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# In Vivo Efficacy of Trovafloxacin (CP-99,219), a New Quinolone with Extended Activities against Gram-Positive Pathogens, *Streptococcus pneumoniae*, and *Bacteroides fragilis*

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**The interesting in vitro antimicrobial activity and pharmacokinetics of the new quinolone trovafloxacin (CP-99,219) warranted further studies to determine its in vivo efficacy in models of infectious disease. The significance of the pharmacokinetic and in vitro antimicrobial profiles of trovafloxacin was shown through efficacy in a series of animal infection models by employing primarily oral therapy. Against acute infections, trovafloxacin was consistently more effective than temafloxacin, ciprofloxacin, and ofloxacin against *Streptococcus pneumoniae* and other gram-positive pathogens while maintaining activity comparable to that of ciprofloxacin against gram-negative organisms. In a model of murine pneumonia, trovafloxacin was more efficacious than temafloxacin, while ciprofloxacin failed against *S. pneumoniae* (50% protective doses, 2.1, 29.5, and >100 mg/kg, respectively). In addition to its inherent in vitro potency advantage against *S. pneumoniae*, these data were supported by a pharmacokinetic study that showed levels of trovafloxacin in pulmonary tissue of *S. pneumoniae*-infected CF1 mice to be considerably greater than those of temafloxacin and ciprofloxacin (twice the maximum drug concentration in serum; two to three times the half-life, and three to six times the area under the concentration-time curve). Against localized mixed anaerobic infections, trovafloxacin was the only agent to effectively reduce the numbers of recoverable CFU of *Bacteroides fragilis* (>1,000-fold), *Staphylococcus aureus* (1,000-fold), and *Escherichia coli* (>100-fold) compared with ciprofloxacin, vancomycin, metronidazole, clindamycin, cefoxitin, and ceftriaxone. The in vitro and in vivo antimicrobial activities of trovafloxacin and its pharmacokinetics in laboratory animals provide support for the ongoing and planned human phase II and III clinical trials.**

Several reports that have appeared in the scientific literature indicate that a significant gap exists in the antimicrobial spectrum of most quinolones currently on the market (18, 21). Although this class has exquisite potency against most gram-negative bacteria, quinolones generally have very poor to moderate activity against gram-positive bacteria, particularly the streptococci (21). Numerous clinical failures have been reported with quinolone therapy against *Streptococcus pneumoniae* pneumonia (5, 10, 15, 20, 22, 28). Trovafloxacin and other quinolones under development have enhanced activity against these gram-positive bacteria. Another deficiency in the quinolone antimicrobial spectrum is the lack of effectiveness against *Bacteroides fragilis*, clinically the most significant anaerobic pathogen (18, 29, 30). *B. fragilis* frequently occurs as a mixed infection (polymicrobic) with a facultative organism such as *Staphylococcus aureus* or *Escherichia coli*. In addition to inherent antimicrobial inadequacies, most marketed quinolones require at least twice-daily administration (7).

Many investigators (8, 13, 14, 23) have reported that trovafloxacin (CP-99,219) has potent in vitro activity against gram-positive bacteria, including streptococci, such as *S. pneumoniae* and *Streptococcus pyogenes*, and enterococci (i.e., *Enterococcus faecalis*). This enhanced in vitro activity against gram-positive pathogens has not diminished the compound's gram-negative antimicrobial spectrum. Compared with marketed quinolones, trovafloxacin maintained comparable or better in vitro activity against members of the family *Enterobacteriaceae*. In addition to its activities against facultative gram-positive and gram-negative bacteria, the compound has also shown good in vitro activity against *B. fragilis* (14, 23, 31).

The studies described in this report were performed to determine if the interesting in vitro activities noted in previous reports could be expressed in vivo, particularly those regarding gram-positive pathogens and *B. fragilis*. Since quinolones performed poorly against *S. pneumoniae* pneumonia, some studies focused on the efficacy and pharmacokinetics of trovafloxacin in a murine *S. pneumoniae* model of this critical disease. The compound was also assessed in other forms of localized infections, such as implants of disks contaminated with mixtures of *B. fragilis* and *S. aureus* or *B. fragilis* and *E. coli*.

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## MATERIALS AND METHODS

**Antimicrobial agents.** Trovafloxacin and temafloxacin were provided by Pfizer Central Research Division, Pfizer, Inc., Groton, Conn. All other antimicrobial agents were obtained from the respective pharmaceutical manufacturers. Compounds were given as solutions or suspensions with distilled water as the common vehicle.

**Microorganisms.** Except for *S. pneumoniae* P4241, *S. pyogenes* ATCC 12384, and *Klebsiella pneumoniae* ATCC 43816, all of the organisms used in the acute systemic disease studies were obtained as isolates from human patients in hospitals throughout the United States. All of the *S. pneumoniae* cultures used in these studies were susceptible to penicillins and macrolides, as determined by National Committee for Clinical Laboratory Standards procedures. The culture numbers noted with the data are designations assigned to these organisms in the Pfizer culture collection. The *S. pneumoniae* serotype 3 strain P4241 used in the

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mouse model of bacterial pneumonia was obtained from J.-J. Pocidalo (Hôpital Claude Bernard, Paris, France). *S. aureus* Pfizer 01A0052, *E. coli* Pfizer 51A0266, and *B. fragilis* ATCC 25285 were used in the polymicrobic infections.

