ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1996, p. 314–319 Vol. 40, No. 2
0066-4804/96/$04.00+0

Activity of Trovafloxacin (CP-99,219) against *Legionella* Isolates: In Vitro Activity, Intracellular Accumulation and Killing in Macrophages, and Pharmacokinetics and Treatment of Guinea Pigs with *L. pneumophila* Pneumonia

PAUL H. EDELSTEIN,[1,2]* MARTHA A. C. EDELSTEIN,[1] JIANJUN REN,[1] ROBERT POLZER,[3] AND RONALD P. GLADUE[3]

Department of Pathology and Laboratory Medicine[1] and Department of Medicine,[2] University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-4283, and Central Research Division, Pfizer Inc., Groton, Connecticut 06340[3]

Received 21 August 1995/Returned for modification 3 October 1995/Accepted 11 November 1995

The activity of trovafloxacin against 22 clinical *Legionella* isolates was determined by broth microdilution susceptibility testing. The trovafloxacin concentration required to inhibit 90% of strains tested was ≤0.004 μg/ml, in contrast to 0.032 μg/ml for ofloxacin. In guinea pig alveolar macrophages, trovafloxacin achieved intracellular levels up to 28-fold over the extracellular concentration, which was similar to the levels obtained with erythromycin. Trovafloxacin (0.25 μg/ml) reduced bacterial counts of two *L. pneumophila* strains grown in guinea pig alveolar macrophages by >2 $\log_{10}$ CFU/ml, without regrowth, under drug-free conditions over a 3-day period; trovafloxacin was significantly more active than ofloxacin or erythromycin (0.25 to 1 μg/ml) in this assay. Single-dose (10 mg of prodrug CP-116,517-27 per kg of body weight given intraperitoneally [i.p.], equivalent to 7.5 mg of trovafloxacin per kg) pharmacokinetic studies performed in guinea pigs with *L. pneumophila* pneumonia revealed peak serum and lung trovafloxacin levels to be 3.8 μg/ml and 5.0 μg/g, respectively, at 0.5 h and 4.2 μg/ml and 2.9 μg/g, respectively, at 1 h. Administration of a lower prodrug dose (1.4 mg of trovafloxacin equivalent per kg i.p.) gave levels in lung and serum of 0.4 μg/g and 0.4 μg/ml, respectively, 1 h after drug administration. The terminal half-lives of elimination from serum and lung were 0.8 and 1.1 h, respectively. All 15 infected guinea pigs treated for 5 days with CP-116,517-27 once daily (10 mg/kg/day i.p., equivalent to 7.5 mg of trovafloxacin per kg/day) survived for 10 days after antimicrobial therapy, as did all 15 guinea pigs treated with ofloxacin once daily (10 mg/kg/day i.p.) for 5 days. None of 13 animals treated with saline survived. In a second experiment with animals, trovafloxacin (1.4 mg/kg/day i.p. for 5 days) protected all 16 guinea pigs from death, whereas all 15 animals treated with saline died. Trovafloxacin is an effective antimicrobial agent against *Legionella* in vitro and in vivo, with the ability to concentrate in macrophages and kill intracellular organisms.

Assessment of the in vitro activities of antimicrobial agents against *Legionella pneumophila* is complex because of the need to measure the intracellular activity of the antimicrobial agent and the inactivation of many antimicrobial agents by media optimal for the growth of the organism. The quinolone group of antimicrobial agents has been remarkably active against *L. pneumophila* in vitro and in an animal model of Legionnaires' disease (5, 8, 10, 16, 18, 23, 29–31, 35). Trovafloxacin is a new fluoroquinolone antimicrobial agent that has previously been shown to have potent in vitro and in vivo activities against *Streptococcus pneumoniae* (17, 20, 22) and excellent in vitro activity against *L. pneumophila* (3). We determined the activity of trovafloxacin for *Legionella* isolates using three different testing methods: broth microdilution susceptibility testing, inhibition of bacterial growth within alveolar macrophages, and treatment of guinea pigs with *L. pneumophila* pneumonia. As a prelude to the animal treatment studies, we determined the pharmacokinetics of trovafloxacin in *L. pneumophila*-infected guinea pigs.

* Corresponding author. Present address: Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305-5402. Phone: (415) 723-2671. Fax: (415) 723-1837. Electronic mail address: phe@mail.med.upenn.edu.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All legionellae studied were low-passage clinical isolates. These strains were identical to those used in prior studies and were composed of 2 strains each of *Legionella dumoffii*, *Legionella longbeachae*, and *Legionella micdadei*; 1 strain of *Legionella bozemanii*; and 15 strains of *L. pneumophila* (10, 16). *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used as control organisms for susceptibility testing. To obtain inocula for susceptibility testing, legionellae were grown on locally made buffered charcoal yeast extract medium supplemented with 0.1% α-ketoglutarate (BCYEα), and nonlegionellae were grown on commercial tryptic soy agar containing 5% sheep blood (6). Incubation of all media was at 35°C in humidified air for 24 to 48 h, depending on the organism and the growth rate.

