Pharmacodynamics of trovafloxacin in experimental pneumococcal meningitis: basis for dosage selection in children with meningitis

Cynthia C. McCoig*, Loretta Wubbel, Hasan S. Jafri, Irja Lutsar, Rafael Bastero, Kurt Olsen, Sharon Shelton, Ian R. Friedland and George H. McCracken

Department of Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-9063, USA

Trovafloxacin is a recently approved fluoroquinolone with excellent activity against Gram-positive and Gram-negative organisms that offers a potential alternative for treatment of \( \beta \)-lactam-resistant pneumococcal meningitis. Using the rabbit meningitis model, we sought to characterize the pharmacodynamic properties of trovafloxacin in the cerebrospinal fluid (CSF). Animals were given single doses of trovafloxacin of 10, 15, 20 or 30 mg/kg; 1 h after infusion mean CSF concentrations were 0.59 ± 0.18, 0.74 ± 0.14, 1.12 ± 0.12 and 1.07 ± 0.35 mg/L, respectively. The bacterial killing rate increased with increasing dosages of trovafloxacin, indicating that its activity is concentration dependent. All three pharmacodynamic indices (area under the concentration curve (AUC)/MBC, peak concentration (C_{\text{max}})/MBC, and time above MBC (\( T > \text{MBC} \)) correlated with bacterial killing; however, AUC/MBC correlated best (\( r = 0.71 \)). In a second experiment we found comparable bacterial killing with multiple doses of trovafloxacin given either every serum half-life or every two serum half-lives. In both experiments bacterial regrowth occurred when the concentration of trovafloxacin in CSF fell below the MBC. These data have been used in formulating an appropriate regimen for trovafloxacin treatment of bacterial meningitis in children.

Introduction

Current initial therapy for penicillin- and cephalosporin-resistant pneumococcal meningitis consists of vancomycin and a third-generation cephalosporin. However, penetration of vancomycin into the cerebrospinal fluid (CSF) has been characterized as erratic especially when used with dexamethasone.\(^ 1 \) By contrast, most fluoroquinolones enter CSF well because of their lipophilicity.\(^ 3 \) Trovafloxacin, a fluoroquinolone recently approved for use in adults, has excellent activity against both Gram-positive and Gram-negative organisms. Its activity against \textit{Streptococcus pneumoniae} is independent of the organism’s resistance to \( \beta \)-lactam antibiotics, which makes it a potential option for the treatment of cephalosporin-resistant pneumococcal meningitis.

The activity of quinolones best correlates with area under the concentration curve (AUC)/MIC in experimental sepsis when survival is used as an endpoint.\(^ 5 \) In experimental meningitis, activity, defined by bacterial killing rate in CSF, correlated with peak concentration (\( C_{\text{max}} \))/MBC.\(^ 6 \) However, the pharmacodynamic properties of trovafloxacin in CSF have not been studied in detail. The purpose of this study was to determine the pharmacodynamic profile of trovafloxacin in CSF in experimental penicillin- and cephalosporin-resistant pneumococcal meningitis and to determine the dosing regimen necessary for optimal bacterial killing that could be implemented for treatment of children with bacterial meningitis.

Materials and methods

Bacterial strain

A type 6B strain of \textit{S. pneumoniae} originally isolated from an infant with meningitis was used for all experiments.\(^ 7 \) After intrathecal passage in rabbits, the strain was grown overnight on blood agar plates. The plates were washed with phosphate-buffered saline (PBS), and aliquots of the resultant suspension were frozen at \(-70^\circ\text{C}\). For preparation of the inoculum, aliquots were diluted in PBS to a concentration of approximately \( 5 \times 10^5 \text{ cfu/mL} \) of which 250 \( \mu \text{L} \) was injected intracisternally into each rabbit. The

*Corresponding author. Tel: +1-214-648-3720; Fax: +1-214-648-2961.
inoculum size was confirmed by quantitative cultures in each experiment.

