Comparison of the effect of cefepime with four cephalosporins against pneumococci with various susceptibilities to penicillin, in vitro and in the mouse peritonitis model

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The purpose of this study was to compare the effect, both in vitro and in vivo, of cefepime with those of four other cephalosporins, namely ceftriaxone, cefotaxime, cefuroxime and cephalothin, against penicillin-resistant pneumococci. One hundred pneumococcal strains, 31 penicillin-susceptible, 30 penicillin-intermediate-resistant and 39 penicillin-resistant pneumococci, were used in MIC studies. Time–kill experiments were carried out for four strains. In the mouse peritonitis model, the dose that gave protection to 50% of mice challenged with a lethal dose of pneumococci (ED$_{50}$) was determined for three pneumococci and five cephalosporins. The MICs of all five cephalosporins and penicillin correlated significantly with each other. In vitro, the most potent cephalosporins against pneumococci were cefotaxime, ceftriaxone and cefepime, followed by cefuroxime and cephalothin. In time–kill experiments, carried out for four pneumococci, no differences were found in the killing effect of these five cephalosporins in relation to MICs. In the mouse peritonitis model, there was no significant correlation between log(MIC) and log(ED$_{50}$) for the five cephalosporins against three pneumococci (Spearman’s $\rho = 0.39$, $P = 0.16$). However, if the values for cefepime against the three pneumococci were excluded, there was a significant correlation for the remaining four cephalosporins (Spearman’s $\rho = 0.62$, $P = 0.04$). For all three pneumococci, the ED$_{50}$s of cefepime were lower than expected from the MICs. It was not possible to explain this beneficial difference in the effect of cefepime in terms of in-vitro bactericidal activities, serum protein binding or pharmacodynamic parameters.

Introduction

Treatment of pneumococcal infections has become more difficult in the recent years, since clinical isolates of Streptococcus pneumoniae in many parts of the world have shown an increasing frequency of resistance to various antibiotics. Resistance to $\beta$-lactam antibiotics, caused by changes in the genes encoding $\beta$-lactam-binding proteins, is of great importance since $\beta$-lactam antibiotics are first-choice antibiotics in most cases of pneumococcal disease. We have previously shown that there is a linear correlation between the MIC and the dose of penicillin needed for treatment of penicillin-resistant pneumococci. Increased doses of penicillin may, therefore, be sufficient to treat most infections caused by pneumococci with intermediate resistance to penicillin, but for cases caused by penicillin-resistant pneumococci (MICs > 1 mg/L), cephalosporins are suggested. To study the effect of various cephalosporins against penicillin-resistant pneumococci, we used the mouse peritonitis model and various in-vitro measurements. The four cephalosporins studied were cefepime (fourth-generation), cefotaxime and ceftriaxone (both third-generation; they differ considerably in their pharmacokinetics), cefuroxime (second-generation) and cephalothin (first-generation).

Materials and methods

Bacteria and media

One hundred pneumococcal strains were chosen to represent the different susceptibility groups for penicillin, i.e. susceptible (MIC < 0.1 mg/L; $n = 31$), intermediate resistant (0.1 $\leq$ MIC $\leq$ 1.0 mg/L; $n = 30$) and resistant
The pneumococcal strains were not epidemiologically related, and some of these strains had been tested previously for susceptibility to macrolides, tetracycline and clindamycin. Forty-five strains were isolated from patients in Denmark (serotypes 4 (n = 1), 6A (1), 6B (4), 7F (1), 9N (2), 9V (19), 12F (1), 14 (5), 15A (1), 19A (2), 19F (2), 20 (2), 22F (1), 23F (1) and 31 (1)), 22 from patients in Iceland (serotypes 6B (n = 5), 9V (1), 14 (1), 19A (6), 23F (8) and 36 (1)) and 33 from patients in other parts of the world (serotypes 3 (n = 8), 6A (1), 6B (1), 7F (1), 9L (1), 14 (9), 15B (1), 19A (3) and 23F (7)). Four pneumococci, three penicillin-resistant, but with different susceptibilities to the cephalosporins (serotypes 9V (n = 2) and 14 (n = 1)), and one penicillin-intermediate-resistant strain (serotype 6B), were studied by time–kill experiments. For the mouse peritonitis model, three of these four strains were chosen (serotypes 6B, 9V and 14). S. pneumoniae ATCC 25922 was included in all MIC determinations for five cephalosporins as reference strains.

