ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1993, p. 1826–1830
0066-4804/93/091826-05$02.00/0

Vol. 37, No. 9

In Vitro and In Vivo Activities of Q-35, a New Fluoroquinolone, against *Mycoplasma pneumoniae*

YUKIKO GOHARA,[1] SUMIO ARAI,[1]* AKIRA AKASHI,[1] KOICHI KUWANO,[1] CHENG-CHUANG TSENG,[1] SHUZO MATSUBARA,[2] MASAHIKO MATUMOTO,[2] AND TAKENORI FURUDERA[3]

Department of Microbiology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830,[1] Clinical Research Department II, Research and Development Division, Chugai Pharmaceutical Co., Ltd., Chuo-ku, Tokyo 104,[2] and Japan Bioindustry Association, Minato-ku, Tokyo 105,[3] Japan

Received 1 March 1993/Returned for modification 4 May 1993/Accepted 5 July 1993

The in vitro potency and in vivo efficacy of Q-35, a new fluoroquinolone, against *Mycoplasma pneumoniae* were investigated by pharmacokinetic studies with *M. pneumoniae*-infected hamsters. By using fluoroquinolones, macrolides, and tetracyclines as references, Q-35 was found to possess the greatest mycoplasmacidal activity. The MIC for 90% of strains tested (MIC_{90}) and the MIC_{50} were 0.78 and 0.39 μg/ml, respectively, and the MBC for 90% of strains tested (MBC_{90}) and the MBC_{50} were 3.13 and 0.78 μg/ml, respectively. The MBC_{50}-to-MIC_{50} ratio for Q-35 was 2. Furthermore, only Q-35 continued to be effective against 19 strains of erythromycin-resistant mutants of *M. pneumoniae*. The efficacies of fluoroquinolones against *M. pneumoniae* were also investigated by using an experimental hamster pneumonia model to measure the CFU of *M. pneumoniae* in the lungs. Q-35 and ofloxacin were efficacious following oral administration of 200 mg/kg/day for 5 days, initiated 24 h after infection, while ciprofloxacin was not active. Continuous administration of Q-35 for 10 days significantly reduced numbers of viable *M. pneumoniae* in the lungs. These results suggest that both Q-35 and ofloxacin are effective in the early phase of infection and, moreover, that Q-35 is also effective in the middle stage of infection, when progressive lung alterations and continuous increases in mycoplasmal growth occur. Peak levels of Q-35 in sera and lungs after oral administration were higher than those of ciprofloxacin but lower than those of ofloxacin. On the basis of these results, Q-35 appears to be a promising antimicrobial agent in chemotherapy of mycoplasmal infection.

Q-35 is a new fluoroquinolone with a broad spectrum of activity against various respiratory pathogens, including gram-positive organisms, methicillin-resistant *Staphylococcus aureus*, and gram-negative anaerobes (13, 14). *Mycoplasma pneumoniae* is the causative agent of upper respiratory tract infections and pneumonia in humans and in experimental animals such as hamsters (4, 7, 10). Macrolide and tetracycline antibiotics have been widely used for chemotherapy of *M. pneumoniae* infections because of the susceptibility of the organisms to these antibiotics (5, 15, 20). However, reported incidences of mutants resistant to erythromycin, isolated both from patients treated with antibiotics and from untreated patients, have increased (16, 17). Recently, we and others have demonstrated the efficacy of fluoroquinolones against *M. pneumoniae* in vitro and in vivo (1, 2, 18). In this study, we evaluated the in vitro potency and in vivo efficacy of Q-35 against *M. pneumoniae*.

MATERIALS AND METHODS

***M. pneumoniae* strains.** Forty-nine strains of *M. pneumoniae* isolated from throat swabs of patients with pneumonia and strain FH were used. The isolates were subcultured up to four times in mycoplasma liquid medium (6) (Difco Laboratories, Detroit, Mich.), divided among several tubes, and stocked at −80°C until used for inoculation. Each isolate was identified as *M. pneumoniae* by the growth inhibition test (22).

