Trovafoxacin in Treatment of Rabbits with Experimental Meningitis Caused by High-Level Penicillin-Resistant
Streptococcus pneumoniae

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The fluoroquinolone trovafloxacin was bactericidal (0.47 ± 0.23 Δlog_{10} CFU/ml · h after 10 mg/kg of body
weight and 0.78 ± 0.15 Δlog_{10} CFU/ml · h after 30 mg/kg) in the treatment of experimental meningitis caused
by a highly penicillin-resistant (MIC and minimum bactericidal concentration = 4 and 4 μg/ml) strain of Strepto-
coccus pneumoniae. Combinations with ampicillin and rifampin were indifferent compared to single drugs.

Streptococcus pneumoniae is currently the most common cause of bacterial meningitis in the United States and leads to
significant morbidity and mortality (21). Penicillin and cephalosporins have been the mainstay of therapy; however, the
global spread of multidrug-resistant strains of S. pneumoniae has complicated the antimicrobial therapy of meningitis (4,
12). Vancomycin has been used with success against S. pneumoniae strains with intermediate- and high-level β-lactam
resistance in patients (11) and in animal models of meningitis (3, 13), and it is considered by some to be the drug of choice
for this indication. Vancomycin, however, shows a relatively high degree of variability with regard to its penetration into
the cerebrospinal fluid (CSF), and recent reports have documented vancomycin failures in humans (26). Therefore, most
recommendations for the treatment of penicillin-resistant pneumococcal meningitis involve at least two antibiotics, typ-
ically an expanded-spectrum cephalosporin combined with vancomycin or rifampin (11, 18). Dual antibiotic therapy is
cumbersome and expensive and bears the potential for increased adverse events. The need for new, more effective anti-
biotics for the treatment of meningitis caused by resistant pneumococci is obvious.

Quinolones are highly active in vivo against susceptible organisms. They are lipophilic and enter the CSF better than
other classes of antibiotics (6, 20). Nonetheless, quinolones have not been used routinely in the treatment of meningitis
because of their limited activity against common meningal pathogens such as S. pneumoniae (20). Newer quinolones have
improved activity against gram-positive pathogens (10, 22, 23), and some have been shown to be efficacious in experimental
meningitis caused by pneumococci (16, 17). Trovafoxacin is a representative of this new group of quinolones with improved
activity against many gram-positive pathogens, including S. pneumoniae (9). We tested trovafloxacin in a meningitis model
in rabbits to determine its efficacy in meningitis caused by a highly penicillin-resistant strain of S. pneumoniae.

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Orleans, La., September 1996.)

In vitro studies. S. pneumoniae serotype 23F, used in this study (a generous gift from Alex Tomasz, Rockefeller Univer-
sity, New York, N.Y.), shows high-level penicillin resistance and resistance to other antibiotics (erythromycin and trimeth-
oprism-sulfamethoxazole) and has been shown to spread intercontinentally (1, 14). Trovafoxacin was provided by Pfizer Inc.
(Groton, Conn.). Ampicillin, rifampin, and ceftriaxone (Roche Laboratories, Nutley, N.J.) were obtained from commercial
sources. MICs and minimum bactericidal concentrations (MBCs) were determined in Todd-Hewitt broth (Difco Laborato-
ries, Detroit, Mich.) by the standard tube macrodilution method with an inoculum of 7 × 10⁶ CFU/ml which was chosen
to reflect CSF bacterial titers at the initiation of therapy. The MIC was defined as the lowest concentration inhibiting visible
growth after 24 h of incubation at 37°C in room air with 5% CO₂, and the MBC was defined as the lowest concentration
killing ≥99.9% of the initial inoculum. The MICs and MBCs, respectively, of the drugs studied were as follows (micro-
grams per milliliter): penicillin, 4.0 and 4.0; ampicillin, 4.0 and 8.0; ceftriaxone, 0.25 and 0.5; rifampin, 0.12 and 0.12; and
trovafoxacin, 0.12 and 0.25. The organism was highly resistant to penicillin and ampicillin but was sensitive to ceftriaxone,
either at a relatively high MIC and MBC. The lowest MICs and MBCs were those of trovafloxacin and rifampin. Thus, this
organism was characteristic of penicillin-resistant pneumococci in at least two ways. First, the high-level resistance to penicillin,
mediated by alterations of several penicillin-binding proteins in these organisms, was associated with an increase in the
MICs and MBCs of other β-lactams, such as ceftriaxone. Second, even though β-lactam-resistant pneumococci are often
also resistant to other antibiotics, such as macrolides or trimethoprim-sulfamethoxazole, they typically remain sensitive
in quinolones and rifampin (22, 23). These two classes of antibiotic thus represent potential candidates for the therapy of
infections caused by β-lactam-resistant pneumococci.

Meningitis model in rabbits. The animal studies were approved by the Committee on Animal Research of the Univer-
sity of California, San Francisco. A modification of the rabbit model of bacterial meningitis described by Dacey and Sande
(5) was used. After intramuscular anesthesia with ketamine (30
mg/kg of body weight), xylazine (15 mg/kg), and acepromazine (3 mg/kg), rabbits were infected by direct intracisternal injec-
tion of 10⁶ CFU of S. pneumoniae suspended in 0.3 ml of saline. The inoculum was prepared from bacteria cultured on
blood agar plates after several intrathecal passages in rabbits and was stored at −70°C as a suspension in sterile saline. To
infect animals, the frozen organisms were thawed, grown in
Ampicillin 50 (2

serum were generated in rabbit serum, while standard curves

were determined by Bonferroni tests. Statistical significance

was performed by analysis of variance. To minimize variability, the concentrations of drugs in all sam-

cles containing the same drug were determined on a single day. Assay variability for individual samples was <10%. *Escherichia coli* (ATCC 10536) was used as the indicator strain for ceftri-

axone (limits of detection, 0.25 μg/ml in serum and 0.12 μg/ml in CSF). *Bacillus subtilis* (ATCC 6633) was used as the indica-

tor strain for trovafloxacin, ampicillin, and rifampin. The limits of
detection were 0.12 μg/ml in serum and 0.06 μg/ml in CSF for all three drugs.

