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Comparison of Cotrimoxazole, Ampicillin, and Chloramphenicol in Treatment of Experimental *Haemophilus influenzae* Type B Meningitis

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To evaluate cotrimoxazole in the treatment of bacterial meningitis, we compared its action with that of ampicillin and chloramphenicol in experimental *Haemophilus influenzae* type b meningitis. Both trimethoprim and sulfamethoxazole penetrated well into the cerebrospinal fluid of infected rabbits, reaching 40 and 26%, respectively, of their simultaneous serum levels. Levels measured 30 and 60 min after intravenous injection exceeded the minimum inhibitory concentration of this combination for *H. influenzae* by 10- to 100-fold. The mean ratio of trimethoprim to sulfamethoxazole in cerebrospinal fluid was 1:22. Cotrimoxazole was as effective as ampicillin in therapy of β-lactamase-negative *H. influenzae* meningitis and as effective as chloramphenicol for a β-lactamase positive strain. These findings corroborate favorable preliminary clinical experience reported by others and indicate that cotrimoxazole deserves further study in the therapy of bacterial meningitis.

Haemophilus influenzae type b is the most common cause of bacterial meningitis in early childhood and an occasional but increasingly frequent cause of this disease in older children and adults (15). Mortality ranges from 2 to 10% (21), and survivors may suffer from permanent neurological sequelae (29).

Clinical isolates of *H. influenzae* type b were uniformly susceptible to ampicillin until 1974, when cases of meningitis caused by ampicillin-resistant *H. influenzae* type b were first recognized (7, 9, 30). Because β-lactamase-producing strains presently account for 5 to 18% of the cases of *H. influenzae* type b meningitis and bacteremia in the United States (8), most clinicians now routinely include chloramphenicol in their initial treatment of children with bacterial meningitis (1). This therapeutic approach generally has proved successful. Ampicillin and chloramphenicol have additive or even synergistic activity against many strains of *H. influenzae* type b (12). However, it should be noted that for pneumococci this combination may be antagonistic, both in vitro and in experimental animals (13, 31). Mathies et al. (25) reported that clinical outcome of bacterial meningitis was somewhat worse in patients treated with combined ampicillin, chloramphenicol, and streptomycin than in patients treated with ampicillin alone. Other potential disadvantages of combining ampicillin and chloramphenicol include chloramphenicol toxicity, the occasional occurrence of chloramphenicol-resistant strains (17, 23), and the recent observation that both ampicillin and chloramphenicol resistance may occur simultaneously and be transmissible from one strain of *H. influenzae* to another (5).

These considerations encouraged us to study alternative regimens for treatment of bacterial meningitis. Here we report a favorable comparison of cotrimoxazole (trimethoprim plus sulfamethoxazole) versus ampicillin or chloramphenicol in the therapy of experimental *H. influenzae* type b meningitis.

MATERIALS AND METHODS

Organisms. We used two strains of *H. influenzae* type b. The Eagan strain, kindly supplied by R. E. Moxon, is β-lactamase negative. A second strain, isolated from the cerebrospinal fluid (CSF) of a child at Duke University Medical Center, was determined to be β-lactamase positive by the standard acidometric method.

These strains were stored at −70°C in vials containing equal parts of skimmed milk (Difco Laboratories) and 0.015 M phosphate-buffered saline, pH 7.4 (PBS). Three days before inoculation, this suspension was thawed and streaked on GC medium agar (Difco) supplemented with 1% hemoglobin (Difco) and Isovitalex (BBL Microbiology Systems). After incubation for 2 days at 37°C in air, organisms were subcultured onto the same medium, incubated for 6 h, taken up on sterile cotton swabs, and suspended in PBS at an optical density (OD) of 0.3 at 540 nm. This suspension contained 5×10^9 colony-forming units (CFU) per ml. The minimum inhibitory concentrations (MICs) of ampicillin and of chloramphenicol for each strain were determined by tube dilution, using a final concentra-

