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Experimental Study of Clinafloxacin Alone and in Combination in the Treatment of Ciprofloxacin-Susceptible and -Resistant Pneumococcal Meningitis

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ABSTRACT

The increasing incidence of ciprofloxacin resistance in *Streptococcus pneumoniae* may limit the efficacy of the new quinolones in difficult-to-treat infections such as meningitis. The aim of the present study was to determine the efficacy of clinafloxacin alone and in combination with teicoplanin and rifampin in the therapy of ciprofloxacin-susceptible and ciprofloxacin-resistant pneumococcal meningitis in rabbits. When used against a penicillin-resistant ciprofloxacin-susceptible strain (Clinafloxacin MIC 0.12 μg/ml), clinafloxacin at a dose of 20 mg/kg per day b.i.d. decreased bacterial concentration by −5.10 log cfu/ml at 24 hr. Combinations did not improve activity. The same clinafloxacin schedule against a penicillin- and ciprofloxacin-resistant strain (Clinafloxacin MIC 0.5 μg/ml) was totally ineffective. Our data suggest that a moderate decrease in quinolone susceptibility, as indicated by the detection of any degree of ciprofloxacin resistance, may render these antibiotics unsuitable for the management of pneumococcal meningitis.

INTRODUCTION

THE WORLDWIDE INCREASE in penicillin- and cephalosporin-resistant pneumococci has led to changes in the therapy of pneumococcal infections. The combination of high doses of cefotaxime and vancomycin is recommended by most authorities for pneumococcal meningitis caused by resistant strains, as well as for initial empirical therapy. In patients infected by hypothetical strains with very high cephalosporin resistance and in those with penicillin allergy, the use of non-β-lactam antibiotics may be warranted.[7,12,23–25] Glycopeptides, rifampin, and the new quinolones are among potentially effective antibiotics in this setting. Monotherapy with systemic vancomycin in adults has been associated with clinical and bacteriological failures, better explained by the erratic and borderline vancomycin cerebrospinal fluid (CSF) levels, which fall when dexamethasone is administered concomitantly.[26] Teicoplanin alone as well as the combination of rifampin and vancomycin, with and without dexamethasone, have all been effective in the rabbit model of pneumococcal meningitis, but clinical experience is lacking.[6,13]

Quinolones enter the CSF better than other classes of antimicrobial agents and are rapidly bactericidal for susceptible organisms in a highly concentration-dependent way.[19] Ciprofloxacin has excellent activity against Gram-negative organisms and has been successfully used for the treatment of Gram-negative bacillary meningitis in animal models and patients, but its activity is insufficient for treating meningitis due to Gram-positive organisms.

In recent years, new quinolones with enhanced activity against *S. pneumoniae* (including clinafloxacin, trovafloxacin, moxifloxacin, grepafloxacin, gatifloxacin, and gemifloxacin) have proven to be potentially useful for the treatment of experimental meningitis caused by penicillin- and cephalosporin-resistant pneumococcal strains.[2,8,9,11,15,17,18,21] However, the increasing incidence of ciprofloxacin resistance in *S. pneumoniae* may reduce the efficacy of these new quinolones for treating meningitis, because cross-resistance is likely to occur. In fact, although their activity against pneumococcal strains with high ciprofloxacin MICs remains good, susceptibility to all drugs in the class is decreased to some extent.[3,10]

To date, few studies have assessed the influence of this resistance on the therapeutic efficacy of the new quinolones in experimental pneumococcal meningitis. The aim of the present study was to determine the efficacy of clinafloxacin, alone and in combination with teicoplanin and rifampin, to treat

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ciprofloxacin-susceptible (CS) and ciprofloxacin-resistant (CR) pneumococcal meningitis in rabbits.

MATERIALS AND METHODS

Bacterial strains

Two *S. pneumoniae* strains were used. Strain 2349, belonging to serotype 23 F, was recovered from a patient with pneumococcal meningitis and strain 4371, belonging to serotype 9 V, was recovered from a patient with chronic bronchitis. MICs were determined by microdilution method in cation-supplemented Mueller-Hinton broth with 5% whole defribinated horse blood and the appropriate concentration of antibiotic. The wells of microdilution plates were inoculated to a volume of 100 μl with an inoculum containing 1 million cfu/ml. MICs of strain 2349 were as follows: penicillin, 4 μg/ml; ceftriaxone/cefotaxime, 2 μg/ml; clinafloxacin, 0.12 μg/ml; ciprofloxacin, 2 μg/ml; teicoplanin, 0.03 μg/ml; rifampin, 0.06 μg/ml; vancomycin, 0.25 μg/ml. MICs of strain 4371 were: penicillin, 2 μg/ml; ceftriaxone/cefotaxime, 1 μg/ml; clinafloxacin, 0.5 μg/ml; ciprofloxacin, >32 μg/ml; teicoplanin, 0.12 μg/ml; rifampin, 0.03 μg/ml; vancomycin, 0.25 μg/ml. The CR strain was previously examined within the quinolone-resistance-determining regions, showing mutations in *ParC* (S79Y), *ParE* (I460V), and *GyrA* (S81F). This strain also demonstrated an efflux mechanism.[10] The possibility of a change in MIC during therapy was investigated by re-assessing MICs in the colonies growing in CSF at 24 hr of therapy.

