ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1995, p. 1661–1666 Vol. 39, No. 8
0066-4804/95/$04.00+0

Inhibitory and Bactericidal Activities of Levofloxacin, Ofloxacin, Erythromycin, and Rifampin Used Singly and in Combination against *Legionella pneumophila*

ALDONA L. BALTCH,* RAYMOND P. SMITH, AND WILLIAM RITZ

Department of Medicine, Section of Infectious Diseases, Stratton Veterans Affairs Medical Center, and Albany Medical College, Albany, New York

Received 15 February 1995/Returned for modification 23 March 1995/Accepted 12 May 1995

The susceptibilities of 56 *Legionella pneumophila* isolates (43 clinical and 15 environmental isolates) to levofloxacin, ofloxacin, erythromycin, and rifampin were studied with buffered charcoal yeast extract (BCYE) agar (inoculum, 10^4 CFU per spot), and the susceptibilities of five isolates were studied with buffered yeast extract (BYE) broth (inoculum, 10^5 CFU/ml). The MICs inhibiting 90% of strains tested on BCYE agar were 0.125, 0.25, 1.0, and ≤0.004 μg/ml for levofloxacin, ofloxacin, erythromycin, and rifampin, respectively. The MICs by the BYE broth dilution method were 1 to 3, 2, 1 to 2, and 1 tube lower than those by the agar dilution method for levofloxacin, ofloxacin, erythromycin, and rifampin, respectively. The MBCs were 1 to 2 tubes higher than the broth dilution MICs for levofloxacin, 1 to 3 tubes higher than the broth dilution MICs for ofloxacin, 1 to 3 tubes higher than the broth dilution MICs for erythromycin, and the same as the broth dilution MICs for rifampin. In kinetic time-kill curve studies, at drug concentrations of 1.0 and 2.0 times the MIC, the most active drugs were levofloxacin and rifampin. At 72 h, concentrations of levofloxacin and rifampin of 2.0 times the MIC demonstrated a bactericidal effect against *L. pneumophila*. In contrast, at concentrations of 1.0 and 2.0 times the MICs regrowth was observed with ofloxacin and only a gradual decrease in the numbers of CFU per milliliter was observed with erythromycin. Only a minor inhibitory effect was observed with 0.25 or 0.5 time the MICs of all drugs at 24 to 48 h, with regrowth occurring at 72 h. In kinetic time-kill curve studies, combinations of levofloxacin plus rifampin, ofloxacin plus rifampin, and erythromycin plus rifampin at 72 h demonstrated synergy at 0.5 time the MICs of each drug. However, at these concentrations only levofloxacin plus rifampin demonstrated a synergistic bactericidal effect against *L. pneumophila* at 72 h. In contrast to erythromycin or ofloxacin plus rifampin at 0.25 time the MICs, only levofloxacin plus rifampin demonstrated synergy. Thus, levofloxacin demonstrated the best inhibitory and bactericidal effects against *L. pneumophila* when it was studied alone or in a combination with rifampin.

The treatment of Legionnaires' disease to date includes erythromycin as the first-choice antimicrobial agent (8, 16, 23, 29). Other drugs include azithromycin, clarithromycin, fluoroquinolones, doxycycline, or trimethoprim-sulfamethoxazole. A lack of comparative studies of these drugs in humans is well known. It has been demonstrated that while erythromycin, clindamycin, newer macrolides, and selected other antimicrobial agents are primarily inhibitory in their in vitro activities against *Legionella pneumophila*, fluoroquinolones and rifampin exhibit a rapid inhibitory and bactericidal activities which are concentration dependent (1–6, 9–12, 15, 17–22, 24–26, 31–35, 39). To maximize clinical efficacy and minimize the selection of resistant mutants, rifampin is frequently added to the therapy with erythromycin, especially for the treatment of more severe cases of Legionnaires' disease (8, 16, 23, 29). Few in vitro studies have compared the inhibitory and bactericidal activities of fluoroquinolones with the addition of rifampin (2, 17, 27, 39). A common characteristic of the currently acceptable antimicrobial agents against *L. pneumophila* is their ability to enter human phagocytes, where *L. pneumophila* is known to persist (24, 38).

Levofloxacin is the more active isomer of the two optically active isomers of ofloxacin (14, 37). Studies to date indicate that the inhibitory activity of its DNA gyrase is equal to that of ciprofloxacin. The concentrations of levofloxacin in serum and tissue are higher than those of ciprofloxacin observed in animal models, and the half-life of levofloxacin in serum is longer (14, 37). The inhibitory activity of levofloxacin is greater than those of ofloxacin and ciprofloxacin except against members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa*, against which ciprofloxacin is more active (37).

The study described here was performed to demonstrate the inhibitory activity of levofloxacin compared with those of

TABLE 1. Comparative antimicrobial susceptibilities of 56 *L. pneumophila* isolates to levofloxacin, ofloxacin, erythromycin, and rifampin by the agar dilution technique

Antibiotic	MIC (μg/ml)[a]		
	50%	90%	Range[b]
Levofloxacin	0.125	0.125	0.003–1
Ofloxacin	0.125	0.25	0.06–1
Erythromycin	0.25	1.0	0.06–1
Rifampin	≤0.004	≤0.004	≤0.004–0.008

[a] 50% and 90%, MICs inhibiting 50 and 90% of isolates tested, respectively.
[b] Includes data for two serogroup 4 isolates for which the MICs of levofloxacin, ofloxacin, erythromycin, and rifampin were 1.0, 1.0, 1.0, and <0.004 μg/ml and 0.5, 1.0, 1.0, and <0.004 μg/ml, respectively.