**Acute systemic infections.** All acute systemic infections were produced by intraperitoneal administration of a bacterial challenge to CF1 (Charles River) mixed-sex mice (body weights, 11 to 13 g). The numbers of organisms injected were 1 to 10 times the 100% lethal dose. Except for *S. pyogenes*, *S. pneumoniae* P 4241, and *K. pneumoniae*, which were administered as suspensions in brain heart infusion broth, all challenges were suspended in 5% hog gastric mucin. Survival was recorded over a 4-day period, and the 50% protective dose ($PD_{50}$) was calculated from data obtained from a dose range consisting of four different antibiotic concentrations in a fourfold dilution series. Mice (10 per group) were treated orally at 0.5 and 4 h after challenge. For each of the pathogens used, the trovafloxacin MIC was below the reported MIC at which 90% of the isolates of that genus and species are inhibited (14).

**Bacterial pneumonia.** *S. pneumoniae* was grown on 5% sheep blood agar for 16 to 18 h under an atmosphere of 5% $CO_2$. The growth was removed from the agar surface, suspended in saline, and adjusted to a turbidity of 34 to 36% transmittance at 600 nm. *S. pneumoniae* pneumonia was induced by intranasal administration of 25 μl of the adjusted suspension (approximately $10^6$ CFU) to methoxyflurane-anesthetized 18- to 20-g mixed-sex CF1 mice. Oral treatments were begun 18 h after challenge and continued every 12 h for 3 consecutive days (total of six doses). The number of CFU per lung was approximately $10^5$ at initiation of therapy. Survivors were recorded daily over a 10-day interval, at which time the $PD_{50}$ was calculated from the dose titration data ($n = 10$ per treatment group). This model has been described in detail (1, 3) and reported to mimic the pathogenesis of most bacterial pneumonias in humans. The cause of death is severe pneumonia or septicemia. Although temafloxacin is no longer used in human medicine, it was included in these studies because it was the first quinolone shown to be consistently effective against *S. pneumoniae* in humans and was reported (1, 2) to be effective in this model.

**Experimental anaerobic infections.** Sterilized paper disks, later inoculated with a 1:1 mixture of *S. aureus* and *B. fragilis* (final concentrations of $4 \times 10^5$ *S. aureus* and $1 \times 10^6$ *B. fragilis* CFU per disk) or *E. coli* and *B. fragilis* ($3 \times 10^3$ *E. coli* and $1.6 \times 10^6$ *B. fragilis* CFU per disk), were implanted on the left midbacks of methoxyflurane-anesthetized CF1 mice as previously described (11). It was very important to have a relatively small inoculum of *E. coli* compared with *B. fragilis* in the mixed challenge, since a larger number of *E. coli* bacteria resulted in acute infection with this organism, as reported by other investigators (6). CFU of *S. aureus* and *B. fragilis* were determined from platings on Wilkens-Chalgren agar incubated anaerobically (chamber containing 80% $N_2$, 10% $H_2$, and 10% $CO_2$); the distinct colony morphologies allowed differential counting on the same plate. Counts of *E. coli* and *B. fragilis* mixtures were made by platings on brucella blood agar incubated aerobically (*E. coli*) and bacteroides bile esculin agar incubated anaerobically (*B. fragilis*).

Treatments (15 mice per group for the *S. aureus-B. fragilis* studies and 5 per group for the *E. coli-B. fragilis* studies) were begun at 24 h postchallenge and continued three times a day for three consecutive days; disks were removed 2 days after completion of therapy, dilutions were made, and samples were plated on the appropriate media.

**Statistical analysis.** Where indicated, statistical significance ($P$ value) was determined by the Student $t$ test of independent samples. The $PD_{50}$, expressed as milligrams per kilogram per dose, was calculated by the probit method (9).

**In vitro MIC evaluations.** Except as noted for *S. pneumoniae*, MICs against all facultative pathogens were determined by the broth dilution technique, using brain heart infusion medium in sterile 96-well plates incubated at 37°C. The MICs against *B. fragilis* were determined by the agar dilution technique utilizing Wilkens-Chalgren agar; incubation was done at 37°C under an atmosphere containing 10% $H_2$, 10% $CO_2$, and 80% $N_2$ in an anaerobic chamber.

**Pharmacokinetic studies.** Trovafloxacin, temafloxacin, and ciprofloxacin solutions were prepared in sterile water on the day of pharmacokinetic assessment. Each compound was administered by oral gavage at a dose of 25 mg/kg contained in a total volume of 0.2 ml. The pharmacokinetic profiles of these compounds were characterized in mice given a single dose at 18 h following an intranasal challenge with *S. pneumoniae*.

Mice were euthanized by $CO_2$ asphyxiation, and blood samples were obtained via intracardiac puncture. Blood samples were allowed to clot for 1 h and then centrifuged to separate the serum. Pulmonary tissue was collected following exsanguination by making a midline incision, exposing the chest cavity, and excising both lungs. The lungs were blotted and subsequently placed into previously tared polystyrene tubes, weighed, and maintained at −70°C until analyzed. Blood and lung samples were harvested at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h postadministration of the drug. Hemoglobin levels in blood and tissue were determined by using a spectrophotometric assay, and concentrations in lung tissue were corrected for extraneous blood (34).

