 35.8	9.4 ± 3.6	6.4

[a] Values listed are means ± standard deviations.
[b] Calculated as [(CSF concentration)/(serum concentration)] × 100%.

Inoculation was accomplished by withdrawal of normal CSF and the injection of the inoculum directly into the cisterna magna. The animals were removed from the frame and returned to their cages. The time between inoculation and treatment varied with the infecting organism from 6 to 18 h, as described previously (9, 29). In all models, the animals had meningitis as manifested by fever (>39.6°C), neurological signs, CSF pleocytosis (>95% polymorphonuclear leukocytes), and CSF bacterial counts of 4.0 to >8.0 $\log_{10}$ CFU/ml. All untreated control animals in each group died within 72 h of infection.

Treatment regimens. The dosages (in milligrams per kg body weight per h) of drugs were as follows: penicillin, 30; gentamicin, 2.5; ampicillin, 30; chloramphenicol, 30. Cefoperazone was given at a dosage of 60 mg/kg per h to 58 animals and compared to the standard drug in each of the four models. In addition, eight untreated animals were included in each group. Antibiotics were administered with a constant intravenous infusion pump (Sage model 352) via a femoral venous catheter. An initial bolus loading dose consisting of 20% of the total 8-h dose was given immediately before the start of the infusion.

Assessment of therapeutic results. Serial blood (3 ml) and CSF (0.2 ml) samples were taken from an indwelling femoral arterial catheter and spinal needle, respectively, before treatment and after 4 and 8 h of therapy. Each CSF sample was titered immediately for quantitative bacterial counts. Serial dilutions were performed and added to tryptic soy agar pour plates with 0.5 ml of sheep blood added for *S. pneumoniae* counts. For *H. influenzae*, CSF samples were surface plated on chocolate agar (with IsoVitaleX added). All of the remaining CSF and serum samples were kept at −70° until antibiotic assays were performed (within 2 weeks). This period of storage did not affect the assay results. In addition, antibiotic concentrations were determined on serum and CSF samples drawn 1 and 2 h after initiation of treatment (for a total of five determinations per animal).

Antibiotic assays. Antibiotic levels were determined by agar well diffusion techniques. *Bacillus subtilis* spore solution (0.9 ml) was added to 1,000 ml of antibiotic medium no. 1 (Difco) and no. 11 for the penicillin and ampicillin assays, respectively. Gentamicin concentrations were assayed with a multidrug-resistant strain of *Staphylococcus epidermidis* (ATCC 27626) by the technique described by Alcid and Seligman (1). Chloramphenicol bioassays employed a marine bacterium (*Beneckea natriegens*) in 1.5% salt agar as described previously (4). The cefoperazone assay used 2.5 ml of a suspension containing *Sarcina lutea* (ATCC 9341) in antibiotic medium no. 1. This suspension gave a transmission of 21% at 580 nm. All specimens and standards were tested in triplicate. Wells (4 mm) were cut into the agar and filled with approximately 0.03 ml of specimen. The zone of inhibition was measured (after 18 h of incubation at 37°C) and compared to a standard curve. The standard curve was determined by dissolving known concentrations of antibiotic in pooled rabbit serum and saline (zone sizes for identical concentrations of antibiotic dissolved in rabbit CSF and saline were found to be equal). Thus, all CSF concentrations were calculated from standards that used saline diluent.

Statistical procedures used in data analysis. The percent penetration of drug into the CSF is defined by the formula: percent penetration = (CSF concentration/serum concentration) × 100%. Statistical analysis for change in CSF bacterial concentrations was done on unpaired data by Student's *t* test (two tailed). The degree of CSF sterilization between drugs was compared by Fisher's exact test. In addition, covariance and regression line analysis were performed to assess differences between drugs in each model.