* Corresponding author. Mailing address: Infectious Diseases (111D), Stratton Veterans Affairs Medical Center, Albany, NY 12208. Phone: (518) 462-3311. Fax: (518) 462-3350.

TABLE 2. Inhibitory and bactericidal activities of levofloxacin, ofloxacin, erythromycin, and rifampin against five serogroup 1 *L. pneumophila* isolates by the broth dilution technique

Isolate	Levofloxacin		Ofloxacin		Erythromycin		Rifampin	
	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
LB-428	0.03	0.06	0.03	0.25	0.125	>2.0	0.001	>0.015
LB-360	0.03	0.06	0.03	0.125	0.06	>2.0	0.001	>0.015
L-943	0.03	0.25	0.03	0.125	0.25	1.0	0.001	>0.015
L-1033	0.03	0.25	0.06	0.25	0.5	1.0	0.001	0.015
L-1043	0.06	0.25	0.06	0.125	0.25	1.0	0.0005	0.015

ofloxacin, erythromycin, and rifampin against *L. pneumophila*. Furthermore, the bactericidal activity of levofloxacin with and without rifampin was also compared with that of ofloxacin and erythromycin in kinetic time-kill curve studies.

MATERIALS AND METHODS

Bacterial strains. A total of 56 nonduplicated *L. pneumophila* isolates were obtained from the New York State Health Department and four local hospitals in Albany, N.Y. Forty-one isolates were of clinical origin and 15 isolates were of environmental origin. Species identification and serotyping were performed by the Wadsworth Laboratories for Research, New York State Department of Health. All but nine clinical isolates were serogroup 1; the other nine isolates comprised four, two, two, and one isolate of serogroups 5, 4, 3, and 2, respectively. All environmental isolates were serogroup 1. The isolates were stored at −70°C in skim milk.

Antimicrobial agents. Standard laboratory powders of the following antimicrobial agents were obtained from the indicated companies: levofloxacin and ofloxacin, R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J.; erythromycin and rifampin, Sigma Chemical Co., St. Louis, Mo. Antibiotic solutions were prepared, filter sterilized (pore size, 0.45 μm; Lab Product Sales, Rochester, N.Y.), and used on the same day.

Susceptibility testing. Antimicrobial susceptibilities were determined by agar dilution, broth dilution, and kinetic time-kill curve techniques as recommended by the National Committee for Clinical Laboratory Standards (1990) (28a).

The microorganisms were grown in buffered yeast extract (BYE) broth (BBL Microbiology Systems, Cockeysville, Md.) as described by Liebers et al. (25) for 24 h in a 35°C water bath to a density of a McFarland no. 0.5 standard (10^8 CFU/ml). This bacterial density was diluted with BYE broth to 10^7 CFU/ml. The bacterial inoculum was delivered to the surface of antibiotic-containing buffered charcoal yeast extract (BCYE) agar (BBL Microbiology Systems) with a Steers replicator (10^4 CFU per spot). The plates were incubated at 35°C for 48 h in air. The MIC was recorded as the lowest concentration of the drug at which there was no growth or one discrete colony. A faint haze was disregarded. For five isolates, MICs and MBCs were determined by the broth dilution technique. Tubes containing BYE broth with twofold serial dilutions of four antibiotics were inoculated with *L. pneumophila* (10^5 CFU/ml) and were incubated at 35°C for 48 h. The MIC was recorded as the lowest antibiotic concentration demonstrating no visible growth in BYE broth. The MBC was determined by removing 0.1 ml of the bacterial suspension from subcultures demonstrating no visible growth and inoculating the surface of BCYE agar. The plates were incubated at 35°C for 48 h in air. The MBC was recorded as the lowest antibiotic concentration demonstrating 99.9% killing of the bacterial inoculum. In kinetic time-kill curve studies, the tube macrodilution BYE broth MIC was used, and the effects of levofloxacin, ofloxacin, erythromycin, and rifampin were studied at 0.25, 0.5, 1.0, and 2.0 times the MICs of the drugs. Subsequently, combinations of levofloxacin plus rifampin, ofloxacin plus rifampin, and erythromycin plus rifampin were studied at 0.25 and 0.5 time the MICs of both antibiotics. Polypropylene tubes containing BYE broth were inoculated with *L. pneumophila* obtained from the surfaces of BCYE agar plates previously grown for 48 h. This bacterial suspension was incubated in a shaking 35°C water bath for 2 h to a density of a McFarland no. 0.5 standard (10^8 CFU/ml). Antibiotic-containing tubes with BYE broth were inoculated with the organism at a starting concentration of 2.5×10^5 CFU/ml. All tubes were incubated at 35°C in a shaking water bath. At 6, 24, 48, and 72 h, 100-μl suspensions were removed from individual tubes, serially diluted, and plated onto the surfaces of BCYE agar plates. Following incubation at 35°C for 48 h in air, the plates were read with a colony counter (New Brunswick Scientific Co., New Brunswick, N.J.). Synergy was recorded if at 72 h we observed a 2-log difference in activity with the drug combinations compared with the activity of the most effective single drug. Antibiotic carryover experiments demonstrated no drug carryover for all samples tested (36).