Serum and tissue samples were analyzed for trovafloxacin by a reversed-phase high-performance liquid chromatography procedure using ion pairing; this method was adapted from the method of Nix et al. (24) for the assay of ciprofloxacin. The mobile phase for the separation of trovafloxacin consisted of acetonitrile–26 mM phosphoric acid buffer (17:83 [vol/vol]). The phosphate buffer was made with phosphoric acid, fortified with 10 mM dibutylamine phosphate, and adjusted to pH 3.0 with 40% tetrabutylammonium hydroxide. The mobile phase was passed through a $C_{18}$ reversed-phase radial cartridge (8 by 100 mm; particle size, 4 μm; Radial-pak/Nova-pak; Waters) at a flow rate of 1.5 ml/min. A guard column with 4-μm $C_{18}$ packing was used to maintain analytical integrity. The mobile phase was monitored by UV light detection at 277 nm.

Serum samples (200 μl) were mixed vigorously with 10 μl of an internal standard solution (200 μg of a methyl derivative of trovafloxacin per ml) and placed into disposable borosilicate tubes (16 by 100 mm). Five hundred microliters of 50 mM phosphate buffer (pH 3.0) was added to each tube, vortexed, and applied to a $C_{18}$ Sep-pak solid-phase support that had previously been conditioned with 1 ml of methanol and then 5 ml of phosphate buffer. The cartridge was washed with 10 ml of phosphate buffer, and the sample was eluted with 2 ml of methanol. Samples were evaporated to dryness and subsequently reconstituted with 200 μl of the mobile phase. Lung samples were homogenized with 5 ml of an extraction mixture of 0.15 M $H_3ClO_4$ and 0.15 M $H_3PO_4$ in water-methanol (50/50 [vol/vol]) and fortified with the internal standard solution. The lung extraction buffer homogenate was centrifuged in screw-cap tubes for 10 min at $3,000 \times g$ on a swinging-bucket centrifuge, and the supernatant was decanted and evaporated to near dryness. The residue was dissolved in 2 ml of 0.025 M $KH_2PO_4$, pH 3.0, and extracted twice with 5 ml of ethyl acetate. The extracts were combined and evaporated to dryness, redissolved in 1 ml of the mobile phase, and washed with 2 ml of hexane, and the hexane layer was removed by aspiration. Both the serum and lung samples were filtered through a 0.22-μm-pore-size filter (polyvinylidene difluoride LC 13) and placed into sample vials. Calibration standards were prepared by adding trovafloxacin and the internal standard solution to corresponding blank biological specimens and assayed as described above.

The concentrations of trovafloxacin were calculated by linear regression analysis of the log transform of the peak height ratio of trovafloxacin to the internal standard solution versus the log transform of fortified concentrations of trovafloxacin. The lower limits of quantification of trovafloxacin were 0.1 μg/ml of serum and 0.2 μg/g of lung tissue. The dynamic ranges of the assays are 0.1 to 20.0 μg/ml of serum and 0.2 to 20 μg/g of lung tissue, and the coefficient of variation of inter- and intraday variations for five replicates was ≤5.0%. Ciprofloxacin and temafloxacin concentrations were determined by using previously published methods (16, 24).

**Pharmacokinetic analysis.** Concentration-time data were fitted to a one- or two-compartment open model according to the curves of the plotted data, and the parameters were estimated by standard methods (17). $C_{max}$ is the highest observed concentration; $T_{max}$ is the earliest time at which $C_{max}$ occurred; terminal elimination half-life was calculated as $\ln 2/k_{el}$, where $k_{el}$ is the elimination rate constant derived from the slope obtained by least-squares regression analysis for apparently linear portions of the log serum-versus-time curve; and the area under the concentration-time curve from zero to last ($AUC_{0-last}$) was calculated by the trapezoidal method through the last time point at which the drug could be measured.

## RESULTS

**Acute systemic infections.** Trovafloxacin and temafloxacin were the most effective agents against gram-positive infections; both were particularly active against *S. pneumoniae* and *E. faecalis* (Table 1). Ciprofloxacin and ofloxacin failed against these two pathogens at the highest dose level employed. Except for ofloxacin, all of the quinolones were similarly effective against *S. pyogenes*.

All of the agents protected mice against methicillin-susceptible and -resistant *S. aureus* (MRSA) strains that were quinolone susceptible. Against one of the quinolone-resistant *S. aureus* strains, only trovafloxacin was moderately active; temafloxacin, ciprofloxacin, and ofloxacin were inactive. The moderate activity of trovafloxacin was consistent with its demonstrated in vitro potency against this strain. No antimicrobial agent showed in vivo activity against the *S. aureus* highly resistant to quinolones.

All of the quinolones showed very similar activities against most of the gram-negative pathogens examined (Table 2). The exceptions were in studies with *Salmonella enteritidis* and *Enterobacter aerogenes*. Against *S. enteritidis*, both trovafloxacin and temafloxacin were active, while ciprofloxacin and ofloxacin failed. Temafloxacin, ciprofloxacin, and ofloxacin showed moderate activity against *E. aerogenes*; trovafloxacin was ineffective at the highest dose used.

