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Comparative In Vitro Activities of a New Quinolone, OPC-17116, Possessing Potent Activity against Gram-Positive Bacteria

HIROKAZU WAKEBE†* AND SUSUMU MITSUHASHI

Episome Institute, Fujimimura, Seta-Gun, Gunma 371-01, Japan

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The in vitro antibacterial activity of OPC-17116, a new fluoroquinolone, against a wide variety of clinical isolates was evaluated and compared with those of ciprofloxacin, ofloxacin, and norfloxacin. OPC-17116 showed potent broad-spectrum activity against gram-positive and -negative bacteria. The activity of this compound against gram-positive bacteria was higher than those of other quinolones, and its activity against gram-negative and anaerobic bacteria was roughly comparable to those of other quinolones. OPC-17116 had potent activity against important pathogens of respiratory tract infections such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Branhamella catarrhalis*. The MICs of this compound against 90% of these organisms, except for methicillin-resistant *S. aureus*, ranged from ≦0.006 to 3.13 μg/ml. OPC-17116 at more than one-half the MICs was bactericidal against clinical isolates of *S. aureus*, *Escherichia coli*, *K. pneumoniae*, and *P. aeruginosa*. The activity of OPC-17116 was decreased by several culture conditions such as acidic pH, high concentration of Mg^{2+} ions, and inoculum size of 10^7 CFU/ml. OPC-17116 inhibited the supercoiling activity of DNA gyrases from *E. coli* KL-16 and *S. aureus* SA113 (50% inhibitory concentrations, 0.19 and 23.0 μg/ml, respectively). The amount of OPC-17116 accumulation was higher than that of other quinolones in *S. aureus*.

A number of new quinolone antibacterial agents such as norfloxacin (12), ofloxacin (22), and ciprofloxacin (27) have been developed and introduced into the market. These drugs have broad spectra and potent activities against gram-positive and gram-negative bacteria.

OPC-17116 [(±)-1-cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid hydrochloride] is a new quinolone antibacterial agent synthesized at Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan. This compound has one methyl residue at the 5 position of the quinolone core and one at the 3 position of the piperazine moiety. In an earlier study (10), in which this agent was compared with ofloxacin, enoxacin, ciprofloxacin, and tosufloxacin, good in vitro and in vivo activities were demonstrated. Those researchers also reported that the peak level of OPC-17116 in the lungs of mice was significantly higher than the levels in lungs achieved with an equivalent dose of other quinolones. In this report, we describe the in vitro antibacterial activity, bactericidal activity, and inhibition of DNA gyrase supercoiling activity of OPC-17116 in comparison with those of ciprofloxacin, ofloxacin, and norfloxacin. Furthermore, we report the amounts of uptake of these quinolones in *Staphylococcus aureus*.

MATERIALS AND METHODS

Drugs. OPC-17116 was provided by Otsuka Pharmaceutical Co. The other drugs used in the experiment were obtained from the following sources: ciprofloxacin, Bayer Yakuhin, Ltd., Osaka, Japan; ofloxacin, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan; norfloxacin, Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan; methicillin, Banyu Pharmaceutical Co., Ltd., Tokyo, Japan.

Organisms. Bacterial strains used in this study were reference strains and clinical isolates collected from several hospitals and laboratories in Japan between 1985 and 1990. All isolates were maintained at the Episome Institute.

Susceptibility tests. The antibacterial activities of the drugs were determined by the twofold agar dilution method with Sensitivity Disk Agar-N (SDA; Nissui Pharmaceutical, Tokyo, Japan), which is modified Mueller-Hinton agar, unless specified otherwise. SDA supplemented with 10% defibrinated horse blood and 5% Fildes enrichment (Difco Laboratories, Detroit, Mich.) was used for growth of streptococci and *Haemophilus influenzae*, respectively. GC agar (Difco) supplemented with 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) was used for *Neisseria gonorrhoeae*. GAM agar (Nissui) was used for obligate anaerobes. Overnight broth cultures of the bacterial strains were diluted with corresponding fresh broth to a final concentration of approximately 10^6 CFU/ml, and an inoculum of 10^4 CFU per spot was applied with an inoculating apparatus (Microplanter; Sakuma Seisakusho, Tokyo, Japan) to agar plates containing graded concentrations of drug. The plates were incubated at 37°C for 18 h except for *N. gonorrhoeae*, which was incubated in a candle jar, and obligate anaerobes, which were incubated in an anaerobic chamber. The MIC was defined as the lowest concentration of drug that inhibited visible growth on the plate.

