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In Vitro and In Vivo Antibacterial Activities of a New Quinolone, OPC-17116

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The in vitro and in vivo antibacterial activities of OPC-17116 were compared with those of ofloxacin, enoxacin, ciprofloxacin, and tosufloxacin. The MICs of OPC-17116 for 90% of the strains tested were 0.125 to 8 μg/ml against gram-positive bacteria such as members of the genera *Staphylococcus*, *Streptococcus*, and *Enterococcus*: ≤0.063 to 16 μg/ml against members of the family *Enterobacteriaceae*; and ≤0.063 to 16 μg/ml against glucose-nonfermentative bacilli such as *Pseudomonas aeruginosa*. The activity of OPC-17116 against gram-positive organisms was comparable to that of tosufloxacin and higher than those of other reference drugs. The in vitro activity of OPC-17116 against gram-negative bacteria was similar to those of the reference drugs. In experimental systemic infections in mice with various organisms, the efficacy of OPC-17116 was similar to that of tosufloxacin and greater than those of ofloxacin, enoxacin, and ciprofloxacin. In a pyelonephritic model in mice with *P. aeruginosa* KU-1, OPC-17116 was as active as ciprofloxacin and more active than ofloxacin, enoxacin, and tosufloxacin. In respiratory tract infections in mice with *Staphylococcus aureus* Smith, *Streptococcus pneumoniae* TMS 3, and *Klebsiella pneumoniae* 3K25, the efficacy of OPC-17116 was generally greater than that of tosufloxacin. The peak level of OPC-17116 in the lungs of mice was 10 times higher than that in serum and was significantly greater than levels in lung achieved with an equivalent dose of the other quinolones. The therapeutic efficacy of OPC-17116 may depend not only on its in vitro activity but also on its high concentration in tissue.

Recently, new quinolone derivatives with a broad antibacterial spectrum, such as ofloxacin (9), enoxacin (6), ciprofloxacin (1), and tosufloxacin (2), have been developed and used extensively for the treatment of bacterial infections. However, one important problem with the new quinolones is their comparatively weak activity against gram-positive cocci, including staphylococci and streptococci. The search for quinolones which will overcome the resistance of *Staphylococcus aureus* to the current available quinolone derivatives must be continued.

OPC-17116 is a new quinolone having a methyl group at the 5-position (Fig. 1) which imparts broad-spectrum antibacterial activity. In this report, the in vitro and in vivo antibacterial activities of OPC-17116 are compared with those of ofloxacin, enoxacin, ciprofloxacin, and tosufloxacin.

MATERIALS AND METHODS

Antimicrobial agents. OPC-17116 was synthesized by the Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan. Other antimicrobial agents were provided as follows: ofloxacin, Daiichi Seiyaku Co., Ltd., Tokyo, Japan; enoxacin, Dainippon Pharmaceutical Co., Ltd., Osaka, Japan; ciprofloxacin, Bayer Yakuhin Co., Ltd., Tokyo, Japan; tosufloxacin, Toyama Chemical Co., Ltd., Tokyo, Japan.

Test strains. The bacterial strains used in this study were obtained between 1985 and 1990 from hospitals in several regions in Japan. All organisms were stocked frozen in the Department of Microbiology, Toho University School of Medicine, Tokyo, Japan.

Determination of MICs. MICs were determined by the broth microdilution method by the reference procedure recommended by the Japan Society for Chemotherapy (5). Test inocula were grown overnight in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) at 37°C. Unless otherwise specified, the susceptibilities of the aerobic bacteria were determined by using Mueller-Hinton broth supplemented with calcium and magnesium salts and a final inoculum size of 10^5 CFU/ml. For testing *Streptococcus* spp. and *Haemophilus influenzae*, the medium was supplemented with 5% defibrinated horse blood and with 5% Fildes enrichment (Difco), respectively.