RESULTS

Susceptibilities by agar dilution technique. The comparative antimicrobial susceptibilities of 56 nonduplicate *L. pneumophila* isolates to levofloxacin, ofloxacin, erythromycin, and rifampin are demonstrated in Table 1. The most active drug was rifampin; this was followed by levofloxacin, ofloxacin, and erythromycin (MICs inhibiting 90% of isolates tested, ≤0.004, 0.125, 0.25, and 1.0 μg/ml, respectively).

Inhibitory and bactericidal activities by the broth dilution technique. Table 2 provides the MICs and MBCs of levofloxacin, ofloxacin, erythromycin, and rifampin for five serogroup 1 *L. pneumophila* isolates. The most active inhibitory drug was rifampin; this was followed by levofloxacin, ofloxacin, and erythromycin (MICs, 0.0005 to 0.001, 0.03 to 0.06, 0.03 to 0.06, and 0.06 to 0.5 μg/ml, respectively). The bactericidal activity (MBC) was ≥4 tubes greater than the MIC of rifampin, 1 to 3 tubes greater than the MIC of levofloxacin, 1 to 2 tubes greater than the MIC of ofloxacin, and ≥3 tubes greater than the MIC of erythromycin. Table 3 provides the inhibitory activities of the four test drugs against the same five strains of *L. pneumophila* in both agar and broth dilution tests. Agar dilution MICs were 1 to 3 tubes greater than those measured in broth for all drugs tested.

Bactericidal activity as determined by kinetic time-kill curves studies. A total of 238 kinetic curves were determined in 21 experimental runs for three serogroup 1 clinical isolates of *L. pneumophila* against levofloxacin, ofloxacin, erythromycin, and rifampin at 0.25, 0.5, 1.0, and 2.0 times the MICs. As demonstrated in Fig. 1A to D, at drug concentrations of 1.0 and 2.0 times the MIC, the most active drugs were levofloxacin and rifampin. At 72 h, levofloxacin and rifampin at 2.0 times the MIC demonstrated a bactericidal effect, with no regrowth

TABLE 3. Inhibitory activities of levofloxacin, ofloxacin, erythromycin, and rifampin against five serogroup 1 *L. pneumophila* isolates by BCYE agar and BYE broth dilution techniques

Isolate	Method	MIC (μg/ml)			
		Levofloxacin	Ofloxacin	Erythromycin	Rifampin
LB-428	Agar	0.06	0.125	0.25	0.008
	Broth	0.03	0.03	0.125	0.001
LB-360	Agar	0.06	0.125	0.25	≤0.004
	Broth	0.06	0.03	0.06	0.001
L-943	Agar	0.125	0.125	1	≤0.008
	Broth	0.03	0.03	0.25	0.001
L-1033	Agar	0.125	0.125	1	≤0.008
	Broth	0.03	0.06	0.5	0.001
L-1043	Agar	0.25	0.25	1	≤0.008
	Broth	0.06	0.06	0.25	0.0005

