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NOTES

In Vitro Antibacterial Activity of AM-1155, a Novel 6-Fluoro-8-Methoxy Quinolone

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The in vitro antibacterial activity of AM-1155 against a wide variety of clinical isolates was compared with those of other fluoroquinolones. The MICs of AM-1155 for 90% of *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterococcus faecalis* isolates tested were 0.10, 0.39, and 0.78 μg/ml, respectively. The antibacterial activity of AM-1155 against gram-positive bacteria and anaerobes was comparable to those of sparfloxacin and tosufloxacin. AM-1155 inhibited 90% of most species of the family *Enterobacteriaceae* at a concentration of 0.39 μg/ml. AM-1155 generally had activity comparable to that of sparfloxacin against gram-negative bacteria. AM-1155 showed moderate activity against methicillin- and quinolone-resistant *S. aureus*. AM-1155 demonstrated bactericidal activity at the MIC. The frequency of occurrence of spontaneous mutants resistant to four times the MIC of AM-1155 was $<1 \times 10^9$ for *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. AM-1155 strongly inhibited the supercoiling activities of DNA gyrases purified from *E. coli* and *S. aureus*.

Recently, the introduction of both a piperazine side chain and a fluorine atom to the molecule has resulted in the appearance of a new group of fluorinated quinolones such as norfloxacin (10, 11), ofloxacin (17), ciprofloxacin (20), fleroxacin (5), lomefloxacin (7), tosufloxacin (3), and sparfloxacin (12). These new quinolones have high levels of activity against members of the family *Enterobacteriaceae* and a broad antibacterial spectrum, including *Pseudomonas aeruginosa* and many gram-positive bacteria.

AM-1155 [(±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid] is a novel quinolone compound developed by Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan.

AM-1155 has characteristic structures such as a 3-methyl-1-piperazinyl moiety and a methoxyl moiety and has a broad spectrum of activity against various bacteria, including anaerobes and *Mycoplasma pneumoniae* (8).

In the study described here, AM-1155 was compared with other new quinolone compounds by determining its in vitro antibacterial activity against clinical isolates, bactericidal activity, frequency of spontaneous mutation, and DNA gyrase inhibition.

(Part of this work was presented at the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy [19].)

Antimicrobial agents. AM-1155 and norfloxacin were obtained from Kyorin Pharmaceutical Co., Ltd.; other drugs used were ciprofloxacin, from Bayer Yakuhin, Ltd., Osaka, Japan; ofloxacin, from Daiichi Seiyaku Co., Ltd., Tokyo, Japan; sparfloxacin, from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan; tosufloxacin, from Toyama Chemical Co., Ltd., Toyama, Japan; and methicillin sodium, from Banyu Yakuhin Co., Ltd., Tokyo, Japan.

Organisms. The bacterial strains used in the study were reference strains maintained in our laboratories and recent clinical isolates collected in various hospitals in Japan between 1984 and 1991.

Determination of MICs. MICs were determined by the twofold serial agar dilution method. The media used for preculture and MIC determinations were Sensitivity Test Broth (Nissui Pharmaceutical, Tokyo, Japan) and Sensitivity Disk Agar-N (SDA; Nissui). For streptococci, preculture took place in brain heart infusion broth (Difco Laboratories, Detroit, Mich.), and MICs were determined by using SDA supplemented with 5% defibrinated horse blood. SDA was supplemented with 5% Fildes enrichment (Difco) for *Haemophilus influenzae*. Chocolate agar was used for *Neisseria gonorrhoeae*. Gifu anaerobic medium broth (Nissui) and Gifu anaerobic medium agar (Nissui) were used for obligate anaerobes.

An overnight culture of bacterial suspension was diluted with corresponding fresh broth or buffered saline containing 0.01% gelatin to a concentration of approximately 10^6 CFU/ml. A portion (about 5 μl) of the dilution was inoculated with an inoculation apparatus (Microplanter; Sakuma Seisakusho, Tokyo, Japan) onto agar plates containing various concentrations of drug. The final inoculum size was approximately 10^4 CFU per spot. The plates were incubated at 37°C for 18 h, but *H. influenzae* and *N. gonorrhoeae* were cultured in a candle jar for 18 and 48 h, respectively, and anaerobes were incubated in an anaerobic chamber for 18 h. The MIC was defined as the lowest drug concentration that inhibited visible growth.

