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Synergistic Action of Nafcillin and Ampicillin Against Ampicillin-Resistant *Haemophilus influenzae* Type b Bacteremia and Meningitis in Infant Rats

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Infant rats with bacteremia and meningitis induced by ampicillin-resistant *Haemophilus influenzae* type b were treated with ampicillin and nafcillin, alone or in combination. Neither ampicillin alone (in 19 animals) nor nafcillin alone (in 20 animals) sterilized the blood or cerebrospinal fluid of any treated infant rat. When the combination of ampicillin and nafcillin was used, blood cultures were negative in 18 of 19 infant rats, and cerebrospinal fluid cultures were sterile in 15 of 19 when cultured 30 h after initiation of treatment. In vitro results demonstrated definite synergism between ampicillin and nafcillin against ampicillin-resistant *H. influenzae* type b. The study suggests that such synergism also exists in vivo.

We have recently reported that ampicillin and nafcillin act synergistically in vitro against ampicillin-resistant *Haemophilus influenzae* type b (10). This report is concerned with the capacity of this antibiotic combination to eradicate ampicillin-resistant *H. influenzae* type b from the blood and spinal fluid of experimentally infected infant rats.

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MATERIALS AND METHODS

The strain of *H. influenzae* type b used in this study was isolated from the blood of a child with meningitis and produced β-lactamase as demonstrated by the Escamilla assay (3). By using Mueller-Hinton broth (Difco) supplemented with 1% IsoVitaleX (BBL Microbiology Systems) and hemin (10 μg/ml), twofold serial dilutions of ampicillin and nafcillin in a checkerboard pattern were prepared. The final concentrations of the antibiotics ranged from 0.4 to 50 μg/ml. An inoculum of 5×10^4 colony-forming units (CFU) per ml was added to each broth tube to determine the minimal inhibitory concentration (MIC).

In vivo studies utilized 5-day-old Sprague-Dawley strain COBS/CD rats (Charles River Breeding Lab, Portage, Mich.). From an overnight growth of ampicillin-resistant *H. influenzae* type b on a chocolate agar plate, three colonies were inoculated into 5 ml of Mueller-Hinton broth supplemented with 1% IsoVitaleX and hemin (10 μg/ml). The tube was shaken for 4 h at 37°C. Dilutions of the growth were made to reach a final concentration of 10^5 CFU/ml. The rats were injected intraperitoneally with 0.1 ml of the bacterial suspension. Blood and cerebrospinal fluid (CSF) cultures were obtained 36 h later. Blood samples were taken from the tail vein with a 5-μl capillary pipette. CSF was obtained by percutaneous puncture of the cisterna magna with a 30-gauge needle (Becton Dickinson Co., Rutherford, N.J.). The fluid in the needle was then transferred to a 5-μl capillary pipette by insertion of the pipette tip into the hub of the needle and cultured quantitatively onto a chocolate agar plate. The lower limit of detectable bacteria was 2×10^2 organisms per ml. This method allows repetitive sampling of CSF and minimizes the problem of CSF contamination by blood.

The rats were divided into four treatment groups, as shown in Table 1. All animals received a total of five doses of antibiotics or saline intraperitoneally at 6-h intervals. Six hours after the fifth dose, samples of blood and CSF were again obtained for quantitative culture. The geometric means of the bacterial CFU per milliliter were calculated for each group. In one experiment, a sixth dose of antibiotic was administered to six rats each in groups I and II. Blood and CSF samples (5 μl each) were obtained from these animals 15, 45, 75, and 120 min after the sixth dose to determine blood and CSF concentrations of ampicillin or nafcillin. The antibiotic concentrations were measured by agar diffusion with *Sarcina lutea* as the test organism. The lowest concentration that could be measured reliably was 0.4 μg/ml. Student's *t* test was used for statistical analysis of the data obtained.

RESULTS

The ampicillin-resistant *H. influenzae* type b strain used in this study demonstrated MICs of 12.5 μg/ml for ampicillin and 12.5 μg/ml for nafcillin. However, a combination of only 0.78 μg of ampicillin per ml with 0.78 μg of nafcillin per ml was inhibitory. Other combinations were also inhibitory, for example, 1.56 μg of ampicillin with 0.4 μg of nafcillin per ml or 1.56 μg of nafcillin with 0.78 μg of ampicillin per ml.

