Pharmacokinetics and Therapeutic Efficacy of Imipenem, Ceftazidime, and Ceftriaxone in Experimental Meningitis Due to an Ampicillin- and Chloramphenicol-Resistant Strain of Haemophilus Influenzae Type b

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The pharmacokinetics and therapeutic efficacy of imipenem, ceftazidime, and ceftriaxone were compared with those of ampicillin and chloramphenicol in rabbits with experimental meningitis due to an ampicillin- and chloramphenicol-resistant strain of Haemophilus influenzae type b. The mean bacterial colony counts in cerebrospinal fluid were reduced by 49% (−1.85 log₁₀ CFU/ml), 92% (−3.37 log₁₀ CFU/ml), and 92% (−4.30 log₁₀ CFU/ml) after a single dose of imipenem, ceftazidime, and ceftriaxone, respectively. The median peak cerebrospinal fluid bactericidal titers against this multiply resistant strain of H. influenzae were 1:4 for thienamycin, 1:16 for ceftazidime, and 1:256 for ceftriaxone. By contrast, no bactericidal activity was observed in cerebrospinal fluid and the mean concentrations of H. influenzae were either unchanged or slightly increased in animals treated with ampicillin or chloramphenicol.

Haemophilus influenzae type b is the principal pathogen causing serious infections, especially meningitis, in infants and children. Since ampicillin-resistant H. influenzae was reported in 1974 (15), the combination of ampicillin and chloramphenicol has been considered the initial treatment of choice for meningitis (1). Recently, strains of ampicillin- and chloramphenicol-resistant H. influenzae type b have been isolated from cerebrospinal fluid (CSF) of patients with meningitis (4, 12, 16), and it is possible that the incidence of these multiply resistant H. influenzae strains may substantially increase in the future (13). Because there are only a few reports on management of patients with meningitis due to multiply resistant strains, there are no guidelines for selecting antimicrobial therapy.

In the present study we determined the pharmacokinetics and therapeutic efficacy of three new β-lactam antibiotics (imipenem, ceftazidime, and ceftriaxone) and compared these results with those of ampicillin or chloramphenicol in experimental meningitis due to a multiply resistant H. influenzae strain.

MATERIALS AND METHODS

Test organism. The strain of H. influenzae type b used to produce meningitis in rabbits was obtained from CSF of a 13-month-old boy with meningitis who was treated in Dallas. The organism produced β-lactamase and acetylmethytransferase (determined by A. L. Smith, Seattle). The minimal bactericidal concentrations of ampicillin and chloramphenicol against this strain were 4.0 and 32 μg/ml, respectively. The organism was grown overnight in brain-heart infusion with Levinthal supplement and, after centrifugation (3,500 rpm, 15 min), suspended in phosphate-buffered saline (0.01 M PO₄−0.15 M NaCl, pH 7.4) to a concentration of 5 × 10⁸ CFU/ml for inoculation.

Rabbit model. New Zealand white male rabbits weighing 2 to 3 kg each were prepared by the method detailed previously (2). Light anesthesia was induced by intravenous administration of sodium pentobarbital. For inoculation, 0.5 ml of the H. influenzae suspension (2.5 × 10⁸ CFU) was injected intracisternally. The rabbits were lethargic at 14 to 16 h after inoculation, at which time CSF was removed for culture and antimicrobial therapy was started. Quantitative CSF cultures yielded 5.0 × 10² to 8.9 × 10⁵ CFU of H. influenzae per ml. The mean concentrations for each therapy group of animals was from 4 × 10³ to 5 × 10⁴ CFU/ml. The mean CSF leukocyte count was 828 (range, 82 to 3,200) cells per mm³, and the mean protein value was 147 (range, 60 to 420) mg/dl. Untreated rabbits were observed until they died within 18 to 96 h after inoculation, and treated animals were sacrificed with an overdose of pentobarbital after the experiments were completed, which was approximately 20 to 22 h after inoculation and 5 h after an antibiotic dose was given.