Antimicrobial agents. Standard powders of trovafloxacin and ofloxacin were obtained from Pfizer Central Research, Groton, Conn., and Roussel UCLAF, Romainville, France, respectively. The mesylate salt of the parenteral prodrug ester of trovafloxacin (CP-116,517-27) was used for in vivo studies and was also obtained from Pfizer Central Research. CP-116,517-27 is rapidly hydrolyzed in guinea pig and human serum to the parent compound trovafloxacin (4); 10 mg of the prodrug is equivalent to 7.5 mg of the parent compound. Immediately before use, CP-116,517-27 was dissolved in sterile saline for injection, USP. The prodrug concentration used was 4 mg/ml for the pharmacokinetic studies and ≤3.5 mg/ml for the treatment studies (expressed as the concentration of CP-116,517-27). CP-116,517-27 solution for injection was made fresh daily and was used within 1 h of preparation. Sterile ofloxacin for injection was diluted in normal saline for injection, USP, and was obtained from McNeil Pharmaceuticals, Springhouse, Pa.

Antimicrobial susceptibility testing. Broth microdilution susceptibility testing was performed with buffered yeast extract (BYEα) broth (*Legionella* bacteria) or Mueller-Hinton broth (non-*Legionella* bacteria), with a final volume of 100 μl and a final bacterial concentration of 5×10^5 CFU/ml (7). Otherwise, the broth microdilution method was performed exactly as described previously for a broth

macrodilution method (10). The plates were read after 18 to 24 h of incubation for the non-*Legionella* bacteria and after 42 to 48 h of incubation for the *Legionella* bacteria. All testing was done in duplicate; in the case of disagreement, the geometric mean value was used as the MIC. Antimicrobial susceptibility testing with erythromycin of the same bacteria used in the present study has been reported previously (10). The erythromycin MIC for bacterial strains F889 and F2111 is 0.125 μg/ml.

Intracellular accumulation studies. The intracellular accumulation of trovafloxacin and erythromycin was determined as described previously (21, 32). Briefly, alveolar macrophages were obtained from guinea pigs by lavage. Cells were collected, washed, and incubated in 48-well plates for 2 h. After this time, the adherent cells were washed and incubated in triplicate with 10 μg of ^{14}C-trovafloxacin or ^{14}C-erythromycin per ml for various times. ^{14}C-trovafloxacin was synthesized by the Medicinal Chemistry Department at Pfizer and had a specific activity of 26 mCi/mmol. ^{14}C-erythromycin was purchased from Dupont, NEN Research Products, Boston, Mass., and had a specific activity of 15.4 mCi/mmol. The intracellular concentration of drug was then determined by washing the cells, lysing them in 0.05% Triton X-100, and assessing the radioactivity with a MicroBeta scintillation counter (Wallac Inc., Gaithersburg, Md.). The intracellular concentration of drug was determined from a standard curve and by using a cell volume, determined as described previously (21), of 4 μl/10^7 cells. In addition, the release of drug over a 1-h period was assessed in cells that were loaded with the antibiotic for 1 h, washed, and then reincubated at 37°C. All evaluations were done in RPMI 1640 (BioWhittaker, Walkersville, Md.) containing 10% fetal calf serum.

Growth inhibition in alveolar macrophages. Guinea pig pulmonary alveolar macrophages were harvested and purified as described previously (10). The final concentration of macrophages was approximately 10^5 cells per well. Incubation conditions for all macrophage studies were 5% CO_2 in air at 37°C.

L. pneumophila F889 and F2111 grown overnight on BCYEα agar were used to infect the macrophages. Approximately 10^4 bacteria were added to each well. The bacteria were incubated with the macrophages for 1 h in a shaking incubator and then for 1 day in stationary culture as described previously (10). One set of replicate wells was washed (500 μl) three times with tissue culture medium and was then sonicated at low energy to release the intracellular bacteria, which were quantified with BCYEα agar. Antimicrobial agents were then added to the washed, nonsonicated wells; no antimicrobial agent was added to several wells, which served as growth controls. The infected tissue cultures were then incubated for 2 days, after which supernatant samples were taken for quantitative culture. The antimicrobial agents were then removed by washing, and the experiment was continued for 4 more days, with daily quantification of the *L. pneumophila* isolates in the well supernatants. All experiments were carried out in duplicate or triplicate, and quantitative plating was done in duplicate. All wells were observed microscopically daily to detect macrophage infection and to roughly quantify the numbers of macrophages in the wells. In this system there is no extracellular growth of *L. pneumophila*, so all increases in the bacterial concentration in the supernatant are the result of intracellular growth.

Guinea pig pneumonia model. Hartley strain male guinea pigs (weight, ≈320 g) were used for the pneumonia model, as described previously (9). The animals were observed for illness 1 week prior to infection; in the case of the animals used for the treatment study, temperatures and weights were obtained during the preinfection period. The guinea pigs were infected with *L. pneumophila* serogroup 1 strain F889, which was administered intratracheally. The bacterial inoculum was suspended in normal saline for injection, and 0.3 ml of the suspension was administered to each animal. About 6×10^6 CFU was administered for both the pharmacokinetic and the treatment studies.

Pharmacokinetic study. Serum and lung trovafloxacin concentrations were measured in guinea pigs with *L. pneumophila* pneumonia as described previously (16). CP-116,517-27 was given as a single intraperitoneal (i.p.) dose (7.5 mg/kg of body weight as trovafloxacin equivalent in ≈0.9 ml) to guinea pigs 1 day after infection; the mean guinea pig weight was 345 g. At timed intervals after drug injection, anesthetized animals in groups of two to four each were exsanguinated by removal of heart blood under direct vision. The lungs were then removed, rinsed in sterile phosphate-buffered saline to remove adherent blood, blotted dry on gauze, placed in a sterile plastic container, and then placed immediately on ice before being frozen at −70°C. Heart blood was collected with a syringe and a needle and was then transferred immediately to sterile evacuated tubes (Vacutainer; Becton-Dickinson, Rutherford, N.J.). The blood was allowed to clot at room temperature (20 to 24°C) for ≈1 h and was then refrigerated at 5°C. Within 2 h, the serum was separated from the cellular blood components by centrifugation at 5,000 × *g* at 5°C for 10 min and was then frozen at −70°C. Negative controls included guinea pig plasma and lung that had been collected identically from normal guinea pigs given identical anesthesia but no antimicrobial agents.