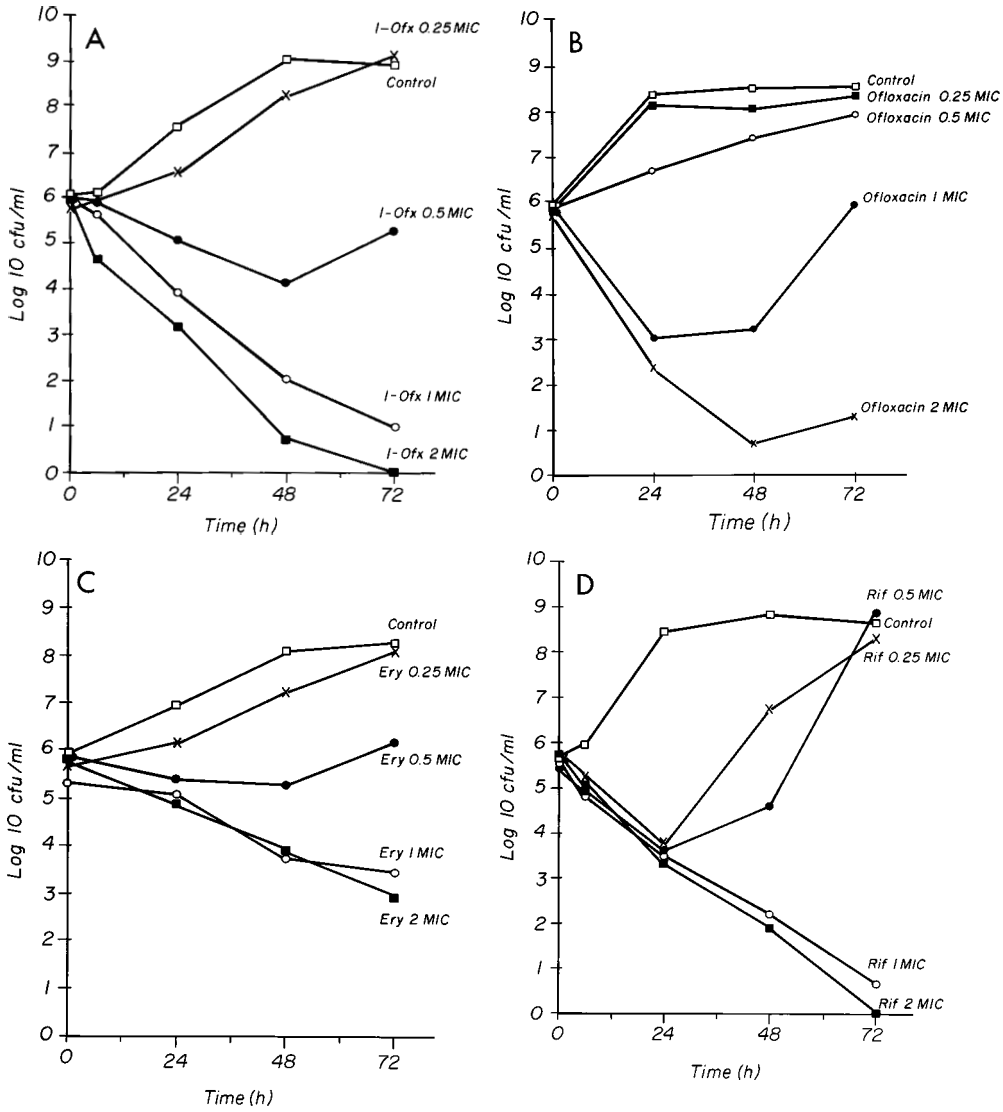

FIG. 1. Kinetic time-kill curves for an *L. pneumophila* serogroup 1 isolate against levofloxacin (1-Ofx) (A), ofloxacin (B), erythromycin (Ery) (C), and rifampin (Rif) (D). All antimicrobial agents were studied at 0.25, 0.5, 1.0, and 2.0 times the MICs. The MICs of levofloxacin, ofloxacin, erythromycin, and rifampin for this *L. pneumophila* isolate were 0.03, 0.03, 0.125, and 0.001 μg/ml, respectively.

of *L. pneumophila* (Fig. 1A and D). In contrast, in studies with 1.0 and 2.0 times the MICs, regrowth was observed with ofloxacin at 72 h and only a gradual decrease in the number of CFU per milliliter was observed with erythromycin (Fig. 1B and C). At lower drug concentrations (0.25 and 0.5 time the MIC), only a minor inhibitory effect or no effect was demonstrated by all drugs at 24 to 48 h and regrowth occurred at 72 h (data not shown).

Combinations of levofloxacin plus rifampin, ofloxacin plus rifampin, and erythromycin plus rifampin demonstrated synergy at 0.5 time the MICs of each drug (Fig. 2A to C). It was noted, however, that the numbers of CFU per milliliter after treatment with ofloxacin plus rifampin at 72 h showed only a 1-log difference from the numbers of CFU per milliliter of the starting inoculum. At these concentrations, only levofloxacin plus rifampin demonstrated a synergistic effect, with no regrowth of *L. pneumophila* at 72 h. In contrast to erythromycin or ofloxacin plus rifampin, at 0.25 time the MICs, only levofloxacin plus rifampin demonstrated synergy (Fig. 3A to C). Thus, levofloxacin demonstrated the best inhibitory and bactericidal effects against *L. pneumophila* when it was studied alone or in a combination with rifampin.

DISCUSSION

Fluoroquinolones have been widely studied in vitro (1–6, 9–12, 15, 17–22, 24–26, 31–35, 39) and in systems including macrophages (1, 9–11, 17–18, 24, 38) against *L. pneumophila*. Our data support the evidence of the in vitro effectiveness of fluoroquinolones against this microorganism. Furthermore, our time-kill curve studies indicated the bactericidal effects of levofloxacin and rifampin. However, this effect was observed only at 2.0 times the MIC of either drug. Erythromycin and ofloxacin at similar concentrations had inhibitory effects, with evidence of regrowth of the organism at 72 h. Because of the frequent addition of rifampin to patients seriously ill with infections caused by *L. pneumophila*, our time-kill curve studies included fluoroquinolone or erythromycin combinations with