For *Neisseria gonorrhoeae*, GC medium base (Difco) was supplemented with a 2% solution containing the following (in grams) in 100 ml of distilled water: carboxylase (Wako Pure Chemical Co., Ltd., Osaka, Japan), 0.001; glucose (Wako), 20; glutamine (Wako), 0.5. The MICs were determined by the agar dilution method with inocula of 10^4 CFU per spot

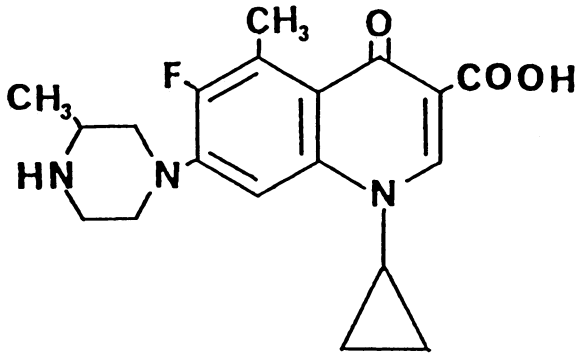

FIG. 1. Chemical structure of OPC-17116.

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TABLE 1. Comparative in vitro activities of OPC-17116 and other drugs against clinical isolates

Organism (no. of isolates)	Drug	MIC (μg/ml)			MBC_{90} (μg/ml)
		Range	50%	90%	
S. aureus, methicillin susceptible (41)	OPC-17116	≦0.063–32	0.125	0.25	0.25
	Ofloxacin	0.25–>128	0.5	1.0	1.0
	Enoxacin	0.25–>128	1.0	8.0	8.0
	Ciprofloxacin	0.125–>128	0.25	2.0	2.0
	Tosufloxacin	≦0.063–>128	≦0.063	0.5	0.5
S. aureus, methicillin resistant (31)	OPC-17116	≦0.063–0.25	≦0.063	0.125	0.25
	Ofloxacin	0.25–0.5	0.5	0.5	0.5
	Enoxacin	1.0–4.0	1.0	2.0	2.0
	Ciprofloxacin	0.25–1.0	0.25	0.5	0.5
	Tosufloxacin	≦0.063–0.25	≦0.063	0.125	0.125
S. epidermidis (34)	OPC-17116	≦0.063–0.5	0.125	0.25	0.25
	Ofloxacin	0.125–1.0	0.5	0.5	0.5
	Enoxacin	0.125–8.0	1.0	1.0	1.0
	Ciprofloxacin	0.125–1.0	0.25	0.5	0.5
	Tosufloxacin	≦0.063–0.25	≦0.063	0.125	0.25
S. pneumoniae (31)	OPC-17116	≦0.063–0.25	≦0.063	0.125	0.25
	Ofloxacin	≦0.063–4.0	1.0	2.0	4.0
	Enoxacin	≦0.063–16	2.0	8.0	8.0
	Ciprofloxacin	≦0.063–2.0	0.25	1.0	2.0
	Tosufloxacin	≦0.063–0.25	≦0.063	0.125	0.25
S. pyogenes (39)	OPC-17116	≦0.063–0.25	0.125	0.25	0.25
	Ofloxacin	0.5–2.0	1.0	2.0	2.0
	Enoxacin	0.5–4.0	2.0	4.0	4.0
	Ciprofloxacin	≦0.063–0.5	0.25	0.5	0.5
	Tosufloxacin	≦0.063–2.0	≦0.063	0.125	0.25
E. faecalis (25)	OPC-17116	≦0.063–16	0.25	0.5	0.5
	Ofloxacin	0.25–64	2.0	2.0	2.0
	Enoxacin	1.0–32	4.0	8.0	8.0
	Ciprofloxacin	0.25–32	1.0	2.0	2.0
	Tosufloxacin	≦0.063–16	0.25	0.5	1.0
Enterococcus faecium (30)	OPC-17116	≦0.063–16	2.0	8.0	8.0
	Ofloxacin	0.25–16	4.0	16	16
	Enoxacin	0.5–128	8.0	32	32
	Ciprofloxacin	0.125–8.0	2.0	4.0	8.0
	Tosufloxacin	≦0.063–8.0	2.0	8.0	8.0
Enterococcus avium (28)	OPC-17116	≦0.063–2.0	0.5	1.0	1.0
	Ofloxacin	0.125–4.0	4.0	4.0	4.0
	Enoxacin	2.0–32	8.0	16	16
	Ciprofloxacin	0.125–2.0	1.0	1.0	2.0
	Tosufloxacin	≦0.063–4.0	0.5	1.0	1.0
E. coli (34)	OPC-17116	≦0.063–1.0	≦0.063	≦0.063	≦0.063
	Ofloxacin	≦0.063–8.0	≦0.063	0.125	0.125
	Enoxacin	≦0.063–32	0.125	0.125	0.25
	Ciprofloxacin	≦0.063–4.0	≦0.063	≦0.063	≦0.063
	Tosufloxacin	≦0.063–2.0	≦0.063	≦0.063	≦0.063
C. freundii (29)	OPC-17116	≦0.063–8.0	≦0.063	1.0	1.0
	Ofloxacin	≦0.063–8.0	0.125	1.0	1.0
	Enoxacin	0.125–8.0	0.25	2.0	2.0
	Ciprofloxacin	≦0.063–2.0	≦0.063	0.5	0.5
	Tosufloxacin	≦0.063–4.0	≦0.063	0.5	1.0
K. pneumoniae (33)	OPC-17116	≦0.063–1.0	≦0.063	0.25	0.25
	Ofloxacin	≦0.063–1.0	0.125	0.5	0.5
	Enoxacin	0.125–4.0	0.25	1.0	1.0
	Ciprofloxacin	≦0.063–0.5	≦0.063	0.125	0.125
	Tosufloxacin	≦0.063–0.5	≦0.063	0.25	0.25
E. cloacae (30)	OPC-17116	≦0.063–0.25	≦0.063	0.125	0.25
	Ofloxacin	≦0.063–0.25	0.125	0.25	0.25
	Enoxacin	≦0.063–1.0	0.25	0.5	1.0
	Ciprofloxacin	≦0.063–0.25	≦0.063	≦0.063	0.125
	Tosufloxacin	≦0.063–0.25	≦0.063	≦0.063	0.125