Antimicrobial agents. The following antimicrobial agents were used: Q-35 (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), ofloxacin (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan), ciprofloxacin (Bayer Pharmaceutical Co., Ltd., Osaka, Japan), erythromycin (Sigma Chemical Co., Ltd., St. Louis, Mo.), and josamycin (Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan).

Selection of erythromycin-resistant mutants. For selection of macrolide-resistant mutants of *M. pneumoniae*, mycoplasma liquid medium containing erythromycin or josamycin in twofold dilutions of 12.5, 25.0, and 50.0 μg/ml were prepared as described previously (16). Samples of medium containing each concentration of the drugs were distributed in 1-ml amounts into each of three tubes. These tubes were inoculated with the microorganisms (10^7 CFU/ml), incubated at 37°C, and observed for 1 month. Cultures grown in the highest concentration of erythromycin that continued to change to yellow were serially subcultured in two series of media with a twofold greater concentration of each macrolide. The strains thus obtained were subcultured in antibiotic-free broth medium and tested for their susceptibility to macrolides and Q-35.

MICs and MBCs. MICs were defined as the lowest concentration of an antimicrobial agent inhibiting more than 99% of the mycoplasmal colonies compared with the control. The definition of MBC most often used in clinical microbiology is the lowest drug concentration that kills 99.9% of a mycoplasmal population in a mycoplasma liquid medium (12, 19, 23). The same 50 strains of *M. pneumoniae* as used in the previous experiment were employed for the determination of MICs and MBCs of Q-35. Each stocked strain of *M. pneumoniae* was diluted to 10^5 CFU/ml. A 200-μl volume of each sample was cultured in a sealed microtiter plate (Nunc Co., Ltd., Roskilde, Denmark) at 37°C. After 2 days of cultivation, twofold dilutions of antimicrobial agents were added to the culture of each strain. After an additional 2 days

* Corresponding author.

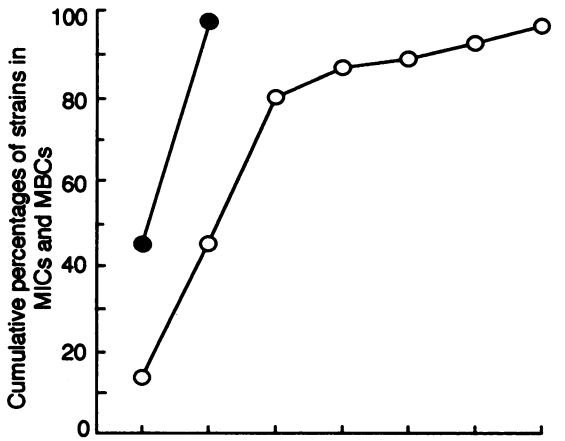
of cultivation, 10-fold dilutions of each sample were used for plating on agar plates to provide a range of 500 to 1,000 CFU/10 μl per sample. FH strains of *M. pneumoniae* were used as references. The number of mycoplasmas in broth without antibiotics reached approximately 10^7 CFU/ml during this period.

Pharmacokinetics in hamsters. The concentrations of Q-35, ofloxacin, and ciprofloxacin in sera and lungs of female Syrian hamsters weighing 70 to 80 g were determined. Blood and tissue samples were collected from groups of five hamsters at 0.125, 0.25, 0.5, 1, 2, 4, and 6 h after oral administration of each drug. Blood and lung samples were collected as described previously (1, 9). Briefly, the thorax of each animal, which had been anesthetized with 0.3 ml of 2% sodium pentobarbital, was opened and the blood was pooled in the axillar space and removed with a capillary pipette. The lungs were dissected free from the trachea and other structures. The lungs were then rinsed in sterile saline to remove adherent blood, blotted dry on gauze, weighed and homogenized in 3 volumes of 0.1 M phosphate buffer, and centrifuged at 1,800 × *g* for 20 min. Antibiotic concentrations in serum and lung samples were determined by a disk agar dilution bioassay procedure with *B. subtilis* 6633 kp used as assay organism and heart infusion agar used as the growth medium, as described previously (11). For pharmacokinetic studies with serum and lung samples, peak antibiotic concentration (C_{max}) was determined. The area under the concentration-time curve (AUC) was calculated by using the trapezoidal rule from zero to infinity. The elimination half-life was determined by linear regression analysis.