**Mouse lung infections.** Trovafloxacin was the most active agent in the *S. pneumoniae* model of murine pneumonia, protecting 90 to 100% of the challenged animals with oral thera-

TABLE 1. In vivo activity of orally delivered trovafloxacin against gram-positive pathogens

| Challenge (strain) | Compound | MIC (μg/ml) | $PD_{50}$ (mg/kg/dose) |
|---|---|---|---|
| *Streptococcus pneumoniae* 02J0025 | Trovafloxacin | 0.20 | 1.3 (0.6–2.6)[a] |
| | Temafloxacin | 0.39 | 10.4 (5.4–20.1) |
| | Ciprofloxacin | 0.78 | >50 |
| | Ofloxacin | 1.56 | >50 |
| *Streptococcus pneumoniae* Pocidalo 4241 | Trovafloxacin | 0.20 | 9.7 (5.8–16.1) |
| | Temafloxacin | 0.78 | 21.5 (11.7–39.5) |
| | Ciprofloxacin | 1.56 | >50 |
| | Ofloxacin | 1.56 | >50 |
| *Streptococcus pyogenes* ATCC 12384 | Trovafloxacin | 0.39 | 3.0 (1.7–5.4) |
| | Temafloxacin | 0.39 | 6.3 (4.1–9.6) |
| | Ciprofloxacin | 0.78 | 8.7 (4.8–15.5) |
| | Ofloxacin | 1.56 | >50 |
| *Enterococcus faecalis* 03A0131 | Trovafloxacin | 0.39 | 6.4 (3.7–11.0) |
| | Temafloxacin | 1.56 | 11.7 (4.7–28.9) |
| | Ciprofloxacin | 1.56 | >50 |
| | Ofloxacin | 0.39 | >50 |
| *Staphylococcus aureus* 01A0400[b] | Trovafloxacin | 0.05 | 2.9 (3.7–11.0) |
| | Temafloxacin | 0.20 | 10.5 (4.9–22.5) |
| | Ciprofloxacin | 0.39 | 13.1 (8.2–21.1) |
| *Staphylococcus aureus* 01A0129[c] | Trovafloxacin | 0.05 | 2.0 (1.1–3.6) |
| | Temafloxacin | 0.20 | 2.1 (1.2–3.8) |
| | Ciprofloxacin | 0.39 | 1.1 (1.0–3.4) |
| *Staphylococcus aureus* 01A1080[c] | Trovafloxacin | 3.12 | 42.6 (40.8–69.1) |
| | Temafloxacin | >50 | >50 |
| | Ciprofloxacin | >50 | >50 |
| | Ofloxacin | >50 | >50 |
| *Staphylococcus aureus* 01A1063[c] | Trovafloxacin | 25 | >50 |
| | Temafloxacin | 50 | >50 |
| | Ciprofloxacin | >50 | >50 |
| | Ofloxacin | >50 | >50 |

[a] 95% confidence limits.
[b] Methicillin susceptible.
[c] MRSA.

TABLE 2. In vivo activity of orally delivered trovafloxacin against gram-negative pathogens

| Challenge strain | Compound | MIC (μg/ml) | $PD_{50}$ (mg/kg/dose) |
|---|---|---|---|
| *Salmonella enteritidis* 58D0079 | Trovafloxacin | 0.03 | 3.1 (1.2–8.0)[a] |
| | Temafloxacin | 0.05 | 8.7 (4.2–17.8) |
| | Ciprofloxacin | 0.20 | >50 |
| | Ofloxacin | 0.20 | >50 |
| *Klebsiella pneumoniae* ATCC 43816 | Trovafloxacin | 0.39 | 1.1 (0.4–1.8) |
| | Temafloxacin | 0.39 | 2.3 (1.0–3.5) |
| | Ciprofloxacin | 0.05 | 2.5 (1.8–3.3) |
| *Klebsiella oxytoca* 53D0024 | Trovafloxacin | 0.10 | 6.8 (3.7–12.6) |
| | Temafloxacin | 0.05 | 6.6 (3.9–11.2) |
| | Ciprofloxacin | 0.05 | 11.8 (6.3–21.8) |
| | Ofloxacin | 0.39 | 36.2 (16.7–78.4) |
| *Serratia marcescens* 63A0017 | Trovafloxacin | 0.39 | 31.7 (14.6–68.6) |
| | Temafloxacin | 0.39 | 34.3 (17.2–68.3) |
| | Ciprofloxacin | 0.05 | 14.6 (6.7–31.8) |
| | Ofloxacin | 0.39 | 40.7 (12.1–136.2) |
| *Escherichia coli* 51A0266 | Trovafloxacin | 0.20 | 2.3 (0.8–6.1) |
| | Temafloxacin | 0.39 | 1.8 (1.2–7.2) |
| | Ciprofloxacin | 0.03 | <0.8 |
| | Ofloxacin | 0.20 | <0.8 |
| *Pseudomonas aeruginosa* 52A0104 | Trovafloxacin | 0.78 | 6.5 (3.5–12.0) |
| | Temafloxacin | 12.5 | 15.1 (7.1–31.9) |
| | Ciprofloxacin | 1.56 | 1.2 (0.5–2.6) |
| | Ofloxacin | 3.12 | 1.9 (0.8–4.7) |
| *Proteus vulgaris* 57A0031 | Trovafloxacin | 0.20 | 23.4 (11.7–46.5) |
| | Temafloxacin | 0.20 | 15.2 (7.1–31.9) |
| | Ciprofloxacin | 0.03 | 10.3 (3.9–26.9) |
| | Ofloxacin | 0.20 | 11.8 (6.3–21.8) |
| *Proteus mirabilis* 57C0175 | Trovafloxacin | 0.39 | 5.6 (3.0–10.3) |
| | Temafloxacin | 0.39 | 4.8 (2.5–9.0) |
| | Ciprofloxacin | 0.39 | 6.9 (3.8–12.9) |
| | Ofloxacin | 0.39 | 17.5 (7.7–39.8) |
| *Morganella morganii* 97A0096 | Trovafloxacin | 0.39 | 16.8 (7.6–37.3) |
| | Temafloxacin | 0.78 | 6.2 (2.4–16.6) |
| | Ciprofloxacin | 0.20 | 4.6 (2.3–9.2) |
| *Providencia stuartii* 77A0039 | Trovafloxacin | 0.20 | 6.3 (3.3–11.8) |
| | Temafloxacin | 0.20 | 3.4 (1.3–9.2) |
| | Ciprofloxacin | 0.20 | 14.2 (6.3–32.1) |
| *Enterobacter cloacae* 67B0153 | Trovafloxacin | 0.20 | 12.5 (6.7–23.1) |
| | Temafloxacin | 0.78 | 11.0 (5.9–20.4) |
| | Ciprofloxacin | 0.78 | 18.1 (7.6–42.8) |
| *Enterobacter aerogenes* 67A0002 | Trovafloxacin | 1.56 | >50 |
| | Temafloxacin | 0.78 | 40.3 (16.8–94.6) |
| | Ciprofloxacin | 0.20 | 19.0 (8.5–42.5) |
| | Ofloxacin | 0.39 | 31.8 (10.4–96.9) |