tion of 10^4 CFU/ml in Schaedler broth (BBL) supplemented with 5% Fildes reagent. For controls, we used ampicillin-sensitive *H. influenzae* CDC 77-62541, β-lactamase-producing strain CDC 77-3353, and *Escherichia coli* ATCC 25922. After 24 h of incubation in air at 35°C, tubes were inspected for visible growth to determine the MIC. Minimal bactericidal concentrations (MBCs) were measured by subculture onto chocolate agar, using a 1:100 standard loop. After incubation for 48 h the MBC was read as the lowest concentration that permitted growth of five or fewer colonies. MICs and MBCs of trimethoprim plus sulfamethoxazole for both strains were kindly performed by Lynn Elwell at Burroughs Wellcome Research Laboratories, Research Triangle Park, N.C.

Production of meningitis. We injected 0.2 ml containing 1×10^9 CFU of *H. influenzae* type b intracisternally into male New Zealand White rabbits weighing 2 to 3 kg which had been sedated with Innovar (McNeil Laboratories, Irvine, Calif.) at 0.3 ml/kg intramuscularly. Six hours after injection of *H. influenzae* type b, the rabbits were severely ill, and antimicrobial therapy was begun. CSF was withdrawn from infected, treated rabbits and infected, untreated controls at 6, 12, and 18 h after inoculation of *H. influenzae* type b. The experiment was terminated at 18 h.

Administration of antimicrobial agents. Ampicillin (Wyeth Laboratories) at 100 mg/kg or chloramphenicol (Parke-Davis) at 25 mg/kg, or a parenteral preparation of cotrimoxazole (Burroughs Wellcome) at 10 mg of trimethoprim and 50 mg of sulfamethoxazole per kg were injected into a marginal ear vein over a period of 15 s, 6 h after inoculation of *H. influenzae* b. A second dose was given 6 h later.

Antimicrobial assay and quantitative cultures. Paired serum and CSF samples were drawn from infected rabbits 30 and 60 min after beginning treatment. In the case of cotrimoxazole, paired samples were also obtained from rabbits without meningitis to measure penetration of both components into normal CSF. Occasional CSF samples that were significantly contaminated by blood (indicated by >60,000 erythrocytes per ml) were excluded. The ampicillin assay was performed by the agar diffusion method of Bennett and King (2), the chloramphenicol assay was performed by an enzymatic method (22), the trimethoprim assay was by agar diffusion using *Bacillus pumilus* WRL-CN607 as the test organism (6), and the sulfamethoxazole assay was by the Bratton-Marshall method (4).

For quantitative culture of CSF, 0.1-ml portions from serial 10-fold dilutions in PBS were spread on chocolate agar plates, and colonies were counted after overnight incubation at 35°C. The reduction in viable count of *H. influenzae* type b after 12 h of treatment was calculated for each animal by reference to the count of *H. influenzae* type b in its CSF immediately before treatment was started.

Statistical methods. Individual treatment groups were compared by the two-tailed *t*-test for unpaired means.

RESULTS

Table 1 lists the MICs and MBCs of ampicillin, chloramphenicol, and cotrimoxazole for the β-lactamase-negative and β-lactamase-positive strains of *H. influenzae* type b.

Although trimethoprim and sulfamethoxazole were synergistic for both strains of *H. influenzae* type b, this combination was bacteriostatic rather than bactericidal at concentrations achievable in vivo. Chloramphenicol was bactericidal for both strains at 1 μg/ml. Ampicillin was bactericidal at 0.5 μg/ml for the Eagan strain, whereas the β-lactamase-producing strain was ampicillin resistant.

The levels of ampicillin, chloramphenicol, trimethoprim, and sulfamethoxazole in serum and in the CSF of rabbits with meningitis at 30 and 60 min are shown in Table 2. Penetration into CSF was 15% for ampicillin, 65% for chloramphenicol, 40% for trimethoprim and 26% for sulfamethoxazole. The mean ratio of trimethoprim to sulfamethoxazole in CSF for all determinations was 1:22 (range, 1:13 to 1:34). In three uninfected rabbits without inflamed meninges, the mean concentrations of trimethoprim and sulfamethoxazole in CSF 30 to 60 minutes after intravenous injection of cotrimoxazole were 0.38 μg/ml (16% of the corresponding serum level) and 13.3 μg/ml (21% of the corresponding serum level), respectively.