Determination of bactericidal activity. Bactericidal activities of drugs were determined by MBC and time-kill studies as described previously (14). Overnight cultures of bacterial strains, for which sensitivity test broth (Nissui) was used, were diluted with fresh sensitivity test broth to about 10^6 CFU/ml, and 1.8 ml of the dilution was added to 0.2 ml of the drug solution in a clear tube and incubated without shaking for 18 h. After determination of the MIC, 0.1 ml of the

* Corresponding author.
† Present address: Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-01 Japan.

TABLE 1. Antibacterial activities of OPC-17116 and other quinolones against aerobic and anaerobic bacteria

Organism (no. of isolates)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (106)	OPC-17116	0.0125–0.2	0.05	0.1
	Ciprofloxacin	0.1–6.25	0.39	0.78
	Ofloxacin	0.2–1.56	0.39	0.39
	Norfloxacin	0.2–25	1.56	3.13
Staphylococcus aureus, methicillin resistant (147)	OPC-17116	0.0125–25	0.2	12.5
	Ciprofloxacin	0.2–>100	6.25	50
	Ofloxacin	0.2–100	1.56	25
	Norfloxacin	0.39–>100	50	>100
	Methicillin	12.5–>100	>100	>100
Staphylococcus epidermidis (110)	OPC-17116	0.025–0.2	0.05	0.1
	Ciprofloxacin	0.1–1.56	0.2	0.39
	Ofloxacin	0.2–0.78	0.39	0.39
	Norfloxacin	0.39–12.5	0.78	0.78
Streptococcus pyogenes (100)	OPC-17116	0.1–0.78	0.39	0.39
	Ciprofloxacin	0.2–3.13	0.39	0.78
	Ofloxacin	0.39–3.13	1.56	1.56
	Norfloxacin	0.78–25	1.56	3.13
Streptococcus pneumoniae (44)	OPC-17116	0.05–12.5	0.39	0.39
	Ciprofloxacin	0.39–50	1.56	6.25
	Ofloxacin	0.78–50	3.13	6.25
	Norfloxacin	3.13–>100	12.5	50
Enterococcus faecalis (103)	OPC-17116	0.1–0.78	0.2	0.39
	Ciprofloxacin	0.39–3.13	0.78	1.56
	Ofloxacin	0.78–6.25	1.56	3.13
	Norfloxacin	1.56–25	3.13	6.25
Enterococcus faecium (95)	OPC-17116	0.1–50	3.13	12.5
	Ciprofloxacin	0.2–>100	3.13	50
	Ofloxacin	0.78–>100	6.25	100
	Norfloxacin	0.78–>100	6.25	>100
Escherichia coli (89)	OPC-17116	0.025–3.13	0.05	0.1
	Ciprofloxacin	≦0.006–1.56	0.025	0.025
	Ofloxacin	0.025–12.5	0.1	0.1
	Norfloxacin	0.05–6.25	0.1	0.2
Klebsiella pneumoniae (108)	OPC-17116	0.025–0.78	0.05	0.1
	Ciprofloxacin	≦0.006–0.39	0.05	0.05
	Ofloxacin	0.05–1.56	0.1	0.2
	Norfloxacin	0.05–3.13	0.2	0.39
Klebsiella oxytoca (104)	OPC-17116	0.0125–0.2	0.05	0.1
	Ciprofloxacin	≦0.006–0.1	0.05	0.05
	Ofloxacin	0.025–0.39	0.1	0.2
	Norfloxacin	0.025–0.39	0.2	0.39
Enterobacter cloacae (102)	OPC-17116	≦0.006–6.25	0.05	0.2
	Ciprofloxacin	≦0.006–6.25	0.025	0.2
	Ofloxacin	0.025–12.5	0.1	0.78
	Norfloxacin	0.05–25	0.1	1.56
Serratia marcescens (108)	OPC-17116	0.1–>100	0.78	25
	Ciprofloxacin	0.0125–50	0.39	12.5
	Ofloxacin	0.1–>100	1.56	25
	Norfloxacin	0.05–>100	1.56	100
Citrobacter freundii (97)	OPC-17116	0.025–50	0.1	1.56
	Ciprofloxacin	≦0.006–25	0.025	0.39
	Ofloxacin	0.05–50	0.2	1.56
	Norfloxacin	0.05–50	0.1	1.56
Shigella spp. (108)	OPC-17116	≦0.006–0.39	0.025	0.05
	Ciprofloxacin	≦0.006–0.39	0.0125	0.025
	Ofloxacin	0.025–0.78	0.05	0.1
	Norfloxacin	0.025–0.78	0.05	0.1
Salmonella spp. (108)	OPC-17116	0.0125–0.2	0.05	0.1
	Ciprofloxacin	0.0125–0.05	0.025	0.025
	Ofloxacin	0.05–0.2	0.1	0.2
	Norfloxacin	0.05–0.39	0.1	0.2
Proteus mirabilis (103)	OPC-17116	0.05–50	0.39	0.39
	Ciprofloxacin	0.0125–6.25	0.05	0.05
	Ofloxacin	0.05–12.5	0.2	0.2
	Norfloxacin	0.05–12.5	0.1	0.1