[a] 95% confidence limits.

pies of 12.5 to 50 mg/kg and an estimated $PD_{50}$ of 2.1 mg/kg (Fig. 1A). Temafloxacin, although less active than trovafloxacin, was also successful in this model with doses of 75 to 100 mg/kg protecting 90 to 100% of the mice, resulting in an estimated $PD_{50}$ of 29.5 mg/kg (Fig. 1B). Ciprofloxacin performed poorly, protecting only 20 to 30% of challenged animals at treatment levels of 75 to 100 mg/kg at the conclusion of the study with an estimated $PD_{50}$ of >100 mg/kg. Untreated animals began dying (50% mortality) by 72 h after intranasal challenge, and 100% mortality occurred by day 6 postchallenge.

**Serum versus lung tissue pharmacokinetics.** The key serum and pulmonary tissue pharmacokinetic parameters determined following administration of a single 25-mg/kg oral dose of trovafloxacin, temafloxacin, or ciprofloxacin to intranasally *S. pneumoniae*-infected mice are shown in Table 3. The $C_{max}$ and AUC values of all compounds were much greater in infected lungs than in serum, yielding pulmonary distribution ratios of 2.3 to 3.9. Treatment with trovafloxacin yielded the highest $C_{max}$; half-life, and AUC values for lung tissue; temafloxacin and ciprofloxacin yielded significantly ($P < 0.05$ by analysis of variance of pooled data) lower values. Specifically, the AUC of trovafloxacin for pulmonary tissue was three- to sixfold greater than the comparable temafloxacin and ciprofloxacin values (analysis of variance, $P < 0.25$). The AUC of temafloxacin in pulmonary tissue was twice that of ciprofloxacin. Detectable levels of trovafloxacin in lung tissue (1.5 μg/g) were present 24 h postadministration, while there were no detectable levels temafloxacin or ciprofloxacin in lung tissue at that time.

