Pharmacodynamics and Bactericidal Activity of Ceftriaxone Therapy in Experimental Cephalosporin-Resistant Pneumococcal Meningitis

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Recent animal experiments and clinical trials with bacterial meningitis have focused on new antimicrobial agents and combination therapy in an effort to enhance eradication of organisms and to improve outcome. Few studies have addressed the pharmacodynamic properties of antibiotics in cerebrospinal fluid (CSF). Täuber et al. (20), using ampicillin therapy in the rabbit meningitis model, showed that only antibiotic dosages that produced concentrations in the CSF 10- to 30-fold greater than the MBC were effective. A similar concentration/MBC effect was demonstrated with ceftriaxone in experimental Escherichia coli meningitis (6).

The increased prevalence of penicillin- and cephalosporin-resistant strains of Streptococcus pneumoniae has complicated treatment of meningitis (15). In patients with meningitis caused by a cephalosporin-resistant pneumococcal strain, attaining antibiotic concentrations that are 10- to 30-fold greater than the MBC in CSF is difficult. It has been demonstrated in animal models that the bactericidal activity of β-lactam antibiotics is concentration independent, indicating that it is the time that an antibiotic concentration exceeds the MIC during the dosing interval that correlates best with bacteriologic effectiveness (10, 11, 13, 22). The amount of time required for an antibiotic to remain above the MIC to achieve maximum efficacy varied from 30 to 100% for the different drug-organism combinations in these models of lung and thigh infections.

Although large dosages of cefotaxime and ceftriaxone have been successfully used for treatment of pneumococcal meningitis caused by intermediate-cephalosporin-resistant (MIC, 1 µg/ml) S. pneumoniae (17, 21), there have been failures as well (2), indicating the need for evaluation of pharmacodynamic profiles of these agents in CSF. Thus, the aim of the present study was to compare the pharmacodynamics and bacteriologic effectiveness of different dosing regimens of ceftriaxone in experimental meningitis caused by a highly cephalosporin-resistant strain of S. pneumoniae in order to establish which of the pharmacodynamic indices best predicts the bacteriologic response in this model.

MATERIALS AND METHODS

Bacterial strain. A highly penicillin- and cephalosporin-resistant strain of S. pneumoniae (MIC and MBC of 4 µg/ml for ceftriaxone), originally isolated from a patient with meningitis (8), was used in all experiments. The strain was grown overnight on blood agar. The plates were flooded with phosphate-buffered saline (PBS), and aliquots of the resultant suspension were frozen at −70°C. Aliquots were thawed and diluted with endotoxin-free PBS to concentration of 10² to 10⁶ CFU/ml.

Meningitis model. The well-characterized meningitis model in rabbits, originally described by Dacey and Sande, was used (5). Briefly, young male New Zealand White rabbits weighing 2 to 2.2 kg were anesthetized with ketamine (40 mg/kg of body weight) and acepromazine (1 mg/kg), given intramuscularly, and were immobilized in stereotactic frames for induction of meningitis and CSF sampling. Animals had free access to water and food while out of the frames. A marginal ear vein once or twice daily for 24 to 72 h. Treatment groups consisted of six to eight animals. Ceftriaxone was diluted with 0.9% NaCl to concentrations of 30 to 40 mg/ml. Animals received 3- to 5-min infusions of ceftriaxone (150 or 400 mg/kg of body weight per day) through the marginal ear vein once or twice daily for 24 to 72 h.

Adequate concentrations of β-lactam antibiotics in cerebrospinal fluid (CSF) are difficult to achieve for meningitis caused by drug-resistant Streptococcus pneumoniae. Ceftriaxone in dosages of 150 or 400 mg/kg of body weight per day, given in one or two doses, was used for the treatment of experimental highly cephalosporin-resistant (MIC and MBC, 4 µg/ml) pneumococcal meningitis. The bacterial killing rate (Δlog₁₀ CFU per milliliter per hour) and pharmacokinetic indices, including percentage of time the antibiotic concentration exceeded the MBC during a 24-h period (T>MBC), CSF peak concentration above the MBC, and area under the concentration-time curve from 0 to 24 h above MBC, were measured and correlated. By multiple stepwise regression, only T>MBC independently predicted the bacterial killing rate. There was a direct linear correlation between T>MBC in CSF and the bacterial killing rate during the first 24 h of therapy (r = 0.87; P = 0.004). Sterilization of CSF was achieved only when the T>MBC was 95 to 100%. In the first 24 h, the 200-mg/kg/12-h regimen, compared with the 400-mg/kg/24-h regimen, was associated with a greater T>MBC (87% ± 10% versus 60% ± 22%; P = 0.03) and greater bacterial killing rate (0.2 ± 0.04 versus 0.13 ± 0.07; P = 0.003), confirming that ceftriaxone exhibits time-dependent bactericidal activity. After 24 h, the T>MBC and the CSF sterilization rates were similar whether ceftriaxone was given once or twice daily.

