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Comparison of Cefamandole, Cephalothin, Ampicillin, and Chloramphenicol in Experimental *Escherichia coli* Meningitis

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The activities of cefamandole, cephalothin, ampicillin, and chloramphenicol were compared in fulminant and temperate *Escherichia coli* meningitis in rabbits. Intensive dosing schedules were employed to achieve maximal therapeutic benefits with short-term treatment. In an 8-h schedule chloramphenicol was significantly more effective in sterilizing the cerebrospinal fluid and curing both fulminant and temperate infections than cefamandole or ampicillin. Cephalothin was without effect in fulminant meningitis. Cefamandole and ampicillin were equivalent in activity in this and longer (12- and 24-hr) treatment schedules. The therapeutic benefits of chloramphenicol were purchased via use of doses above those generally regarded as safe for human use. The mean serum, cerebrospinal fluid, and brain concentrations of chloramphenicol, cefamandole, and ampicillin were significantly greater in rabbits with fulminant meningitis than in those with temperate meningitis. The difference was of such magnitude as to support the need to monitor drug concentrations.

Even with the best of the existing therapeutic regimens, meningitis of gram-negative etiology is still characterized by prolonged culture positivity and high morbidity and mortality (10). The high bacterial population in such infections may contribute to poor therapeutic results (3). There is clearly a need for more effective drugs and treatment schedules.

In current studies, the activity of cefamandole, a relatively new cephalosporin antibiotic, was compared with the activities of chloramphenicol and ampicillin against both fulminant and temperate *Escherichia coli* meningitis in rabbits. Cephalothin, a cephalosporin with very limited clinical efficacy, was included in this study for drug contrast purposes. A wide range of doses and treatment periods ranging from 8 to 24 h was employed in these studies.

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MATERIALS AND METHODS

Both male and female adult New Zealand white rabbits, ranging from 2.0 to 3.5 kg in weight, were used in these studies. Animals were restrained by physical means during infusion, thereby avoiding the effects of barbiturate "restraint" on choroidal cerebrospinal fluid (CSF) production (2). Space was sufficient for the animals to lie or to stand. They were tethered by 2 feet (ca. 0.6 m) of plastic tubing connecting a butterfly needle, which cannulated a marginal ear vein, to a Harvard infusion/withdrawal pump (model 940; Harvard Apparatus, Millis, Mass.). Untreated controls had unlimited access to food and water; intakes were negligible after inoculation. Treated animals had access to water but no food. Except for the modifications summarized below, methods of procedure were those detailed previously (1).

E. coli C13NK1, isolated from a neonate with meningitis, was provided for this study by George McCracken, Jr., University of Texas Health Science Center, Dallas. After overnight growth on Mueller-Hinton (MH) agar (Difco), a suspension of organisms in phosphate-buffered saline was prepared and adjusted with the help of nephelometry (Coleman 6A Spectrophotometer; Coleman Instruments, Maywood, Ill.) to a density of 7.0×10^7 to 8.0×10^7 colony-forming units (CFU)/ml. A cisternal puncture was performed with a 20-gauge needle and a 1.0-ml tuberculin syringe; 0.5 ml of CSF was withdrawn, and 0.5 ml of the bacterial suspension was injected. To produce fulminant infections, 0.5-ml quantities of undiluted suspension were injected intracisternally; temperate infections were induced by injecting 0.5 ml of a 1:100 dilution. If visible blood contamination resulted, the animal was not included in the study. Gentle reaspiration and injection were performed to insure intracisternal inoculation. The needle was withdrawn, and the animal was returned to its cage for 14 h.

Untreated controls were included every other week to insure constancy of virulence (11). Treated animals received an antibiotic in groups of four. Antibiotics were chosen in a nonrandom sequence before therapy. At the completion of therapy, rabbits with fulminant meningitis were sacrificed by an overdose of pentobarbital, at which time serum, CSF, and brain were obtained for assay. Pairs of treated rabbits with temperate meningitis were either sacrificed or anesthetized

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with sodium pentobarbital at the conclusion of therapy. Serum, CSF, and brain were obtained from the group sacrificed; blood and CSF were obtained from the anesthetized group, which was kept under observation for an additional 72 h, then sacrificed for analysis.

A portion of aspirated CSF was used for immediate cell count and quantitative determination of bacterial population via incubation for 24 h on MH agar of 0.1-ml samples of serial 10-fold dilutions in phosphate-buffered saline. The balance of the sample was quick-frozen in a Dry Ice-alcohol bath and stored at −20°C for analysis of drug content.

