Efficacy of Ceftaroline Fosamil against Penicillin-Sensitive and -Resistant *Streptococcus pneumoniae* in an Experimental Rabbit Meningitis Model

P. Cottagnoud, M. Cottagnoud, F. Acosta, A. Stucki

Clinic of Internal Medicine, Clinic Sonnenhof, Bern, Switzerland; Zieglerspital, Bern, Switzerland; Berner Reha Zentrum, Heiligenschwendi, Switzerland

Ceftaroline is a new cephalosporin with bactericidal activity against resistant Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant *Streptococcus pneumoniae*, as well as common Gram-negative organisms. This study tested the prodrug, ceftaroline fosamil, against a penicillin-sensitive and a penicillin-resistant strain of *S. pneumoniae* in an experimental rabbit meningitis model. The penetration of ceftaroline into inflamed meninges was approximately 14%. Ceftaroline fosamil was slightly superior to ceftriaxone against the penicillin-sensitive strain and significantly superior to the combination of ceftriaxone and vancomycin against the penicillin-resistant strain.

The continuous spread of penicillin-resistant pneumococci remains a major challenge for clinicians and infectious disease specialists worldwide. In Spain and Portugal, approximately 10% to 25% of the pneumococcal strains are resistant to penicillin. In France, the resistance rates have reached 25% to 50% based on a European Antimicrobial Resistance Surveillance System survey in 2007 (1).

The guidelines suggest treating meningitis caused by penicillin-resistant pneumococci with a combination of ceftriaxone and vancomycin (2). However, because of the variable penetration of vancomycin into meninges, antimicrobial monotherapy would represent a major treatment advantage. The aim of this study was to test ceftaroline as monotherapy against a penicillin-sensitive and a penicillin-resistant strain of *Streptococcus pneumoniae* in the rabbit experimental meningitis model.

**MATERIALS AND METHODS**

**Strains and microbiology.** The penicillin-sensitive strain (13.05.42) and the penicillin-resistant strain (WB4) of *S. pneumoniae* were kindly provided by the Institute for Infectious Diseases, University of Bern (Bern, Switzerland). The strains were isolated in blood cultures from patients at the university hospital. All strains were grown in Mueller-Hinton broth and stored at −80°C after several passages in rabbits. MICs were determined by agar dilution according to the published literature (3). Inocula for use in rabbit infection studies were prepared from stocks stored at −80°C.

**Study drugs.** The prodrug form of ceftaroline, ceftaroline fosamil (602 µg/mg), was used for *in vivo* studies, and microbiologically active ceftaroline (lot number FMD-CEF-019; 813 µg/mg) was used for *in vitro* studies. Ceftaroline and ceftaroline fosamil were obtained from Forest Laboratories, Inc., New York, NY. Vancomycin and ceftriaxone were commercially obtained and were used according to the manufacturer’s recommendations and guidelines.

**Experimental meningitis model.** The experimental rabbit meningitis model described by Dacey and Sande (4) was employed in this study. The experimental protocols were approved by the Kantonges Veterinärarm des Kantons Bern. Pathogen-free New Zealand rabbits were provided by the Zentral Tierstelle der Medizinischen Fakultät der Universität Bern, where all the experiments were performed. One day before the experiment, the rabbits were anesthetized with intramuscular injections of ketamine (30 mg/kg of body weight) and xylazine (15 mg/kg) and fitted with prostheses on their calvaria to facilitate subsequent placement within a stereotactic frame. On the day of the experiment, the rabbits received ethylcarbamate (1.75 g/kg) by subcutaneous injection and pentobarbital (10 mg/kg) by intravenous (i.v.) injection to induce deep anesthesia. The animals were fixed in a stereotactic frame, and a 3.5-in. (25G) spinal needle was introduced into the cisterna magna. Following the withdrawal of 0.2 ml of cerebrospinal fluid (CSF), the test bacteria (penicillin-sensitive or -resistant *S. pneumoniae*; 1 × 10⁵ CFU in 0.2 ml of saline solution) were injected into the subarachnoid space. After inoculation, the animals were brought back to the cages for the night. The next day, the rabbits were again fitted in the frames using the techniques and anesthesia described above. A catheter was fixed in the femoral artery for serum sampling. A spinal needle was fixed again in the subarachnoid space. Eight hours after inoculation, ceftaroline fosamil 40 mg/kg i.v. (*n* = 10) was injected at hours 0 and 4. The comparator regimen, ceftriaxone 100 mg/kg i.v. (*n* = 10), was administered at hour 0 for the penicillin-sensitive strain. For the penicillin-resistant strain, ceftriaxone 100 mg/kg i.v. at hour 0 plus vancomycin 20 mg/kg i.v. (*n* = 10) at hours 0 and 4 were administered as previously described (5, 6). There was an untreated control group for each strain (*n* = 5).

**Determination of antibiotic levels and CFU titers.** CSF (0.2 ml) was sampled at 0, 1, 2, 4, 6, and 8 h after initiation of therapy. Blood was sampled at 0.25, 0.5, 1, 2, 3, 4, 4.25, 4.5, 5, 6, 7, and 8 h. The ceftaroline concentrations in serum and CSF were determined by diffusion microbioassays using agar plates containing *Bacillus subtilis* (sus-1-A) at 10⁶ CFU/0.1 ml (Raven Biological Laboratories, Inc., Omaha, NE). The limit of detection was 0.2 mg/liter for ceftaroline. The number of CFU per milliliter was determined by serial dilution of CSF and plating on agar plates with incubation overnight at 37°C. The limit of bacterial detection of this assay was estimated to be 50 CFU/ml (1.7 log₁₀). Penetration into the CSF was determined by comparing areas under the concentration versus time curves (AUC) for serum and CSF, using GraphPad Prism software (GraphPad Software Inc., San Diego, CA).
Statistical analysis. The Student t test and one-way analysis of variance (Tukey-Cramer multiple-comparison test) were used for parametric data. Comparison of positive and negative cultures was analyzed by the two-tailed Fisher exact test. A P value of < 0.05 was considered significant.

