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# Cefepime is efficacious against penicillin- and quinolone-resistant pneumococci in experimental meningitis

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**In experimental rabbit meningitis, cefepime given at a dose of 100 mg/kg was associated with concentrations in the cerebrospinal fluid of between 5.3 and 10 mg/L and a bactericidal activity of $-0.61 \pm 0.24$ $\Delta\log_{10}$ cfu/mL·h, similar to the standard regimen of ceftriaxone combined with vancomycin ($-0.58 \pm 0.14$ $\Delta\log_{10}$ cfu/mL·h) in the treatment of meningitis due to a penicillin- and quinolone-resistant pneumococcal mutant strain (MIC 4 mg/L). Compared with the penicillin-resistant parental strain, the penicillin- and quinolone-resistant mutant was killed more slowly by cefepime and ceftriaxone in time-killing assays *in vitro* over 8 h.**

## Introduction

Before the emergence of penicillin-resistant pneumococci, penicillin was usually the first-line antibiotic in the treatment of pneumococcal infections. The global increase of resistant pneumococci has jeopardized the treatment of pneumococcal infections.[1] Additional resistance to cephalosporins has further limited the treatment options against penicillin-resistant isolates. Furthermore, pneumococcal strains resistant to quinolones have already been isolated.[2] $\beta$-Lactam antibiotics remain the first-line drugs for pneumococcal diseases, except when penetration into infected tissues is limited, as is the case in meningitis. A combination of vancomycin and a cephalosporin is recommended currently for meningitis due to resistant strains.[1,3] An alternative regimen based on a monotherapy would represent significant progress, especially when quinolone-resistant strains are suspected.

Cefepime is a broad-spectrum fourth-generation cephalosporin with good activity against a variety of human bacterial pathogens, including penicillin-resistant pneumococci, and has good penetration into the cerebrospinal fluid (CSF).[4,5] The aim of this study was to test the bactericidal activity of cefepime against a pneumococcal strain resistant to penicillin and quinolones in the rabbit meningitis model. The standard regimen consisted of ceftriaxone combined with vancomycin.

## Materials and methods

### Strains

The pneumococcal strain (WB4) was originally isolated from a patient with pneumonia at the University Hospital of Bern, Switzerland, and the quinolone-resistant mutant was obtained by sequential exposure of this strain to trovafloxacin *in vitro*. MICs were as follows—penicillin-resistant strain: penicillin 4 mg/L, ceftriaxone 0.5 mg/L, cefepime 0.5 mg/L, vancomycin 0.12–0.25 mg/L, trovafloxacin 0.12 mg/L, ciprofloxacin 0.5 mg/L; penicillin- and quinolone-resistant mutant strain: penicillin 4 mg/L, ceftriaxone 0.5 mg/L, cefepime 0.5 mg/L, vancomycin 0.12–0.25 mg/L, trovafloxacin 4 mg/L, ciprofloxacin 32 mg/L.

### Rabbit meningitis model

The meningitis model, originally described by Dacey & Sande,[6] was slightly modified. The experimental protocol was accepted by the Veterinäramt des Kantons Bern. Young New Zealand white rabbits weighing 2–2.5 kg were anaesthetized by intramuscular injections of ketamine (30 mg/kg) and xylazine (15 mg/kg) and were immobilized in stereotactic frames for induction of meningitis and CSF sampling. An inoculum containing *c.* $1 \times 10^5$ cfu of penicillin- and quinolone-resistant pneumococci serotype 6