Bacterial titers. Bacterial concentrations in CSF were determined in all samples at 0, 12, 24, 36, 48, 60, and 72 h by plating of undiluted and serial 10-fold dilutions of CSF on sheep blood agar and incubation of the plates for 24 h at 35°C in a 5% CO₂ atmosphere. The lowest bacterial concentration detectable was 10 CFU/ml. Bacterial killing rates were expressed as the change in log₁₀ CFU per milliliter per hour for 24 h.
Ceftriaxone concentration. The concentration of ceftriaxone was determined by reverse-phase high-performance liquid chromatography with CSF and blood taken simultaneously at various times during therapy. The assay was performed, and the serum samples were prepared as described by Jangbluth and Jusko (9). The CSF samples were filtered through 0.2-μm-pore-diameter filters before analysis. Ceftriaxone standards for CSF were prepared between 1 and 10 μg/ml. Samples with higher concentrations were diluted into the standard curve range.

Pharmacokinetic indices. Peak concentrations in CSF (C_{peak}) and time from the injection that concentrations exceeded the MBC were extrapolated from the ceftriaxone concentration-time curve. For pharmacokinetic analyses, the computer program PCNONLIN was used. Elimination rate constants (k_e) were determined by nonlinear regression analysis, and half-lives were calculated as ln2/k_e with a one-compartment model. The area under the concentration-time curves from 0 to 24 h (AUC_{0–24}) for serum and CSF was estimated by the linear trapezoidal rule. The percentage of time that CSF ceftriaxone concentrations exceeded the MBC (T>MBC) and the AUC_{0–24} above the MBC (AUC/MBC) were calculated as described by Schentag et al. (16) and expressed as a fraction of the 24-h treatment period.

In vitro time-kill studies. In vitro time-kill studies were performed as described by Friedland et al. (7). Briefly, bacteria (~10^6 CFU/ml) from an overnight culture and various concentrations of ceftriaxone (1- to 100-fold of MIC) were inoculated into 10 ml of Mueller-Hinton broth with 3% lysed horse blood and incubated at 35°C in ambient air. The time-kill kinetics were studied over an 8-h period. The lowest limit of detection was 10 CFU/ml.

Statistics. Continuous variables were expressed as means ± standard deviations. Penetration of ceftriaxone into CSF was calculated by the formula (AUC_{CSF}/AUC_{serum}) × 100. Differences between categorical data were compared by the Fisher exact test. The Student t test was used to compare continuous variables. The pharmacokinetic profiles (T>MBC, C_{peak}/MBC, and AUC/MBC) were correlated with bacterial killing rate by univariate linear and stepwise multiple regression analysis.

Results

In vitro time-kill curve. The in vitro bacterial killing rate of S. pneumoniae was not concentration dependent and ranged from 0.21 to 0.35 CFU/ml/h for ceftriaxone concentrations of 1- to 100-fold above the MIC (Fig. 1).

Pharmacokinetics. The pharmacokinetic indices of ceftriaxone in CSF and serum are demonstrated in Table 1. A nonlinear pharmacokinetic pattern was seen; with dosages of 150 and 300 mg/kg, the higher dosage resulted in more than two-fold greater concentrations in blood and CSF and AUCs. After dosages of 200 and 400 mg/kg, the AUCs and peak concentrations were similar. The half-life values of ceftriaxone in CSF were 2.1 to 3.7-fold longer than those in serum after dosages of 150 to 400 mg/kg (Table 1). The penetration of ceftriaxone into CSF ranged from 6.3 to 14.5% and was highest in animals treated with 400 mg/kg. Penetration did not change significantly on the second day of therapy.

Ceftriaxone concentrations in CSF exceeded the MBC within 30 min of administration of 150 to 400 mg/kg in all animals (Fig. 2). The concentrations of ceftriaxone in CSF were greater after the second dose than after the first: 16.8 versus 11.6 μg/ml in animals given 200 mg/kg/12 h, respectively, and 22.1 versus 14.5 μg/ml in those treated with 400 mg/kg/24 h, respectively.

