Evaluation of Pefloxacin in Experimental Escherichia coli Meningitis

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The therapeutic efficacy of the fluoroquinolone pefloxacin mesylate was compared with those of cefotaxime and chloramphenicol in a rabbit model of Escherichia coli meningitis. The mean percent penetration (± the standard deviation) of pefloxacin (range, 1 to 30 mg/kg per h) into cerebrospinal fluid of infected rabbits was 51.3 ± 14.0 compared with 11.1 ± 1.0 for cefotaxime (100 mg/kg per h) and 22.3 ± 1.5 for chloramphenicol (60 mg/kg per h). The rate of bacterial killing (Δlog10 CFU/ml per h) did not change over a dosage range of 1 to 15 mg/kg per h (−0.37 ± 0.15, 20% sterile). At 30 mg/kg per h, the rate achieved (−0.77 ± 0.18, 100% sterile) was comparable to that of cefotaxime (−0.88 ± 0.23, 100% sterile) and superior to that of chloramphenicol (−0.10 ± 0.14, 0% sterile).

Pefloxacin mesylate, a new fluoroquinolone, has been shown to have a wide spectrum of activity both in vitro and in vivo (4, 8, 12, 13). Because of its excellent activity against members of the family Enterobacteriaceae and because in vitro tests may not reliably predict the bactericidal activity of a drug against meningitis (9), we evaluated the therapeutic efficacy of pefloxacin with a well-standardized rabbit model of Escherichia coli meningitis. The specific purposes of this investigation were to determine the percent penetration of pefloxacin into the cerebrospinal fluid (CSF) and to compare the in vivo efficacy of the drug with those of cefotaxime and chloramphenicol.

MATERIALS AND METHODS

Experimental strain. E. coli 492 was kindly supplied by George McCracken. It was originally isolated from the CSF of a patient with meningitis and is serum resistant. Stock cultures were stored frozen on glass beads at −70°C until the beginning of each experiment. At that time, a single bead was removed and incubated overnight in trypto soy broth at 35°C. The inocula were prepared from these overnight cultures.

Antimicrobial agents. Pefloxacin was supplied by Rhone-Poulenc Sante, Paris, France. Commercially available cefotaxime (Hoechst-Roussel Pharmaceuticals, Somerville, N.J.) and chloramphenicol (Parke, Davis & Co., Morris Plains, N.J.) were also used.

Susceptibilities. MICs and MBCs were measured in Mueller-Hinton broth according to standard macrotube dilution methods (7), against an inoculum of 2 × 103 to 4 × 104 CFU/ml. The MBC was defined as the lowest concentration of drug that prevented visible turbidity. The MBC, defined as the concentration that killed ≥99.9% of the original inoculum, was measured by streaking 0.01 ml from each tube not showing turbidity onto a blood agar plate, incubating the plates overnight at 35°C, and counting the colonies thereon at that time.

Time-kill curves. The in vitro bactericidal activity in Mueller-Hinton broth or in CSF of normal rabbits was examined over time in tubes containing pefloxacin (0.125, 1.25, 12.5, and 25.0 μg/ml) or cefotaxime (0.25, 2.5, and 25.0 μg/ml). Tubes were inoculated with 1 × 106 to 2 × 106 CFU and incubated at 35°C. Samples were quantitatively cultured at 0, 2, 6, and 24 h.

In vivo experiments. To determine the percent penetration of pefloxacin into the CSF of normal uninfected rabbits, six rabbits received a 3-h constant infusion of 5, 15, or 30 mg/kg per h. Simultaneous blood and CSF samples were obtained at 30 min and 1, 2, and 3 h during the infusion. The CSF and the serum samples were stored at −70°C until drug assays were performed.

E. coli meningitis was induced in New Zealand White rabbits (2 to 3 kg) by previously described methods (1). Briefly, animals were anesthetized with sodium pentobarbital (30 mg/kg) and inoculated intracisternally with 2 × 105 E. coli in 0.2 ml of 0.85% saline. They were then returned to their cages. Sixteen h later, they exhibited such evidences of meningitis as fever (≥39°C), CSF pleocytosis of ≥1,000 leukocytes per mm3, and a mean CSF bacterial titer of 4 × 108 CFU/ml. If untreated, this disease is 100% fatal within 48 to 72 h of induction.

At the beginning of therapy (16 h after intracisternal inoculation), the animals were given a slow intravenous infusion of urethane (1.75 g/kg) as a long-acting anesthetic. Pefloxacin, cefotaxime, and chloramphenicol were infused through a peripheral ear vein at a constant rate of 10 ml/h. The drugs were administered over a 7-h period, with each animal receiving one drug at one concentration. A 10-ml bolus was given at the beginning of the treatment, and simultaneous blood and CSF samples were obtained at 0, 1, 3, 5, and 7 h of therapy. CSF bacterial titers were determined by quantitative cultures onto blood agar plates which were then incubated at 37°C for 24 h. The remainder of the CSF and the serum samples were stored at −70°C until drug assays were performed.

At the end of the infusion, the animals were returned to their cages. The next morning, CSF samples were again obtained for bacterial culture and drug concentration as described above. At this time, a volume of 0.05 ml was also streaked directly onto a blood agar plate containing 5 μg of pefloxacin per ml, and the plates were incubated at 35°C for 24 h.