Inocula were prepared immediately before use by suspending colonies in sterile beef broth and adjusted to an optical density of 0.5 at 540 nm, giving a density of approximately 10^8 cfu/mL, followed by appropriate dilution. Dilutions after making ten-fold dilutions in beef broth, of which 20 μL was plated on 5% blood agar plates in spots in duplicate with subsequent counting of colonies after incubation overnight at 35°C in ambient air.

Beef broth, 5% blood agar plates, and Mueller–Hinton agar plates with 5% horse blood and antibiotics, were produced at the Statens Serum Institut (Copenhagen, Denmark). Mucin (Sigma Chemical Co., St Louis, MO, USA), an enzyme extract of porcine stomach, was used as adjuvant for inoculating mice and was prepared as a saline stock solution of 10% (w/v). Immediately before inoculation, the mucin solutions were diluted 1:1 with pneumococcal suspensions, giving a final mucin concentration of 5% (w/v).

Antimicrobial agents

Cefepime was obtained from Bristol-Myers Squibb (Princeton, NJ, USA), cefotaxime from Hoechst A G (Frankfurt am Main, Germany) and ceftriaxone, ceftroxime and cephalothin from Sigma. The penicillin used for characterizing penicillin susceptibility was penicillin G (Leo, Bollerup, Denmark). All antimicrobial agents were dissolved in phosphate-buffered saline immediately before use.

MIC determinations

MICs were determined using the agar plate dilution method, as recommended by the National Committee for Clinical Laboratory Standards using Mueller–Hinton agar plates supplemented with 5% horse blood, containing drugs in two-fold dilutions from 128 mg/L to 0.004 mg/L and two control plates. Fresh overnight cultures of pneumococci from 5% blood agar plates were suspended in Mueller–Hinton broth and 1 μL containing 10^6 cfu per spot was inoculated on the plates using a multipoint inoculator (A 400; Denley Instruments Ltd, Billingham, Sussex, UK). The plates were incubated overnight, and the MIC was defined as the lowest concentration where fewer than four colonies per spot were observed.

Time–kill experiments

Time–kill curves for the four strains of pneumococci were performed for all five cephalosporins. We used concentrations corresponding to 1 × MIC and 10 × MIC in flasks containing 20 mL beef broth with an inoculum of 1 × 10^5 cfu/mL in a shake stand. A flask incubating for 1 h at 35°C, samples of 1 mL were drawn and replaced by antibiotic solutions of 20 times the final concentration or, for the antibiotic-free control, pure beef broth. Samples were taken for colony counting after 1, 4 and 24 h of incubation.

Serum protein binding

A flask incubation of the media, human serum, mouse serum or beef broth, respectively, in ambient air at 35°C, the pH was adjusted to 7.0–7.5 by bubbling CO₂ through the media. The cephalosporins at concentrations of 150 and 50 mg/L in the sera or media were incubated for 2 h at 35°C. The samples were divided into two parts, one part was centrifuged in tubes with filters with a cut-off at approximately 30 kDa (Centricon 30, no. 4208, Amicon, Beverly, MA, USA) in a fixed-angle rotor at 3000g for 20 min. The antibiotic concentrations in both the centrifuged and the uncentrifuged parts were determined using the agar cup method (with a non-haemolytic streptococcus, strain EB-68; Statens Serum Institut). The in-house method was based on a previously described assay. Variation coefficients were determined for eight samples (four different concentrations in human or mouse serum) as follows (median and range): cefepime, 5.7% (3.6–7.5%); cefotaxime, 6.4% (5.5–8.6%); cefalothin, 7.1% (4.4–11.2%); ceftriaxone, 6.9% (3.1–10.9%); ceftroxime, 6.7% (1.7–11.8%). A standard curve was produced using antibiotic concentrations dissolved in the same serum or medium. Thus, the protein-bound fraction could be calculated.

The mouse peritonitis model

For each of three pneumococci, the effect of the five different cephalosporins was studied in the mouse peritonitis model.
Cephalosporins and pneumococci throughout the study. The mice were kept five to a cage, and had free access to food and water. Inoculation was performed by intraperitoneal injection of an inoculum of 0.5 mL of a pneumococcal suspension via a 25 gauge syringe. The inoculum was 1–5 × 10^6 cfu/mL, with 5% (w/v) mucin in beef broth; this inoculum was known to be 100% lethal within 2 days and to provide a detectable growth phase of bacteria in the peritoneal cavity and in the blood of the mice. Treatment of mice in groups of five with different doses of the cephalosporins was performed as a single subcutaneous injection in the neck region with a volume of 0.5 mL per dose, 1 h after inoculation. Mice were observed for 6 days and the mortality was noted.