Inoculum preparation

Colonies from fresh overnight cultures on 5% blood agar plates were resuspended and grown for 4–6 hr at 37°C in BHI broth medium. Immediately before inoculation, cultures in BHI were centrifuged and resuspended in sterile saline, adjusted to an optical density of 0.5 McFarland ($\sim 10^8$ cfu/ml), and then diluted to the appropriate size. A final inoculum of 10^6 cfu/ml was used. The inoculum size was confirmed by quantitative cultures.

Rabbit model of meningitis

The rabbit model of meningitis was based on an established protocol.[4] The study was approved by the Ethical Committee for Animal Experiments at the University of Barcelona (Bellvitge Campus). Treatment groups were formed, each comprising 8 rabbits, along with a control group, which was inoculated but not treated. Two-kilogram female New Zealand white rabbits were anesthetized intramuscularly with 35 mg of ketamine (Ketolar®, Parke-Davis, El Prat de Llobregat, Spain) and 5 mg of xylazine (Rompun®, Bayer AG, Leverkusen, Germany) per kg of body weight, and a dental acrylic helmet was affixed to the calvarium. Twenty-four hours later, the animals were anesthetized again and placed in a stereotaxic frame. A spinal needle was introduced into the cisterna magna, 200 μl of CSF were withdrawn, and 200 μl of a 10^6 cfu/ml inoculum of one of the infecting strains were instilled. Eighteen hours later, the rabbits were anesthetized again with urethane 1.75 g/kg, subcutaneously (s.c.) (Sigma Chemical Company, St. Louis MO) and thiopental 5 mg/kg, intravenously (i.v.) (Penthotal® sodico, Abbott Laboratories, Madrid, Spain), and a baseline CSF sample was taken. In the experiments with the CS strain, at this point (0 hr), an i.v. dose of either clinafloxacin (Parke-Davis, Ann Arbor, MI) 10 mg/kg per day, 20 mg/kg per day in a single dose or 20 mg/kg per day in two divided doses (b.i.d.), or a combination of clinafloxacin 20 mg/kg per day b.i.d. with teicoplanin 15 mg/kg per day (Targocid®, Hoechst-Marion-Roussell, Barcelona, Spain) or rifampin 15 mg/kg per day (Rifaldin®, Hoechst-Marion-Roussell, Barcelona, Spain) was administered. Saline (Suero fisiológico Braun, Braun S.A. Rubí, Barcelona, Spain) was given to the controls. Teicoplanin and rifampin were administered every 24 hr. In the experiments with the CR strain, only the last dose, 20 mg/kg per day b.i.d., was used alone and in combination. Hydration was ensured with 20 ml of saline injected s.c. three times throughout the experiment. Serial CSF samples were taken at 2 (peak), 6, 24 (trough), and 26 (peak) hr of treatment. CSF samples were used to perform direct and quantitative bacterial cultures and white blood cell (WBC) counts, as well as to determine concentrations of lactic acid, total protein, and CSF clinafloxacin at trough and peak time points.

WBC counts were performed by optical microscopy (40×) with a Neubauer chamber after WBC had been lysed with Turk solution (1:1, self-laboratory made). CSF lactic acid concentrations were determined by the Lactate PAP technique (Biomérieux SA, 69280 Marcy l'Etoile, France) and read by spectrophotometer (505 nm). CSF total protein concentrations were determined by the Bio-Rad Protein Assay technique (BioRad Laboratories, München, Germany) using bovine serum albumin as the standard protein, and also read by spectrophotometer (595 nm). Serial 10-fold dilution cultures of CSF were made to determine bacterial counts at each time point (the detection limit by this method was 10^2 cfu/ml; a value of 1.9 log cfu/ml was assigned to the first sterile culture and a value of 0 to the subsequent ones). To avoid carryover antimicrobial agent interference, the sample was placed onto the plate in a single streak down the center and allowed to be absorbed into the agar until the plate surface appeared dry; the inoculum was then spread over the plate. Plates were incubated overnight at 37°C.

CSF clinafloxacin levels were measured by the disk diffusion bioassay method.[1] The assay organism was *Escherichia coli* ATCC 25922, and the assay medium was DIFCO antibiotic medium No. 1. The minimal detectable concentration was 0.25 μg/ml. Teicoplanin and rifampin controls were performed in *E. coli* plates to ensure that no teicoplanin or rifampin activity would be found when assaying clinafloxacin-teicoplanin and clinafloxacin-rifampin samples. The area under the concentration-time curve (AUC) was calculated by a computer-assisted method according to the trapezoidal rule.