Drug assays. Drug concentrations in serum and CSF were determined by bioassay by the agar well diffusion technique (3), using E. coli (ATCC 10536) as the test strain for
TABLE 1. Pefloxacin concentrations in serum and CSF of uninfected rabbits

<table>
<thead>
<tr>
<th>Dosage (mg/kg per h)</th>
<th>Concentration (ug/ml) in:</th>
<th>Percentage Penetrationa (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>CSF</td>
</tr>
<tr>
<td>30</td>
<td>29.8 ± 5.8</td>
<td>10.0 ± 1.6</td>
</tr>
<tr>
<td>15</td>
<td>14.2 ± 2.8</td>
<td>5.5 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>6.1 ± 0.7</td>
<td>1.6 ± 0.8</td>
</tr>
</tbody>
</table>

a Mean ± standard deviation.

cefotaxime and pefloxacin and Sarcina lutea (ATCC 9341) for chloramphenicol. For each rabbit, the percent penetration was calculated as (mean drug concentration in CSF/mean drug concentration in serum) × 100.

RESULTS

The MIC/MBC of pefloxacin for the E. coli strain was 0.125/0.125 μg/ml. The MICs/MBCs of cefotaxime and of chloramphenicol were 0.25/0.5 and 8/±64 μg/ml, respectively.

In normal uninfected rabbits, pefloxacin penetrated remarkably well into the CSF (Table 1). The percent penetration ranged from 26 to 33% at doses of 5, 15, and 30 mg/kg. The drug penetration increased progressively with increasing dose, ranging from 1.0-2.5, with a mean ± standard deviation of 2.3 ± 1.5.

At the start of the experiment, the bacterial titer in the serum of the rabbits was 4.3 ± 0.8, rising to 6.0 ± 0.5 at the end of the 7-h infusion period if the animals were left untreated. The rates of killing over 7 h for the 1.0-, 2.5-, 5.0-, and 15.0-mg/kg pefloxacin dosages were similar (Table 3). For some unrelated reasons, the killing rate of a dose of 2.5 mg/kg per h was greater than at doses of 5.0 or 10.0 mg/kg per h, although not significantly different. The greatest killing rate attained with the pefloxacin regimen of 30 mg/kg per h (~0.77 ± 0.18) was comparable to that attained with cefotaxime (~0.88 ± 0.2; \( P > 0.1 \)). All recipients of this dose of pefloxacin and of cefotaxime had sterile CSF cultures by the end of the 7-h infusion period. Of the remaining 20 pefloxacin-treated rabbits, only 4 had sterile CSF cultures at the end of the drug infusion.

All recipients of 1.0 mg of pefloxacin per kg per h still had positive CSF bacterial cultures (range, 2.3 to 3.8 log10 CFU/ml) the day after the end of dosing with no growth, however, on the blood agar plates that contained 5 μg of pefloxacin per ml. The remaining CSF samples from pefloxacin-treated rabbits showed no bacterial growth. The mean concentration of pefloxacin in CSF at 24 h was 1.3 ± 1.2 μg/ml. All of the cefotaxime-treated animals had sterile CSF at this time, and no drug was detectable by bioassay. Chloramphenicol reduced bacterial titers by 1 log over the 7-h infusion period, and neither of the chloramphenicol-treated rabbits that survived overnight had sterile CSF.

The killing rates were comparable at concentrations of 0.125, 1.25, and 12.5 μg/ml. The killing curves in pooled, normal CSF (Fig. 1B) showed regrowth when pefloxacin concentrations were ≤1.25 μg/ml. (In both media, the killing curves for 0.125 μg of pefloxacin per ml were similar to those for 1.25 μg of pefloxacin per ml.) The killing effect of cefotaxime in broth was maximal at the MBC for the organism (0.25 μg/ml), while in ex vivo CSF, this was not achieved until a concentration of 2.5 μg/ml was reached (data not shown).

DISCUSSION

Remarkably good penetration of pefloxacin into the CSF of both normal and infected rabbits (34 versus 51%) was obtained, similar in extent to the 52% penetration that Wolff et al. found in humans (14). Armengaud et al. (M. Armengaud, V. Thotran, and D. Costazo, Proc. 13th Int. Congr. Chemother., p. 11023–11028, 1983) also found that pefloxacin readily diffuses into the CSF of both normal dogs (44%) and those with experimentally induced meningitis (75%).

Our results indicate that pefloxacin is active in vitro and in vivo against the strain of E. coli tested. The in vivo killing rate attained with this agent was comparable to that attained with other antimicrobial agents examined in the same model (2, 5, 6, 10, 11). At a dosage of 30 mg/kg per h, pefloxacin was as effective as cefotaxime and superior to chloramphenicol. Pefloxacin-resistant strains were not detected for any of the dosing regimens.
The CSF concentrations of cefotaxime productive at this high killing rate were 10 to 20 times the MBC, a result comparable to that attained with other beta-lactams in the same experimental system (2, 6). Although maximal bacterial killing in the CSF did not occur until pefloxacin concentrations were ~200 times the MBC, because of its excellent ability to cross the blood brain barrier, these concentrations are obtainable.

These studies in animals, along with preliminary data in humans and the excellent in vitro activity of the drug, indicate that pefloxacin should be evaluated further for possible therapy of meningitis caused by E. coli.

FIG. 1. In vitro killing curves of E. coli with pefloxacin. (A) In Mueller-Hinton broth; (B) in ex vivo CSF. Symbols: *, control (no drug); O, 1.25 µg of pefloxacin per ml; Δ, 12.5 µg of pefloxacin per ml; ×, 25.0 µg of pefloxacin per ml.

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LITERATURE CITED