Continued on following page

TABLE 1—*Continued*

Organism (no. of isolates)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Proteus vulgaris (95)	OPC-17116	0.05–3.13	0.2	0.78
	Ciprofloxacin	0.025–0.39	0.05	0.1
	Ofloxacin	0.05–1.56	0.1	0.39
	Norfloxacin	0.05–0.39	0.05	0.1
Providencia rettgeri (54)	OPC-17116	0.025–12.5	0.2	3.13
	Ciprofloxacin	≦0.006–6.25	0.1	0.78
	Ofloxacin	0.05–25	0.39	3.13
	Norfloxacin	0.05–6.25	0.2	1.56
Providencia stuartii (75)	OPC-17116	0.025–3.13	0.2	0.39
	Ciprofloxacin	0.025–1.56	0.2	0.78
	Ofloxacin	0.05–3.13	0.39	1.56
	Norfloxacin	0.05–6.25	0.78	3.13
Morganella morganii (71)	OPC-17116	0.05–12.5	0.2	0.39
	Ciprofloxacin	≦0.006–25	0.0125	0.025
	Ofloxacin	0.05–25	0.1	0.39
	Norfloxacin	0.025–100	0.05	0.1
Pseudomonas aeruginosa (108)	OPC-17116	0.2–>100	0.78	3.13
	Ciprofloxacin	0.05–100	0.2	0.78
	Ofloxacin	0.39–>100	1.56	6.25
	Norfloxacin	0.39–>100	0.78	3.13
Pseudomonas cepacia (48)	OPC-17116	0.78–100	12.5	25
	Ciprofloxacin	3.13–>100	12.5	25
	Ofloxacin	6.25–>100	25	25
	Norfloxacin	12.5–>100	50	100
Xanthomonas maltophilia (49)	OPC-17116	0.2–6.25	0.39	0.78
	Ciprofloxacin	1.56–25	3.13	6.25
	Ofloxacin	0.78–12.5	3.13	3.13
	Norfloxacin	6.25–100	25	50
Acinetobacter calcoaceticus (43)	OPC-17116	0.025–0.39	0.05	0.39
	Ciprofloxacin	0.05–3.13	0.2	0.78
	Ofloxacin	0.05–3.13	0.2	0.78
	Norfloxacin	0.78–25	3.13	12.5
Haemophilus influenzae (74)	OPC-17116	≦0.006–0.05	≦0.006	≦0.006
	Ciprofloxacin	≦0.006–0.05	≦0.006	0.0125
	Ofloxacin	≦0.006–0.1	0.025	0.05
	Norfloxacin	0.025–0.2	0.05	0.05
Branhamella catarrhalis (42)	OPC-17116	≦0.006–0.05	0.025	0.025
	Ciprofloxacin	0.025–0.2	0.05	0.1
	Ofloxacin	0.05–0.2	0.1	0.1
	Norfloxacin	0.1–0.39	0.2	0.39
Neisseria gonorrhoeae (47)	OPC-17116	≦0.006–0.2	0.0125	0.05
	Ciprofloxacin	≦0.006–0.2	≦0.006	0.05
	Ofloxacin	≦0.006–0.39	0.025	0.1
	Norfloxacin	0.0125–1.56	0.025	0.2
Bacteroides fragilis (35)	OPC-17116	1.56–50	3.13	12.5
	Ciprofloxacin	6.25–>100	12.5	50
	Ofloxacin	1.56–100	3.13	25
	Norfloxacin	50–>100	100	>100
Clostridium difficile (26)	OPC-17116	25–50	25	50
	Ciprofloxacin	12.5	12.5	12.5
	Ofloxacin	12.5	12.5	12.5
	Norfloxacin	50–100	100	100
Clostridium perfringens (16)	OPC-17116	0.1–3.13	0.39	0.78
	Ciprofloxacin	0.2–3.13	0.39	0.78
	Ofloxacin	0.39–3.13	0.78	0.78
	Norfloxacin	0.78–6.25	1.56	3.13