Determination of ED_{50}

The ED_{50}, the single dose giving protection to 50% of the mice, for each combination of drug and pneumococcus was determined from two trials, and calculated using the Hill equation. In the first trial, five mice in each of five groups were given the drug in ten-fold dilutions from 100 to 0.01 mg/kg. In the second trial, a range of dosages known from the first trial to produce from no effect to full effect were used. For each strain, a group of mice treated with saline was included as a control for the lethality of the infection.

The pharmacokinetic parameters

The pharmacokinetic parameters were determined using healthy mice that were bled in groups of three at different time intervals after treatments with one of two different doses of each drug. The mice were bled by periorbital cuts after anaesthesia with CO_2. A fer collection of blood samples, the blood was centrifuged at 16300g for 10 min, and the serum was stored at −80°C until analysis, which was performed in duplicate. The cup plate or the disc diffusion bioassay method was used to measure the cephalosporin concentration in mouse serum. The lowest measured value used was 1 mg/L for the drugs. For standard curves, the cephalosporins were diluted in pooled normal mouse serum. The serum half-life, t_{1/2}, was calculated as −log2/β, where β is the slope of the serum elimination regression line (time plotted against log(srum concentration)). The time when the serum concentration was above the MIC, T_{>MIC}, and the time when the unbound fraction of the serum concentration was above the MIC, T_{>MIC-free}, were calculated for doses equal to the ED_{50}S for the combinations of pneumococcal strains and cephalosporins.

Statistical methods

The Hill equation with variable slope (GraphPad Prism, GraphPad Software Inc., San Diego, CA, USA) was used to calculate the ED_{50}S if possible; otherwise the method of Reed & Muench was used. Spearman’s rank-correlation test was used to test the correlation between MIC values. P values of <0.05 were considered significant.

Results

In-vitro investigations

MIC-determinations. The MICs of penicillin and the five cephalosporins for the 100 pneumococci are given in Table I. The MICs of all five cephalosporins increased with increasing MICs of penicillin (see Table I). The MICs of all five cephalosporins and penicillin correlated significantly with each other (for all correlations, Spearman’s ρ > 0.90, P < 0.01). Cefotaxime, ceftriaxone and cefepime had the lowest MICs.

Time–kill experiments. Time–kill experiments were carried out for four strains. The bacterial kill effects are given as the difference in log(cfu/mL) between controls and antibiotic-containing flasks after 1 and 4 h, for each strain and cephalosporin (Table II). For all four strains, both growth controls and antibiotic-containing flasks were sterile after 24 h of incubation due to autolysis. For all combinations, the killing effect expressed in log(cfu/mL) after 1 h was between 0.33 and 2.05 for 1 MIC, and between 0.74 and 2.20 for 10 MIC. After 4 h, the killing effect was between 1.89 and 5.08 for 1 MIC, and between 3.38 and 5.54 for 10 MIC. No differences were found in killing effect of these five cephalosporins in relation to MICs.

