ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2007, p. 2278–2279 Vol. 51, No. 6
0066-4804/07/$08.00+0 doi:10.1128/AAC.01561-06

Letters to the Editor

Activity of Garenoxacin against Macrolide-Susceptible and -Resistant *Mycoplasma pneumoniae*▽

In the treatment of *Mycoplasma pneumoniae* infection, erythromycin and clarithromycin, 14-membered macrolides, and azithromycin, a 15-membered macrolide, are usually considered to be the first-choice drugs (4, 11). Macrolide antibiotics inhibit protein synthesis by binding to domain II and/or domain V of 23S rRNA (1, 13). We found that ca. 20% of *M. pneumoniae* strains isolated from patients in Japan from 2000 to 2003 were macrolide resistant (6, 9). Lucier et al. (5) and Okazaki et al. (7) found that an A-to-G transition or an A-to-C transversion at position 2063 (corresponding to position 2058 in *Escherichia coli*) or 2064 of the 23S rRNA gene results in a high level of resistance to macrolide antibiotics. Given this background, it was considered that an alternative to macrolides in the treatment of pneumonia caused by *M. pneumoniae* is needed.

Garenoxacin, a des-F(6)-quinolone that exhibits excellent activity against respiratory pathogens such as *Streptococcus pneumoniae* and *Chlamydophila pneumoniae*, is under development for both oral and parenteral administration (10). In the present study, we examined the in vitro activity of garenoxacin against *M. pneumoniae* isolates, including macrolide-resistant strains, and compared it with those of other antibiotics.

The following agents were employed for MIC determinations: garenoxacin (Toyama Chemical Co., Ltd., Tokyo, Japan), gatifloxacin (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan), levofloxacin and clarithromycin (LKT Laboratories, Inc., Saint Paul, MN), and minocycline (Lederle-Japan Ltd., Tokyo, Japan). The purity of each of these agents was above 99.8%. The following isolates of *M. pneumoniae* were tested: three macrolide-susceptible reference strains, FH, Mac, and M129, and four macrolide-resistant clinical isolates, 1 and 6 (isolates carrying the A-to-G mutation at position 2063 in the 23S rRNA gene for which the MIC of clarithromycin is 32 μg/ml) and 2 and 4 (isolates carrying the A-to-G mutation at position 2064 in the 23S rRNA gene for which the MIC of clarithromycin is 8 μg/ml). The PCR amplification and sequencing of domain II of the 23S rRNA genes were performed by a method reported previously (6).

A broth microdilution method was used to determine the MICs. Serial twofold dilutions of antibacterial agents prepared in PPLO broth (Difco Inc., Detroit, MI) containing 10^4 to 10^5 CFU of *M. pneumoniae*/ml were put into 96-well microplates. The microplates were sealed with adhesive sheets and incubated at 37°C for 4 to 8 days. The MIC was defined as the lowest concentration of a drug at which the metabolism of the organism was inhibited, as evidenced by a lack of color change in the medium at the time when the drug-free control first showed a color change.

TABLE 1. Activities of various antibiotics against *M. pneumoniae* strains

Strain	Gene mutation[a]	MIC (μg/ml) of:				
		Garenoxacin	Gatifloxacin	Levofloxacin	Minocycline	Clarithromycin
FH	None	0.016	0.063	0.50	0.50	0.004
Mac	None	0.016	0.063	0.25	0.25	0.002
M129	None	0.016	0.063	0.25	0.25	0.002
1	A2063G	0.016	0.031	0.25	0.25	32
6	A2063G	0.031	0.063	0.50	0.25	32
2	A2064G	0.016	0.063	0.25	0.25	8
4	A2064G	0.016	0.031	0.25	0.25	8

[a] Gene mutation in domain II of 23S rRNA genes.

The MICs of garenoxacin for the *M. pneumoniae* isolates, including the macrolide-resistant isolates, were 0.016 to 0.031 μg/ml, two- to fourfold lower than those of gatifloxacin and 8- to 32-fold lower than those of levofloxacin and minocycline (Table 1). The results for macrolide-susceptible strains such as *M. pneumoniae* FH, a reference strain, accorded with those given in previous reports (2, 8, 12).

The newer fluoroquinolones such as gatifloxacin and moxifloxacin exhibit potent in vitro activity against a broad spectrum of organisms, including *M. pneumoniae* (3). Garenoxacin showed more-potent in vitro activity against *M. pneumoniae* than gatifloxacin and has attracted interest as a potential therapy for community-acquired pneumonia. These data indicate that garenoxacin may be a viable alternative to macrolides, such as clarithromycin, in the treatment of pneumonia caused by *M. pneumoniae*, including macrolide-resistant strains.

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▽Published ahead of print on 26 March 2007.