Determination of MBCs. An overnight culture of test strains was diluted with Mueller-Hinton broth (Difco) containing 50 μg of Ca^{2+} and 25 μg of Mg^{2+} per ml, and 0.03 ml of the dilution was added to 0.12 ml of broth containing various concentrations of drugs in a 96-well microplate. The final inoculum size of all strains was approximately 1×10^5 to 2×10^6 CFU/ml. The plates were incubated at 37°C for 18 h. After determination of the MIC, 0.1 ml of the culture was mixed with 10 ml of SDA. The plates were incubated at 37°C for 48 h. The

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TABLE 1. Antibacterial activities of AM-1155 and the other compounds against clinical isolates

Organism (no. tested)	Compound	MIC (μg/ml)[a]		
		Range	50%	90%
Methicillin-susceptible *Staphylococcus aureus* (152)	AM-1155	0.025–6.25	0.10	0.10
	Norfloxacin	0.39–100	1.56	3.13
	Ciprofloxacin	0.20–50	0.39	0.78
	Ofloxacin	0.20–25	0.39	0.78
	Sparfloxacin	0.025–25	0.05	0.10
	Tosufloxacin	0.013–3.13	0.025	0.05
Methicillin-resistant *Staphylococcus aureus* (271)[b]	AM-1155	0.025–25	0.20	6.25
	Norfloxacin	0.78–>100	25	>100
	Ciprofloxacin	0.20–>100	3.13	100
	Ofloxacin	0.20–>100	1.56	50
	Sparfloxacin	0.025–100	0.10	12.5
	Tosufloxacin	0.013–>25	0.10	6.25
Quinolone-resistant *Staphylococcus aureus* (156)[c]	AM-1155	0.05–25	1.56	12.5
	Norfloxacin	12.5–>100	50	>100
	Ciprofloxacin	3.13–>100	12.5	>100
	Ofloxacin	0.78–>100	12.5	100
	Sparfloxacin	0.05–100	3.13	12.5
	Tosufloxacin	0.05–>25	1.56	12.5
Coagulase-negative staphylococci (159)	AM-1155	0.05–0.20	0.10	0.20
	Norfloxacin	0.20–12.5	0.78	0.78
	Ciprofloxacin	0.10–1.56	0.20	0.20
	Ofloxacin	0.20–1.56	0.39	0.39
	Sparfloxacin	0.05–0.20	0.10	0.20
	Tosufloxacin	0.025–0.39	0.05	0.05
Streptococcus pyogenes (79)	AM-1155	0.10–1.56	0.39	0.39
	Norfloxacin	0.78–25	3.13	6.25
	Ciprofloxacin	0.20–3.13	0.78	3.13
	Ofloxacin	0.78–50	1.56	1.56
	Sparfloxacin	0.10–3.13	0.39	3.13
	Tosufloxacin	0.05–6.25	0.20	0.39
Streptococcus pneumoniae (29)	AM-1155	0.10–6.25	0.39	0.39
	Norfloxacin	3.13–100	12.5	50
	Ciprofloxacin	0.39–50	3.13	3.13
	Ofloxacin	1.56–50	6.25	12.5
	Sparfloxacin	0.10–6.25	0.20	0.78
	Tosufloxacin	0.10–25	0.39	1.56
Enterococcus faecalis (112)	AM-1155	0.20–3.13	0.39	0.78
	Norfloxacin	1.56–25	3.13	6.25
	Ciprofloxacin	0.39–6.25	0.78	1.56
	Ofloxacin	0.78–12.5	1.56	3.13
	Sparfloxacin	0.20–3.13	0.39	0.78
	Tosufloxacin	0.10–12.5	0.39	0.39
Enterococcus faecium (54)	AM-1155	0.20–50	1.56	12.5
	Norfloxacin	1.56–>100	6.25	>100
	Ciprofloxacin	0.39–>100	3.13	>100
	Ofloxacin	1.56–>100	6.25	100
	Sparfloxacin	0.39–>100	1.56	50
	Tosufloxacin	0.20–>50	3.13	25
Escherichia coli (108)	AM-1155	0.013–3.13	0.05	0.10
	Norfloxacin	0.013–3.13	0.10	0.10
	Ciprofloxacin	≦0.006–1.56	0.013	0.025
	Ofloxacin	0.025–12.5	0.05	0.10
	Sparfloxacin	≦0.006–3.13	0.025	0.05
	Tosufloxacin	≦0.006–6.25	0.025	0.05