Serum and lung trovafloxacin concentrations were determined in a second experiment in three uninfected guinea pigs by using a lower CP-116,517-27 dose (1.4 mg/kg of trovafloxacin equivalent given i.p.). Heart blood and lung specimens were collected 1 h after drug administration.

Trovafloxacin concentration determinations. Trovafloxacin concentrations in guinea pig serum were determined as described previously (34). Briefly, 200 μl aliquots were prepared by application to Polysorb C-18 MP-1 solid-phase extraction columns (Interaction Chromatography Inc., San Jose, Calif.). Trovafloxacin was eluted from the columns with 2 ml of high-pressure liquid chromatography (HPLC)-grade methanol which was subsequently evaporated at 55°C under a stream of nitrogen. The residue was redissolved in 1.0 ml of the mobile phase, the samples were vortex mixed and filtered, and aliquots (50 μl) were injected directly onto the HPLC column (Nova-pak C-18; 3.9 by 150 mm; Waters Chromatography, Milford, Mass.). Elution of trovafloxacin was monitored by UV detection at 275 nm.

Lung trovafloxacin concentrations were determined as follows. Approximately 0.5 g of thawed lung tissue was added to 5 ml of extraction buffer (0.15 M $HClO_3$ and 0.15 M H_3PO_4 in distilled H_2O–CH_3OH [50/50; vol/vol]). Internal standard (1 μg of CP-102,372) was then added to each sample, and the components were mixed well and homogenized (Polytron Homogenizer; Brinkmann Instruments, Westbury, N.Y.). The samples were subsequently centrifuged, and the resulting supernatant was aspirated to near dryness at 55°C under a stream of nitrogen. The residue was redissolved in 2 ml of 0.025 M KH_2PO_4 (pH 3.0) and was extracted twice with 5 ml of ethyl acetate. The ethyl acetate layers were combined and were evaporated as described above. This residue was resuspended in 0.5 ml of the mobile phase and was washed with 1 ml of hexane. The hexane layer was evaporated off, and aliquots (10 μl) were injected directly onto the HPLC column as described above.

Animal treatment study. The guinea pigs surviving the surgery and intratracheal infection were randomized into three treatment groups 1 day after infection. Starting on that day, treatment was given each morning for 5 days. One group of 15 animals received CP-116,517-27 (7.5 mg of trovafloxacin activity per kg in 1.0 ml), another 15 animals received ofloxacin (10 mg/kg), and the last group of 13 animals received 1.0 ml of normal saline each morning. All dosing was by the i.p. route. Animal weights were taken periodically during the 14-day postinfection observation period. Necropsies and quantitative lung cultures were performed on all animals that died. All animals surviving for 14 days postinfection were killed with pentobarbital; necropsies, quantitative lung cultures, and histologic examinations of the lungs were performed on the seven animals from each group with the lowest weights (9). Reading of the histologic slides was performed blinded with respect to the antimicrobial treatment group assignments.

A second experiment was conducted with the same animal model and infectious inoculum. Four different treatments were given daily for 5 days by the i.p. route: saline (15 animals) and CP-116,517-27 at three different doses (7.50 [14 animals], 3.75 [15 animals], and 1.88 [16 animals] mg/kg/day). Necropsies and histologic examinations of the lungs were performed on all animals. Quantitative lung cultures were performed for *L. pneumophila* for all animals that died of infection and for the seven lowest-weight survivors in each treatment group.

Statistical analysis. Calculation of mean MICs was done by a geometric method. Comparison of nonparametric values was by the Fisher exact test or the chi-square test with correction for continuity. The nonpaired, two-tailed Student *t* test was used to compare parametric mean values. One-way analysis of variance was used to compare multiple parametric values. The InStat computer program was used for statistical analysis (version 2.02; GraphPAD, San Diego, Calif.). Calculation of drug pharmacokinetic parameters was performed by using a noncompartmental model of analysis and computer software (PK_PARAM) developed at Pfizer Central Research.

RESULTS

Broth dilution susceptibility. All 22 *Legionella* strains tested were susceptible to trovafloxacin at the concentrations readily achievable in serum. The average MIC, the MIC required to inhibit 50% of strains, the MIC required to inhibit 90% of strains, and the range of MICs for trovafloxacin were 0.005, ≤0.004, ≤0.004, and ≤0.004 to 0.008 μg/ml respectively; the respective values for ofloxacin were 0.032, 0.032, 0.032, and 0.016 to 0.064 μg/ml. The trovafloxacin MIC for bacterial strains F889 and F2111 was 0.004 μg/ml; the ofloxacin MIC for the same strains was 0.032 μg/ml. Trovafloxacin and ofloxacin were variably inhibited by BYEα broth medium; the MICs for the *S. aureus* control strain were the same with Mueller-Hinton or BYEα broth. The MICs of trovafloxacin or ofloxacin for the *E. coli* control strain were twofold higher when BYEα rather than Mueller-Hinton broth was used.

Intracellular accumulation. Trovafloxacin readily concentrated in guinea pig alveolar macrophages, achieving levels similar to those obtained with erythromycin (Table 1). In addition, ≈50% of the drug egressed from the cells after 1 h of incubation in antibiotic-free medium.