**In vivo results.** The present study confirmed previous find-
ings that showed good penetration of fluoroquinolones in gen-
eral and trovafloxacin in particular into the CSF of rabbits with meningitis (16). Depending on the dosage of trovafloxacin, concentrations in CSF 1 h after administration varied between 19 and 50% of the simultaneous serum concentrations, reflect-
ing penetration rates similar to those found in a previous study with continuous infusion of the drug (Table 1) (16). Ampicillin showed a comparable CSF-to-serum concentration ratio at 1 h (0.33), while the corresponding values for ceftriaxone (0.07) and rifampin (0.04) were considerably lower. The concentra-
tions of trovafloxacin achieved in the CSF exceeded the MBC for the organism 15-fold at the highest dose examined (30 mg/kg) (Table 1). At the lower, clinically more relevant dose of 10 mg/kg, CSF trovafloxacin concentrations exceeded the MBC for the pneumococci by approximately twofold. CSF ceftriaxone concentrations 1 h after injection were 2.3 times higher than the MBC for the organism, while the ratio was 0.7 for ampicillin and 6.3 for rifampin (Table 1). It is important to note that the differences in pharmacokinetics in serum and CSF among the drugs (time to peak and half-lives) make direct comparisons of CSF penetration rates or CSF concentration/ MBC ratios after the administration of a single bolus dose difficult.

The three dosages of trovafloxacin studied were all bacteri-
cidal compared to untreated controls (Table 2). The high dose of trovafloxacin, 30 mg/kg, produced the highest bactericidal rate (0.78 ± 0.15 Δlog10 CFU/ml · h), which was significantly higher than that for a single dose of 10 mg/kg (0.47 ± 0.23 Δlog10 CFU/ml · h; P < 0.05 for analysis of variance of the three trovafloxacin groups). Two doses of 10 mg of trovafloxa-

cin per kg 3 h apart increased the bactericidal rate slightly compared to a single dose. After the single dose, CSF bacterial titers were progressively reduced during the first 5 h of therapy, at which time trovafloxacin concentrations in CSF reached approximately 0.1 μg/ml, the equivalent of the MIC. Between 5 and 7 h of therapy, titers in CSF did not continue to decline

for CSF were generated in saline containing 5% rabbit serum, approximating the protein content of CSF during meningitis. The three trovafloxacin groups). Two doses of 10 mg of trovafloxa-

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Trovafloxacin 30 8 3.92 ± 1.86 15.7 7.85 ± 2.70 0.23 ± 0.13 0.9 0.87 ± 0.59

10 (2×) 10 0.47 ± 0.30 1.9 2.42 ± 1.43 0.23 ± 0.23 0.9 0.93 ± 0.83

5 (2×) 7 0.37 ± 0.17 1.5 1.62 ± 0.44 0.06 ± 0.05 0.3 0.20 ± 0.28

Ceftriaxone 10 7 1.16 ± 0.42 2.3 16.79 ± 4.24 0.45 ± 0.24 0.9 3.93 ± 0.86

Ampicillin 50 (2×) 7 5.56 ± 1.27 0.7 16.86 ± 2.67 0.62 ± 0.21 0.01 0.71 ± 0.37

Rifampin 10 8 0.75 ± 0.27 6.3 16.89 ± 5.09 0.50 ± 0.18 4.2 6.05 ± 1.93

a Two doses given 3 h apart.

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that of the standard dose of trovafloxacin (Table 1). At these doses, which result in concentrations in CSF on the low end of the range achieved in humans with meningitis, neither ampicillin nor ceftaxone showed significant bactericidal activity compared to untreated controls (Table 2). However, ampicillin (25) and ceftaxone (24) showed a strong positive correlation between concentration in the CSF and bactericidal activity in the CSF. It is therefore very likely that both drugs would have been more effective at higher doses. While a direct comparison of the maximal bactericidal rates for the β-lactams and trovafloxacin is not possible based on the present study, previous data indicate that these rates are very similar (16).

Ampicillin is commonly included in the empirical therapy of meningitis to provide coverage for *Listeria monocytogenes* and is synergistic with trovafloxacin in experimental enterococcal endocarditis (2). Its addition to trovafloxacin in the present study did not improve the killing rates compared to those for trovafloxacin alone. Rifampin has been reported to be either indifferent (8) or antagonistic (7) with ceftaxone in the treatment of penicillin-resistant pneumococcal meningitis. Like ampicillin, rifampin was indifferent in combination with trovafloxacin, similar to the results of a previous study of a quinolone with rifampin in the treatment of experimental pneumococcal meningitis (15).

The excellent CSF penetration and bactericidal activity of trovafloxacin qualify this new quinolone as an attractive anti-biotic for the treatment of meningitis caused by susceptible bacteria. Since cross-resistance between quinolones and β-lactams is very uncommon, trovafloxacin appears to be promising for the treatment of meningitis caused by highly penicillin-resistant *S. pneumoniae* (22, 23). This study and previous studies have identified the peak concentration in CSF (16) and half-life in CSF as parameters that allow the estimation of the usefulness of a drug for the treatment of meningitis. If this assessment indicates that the human CSF pharmacokinetic profile is favorable, then a clinical study will be justified.

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**REFERENCES**