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TABLE 1—*Continued*

Organism (no. tested)	Compound	MIC (μg/ml)[a]		
		Range	50%	90%
Klebsiella pneumoniae (127)	AM-1155	0.013–1.56	0.05	0.10
	Norfloxacin	0.025–6.25	0.01	0.39
	Ciprofloxacin	≦0.006–1.56	0.025	0.05
	Ofloxacin	0.05–3.13	0.20	0.39
	Sparfloxacin	0.013–3.13	0.05	0.10
	Tosufloxacin	0.013–0.78	0.05	0.10
Klebsiella oxytoca (104)	AM-1155	0.013–0.20	0.05	0.10
	Norfloxacin	0.025–0.39	0.05	0.10
	Ciprofloxacin	≦0.006–0.05	0.013	0.025
	Ofloxacin	0.05–0.39	0.10	0.20
	Sparfloxacin	0.013–0.20	0.05	0.05
	Tosufloxacin	≦0.006–0.10	0.025	0.025
Citrobacter freundii (97)	AM-1155	0.025–25	0.20	1.56
	Norfloxacin	0.025–50	0.10	1.56
	Ciprofloxacin	≦0.006–12.5	0.025	0.39
	Ofloxacin	0.025–100	0.20	3.13
	Sparfloxacin	0.025–50	0.05	1.56
	Tosufloxacin	0.013–>25	0.05	0.78
Enterobacter cloacae (105)	AM-1155	≦0.006–3.13	0.05	0.20
	Norfloxacin	0.025–25	0.05	0.78
	Ciprofloxacin	≦0.006–6.25	0.013	0.10
	Ofloxacin	0.025–25	0.10	0.78
	Sparfloxacin	≦0.006–3.13	0.05	0.20
	Tosufloxacin	≦0.006–3.13	0.05	0.20
Proteus mirabilis (101)	AM-1155	0.05–1.56	0.20	0.20
	Norfloxacin	0.05–0.78	0.10	0.20
	Ciprofloxacin	0.025–0.20	0.025	0.05
	Ofloxacin	0.05–1.56	0.20	0.20
	Sparfloxacin	0.05–1.56	0.20	0.78
	Tosufloxacin	0.05–0.78	0.20	0.20
Proteus vulgaris (95)	AM-1155	0.05–1.56	0.10	0.39
	Norfloxacin	0.025–0.20	0.05	0.10
	Ciprofloxacin	0.013–0.20	0.025	0.10
	Ofloxacin	0.05–1.56	0.10	0.39
	Sparfloxacin	0.05–3.13	0.20	0.78
	Tosufloxacin	0.025–1.56	0.10	0.39
Morganella morganii (70)	AM-1155	0.025–12.5	0.10	0.20
	Norfloxacin	0.025–50	0.05	1.56
	Ciprofloxacin	≦0.006–25	0.013	0.39
	Ofloxacin	0.05–25	0.10	0.20
	Sparfloxacin	0.025–25	0.20	0.39
	Tosufloxacin	0.025–25	0.10	0.20
Providencia rettgeri (49)	AM-1155	0.025–6.25	0.20	1.56
	Norfloxacin	0.025–6.25	0.20	1.56
	Ciprofloxacin	≦0.006–3.13	0.05	1.56
	Ofloxacin	0.025–12.5	0.39	3.13
	Sparfloxacin	0.013–12.5	0.20	3.13
	Tosufloxacin	≦0.006–1.56	0.10	1.56
Providencia stuartii (58)	AM-1155	0.025–0.78	0.20	0.39
	Norfloxacin	0.05–3.13	0.39	1.56
	Ciprofloxacin	0.013–0.78	0.10	0.39
	Ofloxacin	0.10–1.56	0.39	0.78
	Sparfloxacin	0.013–0.78	0.10	0.39
	Tosufloxacin	0.013–0.39	0.10	0.20