Table I. MIC of penicillin and five cephalosporins for 100 pneumococci

<table>
<thead>
<tr>
<th>Penicillin status</th>
<th>penicillin</th>
<th>cephalothin</th>
<th>MIC_{50}/MIC_{90} (range) in mg/L</th>
<th>cefuroxime</th>
<th>cefotaxime</th>
<th>ceftriaxone</th>
<th>cefepime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible (n = 31)</td>
<td>0.031/0.031</td>
<td>0.125/0.25</td>
<td>0.016/0.031</td>
<td>0.008/0.016</td>
<td>0.016/0.031</td>
<td>0.016/0.06</td>
<td></td>
</tr>
<tr>
<td>(0.016–0.06)</td>
<td>(0.06–8)</td>
<td>(0.008–0.5)</td>
<td>(0.004–0.125)</td>
<td>(0.008–0.25)</td>
<td>(0.008–0.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediately resistant (n = 30)</td>
<td>0.5/1</td>
<td>1/4</td>
<td>0.25/1</td>
<td>0.125/0.5</td>
<td>0.125/0.5</td>
<td>0.25/0.5</td>
<td></td>
</tr>
<tr>
<td>(0.125–1)</td>
<td>(0.125–16)</td>
<td>(0.016–8)</td>
<td>(0.016–1)</td>
<td>(0.016–2)</td>
<td>(0.031–2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant (n = 39)</td>
<td>2/16</td>
<td>8/64</td>
<td>4/8</td>
<td>1/2</td>
<td>1/2</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>(2–64)</td>
<td>(0.125–64)</td>
<td>(0.016–8)</td>
<td>(0.008–4)</td>
<td>(0.016–4)</td>
<td>(0.016–4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table II. Time-kill experiments. The difference in log(cfu/mL) after 1 h and 4 h compared with controls for four pneumococcal strains and the five cephalosporins. The MICs of each drug and strain are given in mg/L.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cephalothin</th>
<th>Cefuroxime</th>
<th>Cefotaxime</th>
<th>Ceftriaxone</th>
<th>Cefepime</th>
<th>Median (range) for strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1×MIC</td>
<td>10×MIC</td>
<td>1×MIC</td>
<td>10×MIC</td>
<td>1×MIC</td>
<td>10×MIC</td>
</tr>
<tr>
<td>161A95</td>
<td>MIC</td>
<td>0.125</td>
<td>0.016</td>
<td>0.008</td>
<td>0.016</td>
<td>0.016</td>
</tr>
<tr>
<td>1h</td>
<td>2.05</td>
<td>2.16</td>
<td>1.62</td>
<td>2.05</td>
<td>1.50</td>
<td>2.20</td>
</tr>
<tr>
<td>4h</td>
<td>5.01</td>
<td>5.54</td>
<td>3.75</td>
<td>4.19</td>
<td>3.94</td>
<td>4.13</td>
</tr>
<tr>
<td>1064</td>
<td>MIC</td>
<td>1</td>
<td>0.25</td>
<td>0.06</td>
<td>0.125</td>
<td>0.5</td>
</tr>
<tr>
<td>1h</td>
<td>1.91</td>
<td>1.92</td>
<td>1.81</td>
<td>2.11</td>
<td>1.70</td>
<td>2.04</td>
</tr>
<tr>
<td>4h</td>
<td>3.86</td>
<td>4.55</td>
<td>3.90</td>
<td>3.86</td>
<td>3.64</td>
<td>3.84</td>
</tr>
<tr>
<td>189/59</td>
<td>MIC</td>
<td>16</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1h</td>
<td>1.19</td>
<td>1.80</td>
<td>1.39</td>
<td>1.86</td>
<td>1.50</td>
<td>1.71</td>
</tr>
<tr>
<td>4h</td>
<td>1.89</td>
<td>4.23</td>
<td>2.44</td>
<td>3.51</td>
<td>3.01</td>
<td>3.38</td>
</tr>
<tr>
<td>2916</td>
<td>MIC</td>
<td>64</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1h</td>
<td>0.77</td>
<td>1.23</td>
<td>0.39</td>
<td>0.88</td>
<td>0.64</td>
<td>1.01</td>
</tr>
<tr>
<td>4h</td>
<td>4.42</td>
<td>5.23</td>
<td>4.23</td>
<td>4.13</td>
<td>4.33</td>
<td>4.45</td>
</tr>
</tbody>
</table>

Median and range for the antibiotics

|        | 1h   | 1×MIC | 1.55 | 1.86 | 1.51 | 1.96 | 1.57 | 1.88 | 1.60 | 1.87 |
|        | range | 0.77-2.05 | 1.23-2.26 | 0.39-1.81 | 0.88-2.05 | 0.64-1.70 | 1.01-2.20 | 0.33-1.93 | 0.74-2.16 | 0.68-1.94 | 0.82-2.12 |
|        | 4h   | 1×MIC | 4.14 | 4.89 | 3.83 | 4.00 | 3.79 | 3.99 | 4.04 | 3.96 |
|        | range | 1.89-5.01 | 4.23-5.54 | 2.44-4.23 | 3.51-4.19 | 3.01-4.33 | 3.38-4.45 | 2.98-4.33 | 3.88-5.15 | 3.29-4.64 | 3.41-4.35 |

*Pencillin MIC: strain 169/95, 2 mg/L; strain 1064, 0.25 mg/L; strain 189/59, 2 mg/L; strain 2916, 4 mg/L.
Cephalosporins and pneumococci