Antimicrobial inhibition of intracellular growth. Both *L. pneumophila* serogroup 1 strains grown in guinea pig alveolar macrophages were significantly inhibited by trovafloxacin, ofloxacin, and erythromycin (Fig. 1). Trovafloxacin was signif-

TABLE 1. Intracellular accumulation and release of trovafloxacin and erythromycin from guinea pig alveolar macrophages

Drug	Incubation time (min)	Accumulation ($\mu g/10^7$ cells)[a]	C/E[a,b]	Release (%)[a]
Trovafloxacin	10	1.5 ± 0.17	28.0 ± 0.3	0
	30	ND[c]	ND	36 ± 10
	60	1.2 ± 0.02	22.6 ± 1.4	46 ± 5
Erythromycin	10	0.57 ± 0.03	12.5 ± 1.17	19 ± 7
	30	ND	ND	43 ± 12
	60	1.13 ± 0.05	21.9 ± 0.13	ND

[a] Values are means ± standard deviations.
[b] C/E, ratio of cellular concentration to extracellular concentration.
[c] ND, not done.

TABLE 2. Concentrations of trovafloxacin in serum and lung of guinea pigs with *L. pneumophila* pneumonia following i.p. administration of CP-116,517-27[a]

Time post-administration (h)	No. of animals	Concn in serum ($\mu g/ml$)		Concn in lung ($\mu g/g$)		Concn in lung/concn in serum
		Mean	Range	Mean	Range	
0.5	3	3.8	3.7–3.9	5.0	3.9–5.5	1.3
1	4	4.2	3.5–4.8	2.9	2.0–3.6	0.7
2	4[b]	1.9	1.4–2.3	2.1	2.1–2.4	1.1
4	4	0.3	0.2–0.4	0.5	0.4–0.7	1.7
8	4	<0.5	<0.5	<0.5	<0.5	

[a] CP-116,517-27 was given at 10 mg/kg, which is equivalent to a trovafloxacin concentration of 7.5 mg/kg.
[b] Only three serum specimens were tested at this time point.

icantly more inhibitory than either ofloxacin or erythromycin. Erythromycin (1.0 μg/ml) was inhibitory only, with rapid regrowth of *L. pneumophila* after drug washout. In contrast, trovafloxacin (0.1 to 0.25 μg/ml) was bactericidal, without measurable regrowth for up to 4 days after drug washout. Trovafloxacin was significantly more active than ofloxacin. None of the antimicrobial agents tested showed evidence of cytotoxicity.

Pharmacokinetic study. The pharmacokinetic data from the study are given in Table 2 for the 7.5-mg/kg trovafloxacin-equivalent dose. Trovafloxacin was not detected in any of the negative control samples. The mean maximum concentrations of trovafloxacin in serum and lung were 4.2 μg/ml and 5.0 μg/g, respectively, with the respective times to the maximum concentration being 1.0 and 0.5 h. The ratios of the trovafloxacin concentration in lung to that in serum ranged from 0.7 to 1.7 over the course of the experiment. The terminal elimination half-lives in guinea pig serum and lung were calculated to be 0.8 and 1.1 h, respectively. The elimination constants were calculated to be 0.88 and 0.63 h^{-1} for serum and lung, respectively.

Average serum and lung trovafloxacin concentrations were 0.4 μg/ml (range, 0.3 to 0.4 μg/ml) and 0.4 μg/g (range, 0.4 to 0.5 μg/g), respectively, 1 h after administration of 1.4 mg of trovafloxacin equivalent per kg ($n = 3$).

Therapy in guinea pigs. All guinea pigs treated with trovafloxacin (7.5 mg/kg/day) survived, whereas none of the 13 guinea pigs receiving saline alone survived ($P < 0.0001$ by chi-square test) (Fig. 2A). Lung cultures and necropsy results for all saline-treated animals were diagnostic of *L. pneumophila* pneumonia; the mean concentration of *L. pneumophila* was 9.7 $\log_{10}$ CFU/g of lung, with a range of 9.5 to 10.1 $\log_{10}$ CFU/g. Two of the seven lungs that were examined from the survivors in the trovafloxacin treatment group were positive for *L. pneumophila*; these contained 2.5 and 3.5 $\log_{10}$ CFU/g. Three of the seven lungs that were examined from the survivors in the ofloxacin treatment group contained *L. pneumophila*; these contained 2.4, 3.2, and 2.2 $\log_{10}$ CFU/g. No significant differences in lung histology were noted between the lungs from the trovafloxacin and ofloxacin treatment groups for the seven lungs in each group that were examined. All seven lungs from the trovafloxacin-treated animals and six of seven lungs from the ofloxacin-treated animals were ≤10% consolidated. The weights of the animals in the trovafloxacin treatment group were significantly lower than those of the animals in the ofloxacin treatment group on days 3 to 13 postinfection ($P < 0.0001$, two-tailed nonpaired t test) (Fig. 2B). The mean weights of the animals in the three treatment groups were not significantly different on days −4, −1, and 0 (day of infection), but on day 1 postinfection, the mean weight of the animals in the ofloxacin group was significantly greater than that of the animals in the trovafloxacin treatment group ($P = 0.03$ by t test). No significant differences in rectal temperatures were noted between the three treatment groups on days −4, −1, 3, 4, and 8. On postinfection days 1 and 2 the temperatures of the animals in the ofloxacin treatment group were significantly higher than the temperatures of the animals in the trovafloxacin treatment group (95% confidence interval