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TABLE 1—*Continued*

Organism (no. tested)	Compound	MIC (μg/ml)[a]		
		Range	50%	90%
Serratia marcescens (111)	AM-1155	0.05–50	0.78	12.5
	Norfloxacin	0.025–100	3.13	50
	Ciprofloxacin	0.013–50	0.39	12.5
	Ofloxacin	0.05–>100	1.56	25
	Sparfloxacin	0.025–100	1.56	12.5
	Tosufloxacin	0.025–>50	0.78	6.25
Pseudomonas aeruginosa (108)	AM-1155	0.39–100	1.56	6.25
	Norfloxacin	0.39–>100	0.78	6.25
	Ciprofloxacin	0.10–100	0.39	1.56
	Ofloxacin	0.78–>100	1.56	12.5
	Sparfloxacin	0.39–>100	1.56	6.25
	Tosufloxacin	0.10–>25	0.39	1.56
Pseudomonas cepacia (44)	AM-1155	0.05–100	12.5	12.5
	Norfloxacin	3.13–>100	25	25
	Ciprofloxacin	0.39–>100	6.25	12.5
	Ofloxacin	0.20–>100	12.5	25
	Sparfloxacin	0.39–100	6.25	12.5
	Tosufloxacin	0.013–25	6.25	12.5
Xanthomonas maltophilia (44)	AM-1155	0.20–1.56	0.39	1.56
	Norfloxacin	3.13–50	12.5	25
	Ciprofloxacin	0.78–6.25	1.56	3.13
	Ofloxacin	0.78–6.25	1.56	3.13
	Sparfloxacin	0.10–0.78	0.20	0.39
	Tosufloxacin	0.10–0.78	0.39	0.78
Acinetobacter spp. (37)	AM-1155	0.025–0.39	0.05	0.20
	Norfloxacin	0.78–25	3.13	6.25
	Ciprofloxacin	0.05–6.25	0.20	0.78
	Ofloxacin	0.10–3.13	0.20	0.78
	Sparfloxacin	≦0.006–0.10	0.025	0.10
	Tosufloxacin	0.013–0.20	0.05	0.10
Haemophilus influenzae (91)	AM-1155	≦0.006–0.025	≦0.006	0.013
	Norfloxacin	0.025–0.20	0.05	0.10
	Ciprofloxacin	≦0.006–0.05	0.013	0.025
	Ofloxacin	0.013–0.39	0.025	0.05
	Sparfloxacin	≦0.006–0.10	0.013	0.025
	Tosufloxacin	≦0.006–0.025	≦0.006	0.013
Branhamella catarrhalis (42)	AM-1155	0.013–0.05	0.025	0.05
	Norfloxacin	0.05–0.78	0.20	0.20
	Ciprofloxacin	0.013–0.10	0.025	0.05
	Ofloxacin	0.05–0.39	0.10	0.10
	Sparfloxacin	≦0.006–0.05	0.013	0.025
	Tosufloxacin	≦0.006–0.05	0.013	0.025
Neisseria gonorrhoeae (40)	AM-1155	≦0.006–0.05	≦0.006	0.025
	Norfloxacin	≦0.006–0.78	0.025	0.20
	Ciprofloxacin	≦0.006–0.10	≦0.006	0.025
	Ofloxacin	≦0.006–0.39	0.013	0.05
	Sparfloxacin	≦0.006–0.20	≦0.006	0.025
	Tosufloxacin	≦0.006–0.10	≦0.006	0.025
Bacteroides fragilis (35)	AM-1155	0.10–12.5	0.78	3.13
	Norfloxacin	25–>100	50	>100
	Ciprofloxacin	3.13–100	12.5	50
	Ofloxacin	0.78–100	3.13	25
	Sparfloxacin	0.78–6.25	1.56	3.13
	Tosufloxacin	0.20–6.25	0.78	3.13

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TABLE 1—*Continued*

Organism (no. tested)	Compound	MIC (μg/ml)[a]		
		Range	50%	90%
Clostridium difficile (19)	AM-1155	0.78–1.56	0.78	1.56
	Norfloxacin	25–50	25	50
	Ciprofloxacin	6.25–12.5	12.5	12.5
	Ofloxacin	12.5–12.5	12.5	12.5
	Sparfloxacin	6.25–6.25	6.25	6.25
	Tosufloxacin	1.56–1.56	1.56	1.56
Clostridium perfringens (16)	AM-1155	0.20–0.78	0.39	0.39
	Norfloxacin	0.78–25	1.56	6.25
	Ciprofloxacin	0.20–12.5	0.39	1.56
	Ofloxacin	0.78–3.13	0.78	3.13
	Sparfloxacin	0.05–1.56	0.20	1.56
	Tosufloxacin	0.05–0.78	0.20	0.78

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
[b] Methicillin MIC, ≧12.5 μg/ml.
[c] Norfloxacin MIC, ≧12.5 μg/ml; ciprofloxacin MIC, ≧3.13 μg/ml.

MBC was defined as the lowest drug concentration that produced 99.9% or greater killing of the initial inoculum.

Time-kill studies. Mid-logarithmic-phase cells (approximately 10^6 CFU/ml) were exposed to test drugs at a concentration of one-fourth, one-half, one, two, or four times the MIC. Duplicate 50-μl aliquots were removed at fixed times, serial 10-fold dilutions were prepared in saline, and the solution was mixed with 10 ml of drug-free SDA. Drug carryover did not affect colony formation since samples were diluted 200-fold with SDA. The number of colonies was determined after incubation at 37°C for 24 h. The limit of detection for this procedure was 20 CFU/ml.

Frequency of spontaneous mutation. The frequency of occurrence of spontaneous mutation to quinolone resistance in *Staphylococcus aureus* Smith, *Escherichia coli* ML4707, and *P. aeruginosa* GN11189 was determined by spreading each test organism in triplicate onto SDA plates containing drug at concentrations of two and four times the corresponding MIC. After incubation at 37°C for 48 h, the colonies were counted and the mutation frequency was calculated as the ratio of the number of resistant cells to the number of cells inoculated.