Susceptibility studies. The minimal bactericidal concentrations of the study drugs for the H. influenzae type b strain used for these studies were determined by a broth dilution method with Mueller-Hinton broth with 1% supplement C (Difco Laboratories, Detroit, Mich.) and an inoculum of 10⁴ CFU/ml. The minimal bactericidal concentration was taken as the lowest concentration of antibiotic producing 99.9% or greater bacterial killing determined by quantitative subcultures onto chocolate agar of each tube containing no visible growth.

Administration of drugs. The antibiotics were dissolved in sterile water to the desired concentration. The dose of each antimicrobial agent was chosen to produce serum concentrations at 0.25 to 0.5 h in rabbits that are within the suggested therapeutic range in infants and children (i.e., ampicillin, ceftazidime, and ceftriaxone, 200 μg/ml; chloramphenicol, 25 to 30 μg/ml; imipenem, 20 μg/ml). The following single doses were administered intravenously over a 5-min period: 70 mg of ampicillin per kg, 20 mg of chloramphenicol per kg, and 25 mg of imipenem, ceftazidime, or ceftriaxone per kg.

Processing of specimens. Blood and CSF samples were

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collected from an indwelling femoral artery catheter and intracerebral spinal needle, respectively, at 0, 0.25, 0.5, 0.75, 1.0, 2.0, 4.0, and 5.0 h after the administration of a single dose of antibiotics. CSF samples were immediately cultured quantitatively on chocolate agar. Serum and the remainder of the CSF samples were kept at -80°C for imipenem and at -20°C for the other antibiotics until antibiotic assays and bactericidal titers were performed within 3 days.

Antibiotic assay. Concentrations of antibiotic were determined by an agar-disk diffusion microbioassay with Sarcina lutea (ATCC 9341) for ampicillin, Beneckea na-trigens (ATCC 14048) for chloramphenicol, Bacillus subtilis (ATCC 12432) for imipenem, Morganella morganii for ceftazidime, and Escherichia coli (ATCC 10536) for ceftriaxone as test organisms. Serum samples and their standards were diluted in 100% normal rabbit serum for chloramphenicol and ceftazi-
dime, and ceftriaxone, in 0.07 M phosphate buffer at pH 6.0 for ampicillin, and at pH 7.0 for imipenem and ceftazidime. CSF samples and their standards were diluted in 0.1 M phosphate buffer (pH 7.9) for chloramphenicol and in 0.07 M phosphate buffer (pH 6.0) for ceftriaxone. The same phosphate buffer as for serum samples was used for CSF samples in assays of ampicillin, imipenem, and ceftazidime.

Chromatography. High-pressure liquid chromatography (HPLC) was used to determine the in vitro effect of penicil-
linase on ampicillin. The HPLC analyses were performed with a model M45 pump (Waters Associates, Milford, Mass.) with a flow rate of 2.0 ml/min, an RCM C18 reverse-phase column (10-µm particle size; Waters Associates), a UV-50 visible wavelength detector (Varian, Walnut Creek, Calif.), and a model 9176 stripchart recorder (Varian) with the chart speed of 1.0 cm/min. The detector was set at 0.05 absorbance unit as the full-scale sensitivity setting (wavelength, 220 nm). The mobile phase consisted of a 1:9 mixture of acetonitrile and 0.1 M KH2PO4 (pH 3.5 with 1 N HCl). Ampicillin was diluted in pooled CSF from healthy rabbits.

CSF bactericidal titers. CSF bactericidal titers against the test pathogen causing meningitis were determined by a microtiter technique with serial twofold dilutions in Mueller-Hinton broth plus 1% supplement C. The inoculum was approximately 5 x 10⁷ CFU/ml. The inhibitory titer was defined as the smallest dilution of CSF that inhibited growth after 18 h of incubation at 37°C. The bactericidal titer was defined as dilution of CSF that killed 99.9% or greater of the original inoculum as determined by quantitative subcultures on chocolate agar.

Pharmacokinetic determinations. The antibiotic concentrations in CSF and serum measured after the administration of a single dose were fitted to a regression line by the method of least mean squares. The half-life in CSF and serum was calculated by dividing ln 2 by the slope of the line. The area under the concentration-time curve in CSF and serum was obtained by successive trapezoidal approximation from time 0 to time ∞.