Concentrations of cefamandole, cephalothin, and ampicillin were determined by means of an agar-well diffusion technique with a standard curve on each plate. When serum levels exceeded the highest concentration of the standard, dilutions were made in normal rabbit serum until the zone of inhibition of the unknown fell within the limits of the standard curve. This zone was then plotted against the logarithm of the antibiotic concentration, and the results were used to generate a computer program for the calculation of unknowns by the method of least squares. Unknowns were assayed in duplicate, and zone sizes were measured in triplicate.

CSF and brain tissue were corrected for contamination with blood by measurement of the hemoglobin concentration after the method of Lowry and Hastings (9) and a calculation utilizing hematocrit. For the assay of brain and CSF antibiotic levels, the culture of *Bacillus subtilis* used for serum assays was diluted 1:20 to reduce the limits of detectability below 1 μg/ml (1).

Chloramphenicol levels in serum, CSF, and brain tissue homogenate were determined by reversed-phase, high-performance liquid chromatography. After preliminary extraction of 0.1 ml of the sample with acetate containing an internal standard, chromatography was performed with a reversed-phase C18 microparticulate column with an acetonitrile-acetate buffer mobile phase, and detection was measured by ultraviolet absorbance at 270 nm (6).

Minimal inhibitory (MIC) and minimal bactericidal concentrations (MBC) of each antibiotic for the C13NK1 strain of *E. coli*, determined by tube dilution in MH broth using 10^5 CFU/ml, were, respectively, 0.5 and 2.0 μg/ml for cefamandole, 0.5 and 2.0 μg/ml for ampicillin, 2.0 and 2.0 μg/ml for cephalothin, and 0.5 and 400 μg/ml for chloramphenicol (14).

Statistical analysis of significance was assessed by chi-squared technique (with Yates' correction for small sample) or by Student's *t* test where indicated.

RESULTS

Characteristics of untreated infections. The reactions of rabbits inoculated intracisternally with 3.5×10^7 to 4.0×10^7 CFU included diarrhea, conjunctivitis, rhinorrhea, excitability, seizures, nuchal rigidity or opisthotonos, hyperpnea, vasodilatation of ear veins, and preterminal vasoconstriction of ear veins. The clinical course was fulminating and fatal; the mean survival of 13 untreated controls was 22.8 h (range, 16 to 84 h). Leukocyte count in terminal specimens of CSF averaged 29.4×10^3 per ml (range 5.0×10^3 to 115×10^3), with more than 95% polymorphonuclear leukocytes. The numbers of *E. coli* in CSF ranged from 10^{10} to 10^{11} CFU/ml. At 14 h after infection (before start of therapy) there was 20% mortality; there was a mean of 2.6×10^6 CFU/ml of CSF (range, 1.0×10^5 to 3.0×10^7) among survivors.

The clinical characteristics of untreated infections in rabbits inoculated with 3.5×10^5 to 4.0×10^5 CFU were similar to those of fulminant meningitis, except that they developed less rapidly. Such infections were uniformly fatal (seven rabbits), with survival ranging from 32 to 140 h (mean, 61 h). At 14 h after infection (before initiation of therapy), *E. coli* in CSF ranged from 2.0×10^2 to 1.0×10^5 CFU/ml. Leukocyte numbers and bacterial populations in CSF at death were similar to those found terminally in rabbits with fulminant meningitis.

Reponses to treatment. (i) Fulminant meningitis. Initially, the various drugs were infused at rates aimed at producing concentrations in serum similar to those commonly reported in humans. For example, two uninfected control animals infused with chloramphenicol (30 mg/kg per h) had serum concentrations of 15.8, 16.0, 15.2, and 15.4 μg/ml and 16.8, 13.2, 12.0, and 14.0 μg/ml at 1, 2, 3, and 4 h. At 4 h, CSF concentrations were 1.6 and 2.7 μg/ml, respectively, and brain levels were less than 1.0 μg/ml in both animals. Treatment of infected animals at these rates for 8 h (the lowest shown in Table 1) resulted in significant reductions of colony counts for chloramphenicol (−5.9 logs), cefamandole (−5.7 logs), and ampicillin (−3.9 logs), but not cephalothin (−0.3 logs), compared to untreated controls. However, CSF was not sterilized in any animal so treated.