Therapeutic failure was defined as an increase in bacterial concentration of at least 1 log cfu/ml compared with a previous count. A therapy was considered bactericidal when it achieved a reduction of 3 log cfu/ml. A synergistic effect of one combination was defined as a decrease ≥2 log cfu/ml compared with the most active antibiotic alone.

Statistical analysis

Student's *t*-test and analysis of variance (ANOVA) were used to compare continuous normally distributed variables. Nonparametric data were analyzed using the Mann-Whitney test.

Comparisons between treatment groups were performed by ANOVA. Student's *t* or Mann-Whitney tests were used for comparisons between CS and CR strains.

RESULTS

Bacterial concentration

With the CS strain (2349), use of clinafloxacin at 10 mg/kg per day and 20 mg/kg per day (in a single dose) achieved an initial reduction at 6 hr. in mean log cfu/ml of -4.56 ± 0.62 and -4.89 ± 1.5, respectively. Both were bactericidal at this point but presented regrowth at 24 hr, and the final reduction at 24 hr in mean log cfu/ml was -2.30 ± 1.94 and -3.83 ± 2.97.

Clinafloxacin at 20 mg/kg per day bid decreased bacterial concentration by more than 4 log cfu/ml at 2 hr and at 24 hr the reduction in mean log cfu/ml was -5.10 ± 1.30. However, there was a regrowth in 1 of 7 animals at this time point.

Combined therapy of clinafloxacin 20 mg/kg per day bid plus teicoplanin 15 mg/kg per day decreased bacterial counts in more than 2 log cfu/ml at 2 hr. Mean reduction at 24 hr in log cfu/ml was -4.09 ± 1.26. At 24 hr, 6 of 7 animals had CSF cultures with bacterial counts below the level of detection. Combination with rifampin 15 mg/kg per day achieved a slight reduction of 1.3 log cfu/ml at 2 hr, but at 24 hr 100% of animals had cultures below the level of detection with reduction in mean log cfu/ml of -2.95 ± 0.91. Comparisons between bacterial counts of different regimens and controls at several time points are shown in Fig. 1.

With the CR strain (4371), clinafloxacin at 20 mg/kg per day bid did not decrease bacterial titers at any time point. Mean log cfu/ml increased by 1.50 ± 2.49 at 24 hr. Mean log cfu/ml in control animals increased by 0.99 ± 2.10 at 24 hr. Therapy using clinafloxacin 20 mg/kg per day b.i.d. plus teicoplanin 15 mg/kg per day decreased bacterial counts by 2 log cfu/ml at 2 hr and at 24 hr mean log cfu/ml decreased by -2.60 ± 0.26. At 26 hr 100% of animals had cultures below the level of detection. Combined therapy with rifampin 15 mg/kg per day showed a slight reduction in bacterial concentration at 2 hr, and at 24 hr, 100% of CSF cultures were below the level of detection with a mean reduction of log cfu/ml -2.38 ± 1.14.

Comparison between clinafloxacin-treated group, combination groups and control group are shown in Fig. 2. Colonies growing in CSF at 24 hr had the same susceptibility as the initial strain.

Initial CSF bacterial concentrations at 0 hr (baseline) were significantly lower in the CR strain than in the CS strain.