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TABLE 1—*Continued*

Organism (no. of isolates)	Drug	MIC (μg/ml)			MBC_{90} (μg/ml)
		Range	50%	90%	
S. marcescens (30)	OPC-17116	≦0.063–8.0	0.5	4.0	4.0
	Ofloxacin	≦0.063–16	1.0	4.0	4.0
	Enoxacin	0.25–64	4.0	8.0	16
	Ciprofloxacin	≦0.063–8.0	0.5	2.0	4.0
	Tosufloxacin	≦0.063–8.0	0.5	2.0	2.0
P. mirabilis (35)	OPC-17116	0.125–1.0	0.25	0.5	0.5
	Ofloxacin	≦0.063–1.0	0.125	0.5	0.5
	Enoxacin	0.25–4.0	0.25	0.5	0.5
	Ciprofloxacin	≦0.063–0.25	≦0.063	0.125	0.125
	Tosufloxacin	≦0.063–1.0	0.25	0.5	0.5
Proteus vulgaris (33)	OPC-17116	≦0.063–0.5	0.125	0.5	0.5
	Ofloxacin	≦0.063–1.0	≦0.063	0.25	0.25
	Enoxacin	≦0.063–4.0	0.25	0.5	0.5
	Ciprofloxacin	≦0.063–0.5	≦0.063	≦0.063	≦0.063
	Tosufloxacin	≦0.063–0.5	0.125	0.25	0.25
P. rettgeri (31)	OPC-17116	≦0.063–1.0	0.125	0.5	0.5
	Ofloxacin	≦0.063–2.0	0.25	1.0	1.0
	Enoxacin	0.125–2.0	0.25	1.0	1.0
	Ciprofloxacin	≦0.063–0.25	≦0.063	0.125	0.125
	Tosufloxacin	≦0.063–1.0	0.125	0.5	0.5
P. stuartii (25)	OPC-17116	≦0.063–0.25	≦0.63	0.25	0.25
	Ofloxacin	≦0.063–0.5	0.125	0.5	0.5
	Enoxacin	0.125–1.0	0.5	0.5	0.5
	Ciprofloxacin	≦0.063–0.125	≦0.063	0.125	0.125
	Tosufloxacin	≦0.063–0.25	≦0.063	0.125	0.25
M. morganii (33)	OPC-17116	≦0.063–0.25	≦0.063	0.125	0.25
	Ofloxacin	≦0.063–0.25	≦0.063	0.125	0.125
	Enoxacin	≦0.063–0.5	0.125	0.25	0.25
	Ciprofloxacin	≦0.063	≦0.063	≦0.063	≦0.063
	Tosufloxacin	≦0.063–0.25	≦0.063	0.125	0.25
P. aeruginosa (32)	OPC-17116	0.25–64	1.0	8.0	16
	Ofloxacin	1.0–>128	4.0	16	32
	Enoxacin	0.5–>128	4.0	16	16
	Ciprofloxacin	0.125–64	0.5	8.0	8.0
	Tosufloxacin	0.25–>128	1.0	4.0	8.0
P. cepacia (30)	OPC-17116	≦0.063–8.0	0.25	2.0	2.0
	Ofloxacin	0.125–16	0.5	4.0	4.0
	Enoxacin	0.25–16	1.0	8.0	8.0
	Ciprofloxacin	0.125–8.0	0.5	2.0	2.0
	Tosufloxacin	≦0.063–16	0.25	1.0	1.0
X. maltophilia (26)	OPC-17116	≦0.063–4.0	0.25	4.0	4.0
	Ofloxacin	0.25–16	1.0	8.0	8.0
	Enoxacin	1.0–64	4.0	32	32
	Ciprofloxacin	0.25–16	1.0	8.0	8.0
	Tosufloxacin	≦0.063–8.0	0.25	4.0	4.0
A. baumanii (13)	OPC-17116	≦0.063	≦0.063	≦0.063	≦0.063