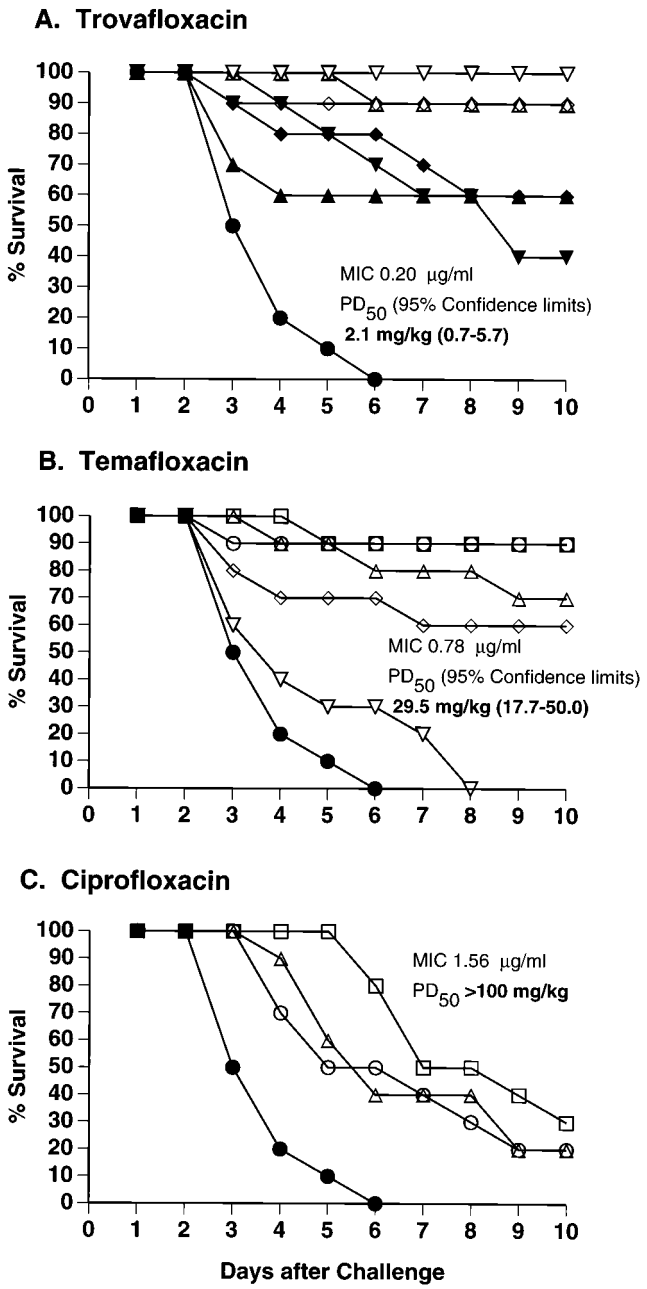


FIG. 1. Survival of intranasally *S. pneumoniae*-challenged mice following oral therapy with trovafloxacin (A), temafloxacin (B), or ciprofloxacin (C) at doses of 100 (□), 75 (○), 50 (△), 25 (◇), 12.5 (▽), 6.25 (▲), 3.12 (◆), and 1.56 (▼) mg/kg and of untreated controls (●).

***S. aureus-B. fragilis* polymicrobic infection.** Trovafloxacin was the most active agent tested against the polymicrobic infection with *S. aureus* and *B. fragilis* (Table 4). Compared with those in untreated controls, trovafloxacin reduced the number of recoverable *S. aureus* CFU by about 1,000-fold and that of *B. fragilis* CFU by >10,000-fold. Vancomycin was also effective against *S. aureus*, reducing the number of CFU 1,000-fold, but was ineffective against *B. fragilis*. Metronidazole was active against *B. fragilis*, reducing the number of CFU 100-fold, and was inactive against *S. aureus*. Ciprofloxacin therapy resulted in recovery of CFU indistinguishable from that in untreated animals.

TABLE 3. Concentrations of trovafloxacin, ciprofloxacin, and temafloxacin in serum and lung tissue after oral administration of 25 mg/kg to *S. pneumoniae*-infected CF1 mice

| Compound | Sample | $C_{max}$ (μg/ml or g) | Half-life (h) | $AUC_{0-last}$ (μg·h/ml) | Tissue/serum AUC ratio[a] |
|---|---|---|---|---|---|
| Trovafloxacin | Serum | 10.7 | 6.8 | 69.9 | |
| | Lung tissue | 25.0 | 5.7 | 163.0 | 2.3 |
| Ciprofloxacin | Serum | 2.0 | 2.5 | 7.0 | |
| | Lung tissue | 12.5 | 1.7 | 27.5 | 3.9 |
| Temafloxacin | Serum | 3.8 | 2.4 | 13.6 | |
| | Lung tissue | 10.2 | 3.5 | 47.2 | 3.5 |

[a] Concentrations in lung tissue at 24 h postadministration (micrograms per gram): trovafloxacin, 1.54; ciprofloxacin, <0.2; temafloxacin, <0.2. Concentrations in serum at 24 h postadministration (micrograms per ml): trovafloxacin, 0.8; ciprofloxacin, <0.1; temafloxacin, 0.5.

A study of the comparative activities of trovafloxacin, clindamycin, and cefoxitin showed that trovafloxacin and clindamycin had similar and most impressive activities (Table 5). Therapy with either agent resulted in a 1,000-fold reduction in the numbers of both *S. aureus* and *B. fragilis* CFU, compared with those in untreated controls. Cefoxitin significantly reduced the number of recoverable *S. aureus* CFU but was not effective in reducing the number of *B. fragilis* CFU compared with that in untreated controls.

Some of the compounds included in these studies served as controls, expressing selective activity against *S. aureus* (vancomycin) and inactivity against *B. fragilis*, while metronidazole provided the reciprocal control, i.e., activity against the anaerobe and inactivity against the facultative organism. The exception was cefoxitin, which was expected to be effective against *S. aureus* and *B. fragilis*. In this system, this agent was only weakly active against *S. aureus* and completely inactive against *B. fragilis*.

***E. coli-B. fragilis* mixed infection.** When antimicrobial agents were given intraperitoneally at 100 mg/kg, trovafloxacin was the only agent that was effective in reducing the numbers of recoverable *E. coli* and *B. fragilis* CFU (Table 6). Therapy with trovafloxacin resulted in a >100-fold decrease in *E. coli* and a >1,000-fold decrease in *B. fragilis*. Ceftriaxone was effective against *E. coli* but showed no activity against *B. fragilis*. Metronidazole was active against *B. fragilis* but not against *E. coli*. A combination of ceftriaxone plus metronidazole resulted in a