All 65 infant rats were bacteremic 36 h after intraperitoneal inoculation of ampicillin-resistant *H. influenzae* type b; 63 of 65 animals had positive CSF cultures at that time. The two

TABLE 1. *Results of antibiotic treatment of infant rats with bacteremia and meningitis*

Antibiotic	Before antibiotic treatment			After 30 h of treatment			
	No. of animals with positive blood and CSF cultures	Mean CFU/ml in blood (range)	Mean CFU/ml in CSF (range)	No. of animals with positive blood cultures	Mean CFU/ml in blood (range)	No. of animals with positive CSF cultures	Mean CFU/ml in CSF (range)
Ampicillin (3.125 mg/kg)	19/19	3.8×10^4 (1.2×10^4–1×10^5)	3.7×10^5 (5×10^4–1×10^6)	19/19	1.2×10^4 (6×10^3–5×10^4)	19/19	1.1×10^5 (1.8×10^4–1×10^6)
Nafcillin (50 mg/kg)	20/20	3.2×10^4 (1×10^4–1×10^5)	3.7×10^5 (8×10^4–1×10^6)	20/20	7.5×10^4 (8×10^3–1×10^6)	20/20	3.4×10^5 (1×10^4–1×10^6)
Ampicillin (3.125 mg/kg) + nafcillin (50 mg/kg)	19/19	2.9×10^4 (1×10^4–1×10^5)	4.4×10^5 (5×10^4–1×10^6)	1/19	2.2×10^2	4/19	8×10^2 (2×10^2–1.7×10^3)
Saline	5/5	2.4×10^4 (1×10^4–6×10^4)	2×10^5 (4×10^4–5.2×10^5)	5/5	3.6×10^4 (2×10^4–6×10^4)	5/5	2.4×10^5 (3.8×10^4–6×10^5)

animals with sterile CSF were excluded from Table 1. There was no significant difference in blood or CSF CFU per milliliter among the groups before therapy. None of the rats receiving ampicillin (group I) or nafcillin (group II) in the doses indicated in the table, nor those receiving saline (group IV), had sterile blood or CSF cultures at 30 h. In contrast, 18 of 19 animals in group III had sterile blood cultures. There was a two-log reduction in CFU per milliliter (from 3.4×10^4 to 2.0×10^2/ml) in the only animal with a positive blood culture after therapy. Post-treatment CSF cultures in 15 of 19 rats in group III were sterile. In the four rats with positive cultures, there was a decline from the mean of 5.5×10^5 to 8×10^2 CFU/ml, a three-log decrease. By Student's t test, the numbers of CFU in blood and CSF in groups I and II were not significantly different from each other or from the numbers in saline controls (group IV). The numbers of CFU per milliliter of these fluids were significantly lower than those in groups I and II ($P < 0.001$).

Blood and CSF levels of ampicillin (after 3.125 mg/kg per dose) and nafcillin (after 50 mg/kg per dose) are shown in Fig. 1. The peak CSF level of ampicillin was 0.82 ± 0.1 μg/ml (range,

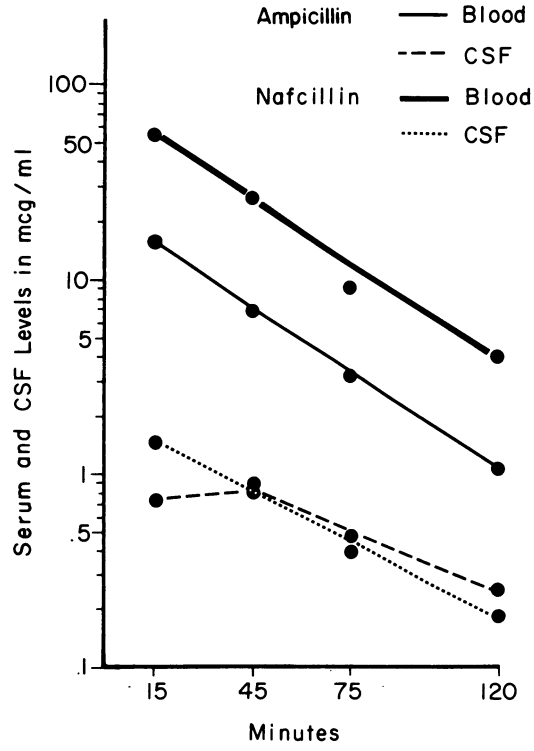

FIG. 1. *Serum and CSF concentrations of ampicillin and nafcillin in infant rats after meningitis.*

0.7 to 0.9 μg/ml), and the peak blood level was 15.2 ± 3.4 μg/ml (range, 12.3 to 21.0 μg/ml). Peak CSF concentrations were 5 to 7% of peak serum levels. The peak CSF level of nafcillin was 1.45 ± 0.53 μg/ml (range, 1.0 to 2.4 μg/ml), and the peak blood level was 55.30 ± 9.13 μg/ml (range, 43 to 67 μg/ml); peak CSF concentrations were approximately 2.5 to 3% those of serum levels.