Doubling the infusion rate of chloramphenicol yielded sterile CSF in two of three animals treated for 8 h. Doubling again produced sterile CSF in four of four animals. Although CSF and brain tissue concentrations never exceeded the MBC of the infecting organism, a progressive antibacterial effect was noted as central nervous system concentrations surpassed the MIC.

Progressive doubling of infusion rates of cefamandole up to 120 mg/kg per h, and of ampicillin up to 80 mg/kg per h, resulted in additional antibacterial effect over results obtained at rates producing clinically relevant serum concentrations, but did not effect bacteriological cure (Table 1). Further increments in dosing schedules did not enhance the antibiotic effects. Cefamandole infusions at 120 mg/kg per h were extended through 12 and 24 h. The mean number of surviving organisms continued to de-

TABLE 1. *Antibiotic treatment, fulminant meningitis*

Therapeutic regimen			No. of rabbits	Drug mean (range) concn in:			Bacteriological status	
Drug[a]	Infusion			Serum (μg/ml)	CSF (μg/ml)	Brain (μg/g)	Negative (no. of animals)	Positive (mean log, no. of organisms [CFU/ml])
	Dose (mg/kg per h)	Duration (h)						
CA	30	8	4	13 (11–29)	2.1 (1.6–2.8)	2.0 (1.6–2.4)	0	4.6
	60	8	3	33 (28–39)	4.6 (2.4–6.2)	4.9 (4.3–5.5)	2	3.0
	120	8	4	239 (162–346)	66 (26–107)	51 (22–97)	4	
CEF	30	8	4	32 (22–39)	3.1 (0.2–5.0)	3.4 (0.1–4.0)	0	4.8
	120	8	4	253 (134–362)	32 (5–76)	39 (16–74)	0	3.1
	120	12	5	288 (119–558)	12 (5–16)	7.3 (4.5–9.3)	1	2.9
	120	24	5	485 (121–878)	18 (5–67)	19 (13–28)	1	2.2
	240	8	5	1,194 (381–2,816)	181 (18–431)	67 (20–151)	1	3.3
AMP	20	8	4	30 (26–38)	2.0 (0.2–2.4)	1.3 (0.3–1.9)	0	6.6
	80	8	4	622 (501–713)	55 (19–76)	75 (44–131)	1	2.3
	80	12	5	350 (305–386)	8.7 (3.8–12)	6.7 (1.2–14)	1	3.1
	80	24	5	395 (140–538)	5.4 (3.7–8.3)	17 (11–24)	2	2.4
	160	8	5	818 (695–1,003)	61 (13–140)	51 (25–107)	1	2.3
CEPH	20	8	4	22 (10–27)	<0.2	<0.2	0	10.1
	80	8	4	412 (189–633)	0.6 (<0.2–1.3)	3.2 (<0.2–8.3)	0	9.9

[a] CA, Chloramphenicol; CEF, cefamandole; AMP, ampicillin; CEPH, cephalothin.

crease, although sterilization of CSF was found in only 20% of animals. Similar results were obtained with ampicillin, 80 mg/kg per h, infused for 12 and 24 h.

Infusion of cephalothin at 80 mg/kg per h for 8 h produced mean serum concentrations in excess of 400 μg/ml. Simultaneous CSF and brain concentrations were less than 1% of these values, and there was negligible antibiotic effect.

Short-term therapeutic success as measured by sterile CSF was not correlated with CSF or brain concentration of antibiotic or with central nervous system drug concentration in excess of the MIC of the infecting strain. For example, after infusion of cefamandole (120 mg/kg per h) for 12 or 24 h, mean CSF concentration among the three animals with sterile CSF was 8.8 μg/ml (range, 4.3 to 15.5); mean brain concentration was 12.7 μg/g (range, 7.2 to 18.0). Mean CSF and brain concentrations of cefamandole among seven animals with positive cultures were 17.8 μg/ml (range, 4.5 to 67.3) and 13.1 μg/mg (range, 4.5 to 27.6). Finally, chloramphenicol infused at 60 and 120 mg/kg per h was significantly more efficacious in rapidly sterilizing CSF than either cefamandole infused at 120 and 240 mg/kg per h ($\chi^2 = 8.18$; $P < 0.001$) or ampicillin at 80 and 160 mg/kg per h ($\chi^2 = 5.16$; $P < 0.05$).