DNA gyrase inhibition. DNA gyrases from *E. coli* KL-16 (4) and *S. aureus* SA113 (13) were prepared by a method described previously. One unit of gyrase activity was defined as the amount of enzyme that catalyzed the conversion of 50% of the relaxed closed-circular pBR322 DNA to the supercoiled form in 1 h at 37°C. The specific activity of DNA gyrase from *E. coli* KL-16 was 1.3×10^3 U/mg of protein, and those of purified subunits A and B from *S. aureus* SA113 were 4.0×10^3 and $>2.0 \times 10^4$ U/mg of protein, respectively. A reaction mixture containing subunits A and B (1 U each), a drug solution, and plasmid pBR322 relaxed by topoisomerase I (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were incubated for 1 h at 37°C. The reaction mixture was analyzed as reported previously (1).

The antibacterial activities of AM-1155 and other new quinolones against recent clinical isolates are given in Table 1. The MICs of AM-1155 for 90% of staphylococci except methicillin-resistant and quinolone-resistant strains, streptococci, and *Enterococcus faecalis* tested (MIC_{90}s) ranged from 0.10 to 0.78 μg/ml. This activity was greater than those of norfloxacin, ciprofloxacin, and ofloxacin and was almost the same as those of sparfloxacin and tosufloxacin. The MIC_{90}s of AM-1155 for methicillin-resistant and quinolone-resistant *S. aureus* were 6.25 and 12.5 μg/ml, respectively. Its activity was comparable to those of sparfloxacin and tosufloxacin. All quinolones tested were inactive against *E. faecium*. The MIC_{90}s of AM-1155 for members of the family *Enterobacteriaceae* were less than or equal to 0.39 μg/ml except for those for *Citrobacter freundii*, *Providencia rettgeri*, and *Serratia marcescens*, for which they were 1.56, 1.56, and 12.5 μg/ml, respectively. The MIC_{90}s of AM-1155 for *P. aeruginosa*, *Pseudomonas cepacia*, and *Xanthomonas maltophilia* were 6.25, 12.5, and 1.56 μg/ml, respectively. AM-1155 inhibited 90% of *Bacteroides fragilis*, *Clostridium difficile*, and *Clostridium perfringens* strains at concentrations of 3.13, 1.56, and 0.39 μg/ml, respectively. *H. influenzae* was highly susceptible to AM-1155 and tosufloxacin in comparison with its susceptibility to other quinolones. The MIC_{90}s of both compounds for *H. influenzae* were 0.013 μg/ml. AM-1155 showed a high degree of activity against *N. gonorrhoeae*, as did ciprofloxacin, sparfloxacin, and tosufloxacin. The MIC_{90}s of the four drugs for this species were 0.025 μg/ml. The antibacterial activity of AM-1155 was similar to those of sparfloxacin and tosufloxacin and was greater than those of norfloxacin, ciprofloxacin, and ofloxacin against gram-positive bacteria. Against gram-negative bacteria, the antibacterial activity of AM-1155 was similar to that of sparfloxacin and was slightly less than those of ciprofloxacin and tosufloxacin. Against anaerobes, AM-1155 was the most active quinolone.

The MICs and MBCs of AM-1155 and reference compounds are given in Table 2. The MBCs of AM-1155 were equal to or twofold greater than the MICs for *S. aureus* and *E. coli* and were two- to fourfold greater than the MICs for *P. aeruginosa*. Similar results were obtained with the other quinolones tested.

Rapid killing was observed in *S. aureus* Smith, *E. coli* NIHJ JC-2, and *P. aeruginosa* GN11189 at one- to fourfold the MIC of AM-1155 (Fig. 1). AM-1155 reduced the proportion of viable cells of all organisms tested to less than 1% of the inoculum within 2 or 6 h at concentrations equal to the MIC. Against *E. coli* NIHJ JC-2, the bactericidal activity of AM-1155 at the MIC was the greatest among the quinolones tested.

The frequency of occurrence of spontaneous mutants resistant to AM-1155, norfloxacin, ciprofloxacin, sparfloxacin, and tosufloxacin was studied. The mutation frequencies in *S. aureus* Smith, *E. coli* ML4707, and *P. aeruginosa* GN11189 at a concentration of two times the MIC of AM-1155 were 2.17×10^{-7}, 1.38×10^{-9}, and 3.11×10^{-7}, respectively. At two