RESULTS

Pharmacokinetics. The serum and CSF concentration-time curves and the mean half-life and area under the curve values for ampicillin, chloramphenicol, imipenem, cef-
tazi-dime, and ceftriaxone are presented in Fig. 1. The mean peak serum concentrations were observed at the completion of a 5-min infusion and ranged from 121 to 122 μg/ml for chloramphenicol and imipenem to 388 μg/ml for ampicillin. The mean values at 0.5 h were from 10 μg/ml for imipenem to 104 μg/ml for ceftriaxone. The mean serum half-life values ranged from 0.36 h for imipenem to 2.14 h for ceftriaxone.

Ampicillin was not detected in CSF in two of six infected animals; in the remaining four animals, it was less than the lowest detectable concentration (0.01 μg/ml) of ampicillin by bioassay at 2 h after the infusion. In vitro studies with HPLC revealed that ampicillin, when added to rabbit CSF, had a retention time of 13.8 min. With the addition of increasing concentrations of penicillinase (penase; Difco) from 1.0 LU/ml to 10 LU/ml, the ampicillin peak decreased remarkably, accompanied by a new and enlarging spike with a retention time of 4.8 min (Fig. 2). Ampicillin was undetectable after addition of 10 LU of penicillinase per ml.

Chloramphenicol concentrations in CSF were undetectable (less than 2.0 μg/ml by bioassay) in three of five animals infected with acetyltransferase-positive strains. The mean peak CSF levels for the remaining two rabbits were 5.3 μg/ml at 0.75 h and 5.12 μg/ml at 2.0 h after a 5-min infusion, respectively. The half-life values were approximately fourfold longer in CSF than in serum for ceftriaxone and ceftazidime and twofold longer for imipenem (Fig. 1). The CSF half-lives of ampicillin and chloramphenicol were indeter-
minal. The ratio of mean CSF area under the curve to mean serum area under the curve was calculated to express the relative penetration of antibiotic into CSF. The values were 0.17 for imipenem, 0.16 for ceftazidime, and 0.10 for ceftriaxone.

Therapeutic effect. The effect of single-dose treatment of
THERAPY FOR EXPERIMENTAL *H. INFLUENZAE* MENINGITIS

FIG. 2. Effect of penicillinase on ampicillin concentration in CSF of healthy rabbits detected by HPLC (see the text). A, Ampicillin peak (20 μg/ml; retention time, 13.8 min); B, new peak after the addition of penicillinase (retention time, 4.8 min).

Experimental meningitis due to an ampicillin- and chloramphenicol-resistant strain of *H. influenzae* was evaluated by quantitative cultures of CSF obtained at various intervals after administration. The mean changes in bacterial colony counts in CSF were compared for the five antibiotics (Table 1). All five untreated rabbits with meningitis had increasing CSF bacterial counts (mean, +1.01 log_{10} CFU/ml at 3 h and +3.14 log_{10} CFU/ml at 5 h) during the 5-h period of observation, and all died by 96 h after inoculation. There was no effect on the concentrations of bacteria in CSF at 5 h after a dose of ampicillin, and there was an increase of +0.78 log_{10} CFU/ml in animals given chloramphenicol. After a single dose of ceftazidime or ceftriaxone, the mean concentrations of *H. influenzae* were reduced by 50% at 2 h and by 91% at 5 h, at which time CSF cultures from four of five animals in each treatment group were sterile. Imipenem reduced the bacterial count -1.85 log_{10} CFU/ml (43%) at 5 h, and only one of five CSF cultures became sterile.

**CSF bactericidal titers.** The mean peak CSF bactericidal titers against the infecting organism are shown for the five test drugs in Table 1. No antimicrobial activity was observed in CSF from animals treated with ampicillin or chloramphenicol. The largest CSF titers were observed in ceftriaxone-treated animals (1:256), whereas those of ceftazidime-treated (1:16) and imipenem-treated (1:4) animals were smaller.