Experimental *M. pneumoniae* model. *M. pneumoniae* 242, freshly isolated from the throat swab of a patient, was passaged five to seven times in mycoplasma liquid medium and used as the challenge strain. Culture samples were kept at −80°C until used. The MIC and MBC of Q-35 for this strain were 1.56 and 12.5 μg/ml, respectively. MBCs of ofloxacin and ciprofloxacin were 3.12 and 12.5 μg/ml, respectively, and the MICs of both of the drugs were 3.12 μg/ml, as described previously (2). Syrian golden hamsters (specific pathogen free, female, 6 weeks old, 70 to 80 g), obtained from the Inoue Animal Center (Kumamoto, Japan), were anesthetized with 0.3 ml of 2% sodium pentobarbital administered subcutaneously (Dainihon Pharmaceutical Co., Ltd., Osaka, Japan) and infected with 2 × 10^7 CFU by the intratracheal peroral route as described by Barile et al. (4). Inoculation with *M. pneumoniae* was carried out as described previously (1).

Pulmonary clearance studies. To evaluate the intrapulmonary killing of mycoplasmas, hamsters were treated orally with Q-35, ofloxacin, or ciprofloxacin at 100 and 200 mg/kg. The durations of therapy were 5 days initiated 24 h after infection and 5 days and 10 days initiated 5 days after infection. Controls received identical treatment with isotonic saline. The thorax of each animal, which had been anesthetized with 0.3 ml of 2% sodium pentobarbital, was opened; blood was pooled in the axillar space and removed with a capillary pipette. The lungs were then dissected free from the trachea and other structures and homogenized in 4 ml of mycoplasma liquid medium. Viable mycoplasmas in the homogenates were quantitated by plating 0.1 ml of serial 10-fold tissue dilutions on agar medium.

Statistical analysis. Means of CFU for each group of five hamsters were compared with those for the control group by Dunnett's multicomparison method (8).



(μg/ml)	0.39	0.78	1.56	3.13	6.25	12.5	25
MIC	28	22[a]					
MBC	7	22	15[a]	3	1	1	1

FIG. 1. MICs and MBCs of Q-35 for 50 strains of *M. pneumoniae*, determined by the broth dilution method. Numbers at the bottom indicate numbers of isolates inhibited. Symbols: ●, MIC; ○, MBC. [a], strain FH.

RESULTS

MICs and MBCs in broth. The MICs and MBCs of Q-35 for the 50 strains of *M. pneumoniae* are shown in Fig. 1. The MIC for 50% of strains tested (MIC_{50}) and the MIC_{90} of Q-35 were 0.39 and 0.78 μg/ml, respectively, and the MBC for 50% of strains tested (MBC_{50}) and the MBC_{90} were 0.78 and 3.13 μg/ml, respectively. The susceptibilities of 19 macrolide-resistant strains of *M. pneumoniae*, induced with erythromycin and josamycin, are presented in Fig. 2. With macrolides, each macrolide-resistant strain showed cross-resistance between erythromycin and josamycin. By contrast, Q-35 was equally active against both erythromycin-susceptible and -resistant strains.

Efficacy of Q-35 for *M. pneumoniae*-infected hamsters. The effects of 5 days of oral administration of Q-35, ofloxacin, or ciprofloxacin given 24 h postchallenge were investigated. Q-35 and ofloxacin were both markedly effective in reducing the counts of viable *M. pneumoniae* in the lungs when administered at 200 mg/kg/day (Fig. 3, columns A). However, they were ineffective when administered at 100 mg/kg/day. Drugs were also administered 5 days after infection to determine their effects on reducing mycoplasmas which had already colonized lung tissue. The effects of 5 and 10 days of oral administration of these drugs on the counts of *M. pneumoniae* when therapy was begun 5 days after challenge are also shown in Fig. 3. Q-35 and ofloxacin were effective when administered orally at 200 mg/kg/day for 5 days (Fig. 3, columns B) or for 10 days (columns C) ($P < 0.05$). Q-35 but not ofloxacin was effective at the dose of 100 mg/kg/day for 10 days of dosing (Fig. 3, columns C) ($P < 0.05$). Ciprofloxacin did not reduce counts of *M. pneumoniae* when administered both 24 h and 5 days after infection.

