J Infect Chemother (2000) 6:148–150

ORIGINAL ARTICLE

Hideaki Ikejima · Hiroyuki Yamamoto · Kazuo Ishida
Mitsuo Kaku · Jingoro Shimada

Evaluation of in-vitro activity of new quinolones, macrolides, and minocycline against *Mycoplasma pneumoniae*

Received: February 23, 2000 / Accepted: May 9, 2000

Abstract We made a comparative study of the in-vitro activities of grepafloxacin (GPFX), ofloxacin (OFLX), erythromycin (EM), clarithromycin (CAM), roxithromycin (RXM), and minocycline (MINO) against 20 strains of *Mycoplasma pneumoniae* (17 clinical isolates and 3 standard strains). The minimum inhibitory concentration (MIC)90-to-minimum bactericidal concentration (MBC)90 ratio showed that the new quinolones have bactericidal effects on *M. pneumoniae*. Thus, it is expected that the new quinolones, especially grepafloxacin, will be clinically useful antimicrobial agents for the treatment of *M. pneumoniae* infection because of their good pharmacokinetic properties and bactericidal action.

Key words *Mycoplasma pneumoniae* · Grepafloxacin (GPFX) · MIC

Introduction

Mycoplasma pneumoniae is a major causative organism of pneumonia, accounting for as many as 20% of all pneumonia cases.[1] The therapeutic drugs recommended for *M. pneumoniae* infections are several quinolones, macrolides, and tetracyclines. Macrolides are effective in reducing the duration of symptoms.[2,3] However, an erythromycin-resistant strain has been reported. After the exposure of strains susceptible to erythromycin in vitro and in vivo, strains resistant to erythromycin and other macrolides occurred spontaneously.[4,5] Therefore, new chemotherapeutic drugs will be needed to achieve another choice of treatment for mycoplasmal infections. Grepafloxacin, one of the new quinolone derivatives, is an antibiotic that can be administered orally. The objective of this study was to assess the in-vitro bacteriostatic and bactericidal actions of grepafloxacin against *M. pneumoniae*, in comparison with these actions of ofloxacin, erythromycin, clarithromycin, roxithromycin, and minocycline.

H. Ikejima (✉) · H. Yamamoto · K. Ishida · M. Kaku · J. Shimada
Department of Microbiology, St. Marianna University School of Medicine, Sugao, Miyamae-ku, Kawasaki, Kanagawa 216-8511, Japan
Tel. +81-44-977-8111; Fax +81-44-977-7818
e-mail: ike@marianna-u.ac.jp.

Materials and methods

Compounds

The following antibiotics were used in this study; grepafloxacin (Otsuka Pharmaceutical, Tokyo, Japan), erythromycin (Dainippon Pharmaceutical Tokyo, Japan), clarithromycin (Taisho Pharmaceutical Tokyo, Japan), roxithromycin (Nippon Hoechst Marion Roussel, Tokyo, Japan), ofloxacin (Daiichi Pharmaceutical Tokyo, Japan), and minocycline (Nihon Lederle Pharmaceutical Tokyo, Japan). All antibiotics were kindly donated by the manufacturers. The new quinolones were dissolved in distilled water with 0.1 N-NaOH. Minocycline was dissolved in pure distilled water, and the macrolides were dissolved in ethanol. These drug solutions (10 mg/ml) were diluted with medium (as outlined below) and then used immediately.

Organisms

Seventeen clinical strains of *M. pneumoniae* were obtained from Nagasaki University Hospital. Three standard strains, M129, FH, and Mac, were obtained from Dr. M. F. Barile (Food and Drug Administration, Bethesda, MD, USA). These strains were cultured in modified Chanock broth medium,[6] which consisted of 70% autoclaved pleuropneumonia-like organism (PPLO) broth (Difco Laboratories, Detroit, Ml, USA), 20% non-activated horse serum, 10% fresh yeast extract (25% solution), 1% glucose, and 0.002% phenol red, adjusted to pH 7.8 with 1 N NaOH.