(ii) Temperate meningitis. Antibacterial effects were again enhanced by increasing infusion rates (Table 2). Chloramphenicol at 120 mg/kg per h sterilized CSF within 8 h in four of four animals, compared to none of four treated with 30 mg/kg per h. Similarly, the mean numbers of surviving organisms were reduced by increasing cefamandole from 30 to 120 mg/kg per h and ampicillin from 20 to 80 mg/kg per h. Two of three animals treated with cefamandole (120 mg/kg per h) and two of two treated with ampicillin (80 mg/kg per h) had sterile CSF after 24 h of therapy. The treatment failure in this group had cefamandole concentrations in serum (326 μg/ml), CSF (71 μg/ml), and brain (26 μg/g) far in excess of the MIC of the infecting organism.

Comparison of concentrations of antibiotics after 8 h of therapy at high rates of infusion in these two models revealed significant differences in the mean values. Thus, chloramphenicol serum ($t = 4.28$; $P < 0.01$), CSF ($t = 2.90$; $P < 0.05$), and brain ($t = 2.49$; $P < 0.05$) levels were greater in fulminant meningitis. Mean cefamandole serum ($t = 2.86$; $P < 0.05$) and brain ($t = 3.45$; $P < 0.02$) but not CSF ($t = 0.82$; $P > 0.05$) levels and ampicillin serum ($t = 11.3$; $P < 0.001$), CSF ($t = 4.0$; $P < 0.01$) and brain ($t = 3.48$; $P < 0.02$) concentrations were also significantly greater in fulminant meningitis. Infusion rates producing serum levels within the range com-

TABLE 2. *Antibiotic treatment, temperate meningitis*

Therapeutic regimen				Drug mean (range) concn in:			Bacteriological status	
Drug[a]	Infusion: Dose (mg/kg per h)	Infusion: Duration (h)	No. of rabbits	Serum (μg/ml)	CSF (μg/ml)	Brain (μg/g)	Negative (no. of animals)	Positive (mean log no. of organisms [CFU/ml])
CA	30	8	4	15 (13–23)	1.4 (0.7–2.9)	2.7 (2.4–3.1)	0	1.6
	120	8	4	63 (56–72)	15 (6–33)	8.6 (3.1–15)	4	
CEF	30	8	4	22 (80–120)	0.6 (0.4–0.7)	0.9 (0.5–1.2)	0	3.9
	120	8	4	91 (53–143)	16 (5–31)	4.5 (3.7–5.8)	1	2.7
	120	24	3	243 (200–301)	35 (13–71)	13 (5–26)	2	6.0
AMP	20	8	4	12 (10–20)	1.7 (1.3–2.1)	0.7 (0.5–1.1)	0	3.6
	80	8	4	74 (37–116)	14 (9–19)	8.2 (5.7–11)	0	2.8
	80	24	2	34 (30–39)	3.3 (2..1–4.5)	2.8 (2.3–3.3)	2	

[a] CA, Chloramphenicol; CEF, cefamandole; AMP, ampicillin.

monly reported in humans or infusions conducted over longer time intervals were not associated with a significant change in body fluid concentrations in fulminant compared to temperate meningitis.

Therapeutic response: temperate meningitis. Four animals were treated for 8 h and two animals were treated for 24 h with chloramphenicol at 60 mg/kg per h. Sterilization of CSF at the conclusion of therapy did not insure bacteriological cure; one animal treated for 8 h and one animal treated for 24 h relapsed. On the other hand, two animals with positive cultures (mean, 3.6×10^1 CFU/ml) after 8 h of therapy were clinically well and had sterile CSF 72 h later. Finally, one animal remained culture positive (2.6×10^2 CFU/ml) both after 24 h of therapy and 72 h later.

Three of four animals treated with chloramphenicol at 120 mg/kg per h for 8 h and four of four treated for 24 h had sterile CSF at the end of the infusion. However, two of the three animals in the former group relapsed, whereas all four animals in the latter group had sterile CSF 72 h after therapy. Results of both treatment regimens were pooled and are presented in Table 3.

Treatment with cefamandole at 120 mg/kg per h resulted in sterilization of CSF in two of four and three of four animals after 8 and 24 h of therapy, respectively. The bacteriological status of the animal at 72 h reflected the findings at the conclusion of therapy. Ampicillin at 80 mg/kg per h failed to sterilize CSF after 8 h, but three of four animals so treated progressed to bacteriological cure. All four animals treated for 24 h maintained sterile CSF through 72 h of observation.

In only one animal (receiving chloramphenicol 60 mg/kg per h for 8 h) was antibiotic measurable in CSF 72 h after treatment. In addition, judgement of clinical status on follow-up correlated well with culture results. Thus, only one animal thought to be infected proved to have a negative culture; however, the CSF leukocyte count was 74,250 per ml. All animals judged clinically well had sterile CSF.