Pharmacokinetic profiles in hamsters. Drug levels in serum and lung samples after oral administration of 50 mg of Q-35, ofloxacin, or ciprofloxacin per kilogram of body weight are shown in Fig. 4. The C_{max}s of Q-35, ofloxacin, and ciprofloxacin in serum were 4.2 ± 0.9, 8.5 ± 0.6, and 0.7 ± 0.1 μg/ml, respectively. The C_{max}s in lungs were 4.1 ± 0.5, 7.6

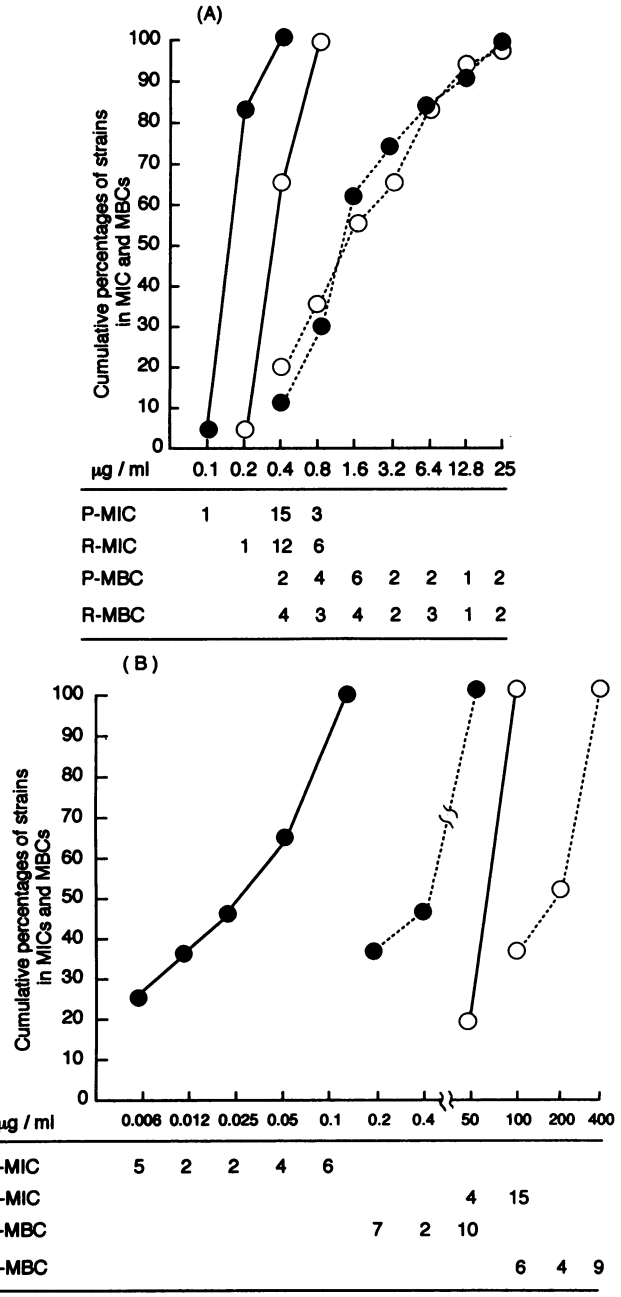


FIG. 2. MICs and MBCs of Q-35 (A) and erythromycin (B) for 19 strains of macrolide-resistant (R) *M. pneumoniae* mutants and their parents (P), determined by the broth dilution method. Numbers at the bottom indicate numbers of strains inhibited. ●, parent strains; ○, macrolide-resistant strains; ———, MIC; – – – –, MBC.