RESULTS

Penetration of antibiotics into CSF. Table 1 shows the mean serum and CSF antibiotic concentration obtained and the percent penetration into the CSF for the agents used in this study. In each case, steady-state serum and CSF levels were obtained within 1 h of initiating infusions. Ampicillin and penicillin (which were given at a dose of 30 mg/kg per h) achieved mean serum levels of 31.9 and 17.0 μg/ml, respectively. Gentamicin, delivered at 2.5 mg/kg per h, gave a mean serum level of 11.5 μg/ml over the 8-h period. The mean serum level of chloramphenicol, delivered at 30 mg/kg per h, was 27.2 μg/ml. The mean serum level of cefoperazone was much higher than that obtained with the other agents, 148 μg/ml, but it was given at a higher dosage (60 mg/kg per h). These levels are similar to those found in humans after standard parenteral therapy. The CSF antibiotic levels reflect the concurrent serum levels and the ability of the drug to penetrate across the inflamed meninges (Table 1). The mean CSF drug concentration exceeded the minimum bactericidal concentra-

TABLE 2. Results of therapy in experimental bacterial meningitis

No.	Infective organism	Therapeutic agent		Bacteriological response in CSF				
		Drug	Minimal bactericidal concentration (μg/ml)	No. rabbit cultures sterile/total at:		No. CFU[a]/ml of CSF at:		
				4 h	8 h	0 h	4 h	8 h
1	*S. pneumoniae*	None		0/8	0/8	5.8 ± 0.5	5.7 ± 0.6	6.0 ± 0.8
		Penicillin	≤0.06	4/11 t	11/11	5.8 ± 1.1	2.5 ± 1.1 t	0 ± 0
		Cefoperazone	≤0.06	8/8 t	8/8	5.7 ± 0.8	0 ± 0 t	0 ± 0
2	*E. coli*	None		0/8	0/8	5.3 ± 0.8	5.9 ± 1.2	6.0 ± 1.0
		Gentamicin	1.0	1/11	2/11 t	5.5 ± 0.7	3.7 ± 1.8	2.1 ± 1.3 t
		Cefoperazone	0.5	0/8	8/8 t	5.6 ± 1.0	2.6 ± 1.3	0 ± 0 t
3	*H. influenzae*	None		0/8	0/8	7.3 ± 1.3	7.7 ± 1.6	7.2 ± 2.0
		Ampicillin	0.5	1/12 t	3/12	7.0 ± 1.6	4.8 ± 1.5 t	2.0 ± 1.6 t
		Cefoperazone	0.125	5/10 t	6/10	7.1 ± 1.3	2.9 ± 1.7 t	0.4 ± 0.8 t
4	*H. influenzae* (β-lactamase producing)	None		0/8	0/8	7.0 ± 1.0	6.8 ± 1.4	6.3 ± 1.0
		Ampicillin	≥32	0/8	0/8 t	7.3 ± 0.8	6.5 ± 1.0	6.2 ± 1.3 t
		Chloramphenicol	1.0	0/8	0/8 t	7.6 ± 1.2	6.7 ± 0.9	6.5 ± 1.6 t
		Cefoperazone	0.5	1/12	7/12 t	7.1 ± 1.3	4.0 ± 2.2	0.6 ± 1.0 t

[a] Mean ± SD (log_{10}). Values connected by t are significantly different at the $P \leq 0.05$ level.

tion for the test strain in each case except for ampicillin in the model of β-lactamase-positive *H. influenzae* meningitis. The mean CSF penetration, expressed as (CSF concentration/serum concentration) × 100%, varied from a low of 2.6% for penicillin to almost 24% for chloramphenicol. The mean CSF cefoperazone concentration did not differ significantly between the experimental models.

Rate of bacterial killing in vivo. The results of therapy are shown in Table 2. The initial CSF bacterial concentrations before therapy were not significantly different between groups in any model. In each case, the mean CSF bacterial concentrations remained relatively unchanged in the untreated control group, and all of these animals died within 72 h of inoculation.

It can be seen in Table 2 (significant differences are indicated) that cefoperazone achieved a significantly more rapid bactericidal effect in vivo than the accepted agent(s) in each model of meningitis employed in these studies. In some infections (e.g., pneumococcal- or ampicillin-sensitive *H. influenzae* meningitis), the differences between cefoperazone and the reference agent were apparent after 4 h of therapy but the results were similar and not significantly different after the full 8-h treatment interval. In other models (e.g., *E. coli* meningitis; Table 2), the reverse was seen, whereas in experimental ampicillin-resistant *H. influenzae* meningitis, cefoperazone was more rapidly bactericidal than ampicillin or chloramphenicol at both time points analyzed.