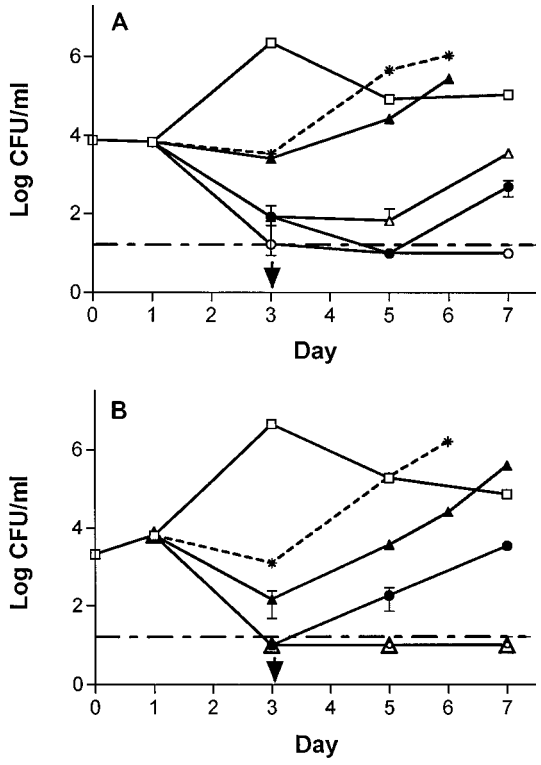

FIG. 1. Growth ($\log_{10}$ CFU per milliliter) of *L. pneumophila* serogroup 1 strains F889 (A) and F2111 (B) in guinea pig alveolar macrophages versus day of incubation after the initiation of infection. See text for experimental details. All points represent the means for triplicate wells counted in duplicate; error bars represent 95% confidence intervals, which, unless shown, were smaller than the height of the symbol representing the mean. The dotted horizontal lines show the lower limit of detection of bacterial growth. Datum points below this line represent undetectable bacterial growth. The large arrows indicate the day that drugs were removed by washing. Symbols: □, no antimicrobial agents; ∗, 1.0 μg of erythromycin per ml; ● and ○, 0.1 and 0.25 μg of trovafloxacin per ml, respectively; ▲ and △, 0.25 and 1.0 μg of ofloxacin per ml, respectively.

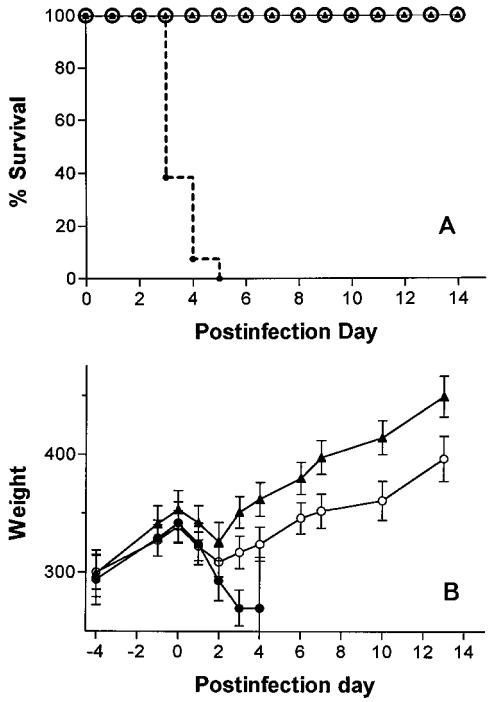

FIG. 2. (A) Percent survival of guinea pigs with *L. pneumophila* pneumonia versus postinfection day. (B) Weight (in grams) versus postinfection day. Animals were treated with trovafloxacin (○; $n = 15$), ofloxacin (▲; $n = 15$), or saline (●; $n = 13$) on postinfection days 1 to 5. Vertical bars represent 95% confidence intervals.

of differences, 0.2 to 0.9°C); this may have been due to slight differences in animal cubicle temperatures on these 2 days.

A second animal experiment studied the effectiveness of three different trovafloxacin dosages, 5.6, 2.8, or 1.4 mg/kg/day given for 5 days. All animals that received trovafloxacin, including all 16 animals receiving the lowest dosage, survived lethal challenge with *L. pneumophila*, whereas none of 15 animals treated with saline survived ($P < 0.0001$ by chi-square analysis). No significant differences in animal weights for animals in the three different trovafloxacin treatment groups were observed on days 1 to 5 and days 9 to 14 postinfection; on day 7 a significant difference was noted between the highest and lowest dosage group, with the latter group being an average of 25 g heavier ($P < 0.05$). On days 4 and 5 postinfection a significant temperature difference was noted between the trovafloxacin treatment groups, but this difference was not noted on days 1, 2, 3, or 7. On these days there were minor (0.3°C) and inconsistent temperature differences that appeared to have no relationship to drug dose. Cultures of lung tissue taken from the 15 saline-treated animals at the time of death were all positive for *L. pneumophila*, with a mean, median, and range of 9.9, 10.0, and 6.5 to 10.2 $\log_{10}$ CFU/g, respectively. Of the seven cultures of lung tissue from animals in the group receiving the highest dosage of trovafloxacin, only one was positive upon culture for *L. pneumophila*; this contained 2.9 $\log_{10}$ CFU/g. Three of seven cultures of lung tissue from animals in the group receiving the intermediate dosage of trovafloxacin grew *L. pneumophila* at concentrations of 2.0, 2.1, and 3.1 $\log_{10}$ CFU/g. Two of seven cultures of lung tissue from animals in the group receiving the lowest dosage of trovafloxacin grew *L. pneumophila*, both at concentrations of 2.6 $\log_{10}$ CFU/g. Histopathology showed that there were no significant differences ($P > 0.1$) between the three different trovafloxacin treatment groups, all of which were significantly different from the saline treatment group ($P < 0.0005$ by chi-square analysis; data not shown).