± 1.2, and 0.8 ± 0.4 μg/ml, respectively. The AUCs of Q-35, ofloxacin, and ciprofloxacin in serum samples were 5.7, 17.2, and 1.0 μg · h/ml, respectively. Those in lung samples were 7.9, 12.8, and 1.1 μg · h/ml respectively. The half-lives of Q-35 and ofloxacin in serum were 1.5 h, and the half-life of ciprofloxacin was 1.6 h. Those in lung samples were 1.3, 1.5, and 1.1 h, respectively. These accumulated data suggest that the most favorable pharmacokinetics in hamsters were obtained with ofloxacin, followed by Q-35 and ciprofloxacin.

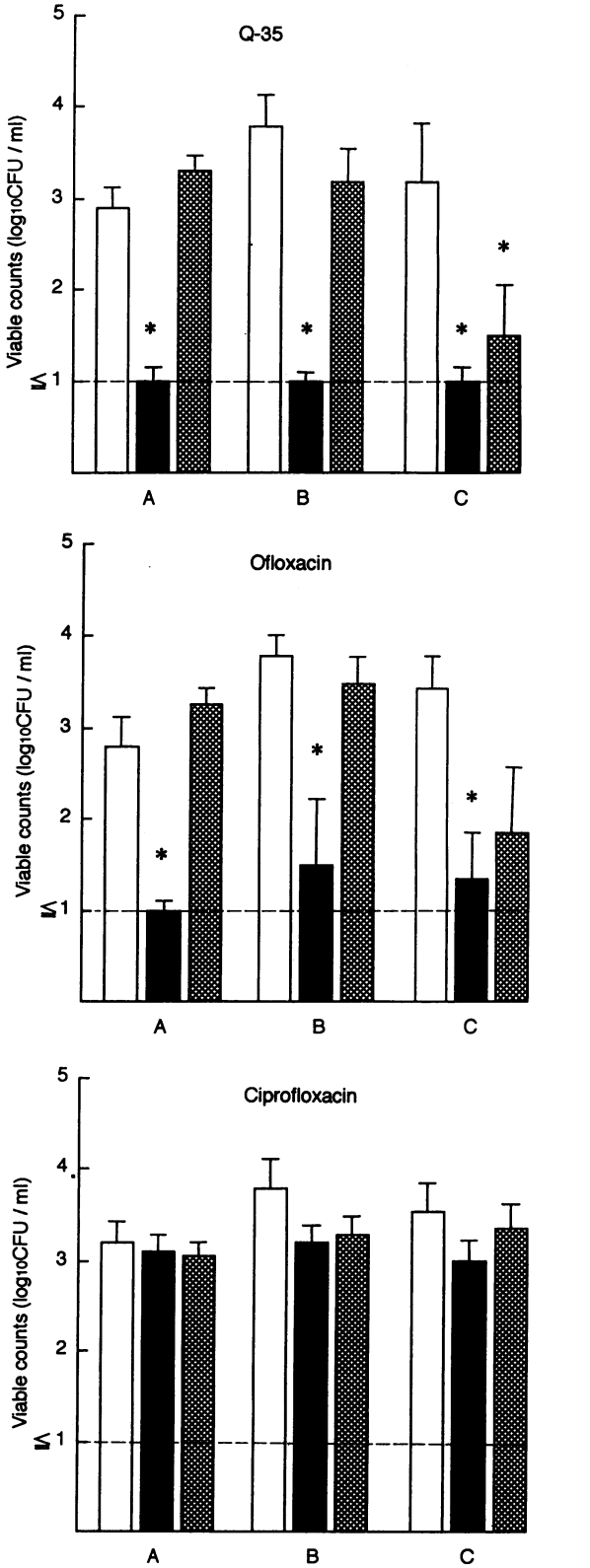


FIG. 3. Activities against *M. pneumoniae* in hamsters of Q-35, ofloxacin, and ciprofloxacin administered orally for 5 days with treatment initiated 24 h after infection (columns A) and administered for 5 days (columns B) or 10 days (columns C) with treatment initiated 5 days after infection. Values are means ± standard deviations (error bars) for five hamsters. Asterisks indicate a significant difference between the drug treatment and the control values ($P < 0.05$). Symbols: □, control; ■ and ▩, 200 and 100 mg of drug per kg per day, respectively.