DISCUSSION

The antibiotic dosages applied in the present study were chosen to simulate blood levels encountered in children treated with conventional dosages. Average peak ampicillin blood levels were reported to range from 6.4 to 38 μg/ml after a 30- to 35-mg/kg dose given intravenously every 6 h in children (8). In comparison, the average peak blood level of infant rats in this study was 15.2 μg/ml (range, 12.3 to 21.0 μg/ml) after intraperitoneal injection of 3.125 mg/kg. The average reported peak blood level of nafcillin in children after intravenous administration of 37.5 mg/kg is 50 μg/ml (4). Such a blood level was attained in infant rats, with administrations of 50 mg/kg every 6 h. At such blood levels, neither ampicillin nor nafcillin alone effected sterilization of blood or CSF in the rat model. Although peak ampicillin blood levels were equal to or exceeded the in vitro MIC of the ampicillin-resistant *H. influenzae* type b, there was minimal reduction in the concentration of viable bacteria in the blood of treated animals in group I. In group II, despite nafcillin levels in the blood which were fourfold greater than the in vitro MIC for the organism, no inhibition was noticed. The reasons for these poor responses are unknown.

In contrast, the same doses of ampicillin and nafcillin in combination were very effective, leading to eradication of the bacteria from the blood in 18 of 19 infant rats within 30 h. In addition, despite CSF antibiotic levels which were well below the MICs of the organism for each antibiotic alone, the combination of drugs eradicated the bacteria in 15 of 19 animals and was associated with significant decrease in concentration of organisms in the remaining four animals.

Previous reports suggested that ampicillin in high dosage (100 mg/kg) was occasionally effective in sterilizing blood and CSF in infant rats with bacteremia and meningitis caused by ampicillin-resistant *H. influenzae* type b (6). When we used ampicillin alone in a dose of 12.5 mg/kg every 6 h (data not shown), we confirmed this observation. However, at a dosage which produces blood levels comparable to those achieved in humans (3.125 mg/kg every 6 h), ampicillin alone failed to eradicate the bacteria.

The present study provided evidence of in vitro synergism between ampicillin and nafcillin against a strain of *H. influenzae* which produced β-lactamase, as was shown previously by us (10) and by others (1). The results in the infant rat model suggest that the combination of ampicillin and nafcillin is also synergistic in vivo.

Several authors recently recommended combination therapy with ampicillin and a penicillinase-resistant penicillin for childhood infections likely to be due to *H. influenzae* type b such as septic arthritis (5, 7) or acute orbital (9) and periorbital (2) cellulitis. The results of this study suggest that the combination of ampicillin and nafcillin may be effective therapy for such conditions even when the etiological agent is ampicillin-resistant *H. influenzae*. The value of such a regimen is that it eliminates the need for chloramphenicol which has potentially fatal side effects.

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LITERATURE CITED

1. **Barson, W. J., M. D. Hilty, R. J. Fass, M. Fleer, and R. L. Brawley.** 1980. In vitro synergy of ampicillin and nafcillin against ampicillin-resistant *Haemophilus influenzae* type b, p. 1032-1033. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease. Proceedings of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
2. **Cho, C. T., and B. Z. Dudding.** 1978. Skin and soft tissue infections, p. 124-132. *In* Pediatric infectious diseases. The Medical Examination Publishing Co., New York.
3. **Escamilla, J.** 1976. Susceptibility of *Haemophilus influenzae* to ampicillin as determined by use of a modified, one-minute beta-lactamase test. Antimicrob. Agents Chemother. **9**:196-198.
4. **Feldman, W. E., J. D. Nelson, and L. R. Stanberry.** 1978. Clinical and pharmacokinetic evaluation of nafcillin in infants and children. J. Pediatr. **93**:1029-1033.
5. **Moffet, H. L.** 1975. Septic arthritis, p. 282-287. *In* Pediatric infectious diseases. The J. B. Lippincott Co., Philadelphia.
6. **Moxon, E. R., A. A. Medeiros, and T. F. O'Brien.** 1977. Beta-lactamase effect on ampicillin treatment of *Haemophilus influenzae* B bacteremia and meningitis in infant rats. Antimicrob. Agents Chemother. **12**:461-464.
7. **Nade, S.** 1977. Choice of antibiotics in management of acute osteomyelitis and acute septic arthritis in children. Arch. Dis. Child. **52**:679-682.
8. **Taber, L. H., M. D. Yow, and F. G. Nieberg.** 1967. The penetration of broad-spectrum antibiotics into the cerebrospinal fluid. Ann. N. Y. Acad. Sci. **145**:473-481.
9. **Walters, E. C., P. H. Wallar, D. A. Hiles, and R. H. Michaels.** 1976. Acute orbital cellulitis. Arch. Ophthalmol. **94**:785-788.
10. **Yogev, R., E. Burkholder, and A T. Davis.** 1980. Synergistic action of ampicillin and nafcillin against ampicillin-resistant *Haemophilus influenzae*. Antimicrob. Agents Chemother. **17**:461-463.