Bacteriologic response. In the first 12 h of therapy, the reduction in CSF concentrations of organisms was independent of the dosages studied (Fig. 3). A second dose of ceftriaxone (whether given after 12 or 24 h) resulted in a further decrease in bacterial CSF concentrations. During the first 24 h of therapy, in animals receiving treatment twice daily, the rate of bacterial killing was greater (0.2 ± 0.04 log_{10} CFU/ml/h) than in those animals given once-daily ceftriaxone therapy (0.14 ± 0.06 log_{10} CFU/ml/h) (P = 0.003). After 24 h, 11 of 20 (55%) animals treated with 200 mg/kg/12 h versus 5 of 20 (25%) treated with 400 mg/kg/24 h had <10 CFU of S. pneumoniae per ml in CSF (P = 0.1). On days 2 and 3, there were no significant differences in bacterial concentrations or in the number of animals with negative CSF cultures, regardless of whether the animals received the daily 400-mg/kg dosage in one or two doses. By 72 h, three of the nine animals treated with 200 mg/kg/12 h had positive CSF cultures compared with none of eight animals given 400 mg/kg/24 h (P = 0.2).

Pharmacodynamic data. The pharmacodynamic indices C_{peak}/MBC, AUC/MBC, and T>MBC were interrelated (r = 0.67; P = 0.01). There was a linear correlation between the bacterial killing rate and T>MBC in CSF for the first 24 h (Fig. 4). By stepwise multiple regression analysis, T>MBC correlated best with bacterial killing rate (P = 0.001) and the other indices (C_{peak}/MBC and AUC/MBC) did not correlate.

### Table 1. Mean (± standard deviation) pharmacokinetic and pharmacodynamic values of single-dose ceftriaxone

<table>
<thead>
<tr>
<th>Ceftriaxone dose [mg/ kg]</th>
<th>CSF</th>
<th>Blood</th>
<th>% Penetration</th>
<th>T&gt;MBC</th>
<th>Bacterial killing rate in first 24 h (ΔCFU/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Half-life (h)</td>
<td>AUC_{0–24} (μg·h/ml)</td>
<td>C_{peak} (μg/ml)</td>
<td>Half-life (h)</td>
<td>AUC_{0–24} (μg·h/ml)</td>
</tr>
<tr>
<td>150 (6)</td>
<td>6.5 ± 0.8</td>
<td>53.1 ± 10.4</td>
<td>5.3 ± 1.4</td>
<td>2.7 ± 0.36</td>
<td>961 ± 234</td>
</tr>
<tr>
<td>200 (7)</td>
<td>7.1 ± 2.7</td>
<td>116.9 ± 39.1</td>
<td>11.6 ± 1.1</td>
<td>3.07 ± 1.1</td>
<td>1.393 ± 273</td>
</tr>
<tr>
<td>300 (8)</td>
<td>5.3 ± 2.2</td>
<td>140.7 ± 59.5</td>
<td>15.8 ± 7.7</td>
<td>2.5 ± 0.44</td>
<td>1.777 ± 158</td>
</tr>
<tr>
<td>400 (7)</td>
<td>7.8 ± 2.6</td>
<td>185.5 ± 66.3</td>
<td>14.5 ± 6.8</td>
<td>2.06 ± 0.08</td>
<td>1.150 ± 146</td>
</tr>
</tbody>
</table>

a After a second dose given at 12 h, T>MBC was 11.6 ± 4.2 h; for the 24-h period, T>MBC was 20.8 ± 2.4 h (P = 0.03 compared with 400 mg/kg once daily).
b Bacterial killing rate was calculated after two doses of 200 mg/kg of ceftriaxone in the first 24 h (P = 0.003 compared with 400 mg/kg once daily).
indicates values below the MBC. CSF measurements were done only at the time points in the first 24 h of therapy. Ceftriaxone dosages of 150 and 400 mg/kg/day were given in one or two doses. CSF cultures were negative (<10 CFU/ml) after 24 h of treatment only in the animals in which the T>MBC was 95 to 100%. Because of the higher CSF concentration of ceftriaxone on the second day, the T>MBC was not different in animals given 200 mg/kg/12 h from that in those treated with 400 mg/kg/24 h (80.7% ≤ 17% versus 68% ± 17.2%; P = 0.14).