Table 3 shows the mean reduction in the

TABLE 1. *MICs and MBCs for the experimental strains of H. influenzae type b*

Antibiotic	MIC (μg/ml)		MBC (μg/ml)	
	β-lactamase negative	β-lactamase positive	β-lactamase negative	β-lactamase positive
Ampicillin	0.5	8	0.5	16
Chloramphenicol	0.5	0.5	1	1
Trimethoprim	0.15	0.15	>50	>50
Sulfamethoxazole	9.5	9.5	>950	>950
Trimethoprim/ sulfamethoxazole	0.05/0.95	0.05/0.95	>5/95[a]	>5/95[a]

[a] Bacteriostatic only at 5 μg of trimethoprim per ml and 95 μg of sulfamethoxazole per ml.

TABLE 2. *CSF and serum levels in rabbits with meningitis*

Antibiotic	CSF at:[a]		Serum at:[a]		CSF/serum (mean % ± SE)
	30 min	60 min	30 min	60 min	
Ampicillin	1.7 ± 0.7 (8)	2.0 ± 1.5 (3)	20.5 ± 3.0 (8)	7.7 ± 0.3 (3)	14.6 ± 5.8
Chloramphenicol	1.1 ± 0.2 (4)	1.0 ± 0.5 (2)	3.6 ± 0.1 (3)	0.8 ± 0.2 (2)	64.6 ± 24
Trimethoprim	1.3 ± 0.1 (6)	0.6 ± 0.1 (5)	3.3 ± 0.4 (6)	1.5 ± 0.2 (5)	39.6 ± 2.6
Sulfamethoxazole	20.7 ± 2.0 (6)	15 ± 1.4 (4)	124.7 ± 15.1 (6)	38.4 ± 5.4 (5)	26.2 ± 4.3

[a] Each value represents mean micrograms per milliliter ± standard error. Each value in parentheses indicates number tested.

TABLE 3. *Reduction of H. influenzae type b during 12 h of treatment*

Antibiotic	Reduction[a]	
	β-lactamase negative	β-lactamase positive
Chloramphenicol	ND	8.7 ± 1.5 (4)
Ampicillin	9.2 ± 0.6 (3)	1.8 ± 1.5 (2)
	$P < 0.01$	$P < 0.03$
Cotrimoxazole	9.2 ± 0.6 (2)	8.8 ± 0.5 (3)
	$P < 0.03$	$P < 0.01$
Control	5.4 ± 0.8 (5)	No survivors

[a] Each value represents [($\log_{10}$ CFU per milliliter immediately before treatment) − ($\log_{10}$ CFU per milliliter 12 h later)] ± standard error. Each value in parentheses represents number tested.

concentrations of viable *H. influenzae* type b in CSF for both strains at the end of 12 h of treatment. For the β-lactamase-negative strain, the reduction in counts was significantly greater in animals treated with ampicillin ($P < 0.01$) or cotrimoxazole ($P < 0.03$) than in untreated controls. For the ampicillin-resistant strain, both chloramphenicol and cotrimoxazole reduced counts better than ampicillin ($P < 0.03$ and $P < 0.01$, respectively), which had little effect.

Figure 1 depicts the mean number of *H. influenzae* type b in surviving animals at 6, 12, and 18 h after inoculation of the β-lactamase-negative strain. Although the number of *H. influenzae* type b fell in control animals, the counts in treated animals were lower at both 12 h ($P < 0.01$) and 18 h ($P < 0.01$) after inoculation. The results of treatment with ampicillin and cotrimoxazole were almost identical. Ten rabbits infected with the Eagan strain survived for 18 h. Five of these had not been treated, and all had positive CSF cultures. The remaining five had been treated with either ampicillin or cotrimoxazole and all had sterile CSF ($P < 0.02$ by χ^2 analysis).