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was injected directly into the cisterna magna. A long-acting anaesthetic (ethylcarbamate = urethane, 3.5 g/rabbit) was injected subcutaneously and animals were returned to their cages. Fourteen hours later the cisterna magna was punctured again for periodic CSF sampling before and 1, 2, 4, 6 and 8 h after initiation of therapy. Anaesthesia was performed by repetitive intravenous injections of nembutal. Antibiotics were administered through a peripheral ear vein as bolus injections at the following dosages: ceftriaxone 125 mg/kg, vancomycin 20 mg/kg, cefepime 100 mg/kg. Ceftriaxone was injected once at 0 h and vancomycin was injected at 0 and 4 h according to Friedland *et al.*[7] and Cottagnoud *et al.*[8] Cefepime was administered twice (at 0 and 4 h) according to Gerber *et al.*[9] Untreated controls received saline. All antibiotics and anaesthetic drugs were purchased commercially. Bacterial titres were measured by 10-fold serial dilutions of CSF samples, plated on blood agar plates containing 5% sheep blood and incubated overnight at 37°C. In parallel, 20 μL of undiluted CSF samples were plated (limit of detectability, 50 cfu/mL). Comparison between different dilutions of CSF was used to exclude significant carry-over effects during therapy. The antimicrobial activity of the regimens during the 8 h treatment was calculated by linear regression analysis and expressed as decrease of $\log_{10}$ cfu per millilitre per hour ($\Delta\log_{10}$ cfu/mL·h) and as killing rate over 8 h. A value of 1.7 ($\log_{10}$ of the limit of detection) was assigned to the first sterile CSF sample and a value of 0 to any following sterile sample. The results are expressed as mean ± S.D. Statistical significance was determined by the Newman–Keuls test.

## *Measurement of antibiotic concentrations in the CSF*

Antibiotic concentrations in the CSF were determined by the agar diffusion method. Standard curves were performed in saline with 5% rabbit serum in order to mimic CSF protein concentration.[10] *Bacillus subtilis* (ATCC 6633) was used for the assay of cefepime.[11] The intra- and inter-day variability of this method was <10%. The limit of detection was 1.5 mg/L for cefepime.

## *In vitro assays*

The pneumococcal strains (a penicillin-resistant strain and a penicillin- and quinolone-resistant mutant) were grown in C+Y medium[12] to optical density 0.3 at 590 nm and then diluted 40-fold to $10^6$ cfu/mL, corresponding to the CSF bacterial titre in rabbits before initiation of therapy. Ceftriaxone and cefepime were added in concentrations corresponding to 5 × and 10 × MIC (2.5 and 5 mg/L, respectively). The MIC was the same for the two strains. Bacterial titres were determined at 0, 2, 4, 6 and 8 h by serial dilution of samples, plated on agar plates containing 5% sheep blood and incubated at 37°C for 24 h. Experiments
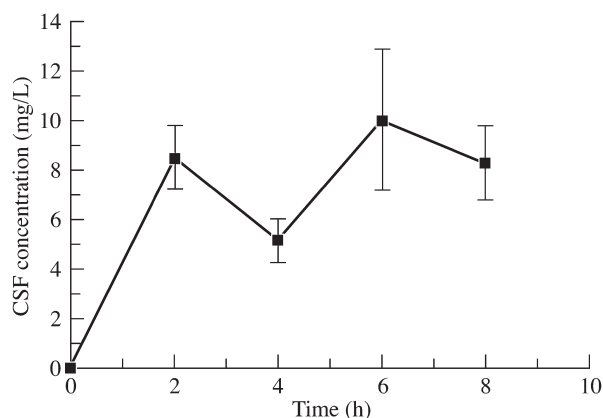


**Figure 1.** Cefepime concentration in the CSF for 8 h after iv injection of 100 mg/kg, given at 0 and 4 h. It remained above the MIC (0.5 mg/L) during the entire treatment period.

were performed in triplicate and results are expressed as mean ± S.D.

## Results

Figure 1 shows the kinetics of cefepime after two doses of 100 mg/kg. After the first dose, cefepime peaked at a mean of 8.5 mg/L, declining slowly to 5.2 mg/L 4 h later. After the second injection, the peak concentration increased slightly to 10 mg/L, decreasing to 8.1 mg/L at the end of the experimental period. During the entire therapy period, CSF concentrations remained above the MIC (0.5 mg/L). The ratio of CSF concentrations to MIC ranged between 24 and 12.