Sterilization of the CSF was analyzed for each drug after 4 and 8 h of treatment (Table 2). The results of Fisher's exact test analysis of potential differences are indicated. Cefoperazone sterilized the CSF more rapidly than the accepted agent in each experimental infection; the time courses to this endpoint generally reflect the differences displayed in Table 2 for actual CSF bacterial counts.

DISCUSSION

This study compared cefoperazone, a new semisynthetic cephalosporin, with accepted antimicrobial agents in the therapy of experimental *S. pneumoniae*, *E. coli*, and *H. influenzae* (both ampicillin susceptible and resistant) meningitis in rabbits. Although differences between experimental models were noted, cefoperazone was significantly more rapidly bactericidal in vivo than the other agents in each type of experimental meningitis in this study.

The results of our experiments and in other recent models of meningitis (20, 23) may suggest a role for cefoperazone in the therapy of meningitis in humans. Penicillin (or ampicillin) is generally preferred in pneumococcal meningitis, but the mortality rate (28% in 1978) (7) has not improved in over 30 years (2, 24). Although cefoperazone was more rapidly bactericidal than penicillin in our studies, it is doubtful that any new cephalosporin will significantly improve the results in penicillin-sensitive pneumococcal meningitis, due to the poor prognostic features (e.g., coma) present in many cases. Since pneumococcal minimal inhibitory concentrations of chloramphenicol may exceed 12.5 μg/ml (21) (difficult to obtain in purulent CSF) and chlor-

amphenicol is less rapidly bactericidal and efficacious in experimental pneumococcal meningitis (W. M. Scheld, R. S. Brown, Jr., D. D. Fletcher, and M. A. Sande. Clin Res. 27:355A, 1979), cefoperazone (or similar agents) may be useful in selected penicillin allergic patients with this disease, provided a low frequency of cross-allergenicity is noted and pending the results of further studies.

The recent emergence of ampicillin-resistant *H. influenzae* isolates (including meningitis cases) in the United States (7) and elsewhere dictates the inclusion of chloramphenicol for serious infections caused by these strains. Although chloramphenicol is bactericidal for these isolates, cefoperazone was much more rapidly bactericidal in vivo in the experiments reported here (Table 2). Although chloramphenicol was only slowly bactericidal against ampicillin-resistant *H. influenzae* in vivo in this study, this agent is curative in humans, and this discrepancy remains unexplained. Cefamandole has been used to treat serious *H. influenzae* infections, but the in vitro activity is poor (minimal inhibitory concentration, ≥128 μg/ml for some strains) (5) and failures in meningitis have been reported (28). Due to its excellent in vitro activity (minimal inhibitory concentrations, ≤0.25 μg/ml for all strains) (3) and in vivo results as reported here, cefoperazone may be useful in these infections. Cefotaxime and moxalactam are also active in vitro against these organisms (3), but data on in vivo efficacy are lacking. An inoculum effect (e.g., increase in minimal inhibitory concentration to 64 μg/ml against 10 of 19 strains at 10^7 CFU inoculum) (30) is observed with cefoperazone against β-lactamase-positive *H. influenzae* in vitro. Since population densities of bacteria of this magnitude are routinely observed in the CSF in both experimental meningitis (as in the present experiments) and in humans (11), this may decrease the in vivo efficacy of cefoperazone, and caution on the use of cefoperazone is advised until more experience accumulates on the treatment of these infections. However, cefoperazone was rapidly bactericidal in our studies, despite CSF bacteria counts of ≥10^7 CFU/ml in 11 of 22 animals.

In addition to the favorable in vitro activity and in vivo efficacy of cefoperazone noted above, the pharmacokinetics of this new agent are an important consideration. The serum half-life of cefoperazone is longer than cefamandole in both rabbits and humans (26, 27). More importantly, the major route of elimination is biliary; no adjustments in dosage are necessary for renal insufficiency (6), a common occurrence in seriously ill patients, including those with meningitis.

Cefoperazone (and other new β-lactam agents) deserves further evaluation for use against bacterial meningitis in humans.

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