Susceptibility tests
The MICs and MBCs of different antibiotics were measured in Mueller–Hinton broth supplemented with 3–5% lysed horse blood by a standard microdilution method.8

Meningitis model
The rabbit meningitis model, modified from the original description by Dacey & Sande,9 was used. New Zealand white male rabbits weighing 2–2.5 kg were anaesthetized with intramuscular ketamine (50 mg/kg) and acepromazine (4 mg/kg) before every procedure. Flunixin meglumine (1.1 mg/kg) was administered intramuscularly every 12 h for analgesia. Animals were immobilized in stereotactic frames, and a spinal needle was introduced into the cisterna magna to withdraw 250 µL of CSF and to inject an equal volume of the bacterial inoculum (approximately 1 × 108). Treatment was initiated 16–18 h after inoculation (0 h) once CSF was withdrawn for quantification of the initial bacterial concentration. Animals were killed with pentobarbital (120 mg/kg) at the end of each experiment or earlier if they appeared severely lethargic or were unable to maintain recumbency.

Treatment
Alatrofloxacin, the prodrug of trovafloxacin (Pfizer Central Research, Groton, CT, USA), was administered as an intravenous bolus to rabbits and protected from light during preparation and infusion. In the first experiment, single doses of 10 mg/kg (n = 6), 15 mg/kg (n = 7), 20 mg/kg (n = 8) or 30 mg/kg (n = 8) were infused at 0 h. In the second experiment, rabbits were given an initial loading dose of 20 mg/kg followed by either 15 mg/kg at 4 h (n = 18) or 10 mg/kg at 2, 4 and 6 h (n = 13). These dosing intervals were chosen on the basis of previous animal studies showing the serum half-life of trovafloxacin to be 2 h.10 Animals in the second experiment were divided into two treatment groups. The first treatment group (treated four times) had CSF samples collected at 1, 2, 3, 4, 5, 6, 7, 24 and 48 h and blood at 0.5, 1, 2, 2.5, 3, 4, 4.5, 5, 6 and 6.5 h. The second treatment group (treated twice) had CSF samples collected at 1, 4, 5, 24 and 48 h and blood at 0.5, 1, 4 and 4.5 h. CSF and blood samples were centrifuged at 5000g for 5 and 10 min, respectively, and the supernatants were stored at −70°C until determination of antibiotic concentrations. CSF specimens visibly contaminated with blood were excluded from analysis.

An additional 100–150 µL aliquot of CSF was collected for quantification of bacterial concentrations at 0, 3, 6, 12 and 24 h (experiment 1) and 0, 4, 24 and 48 h (experiment 2). Bacterial concentrations were quantified by plating undiluted and serial dilutions of CSF (100 µL) on sheep blood agar and incubating in 5% CO2 at 35°C for 24 h. The lowest bacterial concentration detectable by this method was 10 cfu/mL. For purposes of analysis, specimens with <10 cfu/mL were assigned a value of 10 (1 log10) cfu/mL.

Antibiotic assays
Trovafloxacin concentrations were determined by disc diffusion microbioassay using Bacillus subtilis ATCC 6633.11 The lower limit of detection was 0.2 mg/L. Inter- and intra-assay coefficients of variation were <10% for both serum and CSF.

Pharmacodynamic calculations
Pharmacodynamic indices were calculated based on serum and CSF trovafloxacin concentrations measured from 1 to 12 h (experiment 1) after infusion of trovafloxacin. Calculations were performed using Top Fit 2.0 (Karl Thomae, Boehringer Ingelheim, Germany). A two-compartment model (with lag-time) was used for calculations of blood pharmacokinetic indices and a non-compartmental model for CSF pharmacokinetics. Data from each animal were calculated individually for the CSF analyses. Because of infrequent sampling in the α-phase the half-lives (t1/2) and area under the concentration–time curves for serum were calculated based on mean values of each dosage group. The AUCs were estimated to the last quantifiable concentration using the logarithmic trapezoidal rule and extrapolated to infinity using terminal-phase rate constant. Time of concentration above MBC (T > MBC) was calculated using a logarithmic regression line. AUC/MBC was calculated as a ratio, as was Cmax/MBC.