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

growth-negative cultures were mixed with 10 ml of melted SDA. The plates were incubated at 37°C for 48 h, and the MBC was defined as the lowest drug concentration that produced >99.9% killing of the initial cell number in the inoculum. The time-kill study determined the reduction of viable cells during incubation with the drugs in sensitivity test broth. An overnight culture was diluted with fresh broth to about 10^4 CFU/ml. After 2 h of incubation at 37°C with shaking (about 10^6 CFU/ml), drugs at various concentrations were added, and incubation continued. At timed intervals, portions were diluted appropriately with saline, and 0.1-ml portions of the samples or dilutions were mixed with melted SDA. After incubation at 37°C for 48 h, the colonies were counted. Prior to the study, we confirmed that drug carry-

TABLE 2. Bactericidal activities of OPC-17116 and other quinolones against clinical isolates[a]

Organism (n = 10 each)	Drug	MIC (μg/ml)			MBC (μg/ml)		
		Range	50%	90%	Range	50%	90%
S. aureus	OPC-17116	0.025–0.1	0.05	0.1	0.05–0.2	0.1	0.2
	Ciprofloxacin	0.39–6.25	1.56	6.25	0.39–6.25	1.56	6.25
	Ofloxacin	0.39–1.56	0.39	1.56	0.78–1.56	0.78	1.56
E. coli	OPC-17116	0.0125–0.78	0.05	0.78	0.0125–0.78	0.05	0.78
	Ciprofloxacin	0.0125–0.78	0.025	0.78	0.0125–1.56	0.025	1.56
	Ofloxacin	0.1–6.25	0.1	6.25	0.1–6.25	0.2	6.25
K. pneumoniae	OPC-17116	0.1–0.78	0.2	0.78	0.1–0.78	0.2	0.78
	Ciprofloxacin	0.05–1.56	0.1	1.56	0.05–1.56	0.1	1.56
	Ofloxacin	0.2–3.13	0.78	3.13	0.2–3.13	0.78	3.13
P. aeruginosa	OPC-17116	0.2–6.25	0.78	6.25	0.39–6.25	1.56	6.25
	Ciprofloxacin	0.2–1.56	0.39	1.56	0.39–3.13	0.78	3.13
	Ofloxacin	1.56–12.5	3.13	12.5	1.56–25	6.25	25

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

over did not affect colony formation. The effects of culture conditions (pH, magnesium concentrations, and inoculum size) on the MICs were determined by the broth dilution method described above.