Figure 2 shows the mean number of *H. influenzae* type b in surviving animals at 6, 12, and 18 h after inoculation with the β-lactamase-positive strain. No control animals survived for 18 h, and only 2 of ten were alive 12 h after inoculation. Therefore, statistical comparisons of CSF counts between control and treated animals were not performed. The number of *H. influenzae* type b in the CSF of those rabbits treated

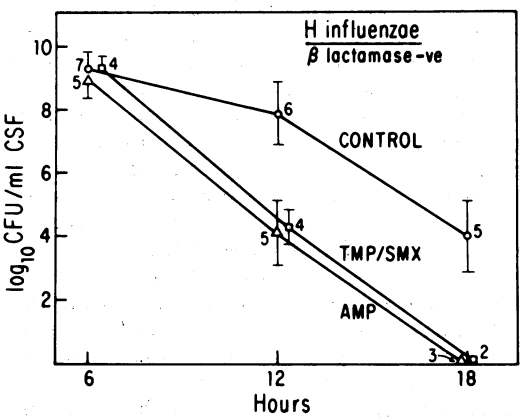

FIG. 1. *Quantitative CSF cultures from treated and untreated rabbits 6, 12, and 18 h after inoculation with β-lactamase-negative H. influenzae type b ($\log_{10}$ CFU per milliliter; mean ± standard error).*

with chloramphenicol or with cotrimoxazole were lower at 12 and 18 h than in those treated with ampicillin, which had no statistically discernible effect. The efficacy of chloramphenicol and that of cotrimoxazole were equal at 12 and 18 h after inoculation, and each was superior to ampicillin at 18 h ($P < 0.05$ and $P < 0.01$, respectively).

DISCUSSION

Cotrimoxazole possesses many properties that recommend it as a potentially valuable agent for

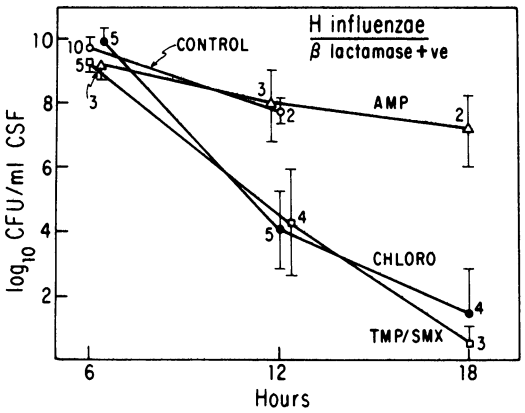

FIG. 2. *Quantitative CSF cultures from treated and untreated rabbits 6, 12, and 18 h after inoculation with β-lactamase-positive H. influenzae type b (log 10 CFU per millilter; mean ± standard error).*

treatment of central nervous system infections. It is active in vitro against most species of bacteria that cause meningitis (26, 27). Both trimethoprim and sulfamethoxazole reach relatively high levels in human CSF (10, 14, 19). The combination is relatively nontoxic, and extensive clinical experience has proven its efficacy in treatment of other bacterial infections in both children and adults. For these reasons, cotrimoxazole has already been used for treatment of meningitis in several uncontrolled case studies (3, 11, 16, 20, 28). Results were encouraging. LaFaix et al. (20) reported treatment of 844 patients with bacterial meningitis with cotrimoxazole, ampicillin, or a combination of penicillin, chloramphenicol, and sulfonamide. Clinical outcome in the 108 patients treated with cotrimoxazole was comparable to results achieved by the other regimens. Sabel and Brandberg (28) reported recovery in nine of ten patients with meningitis and septicemia who were treated with cotrimoxazole after they had failed to improve on conventional antibiotic therapy. Thus, considerable uncontrolled clinical experience has already accumulated, indicating that cotrimoxazole is effective in treatment of central nervous system infections.