	Ofloxacin	≦0.063–0.5	≦0.063	0.25	0.25
	Enoxacin	0.125–2.0	0.25	2.0	2.0
	Ciprofloxacin	≦0.063–0.25	≦0.063	0.25	0.25
	Tosufloxacin	≦0.063–0.125	≦0.063	≦0.063	≦0.063
A. xylosoxidans (19)	OPC-17116	≦0.063–16	0.5	16	32
	Ofloxacin	0.125–32	1.0	32	64
	Enoxacin	≦0.063–64	4.0	64	64
	Ciprofloxacin	≦0.063–32	1.0	16	32
	Tosufloxacin	≦0.063–>128	0.5	>128	>128
H. influenzae (31)	OPC-17116	≦0.063–0.125	≦0.063	≦0.063	≦0.063
	Ofloxacin	≦0.063–0.5	≦0.063	≦0.063	≦0.063
	Enoxacin	≦0.063–8.0	0.125	0.125	0.25
	Ciprofloxacin	≦0.063–0.25	≦0.063	≦0.063	≦0.063
	Tosufloxacin	≦0.063–1.0	≦0.063	≦0.063	≦0.063

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TABLE 1—*Continued*

Organism (no. of isolates)	Drug	MIC (μg/ml)		
		Range	50%	90%
N. gonorrhoeae,[a] non-penicillinase producing (38)	OPC-17116	≦0.006–0.012	≦0.006	≦0.006
	Ofloxacin	≦0.006–0.025	0.012	0.025
	Enoxacin	0.012–0.39	0.10	0.20
	Ciprofloxacin	≦0.006–0.025	≦0.006	0.012
	Tosufloxacin	≦0.006–0.025	≦0.006	0.012
N. gonorrhoeae,[a] penicillinase producing (17)	OPC-17116	≦0.006–0.05	≦0.006	0.025
	Ofloxacin	≦0.006–0.39	0.025	0.20
	Enoxacin	0.025–1.56	0.10	0.78
	Ciprofloxacin	≦0.006–0.20	0.012	0.10
	Tosufloxacin	≦0.006–0.05	0.012	0.05
B. fragilis[a] (30)	OPC-17116	0.39–6.25	1.56	3.13
	Ofloxacin	0.20–3.13	0.78	3.13
	Enoxacin	12.5–50	12.5	50
	Ciprofloxacin	1.56–50	3.13	25
	Tosufloxacin	1.56–3.13	1.56	1.56
C. difficile[a] (15)	OPC-17116	0.20–25	6.25	6.25
	Ofloxacin	0.78–6.25	3.13	6.25
	Enoxacin	0.39–12.5	6.25	12.5
	Ciprofloxacin	0.78–12.5	6.25	12.5
	Tosufloxacin	1.56–3.13	1.56	3.13

[a] Agar dilution method (inoculum size, 10^4 CFU per spot).

after incubation in 5% CO_2 at 37°C for 24 h (4). For anaerobic bacteria, GAM broth (Nissui Seiyaku Co., Ltd., Tokyo, Japan) and GAM agar (Nissui) were used for preculture and determination of the MICs, respectively (3). Incubation was carried out for 48 h at 37°C in an anaerobic chamber (Forma Scientific Co., Cleveland, Ohio). The MICs were determined by the agar dilution method with an inoculum of 10^4 CFU per spot.