The killing rates of the different treatment groups are summarized in the Table. In untreated controls, bacterial titres increased slightly during 8 h (+0.35 ± 0.55 $\log_{10}$ cfu/mL·8 h). Cefepime produced a highly bactericidal activity and managed to sterilize the CSF of five of nine rabbits after 8 h. Cefepime monotherapy produced killing rates similar to the standard regimen based on ceftriaxone combined with vancomycin, which sterilized the CSF of six of nine rabbits after 8 h.

In time-killing assays over 8 h *in vitro*, cefepime and ceftriaxone were bactericidal against the penicillin- and quinolone-resistant strain and produced a decrease in the viable-cell counts of 3.6 and 3.8 $\log_{10}$ cfu/mL over 8 h for cefepime and ceftriaxone, respectively, using concentrations above (10 ×) the MIC (Figures 2 and 3). Cefepime was more bactericidal against the penicillin-resistant parent strain (additional 3 $\log_{10}$ over 8 h) and sterilized cultures after 6 h (Figure 2). Ceftriaxone produced slightly less pronounced bactericidal activity *in vitro* against the penicillin-resistant strain in concentrations above (10 ×) the MIC. Like cefepime, ceftriaxone was less effective against the quinolone- and penicillin-resistant mutant than against the parental strain in the same order of magnitude (*c.* 2.5 $\log_{10}$ over 8 h).

**Table.** Cefepime and combination therapy against penicillin- and quinolone-resistant *Streptococcus pneumoniae* in experimental meningitis

| Antibiotic | $n$ | Initial titre ($\log_{10}$ cfu/mL) (mean ± S.D.) | Killing rate ($\Delta\log_{10}$ cfu/mL·h) (mean ± S.D.) | Killing rate/8 h ($\log_{10}$ cfu/mL) (mean ± S.D.) |
|---|---|---|---|---|
| Controls | 5 | 5.39 ± 0.63 | +0.05 ± 0.11[a] | +0.35 ± 0.55[a] |
| Cefepime | 9 | 5.56 ± 0.85 | −0.61 ± 0.24[b] | −3.94 ± 1.10[b] |
| Ceftriaxone + vancomycin | 9 | 5.75 ± 0.91 | −0.58 ± 0.14[b] | −4.15 ± 0.78[b] |

[a]$P < 0.05$ versus all groups.
[b]$P$ not significant.

## Discussion

Recently the global spread of penicillin-resistant isolates has complicated the treatment of pneumococcal diseases with $\beta$-lactam antibiotics, underlining the need for alternative therapies.[3] Newer quinolones, with an extended activity against Gram-positive microorganisms might be a possible treatment. However, quinolone-resistant pneumococcal strains have been isolated recently, jeopardizing the wide use of this class of antibiotics in pneumococcal infections.[2]

Cefepime is a fourth-generation cephalosporin with good penetration into inflamed meninges[5] and excellent activity against pneumococci.[4] We have demonstrated recently that cefepime is very effective against penicillin-resistant pneumococcal strains in experimental meningitis, mostly due to its excellent penetration into inflamed meninges and its highly bactericidal activity against pneumococci.[9] Because of the lack of guidelines in the literature about the treatment of pneumococcal infections due to penicillin- and quinolone-resistant strains, we arbitrarily chose the combination treatment based on ceftriaxone and vancomycin as standard regimen. The dosages of antibiotics (ceftriaxone, cefepime, vancomycin) used in this experimental model were standard ones that have been used in previous studies in the same model,[7–9] corresponding to high dosages in humans.[13,14] Cefepime (100 mg/kg) administered twice produced CSF concentrations between 5.3 and 10 mg/L, corresponding to levels described previously.[9] The antibiotic concentrations used *in vitro* (5 mg/L, $10 \times$ MIC) are comparable to the concentrations achieved in the CSF of rabbits during meningitis.