DISCUSSION

Trovafloxacin was very active in vitro against the *Legionella* species tested and was substantially more active than ofloxacin. On the basis of susceptibility testing performed previously by us with the same strains and by the same technique, trovafloxacin is one of the most active antimicrobial agents that we have tested against *Legionella* isolates, and it is more active than ciprofloxacin, azithromycin, erythromycin, fleroxacin, Ro 23-9424, RP 74501-RP 74502, clavulanic acid, and tazobactam (11–16). Our in vitro susceptibility results differ from those found by Briggs-Gooding and Jones (3), who reported that the MICs of trovafloxacin at which 50% and 90% of *Legionella* isolates are inhibited was about 10-fold higher than those that we found. We used a broth testing medium, which usually gives MICs lower than those obtained with the agar medium used by Briggs-Gooding and Jones (8).

Trovafloxacin was more active than erythromycin and ofloxacin against the two strains of intracellular *L. pneumophila* studied. Trovafloxacin was bactericidal for intracellular *L. pneumophila* at low extracellular concentrations and prevented bacterial regrowth after the removal of extracellular drug. Since intracellular drug rapidly effluxed from the cell after the removal of extracellular drug, the prolonged period required for bacterial regrowth is explained either by substantial or complete killing of intracellular bacteria or by a very long postantibiotic effect. Prior studies have shown that erythromycin is purely inhibitory in this and other macrophage systems, even at concentrations of 5 μg/ml, and is clearly less active than azithromycin, ofloxacin, ciprofloxacin, fleroxacin, sparfloxacin, clarithromycin, and WIN 57273 (10, 11, 15, 16, 23, 31, 35). On the basis of historical comparisons, for the same bacterial strains and technique, trovafloxacin is among the most active of the other quinolones tested including ciprofloxacin, sparfloxacin, WIN 57273, and fleroxacin (10, 15, 16).

Trovafloxacin is concentrated within macrophages, which accounts in part for its intracellular activity against *L. pneumophila*. Similar levels of intracellular accumulation of trovafloxacin have been observed in human neutrophils and lymphocytes, with intracellular concentrations being 8- to 15-fold greater than extracellular concentrations (unpublished data). The intracellular concentration of erythromycin obtained in this system are similar to those reported previously for this antibiotic (24, 32). These experiments demonstrate that the magnitude of the intracellular concentration alone is not a good measure of relative intracellular activity. Trovafloxacin was significantly more active than erythromycin against intracellular *L. pneumophila*, even though it achieved comparable intracellular levels.

The pharmacokinetic profile of trovafloxacin in guinea pig serum is different from that measured in humans, in which a once-daily oral dose of 200 mg produced peak levels in blood of just over 2 μg/ml and an elimination half-life of 10.5 h (33). We know from the present study that a single 10-mg/kg i.p. dose of CP-116,517-27 (equivalent to 7.5 mg/kg as trovafloxacin) provided concentrations in lung above the extracellular broth MIC at which 90% of strains are inhibited for the *Legionella* strains that we studied for at least 4 h and for possibly as long as 10 h (on the basis of the half-life of elimination from the lung of 1.1 h). Assuming dose-independent kinetics, the lowest effective dose of trovafloxacin (1.4 mg/kg/day) achieved concentrations in lung above the extracellular MIC at which

90% of strains are inhibited for only about 5 h, indicating that effective therapy for Legionnaires' disease does not necessarily require frequent drug dosing. The effectiveness of such a low dose may be accounted for by the prolonged postantibiotic effect or the bactericidal activity, or both, of trovafloxacin against *L. pneumophila*, as demonstrated in the cell infection model.

Trovafloxacin was very effective for the treatment of experimental Legionnaires' disease, as are other quinolone antimicrobial agents (5, 8, 16, 18, 30, 31). No differences were apparent between ofloxacin and trovafloxacin therapy, for animal temperature, necropsy findings, histologic examination of the lung, or lung cultures. The only major difference observed between the two therapies was the slower weight gain for the animals in the trovafloxacin treatment group. This difference could be due to a lower initial weight in the trovafloxacin group immediately prior to therapy on day 1 postinfection. Possible alternative explanations are that trovafloxacin caused gastrointestinal toxicity, decreased appetite directly, or decreased appetite because of peritoneal irritation caused by drug injection at that site. Some direct effect of trovafloxacin appears likely because of the trend toward higher weights in the animals given the lowest dose of trovafloxacin in the second study. The intestines of two trovafloxacin-treated animals from the first experiment appeared abnormal at necropsy, suggesting some gastrointestinal tract toxicity; no such abnormalities were observed in animals in the second experiment. Gastrointestinal tract toxicity of antimicrobial agents for guinea pigs occurs with many drugs, such as penicillin, erythromycin, and rifampin, and does not necessarily indicate the potential for antibiotic toxicity in humans (9). Extensive phase II and III clinical studies with trovafloxacin have revealed little gastrointestinal tract toxicity for humans (33).

Saito and colleagues (31) have previously shown that orally administered ofloxacin (10 mg/kg/day) is effective in a similar guinea pig model of Legionnaires' disease and is more effective than erythromycin (20 mg/kg/day). Also, more than 25 patients with Legionnaires' disease have been successfully treated with oral or intravenous ofloxacin monotherapy given in daily doses of 400 to 800 mg (1, 2, 19, 25–28, 36). Trovafloxacin may be as effective as ofloxacin against *Legionella* isolates, with the added benefit of greater activity against *Streptococcus pneumoniae* (22).