The MIC was defined as the lowest concentration of drug that inhibited visible growth in broth or on agar medium. *Escherichia coli* ATCC 25922 was used as a control strain.

The MBC was determined by a subculture of 0.05 ml of broth in tubes without visible growth and was defined as the concentration which produced a >99.9% reduction in CFU after 18 h of incubation at 37°C.

In vivo studies. In vivo activities were determined against systemic infections, respiratory tract infections, and ascending pyelonephritic infections in mice. Test organisms except for *Streptococcus pneumoniae* for various type infections were cultured on Mueller-Hinton agar at 37°C for 18 h, while *S. pneumoniae* was cultured on blood agar plates at 37°C for 18 h. In the systemic infections, gastric mucin (Difco) was added at a final concentration of 5% to bacterial suspensions made in physiological saline.

(i) Induction of leukopenia. Leukopenic mice were treated with cyclophosphamide (Sigma Chemical Co., St. Louis, Mo.) with a single intraperitoneal dose of 250 mg/kg of body weight 4 days before bacterial challenge.

(ii) Systemic infection. Male ICR strain mice weighing 19 ± 1 g, in groups of six, were inoculated intraperitoneally with 0.5 ml of bacterial suspension, corresponding to a range from 2.3 to 25 times higher than the minimum lethal dose. One hour after infection, mice were treated orally with various dose levels of OPC-17116, ofloxacin, enoxacin, ciprofloxacin, or tosufloxacin. The number of mice surviving at each dose level was recorded 5 days after infection, and the 50% effective dose (ED_{50}) was calculated by the method of van der Waerden (10).

(iii) Pyelonephritic infection. After restriction of water intake for 20 h, a group of six female ICR strain mice weighing 19 ± 1 g were challenged by transurethral inoculation with 0.02 ml of *Pseudomonas aeruginosa* KU-1 suspension (10^5 CFU per mouse) with a polyethylene catheter (7). Immediately after challenge, the urethral meatus was clamped for 1 h, and 4 h after challenge, the mice were given a single oral dose of OPC-17116 or a reference drug. The dosage of each drug was 25 mg/kg. Twenty-four hours after drug administration, the kidneys were removed aseptically under diethyl ether anesthesia, and the tissue was homogenized in 2 ml of saline, after which viable cells were determined by plating aliquots in duplicate on BTB plates.

(iv) Respiratory tract infection. Groups of six control or neutropenic mice were anesthetized with diethyl ether and challenged intranasally by instilling 0.02 ml of bacterial suspension. The challenge doses were 2.6×10^7 CFU for *Klebsiella pneumoniae* 3K25 (control mice), 6.6×10^7 CFU for *S. pneumoniae* TMS 3 (neutropenic mice), and 3.8×10^8 CFU for *S. aureus* (neutropenic mice). Immediately after challenge and then once a day for 3 days, OPC-17116 or tosufloxacin was orally administered at 50 mg/kg. The lungs were then removed aseptically under diethyl ether anesthesia and homogenized in 2 ml of saline, and the viable cells in the lungs of each mouse were counted from duplicate BTB plates.

The therapeutic effects of the test drugs were evaluated by the respective number of viable cells in the lungs 2 and 5 days after challenge.