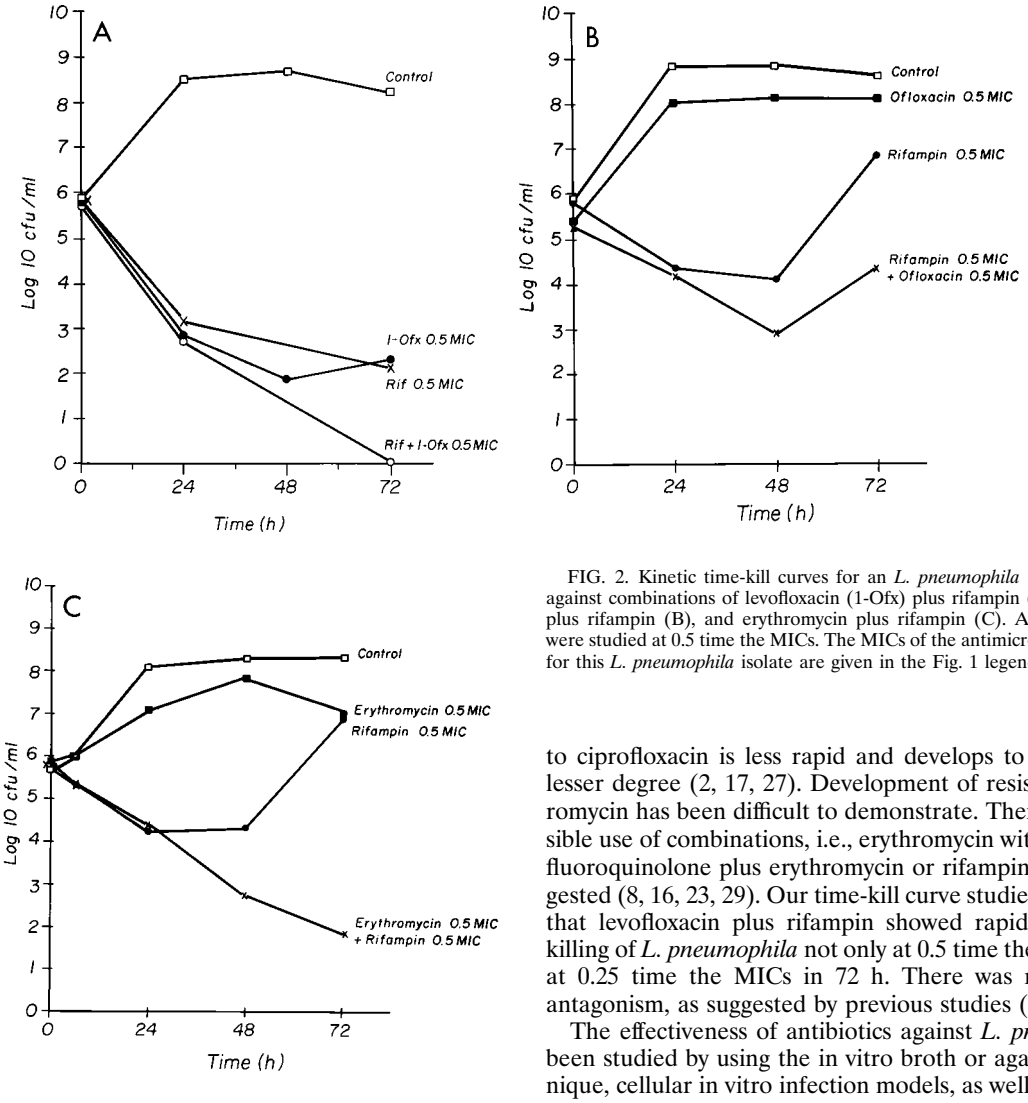

FIG. 2. Kinetic time-kill curves for an *L. pneumophila* serogroup 1 isolate against combinations of levofloxacin (1-Ofx) plus rifampin (Rif) (A), ofloxacin plus rifampin (B), and erythromycin plus rifampin (C). Antimicrobial agents were studied at 0.5 time the MICs. The MICs of the antimicrobial agents studied for this *L. pneumophila* isolate are given in the Fig. 1 legend.

rifampin. Although levofloxacin, ofloxacin, and erythromycin at 0.5 time the MICs demonstrated synergy, only levofloxacin in combination with rifampin at 0.25 time the MICs demonstrated synergy, and at 0.5 time the MICs these combinations showed a synergistic effect, with no regrowth of *L. pneumophila*.

The mortality rate among patients with *L. pneumophila* infections continues to be high, especially in bacteremic, immunocompromised, and intubated patients (8, 16, 23, 29). It is known from previous in vitro studies with and without the inclusion of macrophages that the effect of erythromycin on *L. pneumophila* is an inhibitory one (2, 18, 21, 24, 25, 31, 38). In vivo comparative drug studies of the doses and lengths of therapy needed in patients with different primary diseases are lacking (8, 16, 23, 28–30). Therefore, the possible use of different cell-penetrating drugs other than macrolides, i.e., rifampin or fluoroquinolones, has been investigated. Both rifampin and fluoroquinolones studied in vitro inhibit or kill *L. pneumophila* rapidly and therefore could be considered for use in investigations of different therapies. While resistance to rifampin in vivo during therapy has not been demonstrated (7), it has been shown that in vitro resistance to rifampin develops rapidly and to a high degree, while development of resistance to ciprofloxacin is less rapid and develops to a considerably lesser degree (2, 17, 27). Development of resistance to erythromycin has been difficult to demonstrate. Therefore, the possible use of combinations, i.e., erythromycin with rifampin or a fluoroquinolone plus erythromycin or rifampin, has been suggested (8, 16, 23, 29). Our time-kill curve studies demonstrated that levofloxacin plus rifampin showed rapid and complete killing of *L. pneumophila* not only at 0.5 time the MICs but also at 0.25 time the MICs in 72 h. There was no evidence of antagonism, as suggested by previous studies (17).