Despite such experience cotrimoxazole has been little used for treatment of meningitis in the United States. This is presumably because a parenteral preparation is not generally available, and because treatment with conventional antimicrobial regimens is reasonably successful, at least for the common pathogens. However, if ampicillin and chloramphenicol resistance among *H. influenzae* type b and penicillin resistance among *S. pneumoniae* becomes more prevalent or if *Neisseria meningitidis*, like *N. gonorrhoeae*, were to develop penicillin resistance, the present satisfactory situation could change. These are potential problems in future management of meningitis due to the three most common pathogens, but treatment of gram-negative bacillary meningitis is far from satisfactory even now. With conventional antibiotic therapy, mortality and long-term morbidity are higher in gram-negative bacillary meningitis than in other forms of meningitis (24), and gram-negative bacilli have a propensity to develop resistance during treatment (Z. A. McGee, A. B. Kaiser, C. Rubens, and W. E. Farrar, Jr. Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th. New York, N. Y. Abstr. no. 4, 1977). Cotrimoxazole is of potential value in this setting. It has activity against many species of gram-negative bacilli (other than *Pseudomonas*), and its mechanism of action at separate stages of bacterial metabolism may confer some protection against the emergence of resistant strains. These considerations led us to anticipate increasing interest in cotrimoxazole for the therapy of bacterial meningitis and encouraged us to examine the efficacy of this drug in an experimental model.

In rabbits injected intracisternally with *H. influenzae* type b, both trimethoprim and sulfamethoxazole reached high concentrations in CSF in a ratio favorable for antimicrobial synergy. These CSF levels inhibited both the β-lactamase-negative and β-lactamase-positive strains of *H. influenzae* type b with equal efficacy. Cotrimoxazole was as effective as ampicillin in meningitis due to the ampicillin-sensitive strain, and as effective as chloramphenicol in β-lactamase-positive *H. influenzae* type b meningitis. Thus, in vivo efficacy of cotrimoxazole, ampicillin, and chloramphenicol correlated with the MICs and the CSF penetration of these antimicrobial agents.

It is of particular interest that trimethoprim plus sulfamethoxazole, unlike ampicillin and chloramphenicol, was not bactericidal for either of our strains of *H. influenzae* type b in vitro. Kirven and Thornsberry (18) found that cotrimoxazole could not eradicate nasopharyngeal carriage of *H. influenzae* type b from asymptomatic children unless it had bactericidal activity in vitro against the relevant strains. They further noted that cotrimoxazole was bactericidal for less than one-third of the *H. influenzae* type b isolates tested in their laboratory. Our experimental findings suggest that the clinical relevance of bactericidal activity does not extend to the treatment of *H. influenzae* type b meningitis.

Although we are unaware of any convincing clinical data showing that bactericidal agents are superior to bacteriostatic drugs in the treatment of *H. influenzae* type b meningitis, the authors of a recent experimental study on rabbits (W. M. Scheld, R. S. Brown, and D. D. Fletcher, Clin. Res. 27:355A, 1979) have suggested that bactericidal activity may be necessary for optimal therapy of pneumococcal meningitis. By using a rabbit model similar to ours, they compared the ability of ampicillin, chloramphenicol, and a combination of the two drugs to eradicate *S. pneumoniae* from cerebrospinal fluid. The bactericidal drug, ampicillin, rapidly reduced bacterial counts in the cerebrospinal fluid, and few relapses occurred. Treatment with chloramphenicol, which is bacteriostatic for *S. pneumoniae*, failed to reduce bacterial counts after 8 h, and was associated with relapse in two-thirds of the cases. The combination produced an intermediate effect. The results of our study using *H. influenzae* type b do not support the hypothesis that bactericidal drugs are superior to bacteriostatic in treatment of bacterial meningitis. Chloramphenicol was bactericidal in low concentrations for our β-lactamase-positive strain of HITB, and ampicillin was bactericidal for the non-β-lactamase-producing strain, yet neither of these antimicrobial agents eradicated *H. influenzae* type b faster than cotrimoxazole, which was bacteriostatic at the highest concentation tested. Further studies are required to define the clinical importance, if any, of using a bactericidal drug in preference to a bacteriostatic to treat bacterial meningitis in humans.

The in vitro spectrum of cotrimoxazole, its excellent CSF penetration, and the favorable results obtained in this experimental study, together with preliminary experience in humans, recommend it as an agent worthy of further investigation for treatment of bacterial meningitis.

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