Assay of DNA gyrase inhibition. DNA gyrase from *Escherichia coli* KL-16 (7) was prepared by the method described in a previous report (21), and that from *S. aureus* SA113 (11) was also prepared by the method described in a previous report (19). In short, *S. aureus* cells were treated with lysostaphin and lysozyme (Sigma Chemical Co., St. Louis, Mo.), and 1 M NaCl in cell lysate was added for extraction of the enzyme from DNA. After precipitation of DNA by Polymin P (Bethesda Research Laboratories, Ltd.), the crude cell extract was loaded on a novobiocin-Sepharose column (23), and fractions of DNA gyrase subunits A and B were obtained. The reaction conditions for DNA supercoiling activity were performed according to the modifications described in previous reports (5, 6, 18). One unit of enzyme activity was defined as the amount that converts 50% of relaxed pBR322 DNA to the supercoiled form at 37°C for 60 min as detected by agarose gel electrophoresis. The specific activities 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. The reaction mixture (20 μl) contained 20 mM Tris hydrochloride (pH 7.8), 20 mM KCl, 2 mM $MgCl_2$, 2 mM spermidine, 1.5 mM ATP, 1 mM dithiothreitol, 30 μg of tRNA per ml, 15 μg of bovine serum albumin per ml, 10 μg of relaxed pBR322 DNA per ml, 1 U each of DNA gyrase subunits A and B, and appropriate drug solutions. The concentration of KCl was changed to 100 mM in studies with gyrase isolated from *S. aureus*. After 60 min of incubation at 37°C, the reaction was stopped by the addition of 3 μl of proteinase K (1 mg/ml; Sigma). The mixture was subjected to electrophoresis in a 0.8% agarose gel. The gel was stained in 0.5 μg of ethidium bromide per ml and photographed upon exposure to a UV transilluminator. The 50% inhibitory dose (IC_{50}) for supercoiling activity was determined by using a densitometric assay as described previously (1).

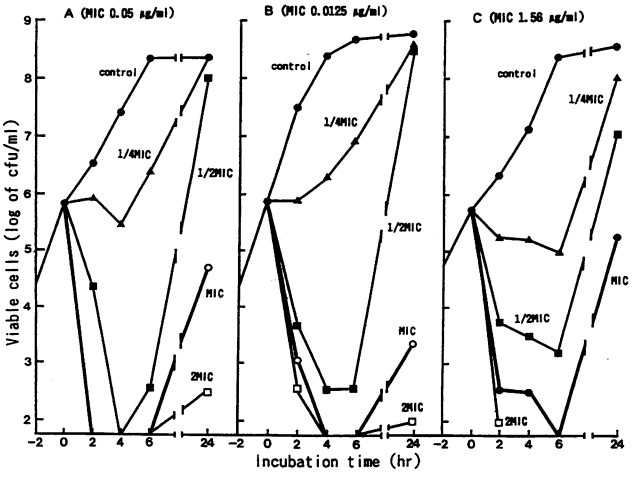

FIG. 1. Bactericidal activity of OPC-17116 against *S. aureus* Smith (A), *E. coli* ML4707 (B), and *P. aeruginosa* GN11189 (C).

Measurement of cell-associated drug in *S. aureus*. The uptake of ciprofloxacin, norfloxacin, ofloxacin, and OPC-17116 by *S. aureus* was examined by the method described in previous reports (8, 29). In this study, we used two strains, *S. aureus* SA113, which is susceptible to all quinolones, and *S. aureus* MS16401, which shows high-level resistance to norfloxacin (MIC, 100 μg/ml) and low-level resistance to ciprofloxacin and ofloxacin (MICs, 12.5 and 3.13 μg/ml, respectively) but susceptibility to OPC-17116 (MIC, 0.39 μg/ml). The quinolones were added to a final concentration of 10 μg/ml and incubated at 37°C for 20 min. Amounts of cell-associated quinolones were measured by high-performance liquid chromatography (Irica Instruments Inc., Kyoto, Japan) with a YMCA-312 column (Yamamura Chemical Laboratories Co., Ltd., Kyoto, Japan). The mobile phases were 5% acetic acid-methanol-acetonitrile (70:15:15 [vol/vol/vol]) for OPC-17116 and 5% acetic acid-methanol (80:20 [vol/vol]) for ciprofloxacin, ofloxacin, and norfloxacin.