The effectiveness of antibiotics against *L. pneumophila* has been studied by using the in vitro broth or agar dilution technique, cellular in vitro infection models, as well as appropriate animal models. Because *L. pneumophila* is an intracellular pathogen, in vitro cellular infection or animal models are important when the effectiveness of new antimicrobial agents is evaluated, and especially if their ability to enter the cell is unknown. In our study all antimicrobial agents tested (fluoroquinolones, erythromycin, and rifampin) are known to enter phagocytic cells, and therefore, their use in therapy has been recommended (8). Levofloxacin is the levorotatory form of ofloxacin. Levofloxacin has considerably greater antibacterial activity, better tissue penetration, a longer half-life, higher concentrations in serum, and longer postantibiotic effect than ofloxacin (13, 14, 37). To date, its side effects have been found to be similar to those of ofloxacin and other fluoroquinolones (37). Our in vitro studies demonstrated that the MICs of levofloxacin for *L. pneumophila* were lower than those of ofloxacin or erythromycin but higher than those of rifampin. Our studies demonstrated that only levofloxacin and rifampin used singly were bactericidal at 2.0 times the MICs in time-kill curve studies and that a combination at 0.5 time the MIC of each of levofloxacin and rifampin was the only drug combination demonstrating not only synergy but also a bactericidal effect in time-kill curve studies. Further in vitro studies on levofloxacin including studies in macrophage systems and in vivo evaluation of this fluoroquinolone are indicated.

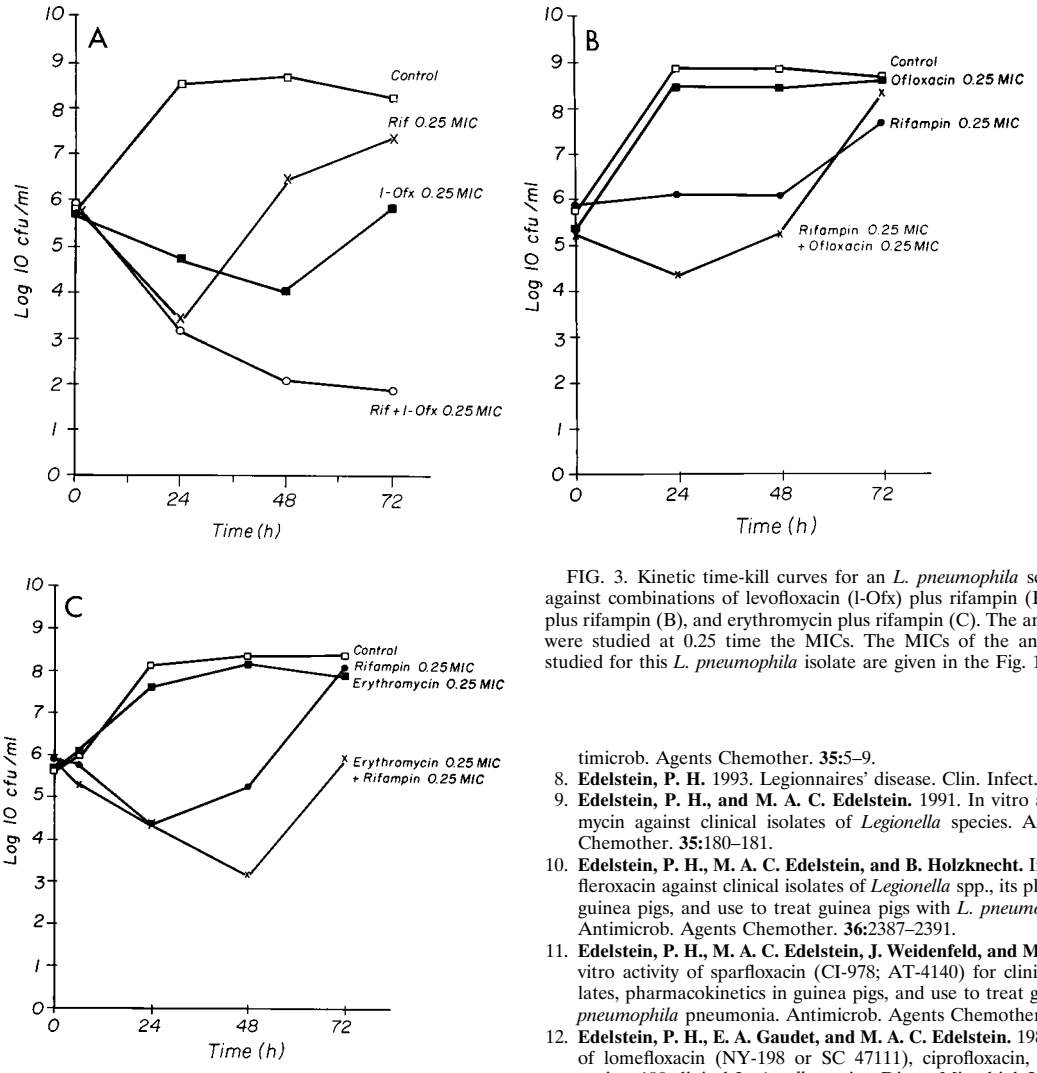

FIG. 3. Kinetic time-kill curves for an *L. pneumophila* serogroup 1 isolate against combinations of levofloxacin (l-Ofx) plus rifampin (Rif) (A), ofloxacin plus rifampin (B), and erythromycin plus rifampin (C). The antimicrobial agents were studied at 0.25 time the MICs. The MICs of the antimicrobial agents studied for this *L. pneumophila* isolate are given in the Fig. 1 legend.