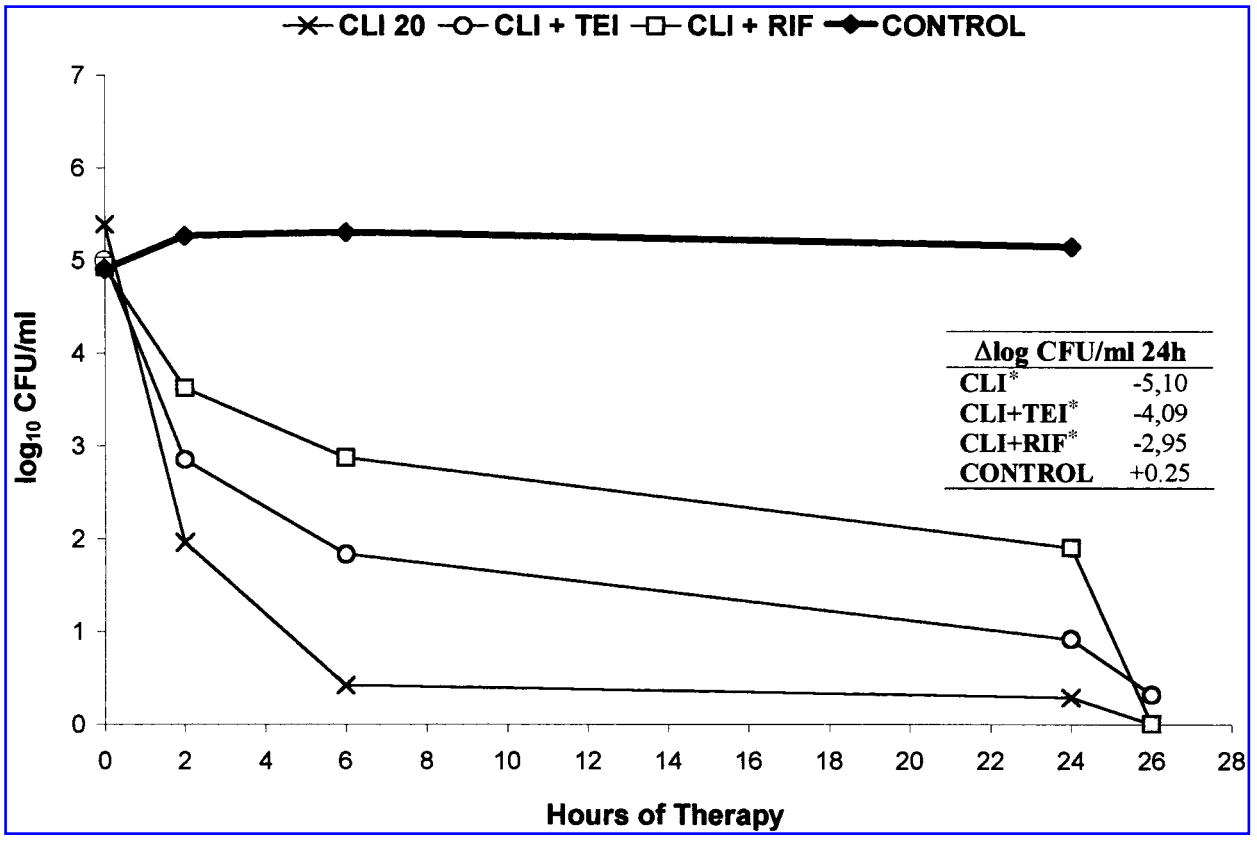

FIG. 1. CSF bacterial counts (expressed as mean log cfu/ml) of rabbits with ciprofloxacin-susceptible pneumococcal meningitis. Therapies: clinafloxacin, 20 mg/kg per day b.i.d. alone (CLI 20) and in combination with teicoplanin 15 mg/kg day (CLI + TEI) or rifampin 15 mg/kg per day (CLI + RIF). *Statistically significant differences were found at 24 hr between all therapeutic groups and the control group ($p < 0.05$).