Statistical analysis
To assess correlations between pharmacokinetic indices (AUC/MBC, Cmax/MBC, T > MBC) and bacterial killing rates, data were fitted to an asymmetrical sigmoid curve.
Trovafloxacin in experimental meningitis using the Hill equation (Sigma Plot, SPSS, Inc., Chicago, IL, USA).

**Results**

**In-vitro susceptibility**

For the pneumococcal strain used, the MICs and MBCs, respectively, of both penicillin and ceftriaxone were 4.0 mg/L and 4.0 mg/L and of trovafloxacin, 0.06 mg/L and 0.125 mg/L.

**Antibiotic concentrations**

Mean serum and CSF trovafloxacin concentrations measured 30 min to 6 h after one dose are presented in Table I. Concentrations at 12 h and 24 h were below the level of detection. The highest CSF and serum concentrations were measured 1 h and 30 min, respectively, after drug administration and were considered the peak concentrations.

**Pharmacokinetic calculations**

The serum and CSF half-lives of trovafloxacin were similar in all treatment groups although there was a trend of increasing CSF half-life values with increased dosages (Table II). The mean values for penetration of trovafloxacin into the CSF (AUC\textsubscript{CSF}/AUC\textsubscript{serum}) were from 21 to 27%.

**Bacteriological effectiveness**

Mean CSF concentrations of *S. pneumoniae* measured in animals treated with single doses of trovafloxacin are shown in Figure 1. Initial mean bacterial concentrations were similar for all groups. Mean maximal reductions in bacterial titres (\(\log_{10}\) cfu/mL) were \(-1.82 \pm 1.68, -2.74 \pm 0.22, -3.27 \pm 1.10\), and \(-4.89 \pm 0.92\) at 12 h for animals receiving 10, 15, 20 and 30 mg/kg, respectively.

When data from all experiments were combined, there was a direct correlation between the bacterial killing rate (\(\Delta \log_{10}\) cfu/mL/h) over 12 h and each of the pharmacokinetic indices, AUC/MBC, \(C_{\text{max}}/\text{MBC}\), and \(T > \text{MBC}\), in CSF; however, the relation between AUC/MBC and bacterial killing rate best fits the sigmoid bacterial killing curve (Figure 2).

Mean CSF concentrations of *S. pneumoniae* measured in animals given multiple doses of trovafloxacin are shown in Figure 3. Mean reductions in bacterial titres were \(-3.84\) and \(-2.80 \log_{10}\) cfu/mL at 4 h for animals treated with 20 mg/kg followed by 10 mg/kg every 2 h for three doses and 20 mg/kg followed by 15 mg/kg at 4 h, respectively. At 24 and 48 h the bacterial titres were similar.

**Discussion**

Like aminoglycosides, fluoroquinolones exhibit concentration-dependent bacterial killing.\textsuperscript{5,12,13} Our study shows that

### Table I. Mean (± s.d.) CSF and serum concentrations of trovafloxacin after a single dose of trovafloxacin in a rabbit model of meningitis

<table>
<thead>
<tr>
<th>Dosage (mg/kg)</th>
<th>Sample</th>
<th>Concentration of trovafloxacin (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>serum</td>
<td>3.48 ± 0.65 2.30 ± 0.57 1.38 ± 0.50 0.81 ± 0.49 0.89 ± 0.61 0.42 ± 0.38</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>CSF</td>
<td>0.59 ± 0.18 0.39 ± 0.17 0.29 ± 0.14 0.24 ± 0.17 0.17 ± 0.06</td>
</tr>
<tr>
<td>15</td>
<td>serum</td>
<td>5.10 ± 1.02 3.29 ± 0.87 2.05 ± 0.55 1.41 ± 0.63 1.17 ± 0.59 0.48 ± 0.32</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>CSF</td>
<td>0.74 ± 0.14 0.54 ± 0.16 0.39 ± 0.15 0.31 ± 0.07 0.19 ± 0.04</td>
</tr>
<tr>
<td>20</td>
<td>serum</td>
<td>6.00 ± 0.94 3.88 ± 1.39 2.76 ± 0.85 1.57 ± 0.72 1.24 ± 0.59 0.61 ± 0.42</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>CSF</td>
<td>1.12 ± 0.12 0.74 ± 0.13 0.48 ± 0.14 0.35 ± 0.09 0.20 ± 0.06</td>
</tr>
<tr>
<td>30</td>
<td>serum</td>
<td>8.21 ± 2.34 5.45 ± 1.39 3.53 ± 1.26 2.32 ± 0.62 1.46 ± 0.43 1.05 ± 0.67</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>CSF</td>
<td>1.07 ± 0.35 0.85 ± 0.18 0.64 ± 0.17 0.48 ± 0.16 0.24 ± 0.11</td>
</tr>
</tbody>
</table>