In this experimental meningitis model, cefepime showed a pronounced antibacterial activity *in vivo* against the penicillin- and quinolone-resistant mutant ($-0.61 \pm 0.24$ $\Delta\log_{10}$ cfu/mL·h), which is comparable to the standard regimen, despite this strain being killed more slowly by cefepime *in vitro*, measured by a decreased bactericidal
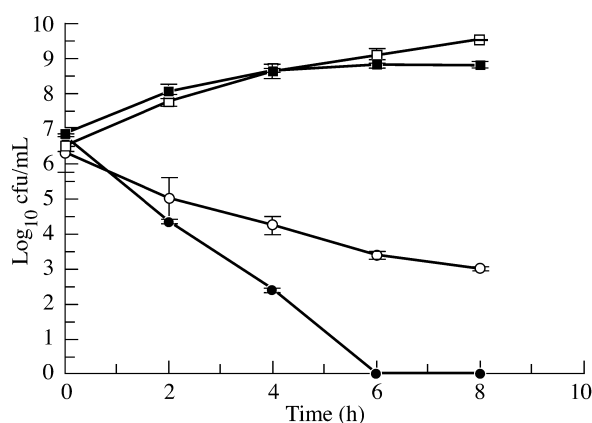


**Figure 2.** Killing rates of cefepime *in vitro* at concentrations corresponding to $10 \times$ MIC (5 mg/L) against a penicillin-resistant strain (●) and against a penicillin- and quinolone-resistant mutant (○). Untreated controls: penicillin-resistant strain (■); penicillin- and quinolone-resistant mutant (□). Experiments were performed in triplicate, and killing rates are expressed as mean ± S.D.
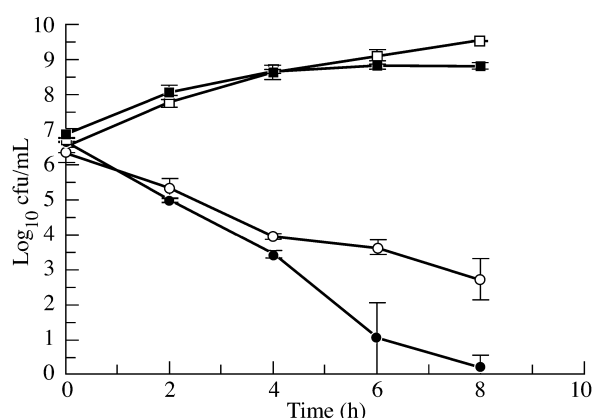


**Figure 3.** Killing rates of ceftriaxone *in vitro* at concentrations corresponding to $10 \times$ MIC (5 mg/L) against a penicillin-resistant strain (●) and against a penicillin- and quinolone-resistant mutant (○). Untreated controls: penicillin-resistant strain (■); penicillin- and quinolone-resistant mutant (□). Experiments were performed in triplicate, and killing rates are expressed as mean ± S.D.

efficacy of *c.* 3 log$_{10}$ cfu/mL in time-killing experiments over 8 h (Figure 2).

In a previous study with the same animal model, cefepime produced similar antibacterial activity ($-0.60 \pm 0.14$ $\Delta$log$_{10}$ cfu/mL·h) against the same penicillin-resistant parent strain used in the present study in time-killing experiments *in vitro*.[9]

Similarly, the penicillin- and quinolone-resistant mutant was killed more slowly by ceftriaxone *in vitro* (Figure 3), whereas the bactericidal activity of vancomycin remained unchanged (data not shown). In addition, this strain selected in the presence of trovafloxacin was also killed less rapidly by trovafloxacin and other quinolones tested in the same experimental setting (data not shown). The underlying mechanism of this common feature (diminution of bactericidal activity) between $\beta$-lactam antibiotics and quinolones is not clear, but it seems to be different from the cross-tolerance observed between vancomycin and $\beta$-lactam antibiotics,[15–17] since the bactericidal activity of vancomycin was maintained *in vitro*. Based on these preliminary data, it is too early to draw conclusions about the clinical significance of this cross-tolerance between quinolones and $\beta$-lactam antibiotics.

In summary, the good penetration of cefepime into the CSF and its efficacy in our animal model mean that cefepime qualifies for further controlled trials, especially with respect to multiresistant strains, e.g. penicillin- and quinolone-resistant strains.

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