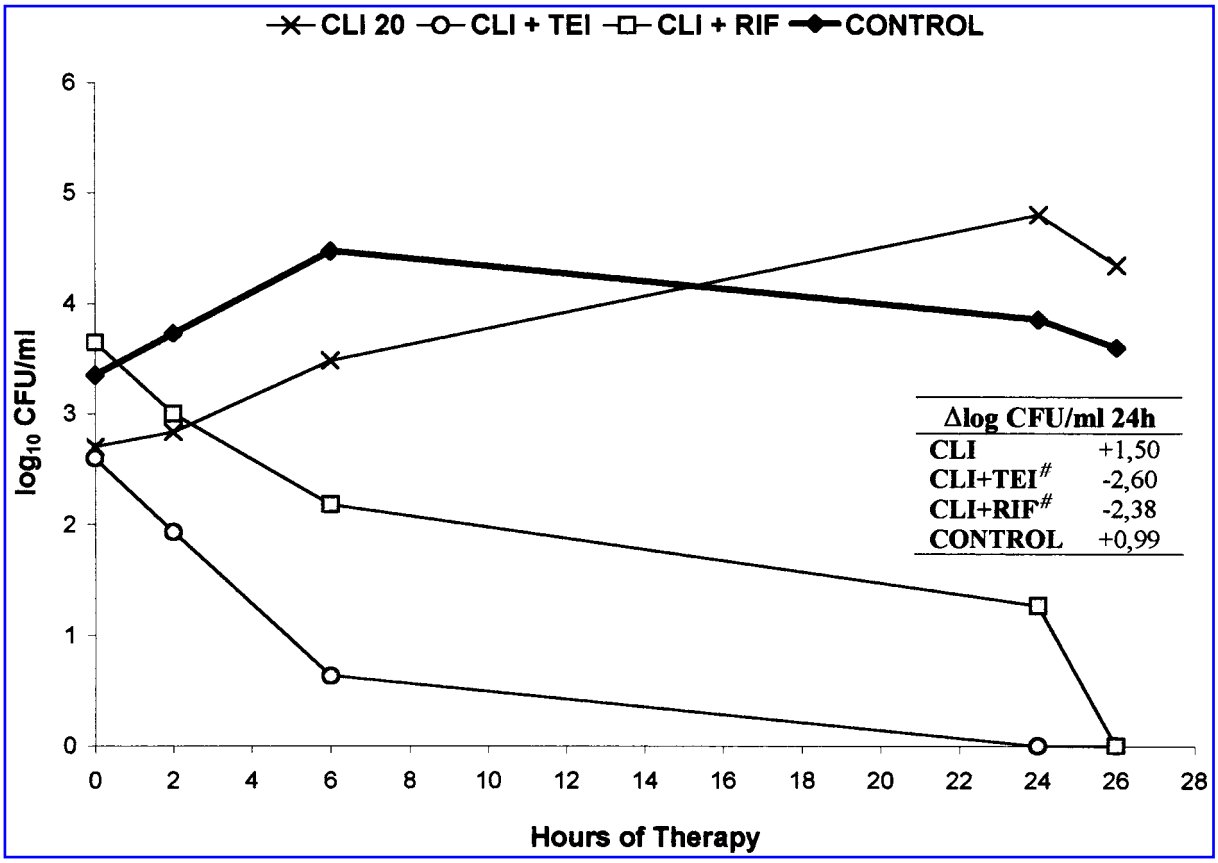

FIG. 2. CSF bacterial counts (expressed as mean log cfu/ml) of rabbits with ciprofloxacin-resistant pneumococcal meningitis. Therapies: clinafloxacin 20 mg/kg per day b.i.d. alone (CLI 20) and in combination with teicoplanin 15 mg/kg per day (CLI + TEI) or rifampin 15 mg/kg per day (CLI + RIF). #Statistically significant differences were found at 24 hr between the CLI + TEI and CLI + RIF groups and the CLI 20 and control groups ($p < 0.05$).

CSF clinafloxacin levels

Mean CSF clinafloxacin peak and trough levels and AUC/MIC parameter of both strains are shown in Table 1. In animals with CS pneumococcal meningitis, mean peak concentration of clinafloxacin was 0.56 ± 0.28 μg/ml and mean trough level was 0.28 μg/ml; both were above the MIC.

Animals infected with the CR strain achieved a mean peak CSF level of clinafloxacin of 0.36 ± 0.14 μg/ml, lower than the mean peak reached with the CS strain. Mean trough concentration was below the level of detection for all samples in this group. Both peak and trough levels were below the MIC.

Inflammatory activity

CSF inflammatory parameters of rabbits with CS and CR pneumococcal meningitis are shown in Table 2. Statistically significant differences between strains were observed for WBC and lactic acid.

Therapeutic failures

With the CS strain, there were no therapeutic failures in the clinafloxacin 20 mg/kg per day b.i.d., clinafloxacin plus teicoplanin and clinafloxacin plus rifampin groups. With the CR

TABLE 1. MEAN ± SD (N) CSF PEAK AND TROUGH LEVELS OF CLINAFLOXACIN FOR BOTH STRAINS: CS 2349 AND CR 4371

Strain	*CSF peak level (μg/ml)*	*CSF trough level (μg/ml)*	*AUC/MIC*[a]
CS 2349	0.56 ± 0.28 (6)	0.28 ± 0.00 (3)	81.67
CR 4371	0.36 ± 0.14 (5)	[b]	8.64

[a]MICs of clinafloxacin are 0.12 and 0.5 for CS and CR strains.
[b]Below the detection limit (0.25 μg/ml).

TABLE 2. INFLAMMATORY PARAMETERS OF CSF OF RABBITS WITH CIPROFLOXACIN-SUSCEPTIBLE AND CIPROFLOXACIN-RESISTANT PNEUMOCOCCAL MENINGITIS