The efficacies of the different regimens are presented as \( \Delta \log_{10} \) CFU/ml/hour and as \( \Delta \log_{10} \) CFU/ml/8 h. A value of 1.7 \( \log_{10} \) CFU/ml was assigned to the first sterile CSF sample (the limit of detection) and a value of 0 to each subsequent sterile CSF sample.

RESULTS AND DISCUSSION

Figure 1 shows the serum levels of ceftaroline in rabbits with inflamed meninges. Approximately 15 min after one injection of 40 mg/kg of ceftaroline fosamil, the peak serum level reached approximately 60 mg/liter, decreasing to 4 mg/liter 4 h later. In the CSF, the ceftaroline levels increased rapidly to 3.2 mg/liter after 1 h and decreased slowly to 1.6 mg/liter at hour 4. After the second injection, ceftaroline levels peaked at approximately 3.8 mg/liter and decreased slowly to 1.8 mg/liter at the end of the treatment period (Fig. 2). Ceftriaxone monotherapy was less efficacious against the same penicillin-resistant strain in two previous studies, with lower killing rates per hour; however, the killing rates of ceftaroline were significantly superior to those of ceftriaxone at the end of the study (P < 0.03) (Table 1). Over the 8-hour treatment period, ceftaroline fosamil demonstrated significantly higher killing rates against the penicillin-resistant strain of S. pneumoniae than the combination of ceftriaxone and vancomycin (P < 0.03) (Table 2).

Ceftriaxone monotherapy was less efficacious against the same penicillin-resistant strain in two previous studies, with lower killing rates per hour (−0.31 \( \log_{10} \) CFU/ml), probably due to the lower MIC of ceftriaxone for the strain (5, 6). As expected, ceftriaxone and ceftaroline produced similar bactericidal activities against the penicillin-sensitive S. pneumoniae strain, despite their different MICs. Ceftriaxone was very efficacious against a penicillin-resistant pneumococcal strain and was superior to the standard regimen of ceftriaxone plus vancomycin in this experimental meningitis model.

In summary, in this rabbit in vivo model, ceftaroline penetration into inflamed meninges and levels in the CSF were sufficient for bactericidal activity against penicillin-sensitive and -resistant S. pneumoniae isolates. Ceftriaxone fosamil may be an option for first-line therapy, especially when penicillin-resistant strains are suspected, and additional studies of ceftaroline in meningitis are warranted.

![FIG 1 Serum levels of ceftaroline after 2 injections of 40 mg/kg i.v. The filled squares represent serum levels of ceftaroline. The error bars represent standard deviations.](image1)

![FIG 2 CSF levels of ceftaroline after 2 injections of 40 mg/kg i.v. The filled squares represent CSF levels of ceftaroline. The error bars represent standard deviations.](image2)

### TABLE 1 Efficacies of drugs against the penicillin-sensitive strain

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Inoculum at 0 h (CFU/ml)</th>
<th>( \Delta \text{Killing/h} ) (CFU/ml)</th>
<th>( \Delta \text{Killing/8 h} ) (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (5)</td>
<td>5.82 ± 0.38 log_{10}</td>
<td>+0.15 ± 0.04 log_{10}</td>
<td>+1.23 ± 0.33 log_{10}</td>
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<tr>
<td>Ceftaroline (10)</td>
<td>6.35 ± 0.47 log_{10}</td>
<td>−0.79 ± 0.14 log_{10}</td>
<td>−6.35 ± 0.47 log_{10}</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>5.91 ± 0.33 log_{10}</td>
<td>−0.67 ± 0.12 log_{10}</td>
<td>−5.54 ± 0.98 log_{10}</td>
</tr>
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</table>

* Ceftriaxone versus ceftaroline: P < 0.06, not significant.

### TABLE 2 Efficacies of drugs against the penicillin-resistant strain

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Inoculum at 0 h (CFU/ml)</th>
<th>( \Delta \text{Killing/h} ) (CFU/ml)</th>
<th>( \Delta \text{Killing/8 h} ) (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (5)</td>
<td>6.04 ± 0.65 log_{10}</td>
<td>+0.12 ± 0.05 log_{10}</td>
<td>+0.95 ± 0.47 log_{10}</td>
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<tr>
<td>Ceftaroline (10)</td>
<td>5.54 ± 0.61 log_{10}</td>
<td>−0.71 ± 0.06 log_{10}</td>
<td>−5.54 ± 0.61 log_{10}</td>
</tr>
<tr>
<td>Ceftriaxone +</td>
<td>5.76 ± 0.54 log_{10}</td>
<td>−0.59 ± 0.11 log_{10}</td>
<td>−4.65 ± 1.00 log_{10}</td>
</tr>
</tbody>
</table>

* Ceftriaxone versus ceftaroline: P < 0.009, highly significant.

* Ceftaroline versus ceftriaxone plus vancomycin: P < 0.03, significant.
ACKNOWLEDGMENT

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REFERENCES