**DISCUSSION**

On the basis of the few published case reports (4, 12, 16), we anticipated that in this experimental meningitis model ampicillin or chloramphenicol would be ineffective in reducing the CSF concentrations of *H. influenzae* resistant to these two drugs. Of interest was the finding of exceedingly small concentrations of these two drugs in purulent CSF despite large concentrations in serum. Other investigators using this same model have detected considerably larger concentrations of ampicillin or chloramphenicol in CSF infected with susceptible bacteria (7, 10, 11, 14). The smaller values observed in this study are most likely explained by degradation of the drugs in CSF by β-lactamase for ampicillin and acetyltransferase for chloramphenicol produced in the CSF by the multiply resistant strain of *H. influenzae*. This was suggested by our in vitro data for which we used HPLC to demonstrate the effect of penicillinase on ampicillin in rabbit CSF. The new spike at 4.8 min of retention time observed after the addition of penicillinase to ampicillin-containing CSF was not detected by HPLC when penicillinase alone was added to rabbit CSF. Furthermore, a peak at 4 to 5 min of retention time was not detected by HPLC in CSF of infected animals treated with ampicillin. This could, in part, be explained by interfering peaks seen in purulent CSF at 4 to 5 min or possibly by metabolism or absorption of the substance that was observed at 4.8 min in our in vitro studies.

We evaluated ceftriaxone, ceftazidime, and imipenem in experimental meningitis because of their excellent in vitro activities against the common pathogens of non-neonatal meningitis: *H. influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis*. Ceftriaxone has been shown to be highly efficacious in infants and children with bacterial meningitis (3, R. W. Steele and R. W. Bradsher, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 317, 1981; B. Congeni, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, abstr. no. 318, 1982), and ceftazidime and imipenem have shown promise in the experimental meningitis model (6, 8). Additionally, imipenem has been shown to have good in vitro activity against multiply resistant strains of *S. pneumoniae* (5, 17).

Ceftriaxone and ceftazidime were comparably effective in reducing the concentrations of *H. influenzae* in CSF after single-dose administration. The bactericidal titers in CSF of 1:254 with ceftriaxone and 1:16 with ceftazidime exceeded the minimum activity (approximately 1:10) in CSF believed to be necessary for a rapid bacteriological effect (9). By contrast, imipenem was less effective in this animal model; the bacterial concentration was reduced by 49% at 5 h, and CSF culture from only one of five animals was sterile after therapy. Imipenem produced a peak CSF bactericidal titer of 1:4 which was lower than the activity shown in previous studies to be necessary for bacteriological efficacy (9, 11).

We interpret these data to indicate that ceftriaxone or ceftazidime should be effective for therapy of meningitis caused by ampicillin- or chloramphenicol-resistant *H. influenzae*. Additional clinical trials are required before either of these agents can be recommended as standard therapy.

<table>
<thead>
<tr>
<th>Drug (dose, mg/kg)</th>
<th>MBC* (μg/ml)</th>
<th>No. of animals</th>
<th>Median peak CSF bactericidal titer</th>
<th>Log 10 CFU/ml in CSF</th>
<th>No. of animals with sterile CSF cultures/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean initial concen</td>
<td>Δ Concen after 5 h (% change)</td>
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<tr>
<td>Ampicillin (70)</td>
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<td>0</td>
<td>-0.02 (-0)</td>
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<tr>
<td>Chloramphenicol (20)</td>
<td>32.0</td>
<td>4</td>
<td>0</td>
<td>+0.79 (+16)</td>
<td>0/4 (0)</td>
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<td>-1.85 (-49)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Ceftazidime (25)</td>
<td>0.03</td>
<td>5</td>
<td>1:16</td>
<td>-3.37 (-91)</td>
<td>4/5 (80)</td>
</tr>
<tr>
<td>Ceftriaxone (25)</td>
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<td>5</td>
<td>1:256</td>
<td>-4.30 (-91)</td>
<td>4/5 (80)</td>
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<td>No therapy</td>
<td>3.3</td>
<td>5</td>
<td>3.14 (+98)</td>
<td>0/5 (0)</td>
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* MBC, Minimal bactericidal concentration.
LITERATURE CITED