(v) Levels in serum and tissue. Groups of six mice each were given a single oral dose of 50 mg/kg of OPC-17116 or the reference drugs. A sample (0.5 ml) of heart blood was collected from mice 0.25, 0.5, 1, 2, 4, and 6 h after administration, and at set intervals the lungs and kidneys were

TABLE 2. Therapeutic effects of OPC-17116 and related drugs on systemic infections in mice

Organism and challenge dose[a] (CFU/mouse)	Drug	MIC (μg/ml)	ED_{50} (95% confidence limit [mg/kg])
S. aureus Smith (4.5×10^7)	OPC-17116	≦0.063	12.5 (12.5–12.5)
	Ofloxacin	0.50	39.7 (31.5–50.0)
	Enoxacin	1.0	79.4 (59.3–106.3)
	Ciprofloxacin	0.50	15.8 (12.5–19.9)
	Tosufloxacin	≦0.063	6.3 (4.6–8.5)
S. aureus TMS 33,[b] methicillin resistant (2.3×10^6)	OPC-17116	0.25	63.0 (43.4–91.5)
	Ofloxacin	0.50	79.4 (59.3–106.3)
	Enoxacin	1.0	158.8 (126.0–200.0)
	Ciprofloxacin	0.25	126.0 (94.1–168.8)
	Tosufloxacin	≦0.063	50.0 (36.1–69.3)
S. pneumoniae TMS 3 (4.7×10^3)	OPC-17116	≦0.063	19.9 (14.8–26.6)
	Ofloxacin	1.0	158.8 (126.0–200.0)
	Enoxacin	4.0	158.8 (126.0–200.0)
	Ciprofloxacin	0.25	126.0 (94.1–168.8)
	Tosufloxacin	≦0.063	15.8 (12.5–19.9)
E. coli C-11 (2.5×10^4)	OPC-17116	≦0.063	0.2 (0.1–0.2)
	Ofloxacin	≦0.063	0.5 (0.4–0.7)
	Enoxacin	≦0.063	1.3 (0.9–1.9)
	Ciprofloxacin	≦0.063	0.6 (0.5–0.8)
	Tosufloxacin	≦0.063	0.3 (0.2–0.3)
K. pneumoniae 3K25 (1.0×10^4)	OPC-17116	≦0.063	6.6 (0.4–1.0)
	Ofloxacin	0.125	7.9 (5.7–10.9)
	Enoxacin	0.125	6.3 (4.6–8.5)
	Ciprofloxacin	≦0.063	2.5 (2.0–3.2)
	Tosufloxacin	≦0.063	2.5 (2.0–3.2)
P. aeruginosa E7 (1.0×10^4)	OPC-17116	0.50	5.0 (3.2–7.7)
	Ofloxacin	2.0	15.8 (12.5–19.9)
	Enoxacin	1.0	31.5 (23.5–42.2)
	Ciprofloxacin	0.25	7.9 (5.4–11.6)
	Tosufloxacin	0.50	12.5 (12.5–12.5)

[a] Challenge doses are 6.9, 10.0, 3.0, 25.0, 10.0, and 18.0 times the minimum lethal doses for *S. aureus* Smith, *S. aureus* TMS 33, *S. pneumoniae* TMS 3, *E. coli*: C-11, *K. pneumoniae* 3K25, and *P. aeruginosa*, respectively.
[b] Neutropenic mice.

removed under diethyl ether anesthesia and lightly washed with saline solution. The drug levels in the serum samples and supernatants obtained from the tissue homogenates were determined by a paper disk method (8).

RESULTS

In vitro activity. The antibacterial activity of OPC-17116 against clinical isolates was compared with those of ofloxacin, enoxacin, ciprofloxacin, and tosufloxacin (Table 1). The MICs for 90% of the strains tested (MIC_{90}s) of OPC-17116 were 0.25, 0.125, and 0.25 μg/ml, respectively, for methicillin-susceptible *S. aureus*, methicillin-resistant *S. aureus*, and *Staphylococcus epidermidis*, which indicated that the activity of OPC-17116 against staphylococci was similar to that of tosufloxacin but better than those of the other drugs. OPC-17116 had a range of MICs from 0.5 to 32 μg/ml for three ciprofloxacin-resistant strains of *S. aureus* (MIC, 8 to >128 μg/ml).

Like tosufloxacin, OPC-17116 was highly active against *S. pneumoniae* and *Streptococcus pyogenes*, with MIC_{90}s of 0.125 and 0.25 μg/ml, respectively.