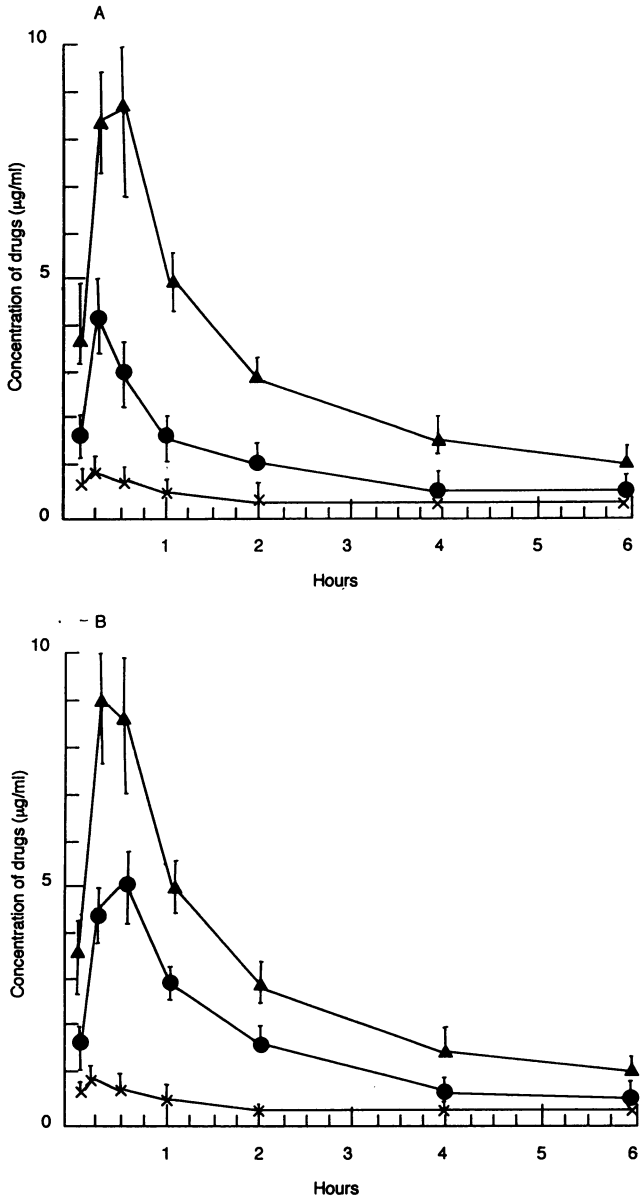


FIG. 4. Concentrations of Q-35, ofloxacin, and ciprofloxacin in serum (A) and lung tissue (B) after oral administration of 50 mg/kg to hamsters. Values are means ± standard deviations (error bars) for five hamsters. Symbols: ●, Q-35; ▲, ofloxacin; ×, ciprofloxacin.

DISCUSSION

Q-35 is a new fluoroquinolone designed to have a broad spectrum of activity. Addition of a 3-methylaminopiperidine and a methoxyl substituent to the quinolone nucleus differentiates Q-35 from other fluoroquinolone compounds. The stereochemical structure of Q-35 closely resembles the tricyclic structural features of ofloxacin (12). Q-35 is active in vitro against gram-positive organisms, including methicillin-resistant *S. aureus* and many of the gram-positive anaerobes (13).

We recently described the antimycoplasmacidal activities of several fluoroquinolones in vitro (2) and their efficacies against *M. pneumoniae* pneumonia in hamsters (1); the purpose of the present study was to evaluate the in vitro and in vivo activities of Q-35 against *M. pneumoniae*.

The current in vitro data, compared with our previously published results (2), showed that the mycoplasmacidal activity of Q-35 was higher than those of ofloxacin and ciprofloxacin as the MBC_{50}-to-MIC_{50} ratios for Q-35, ofloxacin, and ciprofloxacin were 2, 4, and 4, respectively, and the MBC_{50}-to-MIC_{50} ratios for the tetracyclines and macrolides were markedly higher, in the range of 32 to 2,000. As Niitsu et al. have reported, the development of erythromycin resistance in *M. pneumoniae* is accompanied by cross-resistance to other macrolides in vitro (16), and erythromycin-resistant mutants of *M. pneumoniae* were isolated from clinical specimens from a patient who had been treated with erythromycin. Q-35 was highly active against erythromycin- and josamycin-resistant mutant strains of *M. pneumoniae* (0.78 μg/ml). These accumulated data suggest that Q-35 may be useful in treating respiratory tract infections caused by *M. pneumoniae*.