Because the concentrations of trovafloxacin in serum are higher and more sustained in humans than in guinea pigs for equivalent doses and because trovafloxacin has potent activity against *L. pneumophila* in the animal and cell models of infection, therapy with trovafloxacin could be curative in humans with Legionnaires' disease. However, comparative clinical treatment studies with other quinolone antimicrobial agents and macrolides are the only means of determining the relative efficacy of trovafloxacin for humans with Legionnaires' disease as well as the appropriate duration of trovafloxacin therapy for patients with this disease.

ACKNOWLEDGMENTS

This study was funded by Pfizer Central Research, Inc.

Audrey Baylock, Zunxuan Chen, and Donna Riddle provided excellent technical assistance.

REFERENCES

1. **Bertrand, A., F. Janbon, E. Despaux, O. Jonquet, and J. Reynes.** 1987. L'ofloxacine (RU 43280). Etude clinique. Pathol. Biol. (Paris) **35:**629–633.
2. **Bouhaja, B., H. Thabet, L. Slim, F. Aissa, M. Amamou, and M. Yacoub.** 1993. Pneumopathie communautaire mixte à *Legionella pneumophila* et *Staphylococcus aureus*. Presse Med. **22:**1280. (Letter.)
3. **Briggs-Gooding, B., and R. N. Jones.** 1993. In vitro antimicrobial activity of CP-99,219, a novel azabicyclo-naphthyridone. Antimicrob. Agents Chemother. **37:**349–353.
4. **Brighty, K. E., T. D. Gootz, A. E. Girard, R. Shanlter, M. J. Castaldi, D. Girard, S. A. Miller, and J. Faiella.** 1995. Prodrugs of CP-99,219 for intravenous administration; synthesis and evaluation resulting in identification of CP-116,517, abstr. 730. *In* Program and abstracts of the 7th European Congress on Clinical Microbiology and Infectious Diseases. European Society of Clinical Microbiology and Infectious Diseases, Vienna.
5. **Dournon, E., P. Rajagopalan, J. Vilde, and J. Pocidalo.** 1986. Efficacy of pefloxacin in comparison with erythromycin in the treatment of experimental guinea pig legionellosis. J. Antimicrob. Chemother. **17**(Suppl. B)**:**41–48.
6. **Edelstein, P. H.** 1981. Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. J. Clin. Microbiol. **14:**298–303.
7. **Edelstein, P. H.** 1985. Legionnaires' disease laboratory manual, 3rd ed. National Technical Information Service, Springfield, Va.
8. **Edelstein, P. H.** 1995. Antimicrobial therapy for Legionnaires' disease: a review. Clin. Infect. Dis. **21**(Suppl. 3)**:**S265–S276 .
9. **Edelstein, P. H., K. Calarco, and V. K. Yasui.** 1984. Antimicrobial therapy of experimentally induced Legionnaires' disease in guinea pigs. Am. Rev. Respir. Dis. **130:**849–856.
10. **Edelstein, P. H., and M. A. C. Edelstein.** 1989. WIN 57273 is bactericidal for *Legionella pneumophila* grown in alveolar macrophages. Antimicrob. Agents Chemother. **33:**2132–2136.
11. **Edelstein, P. H., and M. A. C. Edelstein.** 1991. In vitro activity of azithromycin against clinical isolates of *Legionella* species. Antimicrob. Agents Chemother. **35:**180–181.
12. **Edelstein, P. H., and M. A. C. Edelstein.** 1992. In vitro activity of Ro 23-9424 against clinical isolates of *Legionella* species. Antimicrob. Agents Chemother. **36:**2559–2561.
13. **Edelstein, P. H., and M. A. C. Edelstein.** 1993. In vitro activity of RP 74501-RP 74502, a novel streptogramin antimicrobial mixture, against clinical isolates of *Legionella* species. Antimicrob. Agents Chemother. **37:**908–910.
14. **Edelstein, P. H., and M. A. C. Edelstein.** 1994. In vitro extracellular and intracellular activities of clavulanic acid and those of piperacillin and ceftriaxone alone and in combination with tazobactam against clinical isolates of *Legionella* species. Antimicrob. Agents Chemother. **38:**200–204.
15. **Edelstein, P. H., M. A. C. Edelstein, and B. Holzknecht.** 1992. In vitro activities of fleroxacin against clinical isolates of *Legionella* spp., its pharmacokinetics in guinea pigs, and use to treat guinea pigs with *L. pneumophila* pneumonia. Antimicrob. Agents Chemother. **36:**2387–2391.
16. **Edelstein, P. H., M. A. C. Edelstein, J. Weidenfeld, and M. B. Dorr.** 1990. In vitro activity of sparfloxacin (CI-978; AT-4140) for clinical *Legionella* isolates, pharmacokinetics in guinea pigs, and use to treat guinea pigs with *L. pneumophila* pneumonia. Antimicrob. Agents Chemother. **34:**2122–2127.
17. **Eliopoulos, G. M., K. Klimm, C. T. Eliopoulos, M. J. Ferraro, and R. C. Moellering, Jr.** 1993. In vitro activity of CP-99,219, a new fluoroquinolone, against clinical isolates of gram positive bacteria. Antimicrob. Agents Chemother. **37:**366–370.
18. **Fitzgeorge, R. B., D. H. Gibson, R. Jepras, and A. Baskerville.** 1985. Studies on ciprofloxacin therapy of experimental Legionnaires' disease. J. Infect. **10:**194–203.
19. **Gentry, L. O., B. Lipsky, M. O. Farber, B. Tucker, and G. Rodriguez-Gomez.** 1992. Oral ofloxacin therapy for lower respiratory tract infection. South. Med. J. **85:**14–18.
20. **Girard, A. E., D. Girard, T. D. Gootz, J. A. Faiella, and C. R. Cimochowski.** 1995. In vivo efficacy of trovafloxacin (CP-99,219), a new quinolone with extended activities against gram-positive pathogens, *Streptococcus pneumoniae*, and *Bacteroides fragilis*. Antimicrob. Agents Chemother. **39:**2210–2216.
21. **Gladue, R. P., G. M. Bright, R. E. Isaacson, and M. F. Newborg.** 1989. In vitro and in vivo uptake of azithromycin (CP-62,993) by phagocytic cells: possible mechanism of delivery and release at sites of infection. Antimicrob. Agents Chemother. **33:**277–282.
22. **Gootz, T. D., K. E. Brighty, M. R. Anderson, B. J. Schmieder, S. L. Haskell, J. A. Sutcliffe, M. J. Castaldi, and P. R. McGuirk.** 1994. In vitro activity of CP-99,219, a novel 7-(3-azabicyclo[3.1.0]hexyl)naphthyridone antimicrobial. Diagn. Microbiol. Infect. Dis. **19:**235–243.
23. **Havlichek, D., L. Saravolatz, and D. Pohlod.** 1987. Effect of quinolones and other antimicrobial agents on cell-associated *Legionella pneumophila*. Antimicrob. Agents Chemother. **31:**1529–1534.
24. **Johnson, J. D., W. L. Hand, J. B. Francis, N. King-Thompson, and R. W. Corwin.** 1980. Antibiotic uptake by alveolar macrophages. J. Lab. Clin. Med. **95:**429–439.
25. **Leroy, O., C. Beuscart, C. Chidiac, B. Sivery, E. Senneville, M. Vincent du Laurier, and Y. Mouton.** 1989. Traitement des pneumonies dues aux légionelles, mycoplasmes, *Chlamydiae* et rickettsies par l'ofloxacine. Pathol. Biol. (Paris) **37:**1137–1140.
26. **Meyer, R. D.** 1991. Role of the quinolones in the treatment of legionellosis. J. Antimicrob. Chemother. **28:**623–625.
27. **Mouton, Y., O. Leroy, C. Beuscart, B. Sivery, E. Senneville, C. Chidiac, G.**