OPC-17116 was more active against *Enterococcus faecalis* than the other drugs tested, except tosufloxacin, with an MIC_{90} of 0.5 μg/ml. Although OPC-17116 exhibited no marked advantages over the other drugs in activity against gram-negative bacteria, it generally exhibited potent activity against members of the family *Enterobacteriaceae*. At ≤0.063 to 0.5 μg/ml, OPC-17116 inhibited 90% of the *E. coli*, *K. pneumoniae*, *Enterobacter cloacae*, *Proteus mirabilis*, *Providencia rettgeri*, *Providencia stuartii*, and *Morganella morganii* isolates, with an activity similar to those of the other drugs. For *Citrobacter freundii* and *Serratia marces-*

TABLE 3. Therapeutic effects of OPC-17116 and other drugs on pyelonephritic infection due to *P. aeruginosa* KU-1 in mice[a]

Drug	MIC (μg/ml)	Viable cells in kidneys[b] (log_{10} CFU/kidney)
OPC-17116	0.5	2.16 ± 2.40
Ofloxacin	4.0	3.47 ± 2.24
Enoxacin	1.0	5.18 ± 0.86
Ciprofloxacin	0.25	1.90 ± 1.90
Tosufloxacin	0.5	4.93 ± 0.50
None (control)		7.06 ± 0.24

[a] Therapy was 25 mg/kg orally 4 h after infection.
[b] Viable cells in kidneys were counted 24 h after administration. Data are given as means ± standard deviations.

TABLE 4. Therapeutic effects of OPC-17116 and tosufloxacin on respiratory tract infections in mice

Organism (challenge dose [CFU/mouse])	Treatment (50 mg/kg)	MIC (μg/ml)	Cell count (log_{10})[a] in lungs at following days after challenge:	
			2	5
K. pneumoniae 3K25 (2.6×10^7)	None		6.92 ± 1.02	6.05 ± 1.12
	OPC-17116	≦0.063	≦1.30	≦1.30
	Tosufloxacin	≦0.063	2.11 ± 0.48	≦1.30
S. aureus Smith[b] (3.8×10^8)	None		—	—
	OPC-17116	≦0.063	3.69 ± 2.12	≦1.30
	Tosufloxacin	≦0.063	5.94 ± 0.95	2.69 ± 0.57
S. pneumoniae TMS 3[b] (6.6×10^7)	None		7.47 ± 0.63	6.82 ± 0.65
	OPC-17116	≦0.063	≦1.30	≦1.30
	Tosufloxacin	≦0.063	2.60 ± 0.34	≦1.30

[a] Data are given as means ± standard deviations. —, mice died.
[b] Neutropenic mice.

cens, the MIC_{90}s were 1.0 and 4.0 μg/ml, respectively, and were similar to those of the other drugs. Like the other drugs, the activity of OPC-17116 against *P. aeruginosa*, *Pseudomonas cepacia*, *Xanthomonas maltophilia*, and *Alcaligenes xylosoxidans* differed among the strains tested, but OPC-17116 was moderately active against these species, with MIC_{50}s of 0.25 to 1.0 μg/ml and MIC_{90}s of 2.0 to 16 μg/ml. OPC-17116 was highly active against both *Acinetobacter baumanii* and *H. influenzae* (MIC_{90}s, ≤0.063 μg/ml) and against *N. gonorrhoeae*, including penicillinase-producing strains (MIC_{90}s, ≤0.006 to 0.025 μg/ml). OPC-17116 was more active than enoxacin and ciprofloxacin against *Bacteroides fragilis* and *Clostridium difficile*, inhibiting 90% of the strains at 3.13 to 6.25 μg/ml.

The MBCs of OPC-17116 for 90% of the isolates tested (MBC_{90}s) for 24 species were compared with their MICs (Table 1). Little difference was observed between the MIC_{90}s and MBC_{90}s for the strains of all species tested.

Animal protection studies. The therapeutic effects of OPC-17116 and comparative quinolones against systemic infections in mice are shown in Table 2. The ED_{50} of OPC-17116 against *S. aureus* Smith was 12.5 mg/kg, which is 3.2 to 6.4 times lower than the doses of ofloxacin or enoxacin needed to obtain a similar effect but somewhat higher than the dose needed for tosufloxacin. Although all the drugs were less active against infection due to *S. aureus* TMS 33, OPC-17116, like ofloxacin and tosufloxacin, was 2.5 times more active than enoxacin and ciprofloxacin.