ACKNOWLEDGMENTS

This study was supported by the R. W. Johnson Pharmaceutical Research Institute and in part by the U.S. Department of Veterans Affairs.

We thank Marie Strouse for excellent secretarial assistance.

REFERENCES

1. **Bacheson, M. A., H. M. Friedman, and C. E. Benson.** 1981. Antimicrobial susceptibility of intracellular *Legionella pneumophila*. Antimicrob. Agents Chemother. **20:**691–692.
2. **Barker, J. E., and I. D. Farrell.** 1990. The effects of single and combined antibiotics on the growth of *Legionella pneumophila* using time-kill studies. J. Antimicrob. Chemother. **26:**45–53.
3. **Chen, S. C. A., M. L. Paul, and G. L. Gilbert.** 1993. Susceptibility of *Legionella* species to antimicrobial agents. Pathology **25:**180–183.
4. **Cherubin, C. E., R. H. K. Eng, S. M. Smith, and E. N. Tan.** 1992. A comparison of antimicrobial activity of ofloxacin, L-ofloxacin, and other oral agents for respiratory pathogens. Diagn. Microbiol. Infect. Dis. **15:**141–144.
5. **Collins, L. A., C. B. Wennersten, M. J. Ferraro, R. C. Moellering, Jr., and G. M. Eliopoulos.** 1994. Comparative activities of piperacillin and tazobactam against clinical isolates of *Legionella* spp. Antimicrob. Agents Chemother. **38:**144–146.
6. **Dubois, J., and J. R. Joly.** 1989. In vitro activity of lomefloxacin (SC 47111 or NY-198) against isolates of *Legionella* spp. Diagn. Microbiol. Infect. Dis. **12:**89S–91S.
7. **Edelstein, P. H.** 1991. Rifampin resistance of *Legionella pneumophila* is not increased during therapy for experimental Legionnaires' disease: study of rifampin resistance using a guinea pig model of Legionnaires' disease. Antimicrob. Agents Chemother. **35:**5–9.
8. **Edelstein, P. H.** 1993. Legionnaires' disease. Clin. Infect. Dis. **16:**741–749.
9. **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.
10. **Edelstein, P. H., M. A. C. Edelstein, and B. Holzknecht.** 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.
11. **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.
12. **Edelstein, P. H., E. A. Gaudet, and M. A. C. Edelstein.** 1989. In vitro activity of lomefloxacin (NY-198 or SC 47111), ciprofloxacin, and erythromycin against 100 clinical *Legionella* strains. Diagn. Microbiol. Infect. Dis. **12:**93S–95S.
13. **Fu, K. P., B. Foleno, and M. E. Rosenthale.** 1992. The postantibiotic suppressive effect of 1-ofloxacin, an optically active isomer of ofloxacin. Diagn. Microbiol. Infect. Dis. **15:**375–378.
14. **Fu, K. P., S. C. Lafredo, B. Foleno, D. M. Isaacson, J. F. Barrett, A. J. Tobia, and M. E. Rosenthale.** 1992. In vitro and in vivo antibacterial activities of levofloxacin (*l*-ofloxacin), an optically active ofloxacin. Antimicrob. Agents Chemother. **36:**860–866.
15. **Furneri, P. M., M. Bazzano, L. Campo, M. Cesana, and G. Tempera.** 1994. In vitro activity of rufloxacin against *Listeria monocytogenes*, *Legionella pneumophila*, and *Chlamydia trachomatis*. Chemotherapy (Basel) **40:**104–108.
16. **Gump, D. W., R. O. Frank, W. C. Foster, C. V. Broome, and W. B. Cherry.** 1979. Legionnaire's disease in patients with associated serious disease. Ann. Intern. Med. **90:**538–542.
17. **Havlichek, D., L. Saravoletz, and D. Pohlod.** 1987. Effect of quinolones and other antimicrobial agents on cell-associated *Legionella pneumophila*. Antimicrob. Agents Chemother. **31:**1529–1534.
18. **Higa, F., A. Saito, J. Inadome, N. Kusano, and K. Kitsukawa.** 1993. Influence of methyprednisolone on the intracellular antimicrobial activity of erythromycin and clindamycin against *Legionella pneumophila*. J. Antimicrob. Chemother. **31:**901–908.
19. **Hohl, P., A. von Graevenitz, and J. Zollinger-Iten.** 1987. Fleroxacin (Ro 23-6240): activity in vitro against 355 enteropathogenic and non-fermentative gram-negative bacilli and *Legionella pneumophila*. J. Antimicrob. Chemother. **20:**373–378.
20. **Hohl, P., A. von Graevenitz, and J. Zollinger-Iten.** 1988. In vitro activity on carumonam (Ro 17-2301; AMA-1080) versus enteropathogenic and nonfermentative gram-negative rods and *Legionella pneumophila*. Antimicrob. Agents Chemother. **32:**1593–1594.
21. **Johnson, D. M., M. E. Erwin, M. S. Barrett, B. Briggs Gooding, and R. N. Jones.** 1992. Antimicrobial activity of ten macrolide, lincosamine and strep-