\*Time after trovafloxacin infusion.
initial bacterial killing in CSF increases as the concentration of trovafloxacin increases. Because bacterial killing rates follow a sigmoid curve we fitted each of the pharmacodynamic indices to such a curve; AUC/MBC correlated best. This is the first demonstration of the association between AUC/MBC and bacterial killing in CSF for trovafloxacin. Studies of fluoroquinolone therapy in systemic infections, other than in the nervous system, have also shown a strong association between AUC/MIC and bacterial killing. Using the rabbit meningitis model, Kim et al. showed that pneumococcal killing by trovafloxacin was dependent on the peak concentration in CSF. However, correlations with CSF AUC/MBC or T > MBC were not assessed, and a specific correlation between C\text{max}/MBC and bacterial killing rate was not reported.

Drusano et al., using lomefloxacin in the neutropenic sepsis rat model, showed that AUC/MIC was most closely
Trovafoxacin in experimental meningitis

Figure 3. Mean concentrations of *S. pneumoniae* in CSF in rabbits treated with different regimens of trovafloxacin: ●, 20 mg/kg at 0 h followed by 15 mg/kg at 4 h; □, 20 mg/kg at 0 h followed by 10 mg/kg at 2, 4 and 6 h. Error bars represent SD.

linked to clinical outcome; however, when $C_{\text{max}}$/MIC ratios were >10:1, $C_{\text{max}}$/MIC was the most accurate indicator.\textsuperscript{14} We were unable to achieve consistently $C_{\text{max}}$/MIC ratios above 10:1 in CSF ($C_{\text{max}}$/MBC > 5 for our strain of *S. pneumoniae*) precluding validation of that observation. Forrest et al., using ciprofloxacin in seriously ill patients, determined that among pharmacodynamic variables the AUC/MIC ratio was the most important predictor of outcome.\textsuperscript{15} This study was conducted in patients with lower respiratory tract, soft tissue, and urinary tract infections. None of the patients studied had meningitis.

In our model of single dosing, we showed that once the concentration of trovafloxacin fell below the MBC, bacterial regrowth occurred, suggesting that trovafloxacin has a minimal post sub-MBC effect in CSF. Because similar results have been reported in this meningitis model,\textsuperscript{1} we believe it is prudent to space the dosing of trovafloxacin in meningitis to ensure that CSF concentrations do not fall below the MBC.

Using multiple trovafloxacin doses we compared regimens in which the doses were given every serum half-life or every two serum half-lives. Dosing intervals larger than two half-lives would allow drug concentrations to fall below the MBC. Both dosing regimens in our experiments resulted in equivalent bacterial killing at 24 and 48 h, suggesting that a dosing interval of every second half-life is sufficient. A recent study in children demonstrated trovafloxacin half-life values of 14.4 h in serum and 10.7 h in CSF.\textsuperscript{16} If data from the rabbit meningitis model are extrapolated to humans, a once-daily dosing regimen (every second half-life) should be effective for treatment of pneumococcal meningitis. Because of possible mild dosage-related adverse reactions with this schedule (A. Arguedas-Mohs, personal communication),\textsuperscript{16} we have chosen a 12 hourly schedule for treatment of meningitis. A multi-centre international collaborative study is currently evaluating trovafloxacin treatment for bacterial meningitis in children using a regimen similar to that used in our model with a loading dose of 5 mg/kg (equivalent to 20 mg/kg in these experiments) followed by 2.5 mg/kg every 12 h (every serum half-life).

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References


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