**DISCUSSION**

Using ceftriaxone for the therapy of experimental highly β-lactam-resistant pneumococcal meningitis, we showed that the most important pharmacodynamic index predicting efficacy was the time that antibiotic concentrations in CSF exceeded the MBC. A direct linear relationship was observed between the bacterial killing rate and the time above the MBC. In the first 24 h, the highest rate of CSF sterilization was achieved when ceftriaxone concentrations in CSF were above the MBC for 95 to 100% of the dosing interval. The dosages used in this study were chosen specifically to test the relationship between T>MBC and bacteriologic efficacy and were considerably larger than those used clinically. The principles we have investigated, however, are expected to be the same if smaller dosages are used against less-resistant pneumococcal strains.

The time-dependent bacterial killing of β-lactam antibiotics is well known and has been demonstrated in vitro and in animal models (10, 11, 22). In a thigh infection model with S. pneumoniae, maximal bacterial killing was achieved when serum penicillin values were constantly maintained above the MBC, whereas with erythromycin therapy, maximal efficacy was achieved when concentrations exceeded the MBC for only 60% of the dosing interval (22). Previous studies of experimental pneumococcal meningitis emphasized the correlation between peak CSF antibiotic concentrations and maximal bactericidal efficacy, but none of these studies analyzed the relationship between T>MBC and bactericidal killing rates (18, 20). Täuber et al. (18), using different β-lactam antibiotic therapies, found that bacterial killing rates increased as peak CSF antibiotic concentrations increased, but this relationship was not consistently linear. Maximal efficacy was achieved once antibiotic concentrations exceeded the MBC by 10- to 30-fold and further increases in CSF concentrations were not more effective. In the aforementioned studies, T>MBC was not specifically measured, but when the same dosages of antibiotics were administered more frequently, thus resulting in greater T>MBCs, bacterial killing rates were greater. Our data indicated that the three pharmacodynamic indices studied (C_{peak}, AUC, and T>MBC) were interrelated and correlated directly with the bacterial killing rate by univariate linear regression analysis. However, by stepwise multiple regression analysis, T>MBC had the strongest correlation with the killing rate.

This observation that T>MBC best predicts the efficacy of β-lactam antibiotic therapy in CSF is similar to results with infections in other body sites (3). The non-concentration-dependent activity of ceftriaxone against the pneumococcal strain used in our study was supported by in vitro time-kill curves; all concentrations used resulted in similar bacterial killing rates over an 8-h period. The peak CSF concentration, which was previously thought to best predict β-lactam efficacy, may be an approximate surrogate measurement for T>MBC, especially...
for ceftriaxone, which has a longer half-life than the other β-lactam antibiotics.

The highest bacterial killing rates we found in animals (0.25 to 0.27 log_{10} CFU/ml/h) and in vitro (0.21 to 0.35 log_{10} CFU/ml/h) were lower than those reported previously (1 to 1.5 log_{10} CFU/ml/h) (18). The slow bacterial killing of the pneumococcal strain we studied is characteristic of the group of slowly killed bacteria described by Liu et al. (12). Such bacteria are lysis defective and are more common among penicillin-resistant strains (1, 12, 14). As observed by Täuber et al. (19) we found that the total duration of therapy was an important factor influencing bactericidal efficacy. After 24 h of high-dose therapy (400 mg/kg/daily), only 40% of the animals had less than 10 CFU/ml, whereas after 72 h, 85% of the animals had negative CSF cultures.

For meningitis caused by susceptible pneumococcal strains, there is unlikely to be a difference in efficacy between once- or twice-daily regimens of ceftriaxone therapy. However, using a highly resistant strain we found that on the first day of therapy, ceftriaxone given at 200 mg/kg/12 h resulted in a significantly greater T>MBC, higher killing rates, and lower bacterial concentrations in CSF at 24 h than did the same total dosage given once daily. These differences can be explained by the short postantibiotic effect of ceftriaxone against pneumococci (4). Beyond the first 24 h, differences between once- and twice-daily regimens were not statistically significant; the T>MBC was similar whether ceftriaxone was given at 200 mg/kg/12 h or 400 mg/kg/24 h.

In summary, T>MBC is an important determinant of bacteriologic efficacy in experimental highly penicillin- and cephalosporin-resistant pneumococcal meningitis. Although, in terms of bacterial killing rates in the first 24 h, twice-daily administration of ceftriaxone was superior to daily administration, CSF sterilization was not significantly greater with twice-daily therapy. Since drug pharmacokinetics in humans and in rabbits differ, recommendations for dosing of humans should be based on further clinical studies.

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REFERENCES