Intratracheal inoculation of hamsters with *M. pneumoniae* results in sequences of pathological and immunological changes resembling those that occur in the natural human disease (4, 7, 10). There is little information on the preclinical efficacy of antimycoplasmal activity of antimicrobial agents in an *M. pneumoniae*-infected model. On the basis of our previous reports (1, 2), we selected Q-35, ofloxacin, and ciprofloxacin as comparative agents since they had low MICs and MBCs against mycoplasmas. Our results show that the efficacies of Q-35 and ofloxacin appear to be dependent on the time after infection at which therapy is initiated (1). At an early stage (1 day after infection), both Q-35 and ofloxacin were active, but 5 days after infection, with increased mycoplasmal growth and inflammatory progress in the lungs at 10 days of dosing, Q-35 appeared to be slightly more efficacious than ofloxacin. The decreased efficacy of ofloxacin beyond the initial stage of the disease has also been reported for pneumococcal infection (3).

Pharmacokinetic data for uninfected hamsters show that absorption and distribution of Q-35 are higher than those of ciprofloxacin but lower than those of ofloxacin. As described previously (1), under fasted conditions, the C_{max}s and AUCs of ofloxacin and ciprofloxacin in serum after oral administration were 25.5 and 1.3 μg/ml and 30 and 1.5 μg · h/ml, respectively. The C_{max}s and AUCs of these drugs in lung samples were 21.3 and 3.6 μg/ml and 36.9 and 3.5 μg · h/ml, respectively. These results were higher than those obtained in the current study. Although the reasons for these discrepancies are not clear, fluoroquinolone absorption might be affected by albumin magnesium in the food (21). The in vitro and in vivo antimycoplasmal activities and pharmacokinetic properties of Q-35 described above suggest that this antimicrobial agent may be useful in treating respiratory tract infections caused by *M. pneumoniae* in humans.

To clarify correlations between the activities of Q-35 against *M. pneumoniae* and the clinical effects of these antibiotics on mycoplasma infections, further work is needed.

ACKNOWLEDGMENTS

We gratefully acknowledge Malcolm Empson for reviewing the manuscript and Kumi Yamada for excellent secretarial assistance in the preparation of the manuscript, and we acknowledge the pertinent suggestions of Hiroki Inuzuka, statistical analyst, Computer Education Center, Kurume University.

This study was partially funded by a grant from Chugai Pharmaceutical Co., Ltd.