TABLE 3. Effect of pH on activity of OPC-17116

Organism	Result (μg/ml) with sensitivity test broth at pH of:					
	6.0		7.0		8.0	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus 209P JC-1	0.2	0.39	0.05	0.1	0.05	0.1
E. coli NIHJ JC-2	0.05	0.05	0.0125	0.025	0.006	0.0125
P. vulgaris OX-19	0.78	1.56	0.1	0.1	0.05	0.05
S. marcescens IAM1184	6.25	6.25	0.78	0.78	0.39	0.39
P. aeruginosa IF03445	3.13	6.25	1.56	3.13	0.78	1.56

TABLE 4. Effect of Mg^{2+} on activity of OPC-17116

Organism	Result (μg/ml) in sensitivity test broth with Mg^{2+} concn of:							
	0 mM		3 mM		6 mM		9 mM	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus 209P JC-1	0.05	0.1	0.2	0.2	0.2	0.39	0.39	0.78
E. coli NIHJ JC-2	0.0125	0.025	0.05	0.05	0.05	0.1	0.1	0.2
P. vulgaris OX-19	0.1	0.1	0.78	0.78	0.78	0.78	0.78	1.56
S. marcescens IAM1184	0.78	0.78	1.56	1.56	1.56	1.56	1.56	1.56
P. aeruginosa IF03445	1.56	3.13	3.13	6.25	3.13	6.25	6.25	12.5

RESULTS

Antibacterial activity. The in vitro activities of OPC-17116, ciprofloxacin, ofloxacin, and norfloxacin against a variety of clinical isolates are shown in Table 1. OPC-17116 showed potent antibacterial activity against gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, and *Enterococcus* spp. in comparison with the other quinolones tested. The MIC of OPC-17116 for 90% of the isolates of *S. aureus* (methicillin susceptible), *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Enterococcus faecalis* were 0.1, 0.1, 0.39, 0.39, and 0.39 μg/ml, respectively. The MICs of OPC-17116 for 90% of these strains were 2- to 128-fold lower than those of the other agents tested. Some isolates were highly resistant to the tested quinolones, including OPC-17116. These isolates included methicillin-resistant *S. aureus* (MRSA), *S. pneumoniae*, and *Enterococcus faecium*. The percentages of quinolone-resistant strains (MIC, ≧6.25 μg/ml) in MRSA (147 strains) were as follows: 17% resistant to OPC-17116, 53% resistant to ciprofloxacin, 18% resistant to ofloxacin, and 74% resistant to norfloxacin. Against members of the family *Enterobacteriaceae* and *Pseudomonas* spp., OPC-17116 was equal to or slightly less active than ciprofloxacin but more active than ofloxacin and norfloxacin. OPC-17116 was less active than other quinolones against *Proteus* spp. and inhibited 90% of *Xanthomonas maltophilia*, *Acinetobacter calcoaceticus*, *H. influenzae*, *Branhamella catarrhalis*, and *N. gonorrhoeae* isolates at concentrations of ≦0.78 μg/ml. Against anaerobic bacteria, the activity of OPC-17116 was roughly equal to those of the other quinolones.

Bactericidal activity. The bactericidal activities of OPC-17116 were compared with those of ciprofloxacin and ofloxacin. For the determination of MBCs, we used 10 clinical strains each of *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (Table 2). The MBCs of OPC-17116, ciprofloxacin, and ofloxacin were equal to or only twice the respective MICs. The killing curve study was performed with *S. aureus* Smith, *E. coli* ML4707, and *P. aeruginosa* GN11189 (Fig. 1). A rapid decrease in the number of viable cells was observed at concentrations above one-half the MIC with all strains; all quinolones tested showed the same result. The effects on activity of alternating pH are shown in Table 3. The activity of OPC-17116 at pH 6.0 was less than that at pH 7.0 against all of the strains tested. The activity at pH 8.0 was the same or slightly better than that at pH 7.0. These results were also obtained with the other quinolones tested (data not shown). The effect of Mg^{2+} on the activity of OPC-17116 is shown in Table 4. The MICs and MBCs of OPC-17116 increased as the Mg^{2+} concentration increased, and the MICs and MBCs at a Mg^{2+} concentration of 9 mM were two- to eightfold higher than those at a Mg^{2+} concentration of 0 mM. The same results were obtained with the other quinolones tested. The effects of inoculum size on the MICs of OPC-17116 are shown in Table 5. There was a slight increase in the MICs at an inoculum of 10^7 CFU/ml compared with MICs at an inoculum of 10^5 CFU/ml.