Therapy	*WBC (0 hr) (wbc/ml)*	*WBC (2 hr) (wbc/ml)*	*WBC (24 hr) (wbc/ml)*	*LACT (0 hr) (mmol/ml)*	*LACT (2 hr) (mmol/ml)*	*LACT (24 hr) (mmol/ml)*	*PROT (0 hr) (g/L)*	*PROT (2 hr) (g/L)*	*PROT (24 hr) (g/L)*	*Brain edema (%)*
Ciprofloxacin-susceptible strain 2349										
CLI	5561 ± 4513[a]	3101 ± 2915	2716 ± 1582	6.98 ± 1.7[a]	4.59 ± 0.92	3.03 ± 0.42	1.6 ± 0.89	1.52 ± 0.73	1.53 ± 0.65	388
CLI + TEI	9107 ± 12199[a]	2373 ± 2292	4593 ± 3362	4.15 ± 2.2[a]	3.47 ± 1.48	1.70 ± 0.57	1.1 ± 0.69	1.33 ± 1.26	1.46 ± 0.84	413
CLI + RIF	7189 ± 7056[a]	8521 ± 8096	6051 ± 5547	7.89 ± 1.80[a]	6.27 ± 2.44	2.83 ± 0.76	2.12 ± 0.66	2.12 ± 0.39	1.97 ± 1.1	405
Control	6540 ± 3632[a]	3850 ± 3317	23787 ± 35017[b]	8.25 ± 1.84[a]	6.19 ± 2.16	13.26 ± 8.41[b]	1.88 ± 0.56	1.33 ± 0.63	1.64 ± 0.39	440
Ciprofloxacin-resistant strain 4371										
CLI	4440 ± 6847[a]	2485 ± 2424	3684 ± 3550	2.86 ± 0.82[a]	2.73 ± 0.28	5.04 ± 2.08	1.03 ± 0.54	1.35 ± 0.69	2.83 ± 2.06	420
CLI + TEI	1641 ± 1933[a]	1584 ± 1454	916 ± 649	3.05 ± 1.22[a]	2.78 ± 0.61	1.62 ± 0.98	1.29 ± 0.82	1.65 ± 0.76	0.83 ± 0.63	419
CLI + RIF	1235 ± 1370[a]	1552 ± 506	1308 ± 1401	2.25 ± 1.19[a]	3.25 ± 0.78	1.63 ± 0.25	0.85 ± 0.52	2.29 ± 2.16	1.08 ± 0.24	420
Control	2470 ± 2086[a]	3216 ± 2330	10197 ± 9816[b]	3.48 ± 0.77[a]	2.86 ± 0.64	6.83 ± 1.61[b]	0.99 ± 0.42	1.27 ± 0.57	3.61 ± 1.39	400

Data expressed in means ± SD at 0 hr (baseline), 2 hr, and 24 hr of treatment. Brain edema is expressed as g H_2O/100 g of dry weight.

[a]Differences in WBC and lactic acid between strain CS 2349 and strain CR 4371 were statistically significant ($p < 0.05$) at 0 hr.

[b]WBC and lactic acid of controls of strain CR 4371 were significantly lower ($p < 0.05$) than those achieved by controls of strain CS 2349 at 24 hr.

strain, there were 8 of 8 therapeutic failures when using clinafloxacin alone at 20 mg/kg per day b.i.d. No therapeutic failures were found with combined therapies.

DISCUSSION

In this rabbit model of meningitis, clinafloxacin showed a very good antimicrobial activity against the penicillin-resistant, ciprofloxacin-susceptible pneumococcal strain 2349 (clinafloxacin MIC 0.12 μg/ml). It was bactericidal at 2 hr. Although there was a regrowth at 24 hr in 1 of 7 rabbits, no therapeutic failures were observed. After performing dose-ranging studies, dose regimen of 20 mg/kg per day administered every 12 hr appeared to be the most effective in this model, achieving CSF levels several times above the MIC over the entire treatment period and an AUC/MIC of 81.67. These results are in accordance with those obtained by Friedland et al.,[7] using doses of 20 mg/kg per day every 5 hr against a pneumococcal strain with a clinafloxacin MIC of 0.06 μg/ml, and by Shapiro et al.,[20] using doses of 21 mg/kg against a pneumococcal strain with a clinafloxacin MIC of 0.03 μg/ml in a meningitis mouse model.

In previous studies, we have shown that both teicoplanin and rifampin have good activity against pneumococcal strain 2349.[6,13] In this study, combined therapy with clinafloxacin and teicoplanin or clinafloxacin and rifampin did not improve the activity of clinafloxacin alone, since no additive or synergistic effect was found.

The intensity of the inflammatory response induced by antibiotics is thought to be an important prognostic factor of pneumococcal meningitis. Highly bacteriolytic antibiotics (especially β-lactams) strongly increase the inflammatory response in the subarachnoidal space because of the massive bacterial lysis produced in early stages of therapy.[22] However, in rabbits treated with clinafloxacin we observed an improvement of the inflammatory parameters at 24 hr in all cases. This feature, also reported in other studies using quinolones,[16] suggests that quinolones have an advantage over betalactam antibiotics in the clinical setting.

Against the penicillin-resistant, ciprofloxacin-resistant pneumococcal strain 4371 (clinafloxacin MIC 0.5 μg/ml), therapy with the same dose regimen of clinafloxacin (20 mg/kg per day in two divided doses) was totally ineffective, as the increase in bacterial counts was similar to that of the control group. In addition, all inflammatory parameters increased between 0 hr and 24 hr, as was the case in the control group. These results are in agreement with the fact that mean CSF peak and trough clinafloxacin levels were below the MIC of the strain, with AUC/MIC values of 8.64. The efficacy observed in rabbits receiving combination therapy was attributable to the bactericidal effect of both teicoplanin and rifampin shown in previous studies. Although failures in CR experiments are clearly related to the inadequacy of the pharmacodynamic parameters, it is surprising that rabbits infected with the CR strain had lower CSF clinafloxacin levels than those infected with the CS strain. We also found significantly lower bacterial counts and lower levels of inflammatory activity in these rabbits (the differences being statistically significant for WBC and lactic acid), both at 18 hr after inoculation (baseline). One might hypothesize that this CR strain, which is distantly related to the wild pneumococcus, induces a lower inflammatory response and lower levels of subsequent blood–brain barrier disruption than the CS strain. However, this disruption is not as important for lipid-soluble drugs like quinolones as for β-lactams. In any event, even with CSF clinafloxacin levels similar to those obtained in CS experiments, the pharmacodynamic parameters were suboptimal.