Table 1. Susceptibilities of *Mycoplasma pneumoniae* strains

Antibiotics	MIC (μg/ml)[a]			MBC (μg/ml)[b]		
	Range	50%	90%	Range	50%	90%
GPFX	0.0625–0.25	0.125	0.25	0.125–2	0.125	0.25
OFLX	1–4	2	2	2–8	2	2
EM	0.0019–0.0078	0.0019	0.0019	0.0019–0.032	0.016	0.032
CAM	<0.0019–0.0039	0.0019	0.0019	0.0019–0.032	0.0078	0.032
RXM	0.0039–0.016	0.0039	0.0039	0.0039–0.125	0.0078	0.0625
MINO	0.0625–8	1	2	0.5–64	32	64

GPFX, Grepafloxacin; OFLX, ofloxacin; EM, erythromycin; CAM, clarithromycin; RXM, roxithromycin; MINO, minocycline; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration

[a] 50% and 90%, MICs for 50% and 90% of strains tested, respectively

[b] 50% and 90%, MBCs for 50% and 90% of strains tested, respectively

Table 2. MIC90/MBC90 ratios of *M. pneumoniae* strains to antibiotics

Antibiotics	MIC90[a]/MBC90[b]
GPFX	1
OFLX	1
EM	0.059
CAM	0.059
RXM	0.062
MINO	0.031

[a] MIC90, MIC for 90% of strains tested

[b] MBC90, MBC for 90% of strains tested

Determination of antibacterial activity

Minimum inhibitory concentrations (MICs) were determined by the micro broth dilution method, using modified Chanock broth medium. Twofold dilutions of drugs in the broth medium were prepared for the following range of concentrations: grepafloxacin, 0.0078 to 8 μg/ml; ofloxacin, 0.0078 to 8 μg/ml; erythromycin, 0.0019 to 2 μg/ml; clarithromycin, 0.0019 to 2 μg/ml; roxithromycin, 0.0019 to 2 μg/ml; and minocycline, 0.0625 to 64 μg/ml. The broth culture of each *M. pneumoniae* strain, after 7 to 14 days of incubation, and containing approximately 10^6 CFU/ml, was used as an inoculum for MIC determination. Each 10-μl aliquot of these broth cultures was inoculated into a well of a 96-well microdilution plate containing 90 μl of the drug solution per well to be inoculated with *M. pneumoniae* at 10^5 CFU/ml. The plates were sealed, and incubated at 37°C. Negative controls without the drugs or the organism were included on each plate. The MIC of *M. pneumoniae* was determined at the time when the color of the no-drug control medium was changed from red to yellow by the organism growth, and the lowest concentration of antibiotic without medium color change by growth inhibition was defined as the MIC. Minimum bactericidal concentrations (MBCs) were determined by the agar method, using the modified Chanock medium with 1.4% agar. An aliquot (0.1 ml) of the broth used for MIC determination was inoculated onto the agar and incubated at 37°C. The concentration of antimicrobial agent without colony formation was defined as the MBC.