TABLE 4. In vivo efficacy of trovafloxacin, ciprofloxacin, vancomycin, and metronidazole against mixed infection with *S. aureus* and *B. fragilis*

| Treatment[a] | *S. aureus* | | *B. fragilis* | |
|---|---|---|---|---|
| | MIC (μg/ml) | $Log_{10}$ CFU/disk (geometric mean ± SD) | MIC (μg/ml) | $Log_{10}$ CFU/disk (geometric mean ± SD) |
| None | | 6.63 ± 0.50 | | 7.28 ± 0.54 |
| Trovafloxacin (p.o.) | 0.01 | 3.79 ± 0.67[b] | 0.19 | 2.65 ± 0.36[b] |
| Ciprofloxacin (p.o.) | 0.39 | 6.58 ± 0.35 | 3.12 | 7.58 ± 0.32 |
| Vancomycin (s.c.) | 1.56 | 3.15 ± 0.32[b] | 25.0 | 6.73 ± 0.85 |
| Metronidazole (p.o.) | >50 | 6.36 ± 0.32 | 0.39 | 5.08 ± 0.55[b] |

[a] Each drug was given at 100 mg/kg. p.o., oral; s.c., subcutaneous.
[b] $P < 0.001$ versus untreated controls; others not significant ($P > 0.05$).

TABLE 5. In vivo efficacy of trovafloxacin, clindamycin, and cefoxitin against mixed infection with *S. aureus* and *B. fragilis*

| Treatment[a] | $Log_{10}$ CFU/disk (geometric mean ± SD) | |
|---|---|---|
| | *S. aureus* | *B. fragilis* |
| None | 6.80 ± 0.26 | 7.34 ± 0.26 |
| Trovafloxacin (p.o.) | 3.92 ± 0.53[b] | 4.97 ± 0.50[b] |
| Clindamycin (p.o.) | 3.64 ± 0.79[b] | 4.63 ± 0.63[b] |
| Cefoxitin (s.c.) | 5.92 ± 0.52[b] | 7.01 ± 0.65 |

[a] Each drug was given at 100 mg/kg. p.o., oral; s.c., subcutaneous.
[b] $P < 0.001$ versus untreated controls; others not significant ($P > 0.05$).

significant reduction in the number of *B. fragilis* CFU but not in the number of *E. coli* CFU. Ciprofloxacin was ineffective against *E. coli* and *B. fragilis*.

## DISCUSSION

Compared with currently marketed quinolones, trovafloxacin showed increased activity against acute infections caused by gram-positive organisms, particularly *S. pneumoniae*, while expressing activity against gram-negative pathogens. The in vitro activities of trovafloxacin correlated well with the efficacy observed in models of acute infection; i.e., those pathogens with the greatest in vitro susceptibility to trovafloxacin yielded the lowest $PD_{50}$s, and those organisms with MICs out of the predicted susceptibility range showed very high $PD_{50}$s or failure. On the basis of the serum pharmacokinetic data presented here and those contained in earlier reports (12, 33), most of the $PD_{50}$s would be projected to yield peak levels in serum and maintenance levels for up to 8 h that are well above the MIC for the challenge organism. With few exceptions, the $PD_{50}$s correlated very well with serum pharmacokinetic and in vitro antibacterial data. Since the pharmacokinetic profile and general characteristics of trovafloxacin, i.e., a longer half-life and increased AUC and $C_{max}$, have been consistently expressed in many species, including humans (32), the data obtained from the mouse infection models may be applied across species with regard to pharmacokinetic impact.

Although all of the quinolones showed in vitro potency against *S. enteritidis* predictive of in vivo success, only trovafloxacin and temafloxacin protected mice against this pathogen. Since the strain used in these studies can produce a classical salmonellosis that is tissue associated (liver and spleen), the success of trovafloxacin and temafloxacin, in contrast to the failure of ciprofloxacin and ofloxacin, may have been due, in part, to the greater concentrations of trovafloxacin and temafloxacin in tissue, as reflected in lung tissue, as well as their vitro potency and serum kinetic advantages.

TABLE 6. In vivo efficacy of parenterally administered trovafloxacin, ciprofloxacin, ceftriaxone, metronidazole, and ceftriaxone plus metronidazole against mixed anaerobic infection with *E. coli* and *B. fragilis*

| Treatment[a] | *E. coli* | | *B. fragilis* | |
|---|---|---|---|---|
| | MIC (μg/ml) | $Log_{10}$ CFU/disk (geometric mean ± SD) | MIC (μg/ml) | $Log_{10}$ CFU/disk (geometric mean ± SD) |
| None | | 6.89 ± 0.07 | | 7.38 ± 0.17 |
| Trovafloxacin | 0.06 | 3.84 ± 0.35[b] | 0.19 | 3.62 ± 0.06[b] |
| Ciprofloxacin | 0.06 | 6.71 ± 0.45 | 3.12 | 6.97 ± 0.40 |
| Ceftriaxone | 0.06 | 5.68 ± 0.62[c] | 50.0 | 7.32 ± 0.29 |
| Metronidazole | >50 | 6.46 ± 0.27 | 0.39 | 5.59 ± 0.35[b] |
| Ceftriaxone-metronidazole | 0.06 | 6.09 ± 0.42 | 0.39 | 6.70 ± 0.20[b] |

[a] Each drug was given at 100 mg/kg.
[b] $P < 0.001$ versus untreated controls.
[c] $P < 0.05$ versus untreated controls; others not significant.

In addition to the in vivo protection data presented here, there has been one previous report of the in vivo activity of trovafloxacin against *S. aureus*. The compound was evaluated for therapy of experimental arthritis caused by an MRSA strain (27). The investigators found that trovafloxacin was effective in sterilizing synovial fluid cultures in this model of suppurative arthritis caused by MRSA. This report is consistent with our observations of the performance of trovafloxacin against MRSA in models of acute infection.