We did not record the emergence of resistance during therapy: MICs of the colonies growing in CSF at 24 hr of therapy were identical to those of the initial isolates. However, this was unlikely to occur in our experiments because the original CS strain had no mutations, while the CR strain already had three (*parC*, *parE*, and *GyrA*). Resistance selection might occur more easily among strains harboring one-step mutation, as has been shown in patients with pneumococcal pneumonia; in this regard, these new quinolones should be used with caution against strains harboring one mutation, signaled by any decrease in ciprofloxacin susceptibility, or in patients with recent quinolone use.[5,14]

It seems reasonable that the findings observed with clinafloxacin, which has been withdrawn from last phase clinical trials, may be generalized to all quinolones. In conclusion, clinafloxacin at doses of 20 mg/kg per day b.i.d. was very effective for the therapy of penicillin-resistant, ciprofloxacin-susceptible pneumococcal meningitis in the rabbit model. Combinations with teicoplanin and with rifampin did not improve activity. The same clinafloxacin schedule was totally ineffective against a penicillin-resistant, ciprofloxacin-resistant pneumococcal strain. A moderate decrease in quinolone susceptibility, as indicated by the detection of any degree of ciprofloxacin resistance, may render these antibiotics unsuitable for the management of pneumococcal meningitis.

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REFERENCES

1. **Chapin-Robertson, K., and S.C. Edberg.** 1991. Measurements of antibiotics in human body fluids: techniques and significance. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. Williams and Wilkins, New York, pp. 295–366.
2. **Cottagnoud, P., F. Acosta, M. Cottagnoud, and M.G. Täuber.** 2002. Gemifloxacin is efficacious against penicillin-resistant and quinolone-resistant pneumococci in experimental meningitis. Antimicrob. Agents Chemother. **46:**1607–1609.
3. **Coyle, E.A., G.W. Kaatz, and M.J. Rybak.** 2001. Activities of newer fluoroquinolones against ciprofloxacin-resistant *Streptococcus pneumoniae.* Antimicrob. Agents Chemother. **45:**1654–1659.
4. **Dacey, M.G., and M.A. Sande.** 1974. Effect of probenecid on cerebrospinal fluid concentrations of penicillin and cephalosporin derivatives. Antimicrob. Agents Chemother. **10:**437–441.
5. **Davidson, R., R. Cavalcanti, J.L. Brunton, D.J. Bast, J.C.S. de Azavedo, P. Kibsey, C. Fleming, and D.E. Low.** 2002. Resistance to levofloxacin and failure of treatment of pneumococcal pneumonia. N. Engl. J. Med. **346:**747–750.