Inhibitory effect on DNA gyrase. The IC_{50}s for supercoiling activities of *E. coli* KL-16 and *S. aureus* SA113 DNA gyrases are shown in Table 6. The inhibitory effect of OPC-17116 against *E. coli* was twofold less than that of ciprofloxacin and about twofold more than those of ofloxacin and norfloxacin. The IC_{50}s of the drugs tested correlated well with their respective MICs. On the other hand, the IC_{50}s of OPC-17116, ciprofloxacin, and ofloxacin in *S. aureus* were 23.0, 20.5, and 27.0 μg/ml, respectively, which were not parallel to the MICs.

Measurement of cell-associated drug in *S. aureus*. The amounts of cell-associated drug are shown in Table 7. The amounts of cell-associated drug in strain MS16401 were lower than those in SA113 with all quinolones tested. The amounts of cell-associated OPC-17116 in both strains were two- to fivefold higher than those of the other quinolones.

DISCUSSION

OPC-17116, a new quinolone antibacterial agent that demonstrated a broad spectrum and potent antibacterial activity against many gram-positive and gram-negative organisms, was tested in this study.

Like other quinolones, OPC-17116 was bactericidal against clinical isolates (Table 2) and rapidly killed the bacterial cells of standard strains at concentrations above one-half the MIC (Fig. 1). OPC-17116 was less active at an acidic pH than at a neutral pH, and its activity was lowered in the presence of high Mg^{2+} concentrations, as was observed with other quinolones (2). Furthermore, we showed that inoculum size affected the activity of OPC-17116 (Table 5). The degree of MIC increase at 10^7 CFU/ml compared with MICs at 10^3 and 10^5 CFU/ml was particularly noticeable with *Serratia marcescens* and *P. aeruginosa*. The same

TABLE 5. Effect of inoculum size on activity of OPC-17116

Organism	MIC (μg/ml) with inoculum size (CFU/ml) of:		
	10^3	10^5	10^7
S. aureus 209P JC-1	0.05	0.1	0.1
E. coli NIHJ JC-2	0.0125	0.025	0.05
P. vulgaris OX-19	0.05	0.05	0.1
S. marcescens IAM1184	0.2	0.39	1.56
P. aeruginosa IF03445	0.39	1.56	3.13

TABLE 6. Inhibitory effect of quinolones on DNA gyrase supercoiling activity

Organism	Drug	MIC (μg/ml)	IC_{50} (μg/ml)
S. aureus SA113	OPC-17116	0.05	23.0
	Ciprofloxacin	0.39	20.5
	Ofloxacin	0.39	27.0
	Norfloxacin	0.78	91.5
E. coli KL-16	OPC-17116	0.025	0.19
	Ciprofloxacin	0.0125	0.11
	Ofloxacin	0.05	0.48
	Norfloxacin	0.05	0.33

result was previously reported by Chin et al. for other quinolones (2).

Although the mode of action of quinolone antibacterial agents has not yet been characterized fully, their activities seem to depend on their inhibition of DNA gyrase. Accordingly, we determined the inhibition of DNA gyrase supercoiling activity with DNA gyrases isolated from *E. coli* KL-16 and *S. aureus* SA113 as representative strains of gram-negative and -positive bacteria. In the case of *E. coli* KL-16, inhibition of DNA gyrase by OPC-17116 and the other quinolones parallelled the respective MICs, as reported previously (4, 9, 21). The differences in inhibitory activities against DNA gyrase should be reflected in the difference in antibacterial activities (MICs) against *E. coli*. Recently, Nakanishi et al. (19) and Tanaka et al. (24) reported the inhibition of DNA gyrase from *S. aureus* by fluoroquinolones and showed that this inhibition was closely related to the antibacterial potencies (MICs) of these drugs. OPC-17116 inhibited the supercoiling activity of *S. aureus* SA113 DNA gyrase, as well as *E. coli* KL-16 DNA gyrase, but contrary to what might be predicted from MIC results, the IC_{50} was not lower than those of ciprofloxacin and ofloxacin. To explain this result, we determined the accumulation of the quinolones tested in two strains of *S. aureus*. OPC-17116 showed a higher amount of cell-associated drug than did the other quinolones tested (Table 7). We infer from this that antibacterial activity against gram-positive bacteria could be affected not only by the inhibition of DNA gyrase but also by the extent of accumulation of drug in the cells.