Beaucaire, and M. Vincent du Laurier. 1990. Efficacy of intravenous ofloxacin: a French multicentre trial in 185 patients. J. Antimicrob. Chemother. **26**(Suppl. D)**:**115–121.
28. **Peugeot, R. L., B. A. Lipsky, T. M. Hooton, and R. E. Pecoraro.** 1991. Treatment of lower respiratory infections in outpatients with ofloxacin compared with erythromycin. Drugs Exp. Clin. Res. **17:**253–257.
29. **Pohlod, D. J., L. D. Saravolatz, and M. M. Somerville.** 1988. Inhibition of *Legionella pneumophila* multiplication within human macrophages by fleroxacin. J. Antimicrob. Chemother. **22**(Suppl. D)**:**49–54.
30. **Saito, A., H. Koga, H. Shigeno, K. Watanabe, K. Mori, S. Kohno, Y. Shigeno, Y. Suzuyama, K. Yamaguchi, and M. Hirota.** 1986. The antimicrobial activity of ciprofloxacin against *Legionella* species and the treatment of experimental *Legionella* pneumonia in guinea pigs. J. Antimicrob. Chemother. **18:**251–260.
31. **Saito, A., K. Sawatari, Y. Fukuda, M. Nagasawa, H. Koga, A. Tomonaga, H. Nakazato, K. Fujita, Y. Shigeno, and Y. Suzuyama.** 1985. Susceptibility of *Legionella pneumophila* to ofloxacin in vitro and in experimental *Legionella* pneumonia in guinea pigs. Antimicrob. Agents Chemother. **28:**15–20.
32. **Stamler, D. A., M. A. C. Edelstein, and P. H. Edelstein.** 1994. Azithromycin pharmacokinetics and intracellular concentrations in *Legionella pneumophila*-infected and uninfected guinea pigs and their alveolar macrophages. Antimicrob. Agents Chemother. **38:**217–222.
33. **Teng, R., S. C. Harris, D. E. Nix, J. J. Schentag, G. Foulds, and T. E. Liston.** 1995. Pharmacokinetics and safety of CP-99,219, a new quinolone antibiotic, following administration of single oral doses to healthy male volunteers. J. Antimicrob. Chemother. **36:**385–394.
34. **Teng, R., T. G. Tesnfeldt, T. E. Liston, and G. Foulds.** Unpublished data.
35. **Vildé, J. L., E. Dournon, and P. Rajagopalan.** 1986. Inhibition of *Legionella pneumophila* multiplication within human macrophages by antimicrobial agents. Antimicrob. Agents Chemother. **30:**743–748.
36. **Wynckel, A., O. Toupance, J. P. Melin, C. David, S. Lavaud, T. Wong, D. Lamiable, and J. Chanard.** 1991. Traitement des légionelloses par ofloxacine chez le transplante renal. Absence d'interférence avec la ciclosporine A. Presse Med. **20:**291–293.