REFERENCES

1. **Arai, S., Y. Gohara, A. Akashi, K. Kuwano, M. Nishimoto, T. Yano, K. Oizumi, K. Takeda, and T. Yamaguchi.** 1993. Effects of new quinolones on *Mycoplasma pneumoniae*-infected hamsters. Antimicrob. Agents Chemother. **37**:287–292.
2. **Arai, S., Y. Gohara, K. Kuwano, and T. Kawashima.** 1992. Antimycoplasmal activities of new quinolones, tetracyclines, and macrolides against *Mycoplasma pneumoniae*. Antimicrob. Agents Chemother. **36**:1322–1324.
3. **Azoulay-Dupuis, E., J. P. Bedos, E. Vallee, D. J. Hardy, R. N. Swanson, and J. J. Pocidalo.** 1991. Antipneumococcal activity of ciprofloxacin, ofloxacin and temafloxacin in an experimental mouse pneumonia model at various stages of the disease. J. Infect. Dis. **163**:319–324.
4. **Barile, M. F., D. K. F. Chandler, H. Yoshida, M. W. Grabowski, R. Harasawa, and S. Razin.** 1988. Parameters of *Mycoplasma pneumoniae* infection in Syrian hamsters. Infect. Immun. **56**: 2443–2449.
5. **Cassell, G. H., K. B. Waites, M. S. Pate, K. C. Canupp, and L. B. Duffy.** 1989. Comparative susceptibility of Mycoplasma pneumoniae to erythromycin, ciprofloxacin, and lomefloxacin. Diagn. Microbiol. Infect. Dis. **12**:433–435.
6. **Chanock, R. M., L. Hayflick, and M. F. Barile.** 1962. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. Proc. Natl. Acad. Sci. USA **48**:41–49.
7. **Clyde, W. A., Jr.** 1971. Immunopathology of experimental *Mycoplasma pneumoniae* disease. Infect. Immun. **4**:757–763.
8. **Dunnett, C. W.** 1980. Pairwise multiple comparisons in the homogeneous variance, unequal sample size case. J. Am. Stat. Assoc. **75**:789–795.
9. **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.
10. **Fernald, G. W., W. A. Clyde, Jr., and J. Bienenstock.** 1972. Immuno-globulin-containing cells in lungs of hamsters infected with *Mycoplasma pneumoniae*. J. Immunol. **108**:1400–1408.
11. **Fernandes, P. B., D. T. W. Chu, R. R. Bower, K. P. Jarvis, N. R. Ramer, and N. Shipkowitz.** 1986. In vivo evaluation of A-56619 (difloxacin) and A-56620: new aryl-fluoroquinolones. Antimicrob. Agents Chemother. **29**:201–208.
12. **Heifets, L., and P. Lindholm-Levy.** 1989. Comparison of bactericidal activities of streptomycin, amikacin, kanamycin, and capreomycin against *Mycobacterium avium* and *M. tuberculosis*. Antimicrob. Agents Chemother. **33**:1298–1301.
13. **Ito, T., M. Otsuki, and T. Nishino.** 1992. In vitro antibacterial activity of Q-35, a new fluoroquinolone. Antimicrob. Agents Chemother. **36**:1708–1714.
14. **Matsumoto, M., K. Kojima, H. Nagano, S. Matsubara, and T. Yokota.** 1992. Photostability and biological activity of fluoroquinolones substituted at the 8 position after UV irradiation. Antimicrob. Agents Chemother. **36**:1715–1719.
15. **Misu, T., S. Arai, M. Furukawa, Y. Yamamoto, and T. Miyazaki.** 1987. Effects of rokitamycin and other macrolide antibiotics on *Mycoplasma pneumoniae* in L cells. Antimicrob. Agents Chemother. **31**:1843–1845.
16. **Niitsu, Y., S. Hasegawa, and H. Kubota.** 1974. In vitro development of resistance to erythromycin, other macrolide antibiotics, and lincomycin in *Mycoplasma pneumoniae*. Antimicrob. Agents Chemother. **5**:513–519.
17. **Niitsu, Y., S. Hasegawa, T. Suetake, H. Kubota, S. Komatsu, and M. Horikawa.** 1970. Resistance of *Mycoplasma pneumoniae* to erythromycin and other antibiotics. J. Pediatr. **76**:438–443.
18. **Osada, Y., and H. Ogawa.** 1983. Antimycoplasmal activity of ofloxacin (DL-8280). Antimicrob. Agents Chemother. **23**:509–511.
19. **Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrob. Agents Chemother. **18**:699–708.
20. **Rasch, J. R., and W. J. Mogabgab.** 1966. Therapeutic effect of erythromycin on *Mycoplasma pneumoniae* pneumonia. Antimicrob. Agents Chemother. **1965**:693–699.
21. **Soergel, F., K. G. Naber, M. Kinzig, G. Mahr, and P. Muth.** 1991. Comparative pharmacokinetics of ciprofloxacin and temafloxacin in humans: a review. Am. J. Med. **91**(Suppl. 6A):51s–66s.
22. **Stanbridge, E., and L. Hayflick.** 1967. Growth inhibition test for identification of *Mycoplasma* species utilizing dried antiserum impregnated paper discs. J. Bacteriol. **93**:1392–1396.
23. **Taylor, P. C., F. D. Schoenknecht, J. C. Sherris, and E. C. Linner.** 1983. Determination of minimum bactericidal concentrations of oxacillin for *Staphylococcus aureus*: influence and significance of technical factors. Antimicrob. Agents Chemother. **23**:142–150.