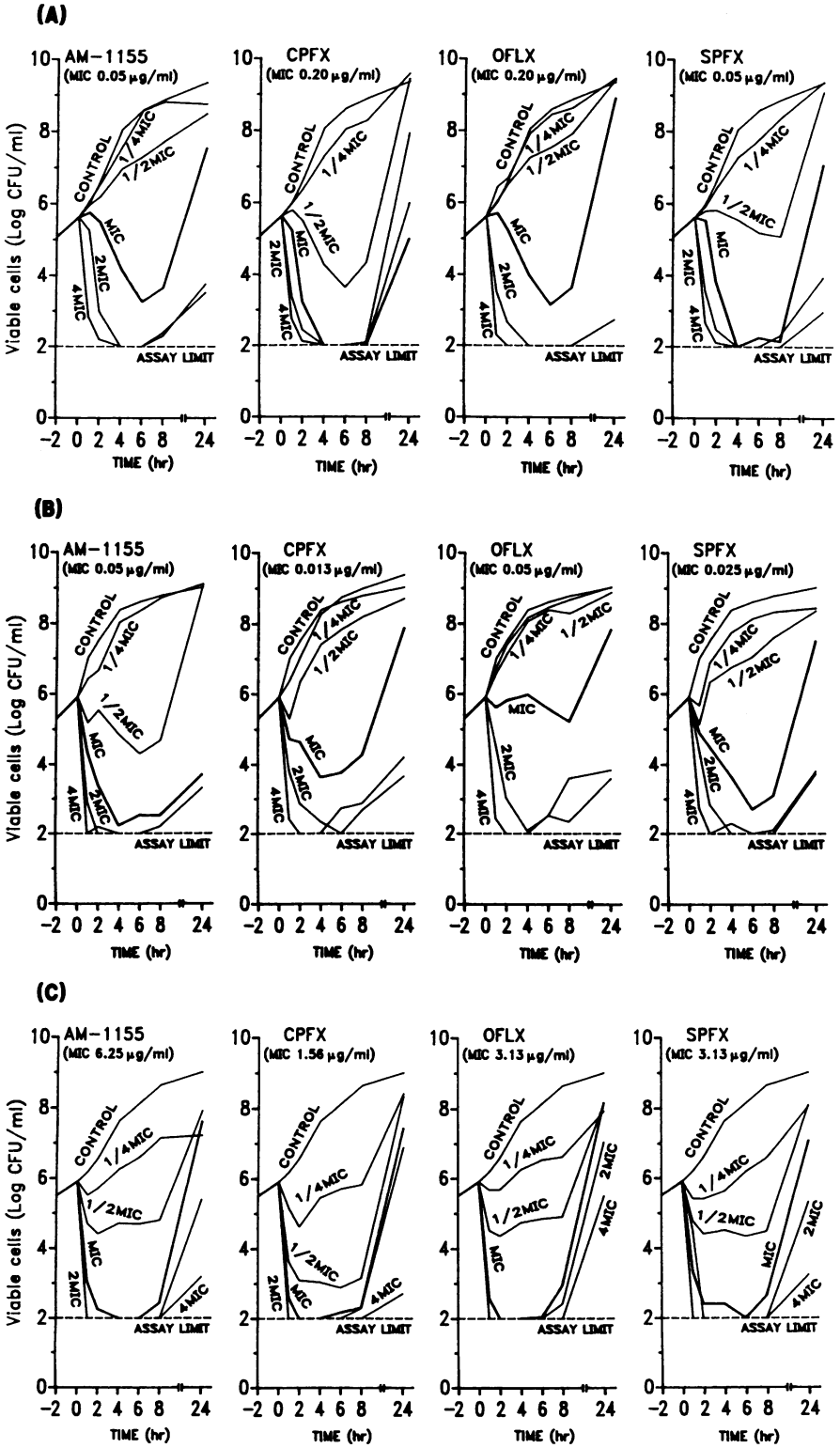

FIG. 1. Bactericidal activities of AM-1155, ciprofloxacin (CPFX), ofloxacin (OFLX), and sparfloxacin (SPFX) against *S. aureus* Smith (A), *E. coli* NIHJ JC-2 (B), and *P. aeruginosa* GN11189 (C).

TABLE 2. Activities of AM-1155 and reference compounds against recent clinical isolates

Organism (no. tested)	Compound	MIC (μg/ml)[a]			MBC (μg/ml)[a]		
		Range	50%	90%	Range	50%	90%
S. aureus (22)	AM-1155	0.05–0.20	0.10	0.20	0.10–0.39	0.20	0.39
	Norfloxacin	0.78–12.5	1.56	3.13	1.56–12.5	3.13	6.25
	Ciprofloxacin	0.20–3.13	0.78	0.78	0.39–3.13	0.78	1.56
	Ofloxacin	0.20–0.78	0.39	0.78	0.39–1.56	0.78	0.78
	Sparfloxacin	0.05–0.20	0.05	0.10	0.05–0.20	0.20	0.20
	Tosufloxacin	0.025–0.10	0.05	0.05	0.025–0.10	0.05	0.10
E. coli (24)	AM-1155	0.025–0.10	0.025	0.10	0.025–0.10	0.05	0.10
	Norfloxacin	0.025–0.39	0.10	0.20	0.025–0.39	0.10	0.20
	Ciprofloxacin	0.006–0.05	0.013	0.05	0.006–0.05	0.025	0.05
	Ofloxacin	0.025–0.20	0.10	0.20	0.05–0.20	0.10	0.20
	Sparfloxacin	0.013–0.10	0.025	0.05	0.025–0.10	0.05	0.10
	Tosufloxacin	0.013–0.10	0.025	0.05	0.013–0.10	0.05	0.05
P. aeruginosa (24)	AM-1155	0.39–6.25	1.56	3.13	0.78–12.5	6.25	12.5
	Norfloxacin	0.78–12.5	1.56	3.13	0.78–12.5	3.13	6.25
	Ciprofloxacin	0.10–3.13	0.39	0.78	0.20–12.5	0.78	3.13
	Ofloxacin	0.78–12.5	3.13	6.25	1.56–50	6.25	12.5
	Sparfloxacin	0.39–6.25	1.56	3.13	0.78–25	6.25	12.5
	Tosufloxacin	0.20–3.13	0.78	1.56	0.39–6.25	1.56	3.13