Table III. The ED\textsubscript{50}S (and 95% confidence intervals) calculated using the Hill equation for three pneumococci and five cephalosporins in the mouse peritonitis model

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Strain 1064</th>
<th>Strain 189/59</th>
<th>Strain 2916</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED\textsubscript{50} (mg/kg)</td>
<td>MIC (mg/L)</td>
<td>ED\textsubscript{50} (mg/kg)</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>40.66\textsuperscript{a}</td>
<td>1</td>
<td>36.67\textsuperscript{a}</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>8.65 (6.05–12.37)</td>
<td>0.25</td>
<td>7.87 (2.88–21.51)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1.07 (0.82–1.38)</td>
<td>0.06</td>
<td>4.33 (3.42–5.48)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.61 (0.50–0.76)</td>
<td>0.125</td>
<td>2.35 (1.65–3.36)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0.66 (0.46–0.96)</td>
<td>0.5</td>
<td>0.60 (0.46–0.77)</td>
</tr>
<tr>
<td>Penicillin\textsuperscript{b}</td>
<td>11.18 (9.43–13.26)</td>
<td>0.25</td>
<td>ND</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The values were calculated using the methods of Reed & Muench.\textsuperscript{12}

\textsuperscript{b}Data formerly published.\textsuperscript{9}

Serum protein binding. The serum protein binding for the five cephalosporins at different concentrations was recorded as median values in mouse serum and human serum: 30% and 60% for cephalothin, 17% and 30% for cefuroxime, 12% and 10% for cefotaxime, 45% and 83% for ceftriaxone, and 20% and 11% for cefepime, respectively.

In-vivo investigations

ED\textsubscript{50} determination. The ED\textsubscript{50} was determined for three pneumococci in the mouse peritonitis model. The values are given in Table III with 95% confidence intervals calculated using the Hill equation with variable slope. It was not possible to calculate the ED\textsubscript{50} using the Hill equation for cephalothin against any of the three strains. For one of the strains, no. 2916, the ED\textsubscript{50} was above the highest dose given. The ED\textsubscript{50} for cefalothin and the two other strains was calculated by the method of Reed & Muench.\textsuperscript{12} The most potent cephalosporin in vivo against pneumococci, i.e. the one with the lowest ED\textsubscript{50}, was cefepime, followed by ceftriaxone, cefotaxime and cephalothin.

There was no significant correlation between log(MIC) and log(ED\textsubscript{50}) (Spearman’s \( \rho = 0.39, P = 0.16 \)). However, if the values for cefepime and the three pneumococci were excluded, a significant correlation appeared for the remaining four cephalosporins (Spearman’s \( \rho = 0.62, P = 0.04 \)). Figure 1 shows the correlation between log(MIC) and log(ED\textsubscript{50}) for cefalothin, cefuroxime, cefotaxime and ceftriaxone for the three strains of pneumococci; log(ED\textsubscript{50}) = 0.60 × log(MIC) + 0.82. The data set for cefalothin and the three pneumococci is also indicated, and shows a lower ED\textsubscript{50} than the other cephalosporins (Figure 1).

Determination of serum half-lives. Values were obtained from mice in groups of three at three times for two concentrations of each drug. Values obtained for cefalothin were not conclusive, since the serum concentrations were below detectable levels at the time point used. The following half-lives were calculated: cefuroxime, 19 min; cefotaxime, 14 min; ceftriaxone, 64 min; cefepime, 22 min. For cefalothin the half-life was taken as 11.5 min\textsuperscript{7} in the following calculations.

Pharmacodynamics. The T\textsubscript{\textgreater M IC} and the T\textsubscript{\textgreater M IC–free} for the different cephalosporins and the three pneumococci at the various ED\textsubscript{50}s are given in Figure 2. The ED\textsubscript{50} of the various cephalosporins represent the same effect in the model, 50% survival of mice, and the corresponding T\textsubscript{\textgreater M IC} or T\textsubscript{\textgreater M IC–free} at this effect are expected to be almost identical, which also seems to be the case for cefepime, cefotaxime, cefuroxime and cephalothin. Ceftriaxone had a relatively
high $T_{>\text{MIC}}$ and $T_{>\text{MIC-free}}$ compared with the other cephalosporins tested.