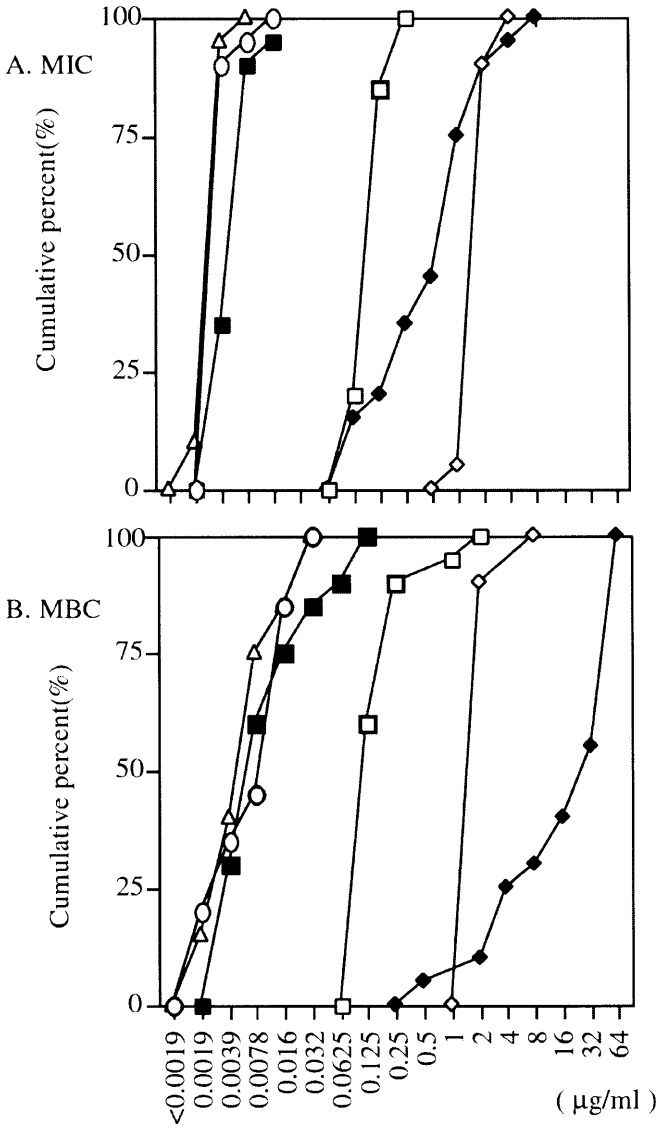

Fig. 1A,B. Distribution of **A** minimum inhibitory concentration (*MIC*)s and **B** minimum bactericidal concentration (*MBC*)s of antibiotics against 20 strains of *Mycoplasma pneumoniae*. *Open squares*, grepafloxacin; *open diamonds*, ofloxacin; *open circles*, erythromycin; *open triangles*, clarithromycin; *solid squares*, roxithromycin; *solid diamonds*, minocycline

Results

As shown in Table 1 and Fig. 1, the MIC50 and MIC90 values for grepafloxacin against *M. pneumoniae* were 0.125 and 0.25 μg/ml, which were higher values than those for the macrolides (0.0019 to 0.0039 μg/ml for erythromycin, clarithromycin, roxithromycin). These MICs of grepafloxacin were lower than the values for ofloxacin and minocycline. The MBCs of macrolides showed values 2 to 16 times higher than their MICs, while the new quinolones had equal MIC and MBC values. The MBC90-to-MIC90 ratios showed the strong bactericidal activity of the new quinolones (Table 2).

Discussion

New quinolone antibacterial agents have a broad spectrum of activity and have been employed for the treatment of various kinds of infections in humans. It has been reported that new quinolones are active against *M. pneumoniae* and that their MICs are comparable to those of tetracyclines, but inferior to those of macrolides.[7–9]

In our study, grepafloxacin demonstrated stronger antibacterial activity against *M. pneumoniae* than ofloxacin and minocycline, although it was less active than macrolides. Comparison of bacteriostatic and bactericidal actions indicated that the new quinolone antibacterial agents had a stronger bactericidal activity than the other drugs tested in this study. In addition, the MBC90 of grepafloxacin was 0.25 μg/ml, lower than that of ofloxacin. These results suggest that grepafloxacin will be another choice for the clinical treatment of mycoplasmal infections, and will be useful to counteract the occurrence of strains that are resistant to macrolides.

References

1. Foy HM, Kenny GE, Cooney MK, D.Allen I. Long-term epidemiology of infections with *Mycoplasma pneumoniae*. J Infect Dis 1979; 139:681–7.
2. Shames JM, George RB, Holliday WB, Rasch JR, Mogabgab WJ. Comparison of antibiotics in the treatment of *Mycoplasmal pneumonia*. Arch Intern Med 1970;125:680–4.
3. Kirst HA, Sides GD. New directions for macrolide antibiotics: pharmacokinetics and clinical efficacy. Antimicrob Agents Chemother 1989;33:1419–22.
4. Lucier TS, Heitzman K, Liu S, Hu P. Transition mutations in the 23S rRNA of erythromycin-resistant isolates of *Mycoplasma pneumoniae*. Antimicrob Agents Chemother 1995;39:2770–3.
5. Niitu Y, Hasegawa S, Suetake T, Kubota H, Komatu S, Horikawa M. Resistance of *Mycoplasma pneumoniae* to erythromycin and other antibiotics. J Pediatr 1970;76:434–43.
6. Chanock RM, Hayflick L, Barile MF. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as PPLO. Proc Natl Acad Sci USA 1962;48:41–9.
7. Ishida K, Kaku M, Irifune K, Mizukane R, Takemura H, Yoshida R, et al. In vitro and in vivo activities of macrolides against *Mycoplasma pneumoniae*. Antimicrob Agents Chemother 1994;38:790–8.
8. Kenny GE, Cartwright FD. Susceptibility of *Mycoplasma pneumoniae* to several new quinolines, tetracycline, and erythromycin. Antimicrob Agents Chemother 1991;35:587–9.
9. Kenny GE, Cartwright FD. Susceptibility of *Mycoplasma hominis, Mycoplasma pneumoniae,* and *Ureaplasma urealyticum* to a new quinoline, OPC 17116. Antimicrob Agents Chemother 1993;37: 1726–7.