DISCUSSION

McCracken has found in clinical *E. coli* meningitis that rapid sterilization of CSF correlates with improved morbidity and mortality rates (10). One potential maneuver to accomplish rapid sterilization is administration of progressively larger quantities of antibiotics. Data from these experiments suggest that an advantage may be gained, up to a certain point, by undertaking this course. Thus, chloramphenicol, cefamandole, and ampicillin all exerted greater antibiotic effect when administered in larger doses. However, infusion of cefamandole, 240 mg/kg per h, and ampicillin, 160 mg/kg per h, did not significantly reduce colony counts when compared to infusions at one-half these rates. Moreover, cephalothin failed to penetrate the central nervous system despite infusion at 80 mg/kg per h; both CSF and brain concentrations remained below the MIC of the infecting organism. These data further support the lack of efficacy of cephalothin in treatment of meningitis (4).

In the current studies chloramphenicol proved significantly more effective in achieving rapid sterilization of CSF than either cefamandole or ampicillin. However, because of chloramphenicol's limited therapeutic index, such dosing is not clinically practical. We also found that central nervous system concentrations of antibiotic in excess of the MIC rather than the MBC were associated with antibacterial effect in vivo. This finding is supported by additional data from experimental meningitis studies of Beam (T.R. Beam, Jr., Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 11th, Boston, Mass., abstr. no. 751, 1979) and Perfect et al. (J.R.

TABLE 3. *Therapeutic response, temperate meningitis*

Drug[a]	Bacteriological status at:				
	End of infusion		72 hr after therapy		
	Culture	No. of animals	Cure	Relapse	Persistence
CA	Sterile	9	7	2	0
	Nonsterile	4	2	0	2
CEF	Sterile	5	5	0	0
	Nonsterile	3	0	0	3
AMP	Sterile	4	4	0	0
	Nonsterile	4	3	0	1

[a] CA, Chloramphenicol; CEF, cefamandole; AMP, ampicillin.

Perfect, S.D.R. Lang, and D.T. Durack, Clin. Res. 27:353A, 1979).

Scheld et al. have emphasized the problem of relapse associated with chloramphenicol therapy (W.M. Scheld, R.S. Brown, Jr., D.D. Fletcher, and M.A. Sande, Clin. Res. 27:355A, 1979). Our findings support the concept that central nervous system concentrations less than the MBC of the infecting strain may be insufficient to guarantee therapeutic success. However, chloramphenicol therapy, which was continued for 24 h and which produced CSF sterilization, was not associated with relapse.

We did not find chloramphenicol accumulation in brain tissues as previously reported (8). Differences in methods may account for this disparity. Kramer et al. administered a single intramuscular injection of chloramphenicol and reported simultaneous serum and tissue concentrations with a variable lag phase between drug administration and tissue sampling. Steady-state kinetics employed in the current study demonstrated that a blood-brain barrier is present for this agent.

The therapeutic index of cefamandole has not been established. Drug failures in therapy of *H. influenzae* meningitis have been reported (12). Although previous clinical (5) and experimental (13) studies suggest that further clinical evaluation may be warranted, a cause for concern is the unexplained failure of cefamandole therapy after 24 h of infusion, with simultaneous CSF and brain concentrations 35- and 13-fold in excess of the MBC in one animal, and a cure rate of 62.5% in the current experiments.

Two features of these models must be emphasized. First, drug persistence, particularly at high infusion rates, may account for eradication of bacteria after conclusion of therapy. Second, host defenses in the rabbit may assist in clearing small numbers of residual bacteria. Spontaneous resolution of infection in rabbits is dependent upon the infecting inoculum (1; Beam, 11th ICAAC, abstr. no. 751). Therefore, extrapolation of these data to the clinical setting cannot be advised.

Finally, there were significant differences between mean serum, CSF, and brain concentrations in animals having malignant and temperate meningitis when treated at the same antibiotic infusion rate for 8 h. Although no toxicity was noted in the experimental animals, altered distribution of these antibiotics is suggested, possibly as a result of decreased hepatic metabolism (chloramphenicol) and renal clearance (cefamandole, ampicillin). These results emphasize the potential importance of monitoring antibiotic concentrations in septic patients, particularly an agent such as chloramphenicol (7). Return of antibiotic concentrations to expected levels after infusions of 12 or 24 h may reflect improved clinical status of the animals and restoration of normal kinetics.

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