togramin drugs tested against *Legionella* species. Eur. J. Clin. Microbiol. Infect. Dis. **11:**751–755.
22. **Jones, R. N., and A. L. Barry.** 1987. Antimicrobial activity of imipenem and SCH34343 against *Legionella* species. Diagn. Microbiol. Infect. Dis. **6:**307–309.
23. **Kirby, B. D., K. M. Snyder, R. D. Meyer, and S. M. Finegold.** 1980. Legionnaire's disease: report of 65 nosocomially acquired cases and review of the literature. Medicine (Baltimore) **59:**188–205.
24. **Kitsukawa, K., J. Hara, and A. Saito.** 1991. Inhibition of *Legionella pneumophila* in guinea pig peritoneal macrophages by new quinolone, macrolide and other antimicrobial agents. J. Antimicrob. Chemother. **27:**343–353.
25. **Liebers, D. M., A. L. Baltch, R. P. Smith, M. C. Hammer, and J. V. Conroy.** 1989. Susceptibility of *Legionella pneumophila* to eight antimicrobial agents including four macrolides under different assay conditions. J. Antimicrob. Chemother. **23:**37–41.
26. **Marshall, A. J., and L. J. V. Piddock.** 1994. Interaction of divalent cations, quinolones and bacteria. J. Antimicrob. Chemother. **34:**465–483.
27. **Moffie, B. G., and R. P. Mouton.** 1988. Sensitivity and resistance of *Legionella pneumophila* to some antibiotics and combinations of antibiotics. J. Antimicrob. Chemother. **22:**457–462.
28. **Morley, J. N., L. C. Smith, A. L. Baltch, and R. P. Smith.** 1994. Recurrent infection due to *Legionella pneumophila* in a patient with AIDS. Clin. Infect. Dis. **19:**1130–1132.
28a. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
29. **Nguyen, M. H., J. E. Stout, and V. L. Yu.** 1991. Legionellosis. Infect. Dis. Clin. N. Am. **5:**561–584.
30. **Parker, M. M., A. M. Macher, J. H. Shelhamer, J. E. Balow, V. Gill, and J. E. Parrillo.** 1983. Unresponsiveness of *Legionella bozemanii* pneumonia to erythromycin administration despite in vitro sensitivity. Am. Rev. Respir. Dis. **128:**955–956.
31. **Rajagopalan-Levasseur, P., E. Dournon, G. Dameron, J.-L. Vilde, and J.-J. Pocidalo.** 1990. Comparative postantibacterial activities of pefloxacin, ciprofloxacin, and ofloxacin against intracellular multiplication of *Legionella pneumophila* serogroup I. Antimicrob. Agents Chemother. **34:**1733–1738.
32. **Reda, C., T. Quaresima, and M. C. Pastoris.** 1994. In-vitro activity of six intracellular antibiotics against *Legionella pneumophila* strains of human and environmental origin. J. Antimicrob. Chemother. **33:**757–764.
33. **Smith, G. M., and R. Sutherland.** 1992. Activity of amoxycillin-clavulanic acid against *Legionella pneumophila* in vitro and in an experimental respiratory infection model. J. Hosp. Infect. **22:**61–67.
34. **Soussy, C. J., J. F. Acar, R. Cluzel, P. Courvalin, J. Duval, J. Fleurette, F. Megraud, M. Meyran, and A. Thabaut.** 1992. A collaborative study of the in-vitro sensitivity of RP 59500 of bacteria isolated in seven hospitals in France. J. Antimicrob. Chemother. **30:**53–58.
35. **Stokes, D. H., M. J. Wilkinson, J. Tyler, B. Slocombe, and R. Sutherland.** 1989. Bactericidal effects of amoxycillin/clavulanic acid against intracellular *Legionella pneumophila* in tissue culture studies. J. Antimicrob. Chemother. **23:**547–556.
36. **Stratton, C. W., IV.** 1991. In vitro testing: correlation between bacterial susceptibility, body fluid levels and effectiveness of antimicrobial therapy, p. 854. *In* V. Lorian (ed.) Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
37. **Takasuna, K., Y. Kasai, C. Usui, M. Takahashi, M. Hirohashi, K. Tamura, and S. Takayama.** 1992. General pharmacology of the new quinolone antibacterial agent levofloxacin. Drug Res. **42:**408–418.
38. **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.
39. **Zanetti, S., D. Ungheri, M. Castellani Pastoris, and G. Fadda.** 1987. Comparative in vitro susceptibility studies of FCE 22250 and rifampicin on *Legionella* and *Chlamydia trachomatis* strains. Drugs Exp. Clin. Res. **13:**631–634.