6. **Fernandez, A., C. Cabellos, J.M. Maiques, F. Tubau, J. Liñares, P.F. Viladrich, and F. Gudiol.** 1998. Experimental study of teicoplanin alone and in combination in the therapy of cephalosporin-resistant pneumococcal meningitis. 38th Interscience Conference on Antimicrobial Agents and Chemotherapy. San Diego (U.S.A). Abstr. B-36, p. 54. American Society for Microbiology, Washington D.C.
7. **Friedland, I.R., M. Paris, S. Ehrett, S. Hickey, K.D. Olsen, and G.H. McCracken, Jr.** 1993. Evaluation of antimicrobial regimens for treatment of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. Antimicrob. Agents Chemother. **37:**1630–1636.
8. **Gerber, C.M., L. Tovar, M. Cottagnoud, K.A. Neftel, M.G. Täuber, and P. Cottagnoud.** 2000. Grepafloxacin against penicillin-resistant pneumococci in the rabbit meningitis model. J. Antimicrob. Chemother. **46:**249–253.
9. **Kim, Y.S., Q.X. Liu, L.L. Chow, and M.G. Täuber.** 1997. Trovafloxacin in treatment of rabbits with experimental meningitis caused by high-level penicillin-resistant *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **41:**1186–1189.
10. **Liñares, J., F. Tubau, R. Pallarés, M.J. Ferrándiz, M.A. Domínguez, F. Manresa, A.G. de la Campa, and R. Martín.** 2000. Prevalence of resistance to fluoroquinolones in clinical isolates of *Streptococcus pneumoniae*. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. Toronto (Canada). Abstr. 2106, p. 128. American Society for Microbiology, Washington, D.C.
11. **Lutsar, I., I.R. Friedland, L. Wubbel, C.C. McCoig, H.S. Jafri, W. Ng, F. Ghaffar, and G.H. McCracken, Jr.** 1998. Pharmacodynamics of gatifloxacin in cerebrospinal fluid in experimental cephalosporin-resistant pneumococcal meningitis. Antimicrob. Agents Chemother. **42:**2650–2655.
12. **Manzor, O., J. Pawlak, and L. Saravolatz.** 1999. In-vitro activity of 29 antimicrobial agents against penicillin-resistant and -intermediate isolates of *Streptococcus pneumoniae*. J Antimicrob Chemother. **43:**31–36.
13. **Martínez-Lacasa, J., C. Cabellos, A. Martos, A. Fernández, F. Tubau, P.F. Viladrich, J. Liñares, and F. Gudiol.** 2002. Experimental study of the efficacy of vancomycin, rifampin and dexamethasone in the therapy of pneumococcal meningitis. J Antimicrob Chemother. **49:**507–513.
14. **Nagai, K., T.A. Davies, B.E. Dewasse, M.R. Jacobs, and P.C. Appelbaum.** 2001. Single- and multi-step resistance selection study of gemifloxacin compared with trovafloxacin, ciprofloxacin, gatifloxacin and moxifloxacin in *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **48:**365–374.
15. **Nau, R., T. Schmidt, K. Kaye, J.L. Froula, and M.G. Täuber.** 1995. Quinolone antibiotics in therapy of experimental pneumococcal meningitis in rabbits. Antimicrob. Agents Chemother. **39:**593–597.
16. **Nau, R., G. Zysk, H. Schmidt, F.R. Fischer, A.K. Stringaris, K. Stuertz, and W. Bruck.** 1997. Trovafloxacin delays the antibiotic-induced inflammatory response in experimental pneumococcal meningitis. J. Antimicrob Chemother. **39:**781–788.
17. **Ostergaard, C., T.K. Sorensen, J.D. Knudsen, and N. Frimodt-Moller.** 1998. Evaluation of moxifloxacin, a new 8-methoxyquinolone, for treatment of meningitis caused by a penicillin-resistant pneumococcus in rabbits. Antimicrob. Agents Chemother. **42:**1706–1712.
18. **Paris, M.M., S.M. Hickey, M. Trujillo, S. Shelton, and G.H. McCracken, Jr.** 1995. Evaluation of CP-99,219, a new fluoroquinolone, for treatment of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. Antimicrob. Agents Chemother. **39:** 1243–1246.
19. **Scheld, W.M.** 1989. Quinolone therapy for infections of the central nervous system. Rev. Infect. Dis. **11**(Suppl. 5):S1194–S1202.
20. **Shapiro, M., K.D. Donovan, and J.W. Gage.** 2000. Comparative therapeutic efficacy of clinafloxacin in a pneumococcal meningitis mouse model. J Antimicrob Chemother. **45:**489–492.
21. **Smirnov, A., A. Wellmer, J. Gerber, K. Maier, S. Henne, and R. Nau.** 2000. Gemifloxacin is effective in experimental pneumococcal menigitis. Antimicrob. Agents Chemother. **44:**767–770.
22. **Stuertz, K., H. Schmidt, H. Eiffert, P. Schwartz, M. Mader, and R. Nau**. 1998. Differential release of lipoteichoic and teichoic acids from *Streptococcus pneumoniae* as a result of exposure to β-lactam antibiotics, rifamycins, trovafloxacin, and quinupristin-dalfopristin. Antimicrob. Agents Chemother. **42:**277–281.
23. **Verhaegen, J., and L. Verbist.** 1999. In-vitro activities of 16 non-beta-lactam antibiotics against penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae*. J. Antimicrob Chemother. **43:**563–567.
24. **Viladrich, P.F., F. Gudiol, J. Liñares, G. Rufi, J. Ariza, and R. Pallares.** 1988. Characteristics and antibiotic therapy of adult meningitis due to penicillin-resistant pneumococci. Am. J. Med. **84:**839–846.
25. **Viladrich, P.F., C. Cabellos, R. Pallares, F. Tubau, J. Martínez-Lacasa, J. Liñares, and F. Gudiol.** 1996. High doses of cefotaxime in treatment of adult meningitis due to *Streptococcus pneumoniae* with decreased susceptibilities to broad-spectrum cephalosporins. Antimicrob. Agents Chemother. **40:**218–220.
26. **Viladrich, P.F., F. Gudiol, J. Liñares, R. Pallares, I. Sabate, G. Rufi, and J. Ariza.** 1991. Evaluation of vancomycin for therapy of adult pneumococcal meningitis. Antimicrob. Agents Chemother. **35:**2467–2472.

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