Evaluation of trovafloxacin as an oral therapeutic agent against *S. pneumoniae* murine pneumonia showed that the compound was successful and, as expressed by $PD_{50}$, 15-fold more potent than temafloxacin, a quinolone recognized for its activity against this organism. The failure of ciprofloxacin and the degree of efficacy obtained with temafloxacin in this model of pneumonia were consistent with published data (1, 2). The *S. pneumoniae* strains used in the acute infection and pneumonia models were susceptible to penicillins and macrolides, but it is expected that trovafloxacin will have equivalent activity against strains resistant to these antibiotics since recent in vitro data have shown trovafloxacin to be equally potent against penicillin-macrolide-susceptible and -resistant *S. pneumoniae* strains (19, 25). When trovafloxacin was evaluated for therapy of experimental meningitis caused by a penicillin- and cephalosporin-resistant *S. pneumoniae* strain, the effectiveness of the compound was comparable to that of other antibiotics, such as vancomycin and ceftriaxone, and therapy with trovafloxacin resulted in a significant reduction of bacterial populations of penicillin-resistant organisms in cerebrospinal fluid (26).

To clarify the data obtained in the efficacy studies beyond the obvious differences in inherent in vitro potency, the pharmacokinetics of trovafloxacin, temafloxacin, and ciprofloxacin in mice with *S. pneumoniae* pneumonia were determined. The AUC and $C_{max}$ of trovafloxacin in lung tissue and serum were much greater than those of the other agents. In addition to these principal predictors of fluoroquinolone efficacy, the concentration of trovafloxacin in lung tissue and serum remained above the MIC for >24 h. The prolonged time for which concentrations stayed above the MIC presumably served to improve the clearance of *S. pneumoniae* from infected tissue and suppressed any subsequent *S. pneumoniae* bacteremia. Therefore, the rank order of these quinolones, based on concentrations in serum and lung tissue, as well as inherent in vitro activities, is in agreement with the in vivo efficacy observed in the *S. pneumoniae* pneumonia model. In addition to providing data for interpretation of the efficacy studies, the concentration of trovafloxacin in lung tissue following oral administration indicated that the compound was readily bioavailable via oral administration. Studies of the oral versus intravenous pharmacokinetics disclosed that the bioavailability of orally administered trovafloxacin in CF1 mice is high (70%; data not shown); this is consistent with values reported (33) for rats, dogs, and monkeys (68, 58, and 85%, respectively).

The results of the mixed anaerobic infections showed trovafloxacin to be effective against polymicrobic infections caused by gram-positive and gram-negative facultative organisms in association with *B. fragilis*, clinically the most significant anaerobic pathogen. Such mixed infections are common in clinical situations (4). Against the *S. aureus-B. fragilis* infection, trovafloxacin was equivalent to clindamycin, an agent recognized for

its antianaerobe activity and effectiveness against gram-positive facultative pathogens. In contrast, ciprofloxacin showed no activity against either organism.

In the mixed infection with *E. coli* and *B. fragilis*, trovafloxacin was active against both organisms. These studies also emphasize an important characteristic of this compound; i.e., unlike many quinolones, it has increased potency against gram-positive bacteria and *B. fragilis* while maintaining the characteristic activity of a quinolone against gram-negative enteric organisms under anaerobic conditions. These data point to the potential usefulness of trovafloxacin as an agent in the treatment of anaerobic infections. A previously published report described similar studies of quinolones given with or without clindamycin against a more robust experimental model of anaerobic mixed infection with *E. coli* and *B. fragilis* yielding a subcutaneous abscess (4). The conclusions of those studies were similar to those presented here; i.e., the data confirmed the importance of the activity of antimicrobial therapy against both components of the mixed infection. The investigator suggested that quinolones with better activity against the *B. fragilis* group held promise for successful single-agent therapy of mixed infections. Otherwise, it was considered necessary that combination therapy be employed with a quinolone, such as ciprofloxacin, plus an antimicrobial agent known to be effective against anaerobic bacteria.

To determine whether or not trovafloxacin's interesting in vitro anaerobic activity would be expressed in vivo, the doses of trovafloxacin used in these initial studies of therapy against anaerobic infections were high (100 mg/kg). However, a later dose titration study of trovafloxacin (data not shown) showed the compound to reduce the numbers of *E. coli* and *B. fragilis* CFU by >1,000-fold when the drug was given at 50 mg/kg, and a significant ($P < 0.05$) reduction in the numbers of CFU of both organisms was observed at a dose of 25 mg/kg. Studies are ongoing to evaluate trovafloxacin at these more relevant levels and with extended periods of therapy, as would be expected in a clinical situation.

The interesting in vitro activities of trovafloxacin against *S. pneumoniae*, other gram-positive and gram-negative organisms, and *B. fragilis* were expressed in in vivo models of acute systemic infection, pneumonia, and localized infection. The in vivo success of this compound rests on its inherent in vitro potency and pharmacokinetics. The extended in vitro and in vivo activities of trovafloxacin against gram-positive and anaerobic organisms, as well as its improved systemic and tissue pharmacokinetics in laboratory animals, support the continued development of this new quinolone for use against human infectious diseases.

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