[a] 50% and 90%, MICs or MBCs for 50 and 90% of recent clinical isolates of each organism tested, respectively.

times the MIC, the frequency of occurrence of spontaneous mutation to resistance to AM-1155 in *S. aureus* Smith was comparable to that to resistance to sparfloxacin. At a concentration of four times the MIC, no mutants of *S. aureus* Smith, *E. coli* ML4707, or *P. aeruginosa* GN11189 resistant to AM-1155 were detected. The mutation frequencies to AM-1155 resistance for all organisms were comparable to or slightly lower than those observed for sparfloxacin and tosufloxacin at a concentration of four times the MIC.

The 50% inhibitory concentrations of AM-1155, ciprofloxacin, and sparfloxacin against the gyrase from *E. coli* KL-16 were 0.64, 1.02, and 0.69 μg/ml, respectively (Table 3). The 50% inhibitory concentrations of AM-1155, ciprofloxacin, and sparfloxacin against the enzyme from *S. aureus* SA113 were 10.5, 55.6, and 35.6 μg/ml, respectively. The inhibitory activity of AM-1155 against the gyrase from *E. coli* KL-16 was similar to those of ciprofloxacin and sparfloxacin. However, AM-1155 inhibited the supercoiling activity of the DNA gyrase purified from *S. aureus* SA113 at a two- to fourfold greater degree than ciprofloxacin and sparfloxacin did.

AM-1155 has a broad spectrum of antibacterial activity against gram-positive and gram-negative bacteria and anaerobes. The in vitro antibacterial activities of AM-1155 described here are in general agreement with those previously reported for this compound (8).

Cross-resistance between AM-1155 and β-lactam antibiotics or aminoglycosides was not observed (data not shown), as reported previously (14). However, partial cross-resistance was observed between quinolones, as reported previously (2, 9, 18).

AM-1155 was bactericidal against organisms in the midlogarithmic growth phase, which is similar to other quinolones. It has been reported that nalidixic acid has a high frequency of mutation and that resistance to nalidixic acid is easily developed (5). However, organisms treated with AM-1155 showed low frequencies of mutation at concentrations of more than two times the MIC for *S. aureus*, *E. coli*, and *P. aeruginosa*. These results were similar to those reported for other quinolones (15).

Sato et al. (16) reported that new quinolones potently inhibit *E. coli* DNA gyrase with a greater degree of activity than nalidixic acid does. Results of our present study show that AM-1155 inhibits the ATP-dependent supercoiling activity of *E. coli* KL-16 and *S. aureus* SA113 DNA gyrases at concentrations lower than those of ciprofloxacin and sparfloxacin. Although the modes of action of quinolone antimicrobial agents have not been characterized fully, their activities seem to be dependent on the inhibition of DNA gyrase. It seems that AM-1155 inhibits DNA gyrase activity at the same level as the reference quinolones do.

AM-1155 demonstrates good absorption and a good pharmacokinetic profile after oral administration (for a 200-mg dose of AM-1155, the maximum concentration of drug in serum is 1.71 μg/ml and the half-life is 7.1 h) (6). These data suggest that AM-1155 may have clinical efficacy against urinary tract and systemic infections caused by various bacteria.

TABLE 3. Inhibition of supercoiling activities of *E. coli* and *S. aureus* DNA gyrases by AM-1155

Compound	*E. coli* KL-16		*S. aureus* SA113	
	MIC (μg/ml)	IC_{50} (μg/ml)[a]	MIC (μg/ml)	IC_{50} (μg/ml)
AM-1155	0.025	0.64	0.05	10.5
Ciprofloxacin	0.006	1.02	0.39	55.6
Sparfloxacin	0.006	0.69	0.05	35.6

[a] IC_{50}, 50% inhibitory concentration.

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REFERENCES

1. **Aoyama, H., K. Sato, T. Fujii, K. Fujimaki, M. Inoue, and S. Mitsuhashi.** 1988. Purification of *Citrobacter freundii* DNA gyrase and inhibition by quinolones. Antimicrob. Agents Chemother. **32:**104–109.