The emergence of MRSA has become a serious problem in the 1980s (13, 15, 25). Furthermore, after introduction of quinolone antibacterial agents for clinical use, MRSA rapidly acquired resistance to those agents as well (3, 16, 17). As OPC-17116 is a new quinolone antibacterial agent possessing potent antistaphylococcal activity, we determined the susceptibilities of 147 clinically isolated strains of MRSA, including quinolone-resistant strains, to this agent. More than 50% of the isolates were resistant (MIC, ≧6.25 μg/ml) to ciprofloxacin and norfloxacin, and more than 80% were susceptible to OPC-17116 and ofloxacin. OPC-17116 showed incomplete cross-resistance compared with ciprofloxacin and norfloxacin. This phenomenon was also previously reported with sparfloxacin (14). Moreover, Yosida et al. (28) reported that decreased quinolone uptake due to efflux of drug is one of the resistance mechanisms found in *S. aureus*, and they also speculated that drug lipophilicity might be related to permeation. Yosida et al. (28) reported that a *norA* gene cloned from a quinolone-resistant *S. aureus* strain (26) conferred relatively high resistance to hydrophilic quinolones such as norfloxacin, ciprofloxacin, and ofloxacin but not to hydrophobic quinolones such as nalidixic acid and sparfloxacin. They also showed that uptake of the hydrophilic quinolone enoxacin by *S. aureus* carrying a plasmid having the *norA* gene was about 50% that of the parent strain lacking the plasmid, whereas uptake of the hydrophobic quinolone sparfloxacin was similar in the two strains. In this study, we determined the uptake of quinolones by strain MS16401, which is susceptible to OPC-17116 but not to other quinolones. The level of OPC-17116 uptake in this strain remained relatively high compared with uptake of other quinolones. OPC-17116 is a moderately lipophilic drug compared with ciprofloxacin, ofloxacin, and norfloxacin (data not shown). This characteristic of OPC-17116 may be related to incomplete cross-resistance, as was observed for sparfloxacin (14) in quinolone-resistant MRSA.

TABLE 7. Cell-associated drug in *S. aureus*

Drug	MIC (μg/ml)[a]		Quinolone uptake at 20 min (μg/mg of cells [dry wt])[b]	
	SA113	MS16401	SA113	MS16401
OPC-17116	0.05	0.39	0.289 ± 0.0300	0.097 ± 0.0037
Ciprofloxacin	0.39	12.5	0.114 ± 0.0008	0.020 ± 0.0001
Ofloxacin	0.39	3.13	0.165 ± 0.0025	0.055 ± 0.0001
Norfloxacin	0.78	100	0.096 ± 0.0006	0.024 ± 0.0015

[a] With inoculum of 10^6 CFU/ml.
[b] Values are means ± standard deviations of the means (n = 3).

In this study, we investigated the in vitro activity of OPC-17116 extensively. We showed that OPC-17116 possesses a potent activity against bacteria known to cause respiratory tract infections, such as *S. aureus*, *S. pneumoniae*, *H. influenzae*, *K. pneumoniae*, *B. catarrhalis*, and *P. aeruginosa*. In addition to its antibacterial activity, another potential advantage of OPC-17116 is its high distribution in lung tissue, as reported previously in a pharmacokinetic study conducted with experimental animals (10, 20). OPC-17116 also showed good therapeutic efficacy in respiratory tract infections in an experimental animal model (10, 20). If this characteristic of OPC-17116 applies to humans, OPC-17116 may become an effective drug against respiratory tract infections. To determine the therapeutic role of OPC-17116, further clinical study is warranted.

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