**Discussion**

Using the mouse peritonitis model, we have shown previously a significant linear correlation between log(MIC) and log($ED_{50}$) for a range of cephalosporins with different MICs for the same pneumococcus and for penicillin against ten pneumococci with varying MIC against penicillin. For cefotaxime, cefuroxime and ceftriaxone, with the same MIC against the pneumococcus used, there was a notable difference in $ED_{50}$; detailed comparative studies of the killing activity of the drugs as well as various pharmacokinetic parameters, including serum protein binding, revealed that the most important factor related to the $ED_{50}$ was the $T_{>\text{MIC}}$. Ceftriaxone had the longest $T_{>\text{MIC}}$ and the lowest $ED_{50}$ measured, confirming earlier results.

In the present study a significant linear correlation was demonstrated between the log(MIC) and log($ED_{50}$) for a number of cephalosporins, but only when the results for cefepime were excluded (Figure 1). For all three pneumococcal strains tested both in vivo and in vitro, the $ED_{50}$s for cefepime were lower than would have been predicted if results from all cephalosporins were pooled. There is no obvious explanation for these results. Other studies comparing the in-vitro and in-vivo activity for cefepime against different bacteria have not reported similar beneficial results for this drug as compared with other cephalosporins. Only two of these studies included pneumococci, but one used two meningitis models where pharmacological parameters were pertinent, and the other included only one pneumococcal strain. No difference was found in the latter study between cefepime, cefotaxime and ceftazidime for the pneumococcus or for four strains of S. aureus in a systemic infection mouse model.

Several factors might explain why the in-vivo activity of cefepime differed from that of the other cephalosporins tested; these include time–kill activity, post-antibiotic effect, better penetration of the drug to the site of infection, less dependence of drug on local inhibitory factors, degree of protein binding, extended $T_{>\text{MIC}}$ and stimulation of local or systemic immune system responses.

The MICs of the cephalosporins against pneumococci were as found by others. No significant differences in time–kill activity in vitro were found for any of the drugs tested in the present study (Table II). Similar results have been reported previously. The time–kill activity in vivo was not determined in the present study, but others have shown that cefepime does not differ in that respect from penicillin and cefotaxime with E. coli and Streptococcus group B, or S. aureus in a rat endocarditis model.

The post-antibiotic effect was not investigated in this study. The post-antibiotic effect of cefepime against Gram-positive bacteria has not been studied but, against Gram-negative bacteria, it does not differ from that of cefotaxime and ceftazidime. A previous study from our laboratory did not suggest any difference in post-antibiotic effect between newer and older cephalosporins.

Wise has reviewed the penetration of fourth-generation cephalosporins into different tissues and cavities and concluded that cefepime penetrated rapidly and completely into the human peritoneal cavity, whether or not inflamed. Presumably the same would be found in mice. The degree of protein binding of cefepime was found to be rather low, 20% and 11% in mouse and human sera, respectively. The low protein binding leads to better penetration into the site of infection than for more highly protein bound drugs such as ceftriaxone. On the other hand, high protein binding may also benefit a drug by binding to proteins at the site of the infection, and thereby extend locally the $T_{>\text{MIC}}$. The serum half-life of cefepime in mice, 22 min, revealed a relatively short $T_{>\text{MIC}}$ for doses at the $ED_{50}$. The $T_{>\text{MIC-free}}$ was also low relative to the other cephalosporins studied (Figure 2). An extended $T_{>\text{MIC}}$ thus could not be the explanation for the in-vivo activity of cefepime.

The activity of an antibiotic in vivo might depend on its
ability to tolerate variation in physicochemical factors (e.g. pH) at the site of the infection, and on the degree to which the antibiotic interacted with the immune system. Although these factors have not been completely elucidated, and although no such studies have been done with cefepime, it is known that the antibiotic activity of other cefalosporins is relatively unaffected by changes in pH. 22 A difference in the interaction of the various cefalosporins with mucin could theoretically be an explanation, but the mucin preparation with pneumococci was inoculated 1 h before the treatment with cefalosporins, and mucin was not detectable in the peritoneum at that time, presumably having been phagocytosed by the macrophages in the peritoneal cavity. 6 Further studies of the interaction of cefepime with the immune system would be necessary to follow this line but are beyond the scope of this study.

In conclusion, we found that the activity of the four cefalosporins, ceftaxime, ceftazidime, ceftriaxone and cephalothin, in vivo was predictable from the activity observed in vitro, but for cefepime, the in-vivo activity was better than expected from the pooled in-vitro data. It was not possible to explain this difference in terms of in-vitro bactericidal activities, serum protein binding or pharmacodynamic parameters.

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