2. **Crumplin, G. C., M. Kenwright, and T. Hirst.** 1984. Investigations into the mechanism of action of the antibacterial agent norfloxacin. J. Antimicrob. Chemother. **13**(Suppl. B):9–23.
3. **Fujimaki, K., T. Noumi, I. Saikawa, M. Inoue, and S. Mitsuhashi.** 1988. In vitro and in vivo antibacterial activity of T-3262, a new fluoroquinolone. Antimicrob. Agents Chemother. **32:**827–833.
4. **Hane, M. W., and T. H. Wood.** 1969. *Escherichia coli* K-12 mutants resistant to nalidixic acid: genetic mapping and dominance studies. J. Bacteriol. **99:**238–241.
5. **Hirai, K., H. Aoyama, M. Hosaka, Y. Oomori, Y. Niwata, S. Suzue, and T. Irikura.** 1986. In vitro and in vivo antibacterial activity of AM-833, a new quinolone derivative. Antimicrob. Agents Chemother. **29:**1059–1066.
6. **Hirai, K., M. Hosaka, E. Wakabayashi, and K. Nishino.** 1992. Antibacterial activity of AM-1155 using an *in vitro* pharmacokinetic model. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 999.
7. **Hirose, T., E. Okezaki, H. Kato, Y. Ito, M. Inoue, and S. Mitsuhashi.** 1987. In vitro and in vivo activity of NY-198, a new difluorinated quinolone. Antimicrob. Agents Chemother. **31:**854–859.
8. **Hosaka, M., T. Yasue, H. Fukuda, H. Tomizawa, H. Aoyama, and K. Hirai.** 1992. In vitro and in vivo antibacterial activity of AM-1155, a new 6-fluoro-8-methoxy quinolone. Antimicrob. Agents Chemother. **36:**2108–2117.
9. **Inoue, S., J. Yamagishi, S. Nakamura, Y. Furutani, and M. Shimizu.** 1982. Novel nalidixic acid-resistance mutations relating to DNA gyrase activity, p. 411–414. *In* S. Mitsuhashi (ed.), Drug resistance in bacteria. Thieme-Stratton, Inc., New York.
10. **Ito, A., K. Hirai, M. Inoue, H. Koga, S. Suzue, T. Irikura, and S. Mitsuhashi.** 1980. In vitro antibacterial activity of AM-715, a new nalidixic acid analog. Antimicrob. Agents Chemother. **17:**103–108.
11. **King, A., C. Warren, K. Shannon, and I. Philips.** 1982. In vitro antibacterial activity of norfloxacin (MK-0366). Antimicrob. Agents Chemother. **21:**604–607.
12. **Kojima, T., M. Inoue, and S. Mitsuhashi.** 1989. In vitro activity of AT-4140 against clinical bacterial isolates. Antimicrob. Agents Chemother. **33:**1980–1988.
13. **Nakanishi, N., S. Yoshida, H. Wakebe, M. Inoue, T. Yamaguchi, and S. Mitsuhashi.** 1991. Mechanisms of clinical resistance to fluoroquinolones in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **35:**2562–2567.
14. **Neu, H. C., and P. Labthavikul.** 1982. In vitro activity of norfloxacin, a quinolinecarboxylic acid, compared with that of β-lactams, aminoglycosides, and trimethoprim. Antimicrob. Agents Chemother. **22:**23–27.
15. **Neu, H. C., A. Novelli, and N. X. Chin.** 1989. Comparative in vitro activity of a new quinolone, AM-1091. Antimicrob. Agents Chemother. **33:**1036–1041.
16. **Sato, K., Y. Inoue, T. Fujii, H. Aoyama, M. Inoue, and S. Mitsuhashi.** 1986. Purification and properties of DNA gyrase from a fluoroquinolone-resistant strain of *Escherichia coli*. Antimicrob. Agents Chemother. **30:**777–780.
17. **Sato, K., Y. Matsuura, M. Inoue, T. Une, Y. Osada, H. Ogawa, and S. Mitsuhashi.** 1982. In vitro and in vivo activity of DL-8280, a new oxazine derivative. Antimicrob. Agents Chemother. **22:**548–553.
18. **Traub, W. H.** 1985. Incomplete cross-resistance of nalidixic acid and pipemidic acid-resistant variants of *Serratia marcescens* against ciprofloxacin, enoxacin, and norfloxacin. Chemotherapy (Basel) **31:**34–39.
19. **Wakabayashi, E., and S. Mitsuhashi.** 1991. Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1493.
20. **Wise, R., J. M. Andrews, and L. J. Edwards.** 1983. In vitro activity of Bay 09867, a new quinolone derivative, compared with those of other antimicrobial agents. Antimicrob. Agents Chemother. **23:**559–564.