Against *S. pneumoniae* TMS 3 (ED_{50}, 19.9 mg/kg) and *E. coli* C11 (ED_{50}, 0.2 mg/kg), OPC-17116 also was more active than the other drugs (except tosufloxacin), and it was the most active drug tested against infections with *K. pneumoniae* 3K25 and *P. aeruginosa* E7, with ED_{50}s of 0.6 and 5.0 mg/kg, respectively.

The therapeutic effect of OPC-17116 against pyelonephritic infection by *P. aeruginosa* is compared with that of the four reference drugs in Table 3. Viable counts in the kidneys at 28 h after challenge increased to about 10^7 CFU

TABLE 5. Levels of OPC-17116 and other drugs after oral administration in mice

Drug[a]	Specimen	Concn[b] after following h:					
		0.25	0.5	1	2	4	6
OPC-17116	Plasma	2.17 ± 0.38	1.09 ± 0.15	0.94 ± 0.09	0.71 ± 0.09	0.53 ± 0	0.38 ± 0
	Lung	20.67 ± 3.45	15.60 ± 0	14.80 ± 0	11.00 ± 0.92	6.20 ± 0	3.03 ± 0.22
	Kidney	26.89 ± 3.91	10.83 ± 0.71	11.00 ± 0	9.17 ± 0	6.16 ± 0.76	3.10 ± 0
Ofloxacin	Plasma	5.10 ± 0	4.60 ± 0	3.85 ± 0.35	2.20 ± 0	1.20 ± 0.07	0.79 ± 0.11
	Lung	17.20 ± 2.50	7.60 ± 0	5.00 ± 0	2.84 ± 0.52	1.84 ± 0	0.86 ± 0.19
	Kidney	35.11 ± 7.35	21.00 ± 0	18.56 ± 3.40	6.83 ± 0.87	3.89 ± 0.48	1.96 ± 0.15
Enoxacin	Plasma	3.23 ± 0.06	4.43 ± 0.42	3.17 ± 0.38	2.30 ± 0	1.12 ± 0.16	0.32 ± 0.05
	Lung	4.31 ± 0.35	7.40 ± 0	6.27 ± 0.95	4.28 ± 0.55	1.92 ± 0.07	ND
	Kidney	27.11 ± 3.15	24.44 ± 4.23	15.22 ± 2.50	12.00 ± 0.58	3.12 ± 0.13	0.83 ± 0
Ciprofloxacin	Plasma	1.68 ± 0.18	1.20 ± 0	0.98 ± 0.37	0.54 ± 0	0.30 ± 0.06	0.27 ± 0.04
	Lung	3.84 ± 0	1.93 ± 0.42	1.12 ± 0.14	0.63 ± 0.13	0.36 ± 0.05	0.30 ± 0
	Kidney	8.56 ± 1.26	4.67 ± 0	3.13 ± 0.90	2.44 ± 0.44	0.98 ± 0	0.70 ± 0
Tosufloxacin	Plasma	1.50 ± 0	1.43 ± 0.47	1.00 ± 0	0.48 ± 0.06	0.40 ± 0.02	0.26 ± 0.02
	Lung	4.19 ± 0.53	2.51 ± 0.47	1.88 ± 0	1.65 ± 0.23	0.98 ± 0	0.76 ± 0.09
	Kidney	12.70 ± 0	4.61 ± 0.77	3.24 ± 0.39	2.97 ± 0	2.09 ± 0.59	1.25 ± 0.33

[a] Mice were given 50 mg of drug per kg.
[b] Data are given as means ± standard deviations. Concentrations in plasma are given as micrograms per milliliter, and those in lung and kidney are given in micrograms per gram. ND, not determined.

per kidney in the untreated mice, whereas OPC-17116 or ciprofloxacin produced a marked decrease in the counts to about 10^2 CFU per kidney 24 h after administration.

Table 4 shows the therapeutic effects of OPC-17116 and tosufloxacin against respiratory tract infections in mice.

In all of the infections (*K. pneumoniae* 3K25, *S. aureus* Smith, and *S. pneumoniae* TMS 3) the viable counts increased markedly in the untreated mice after challenge.

OPC-17116 was potently active in intrapulmonary killing of all strains tested, with complete elimination of the three pathogens from the lungs. In general, the decrease in viable count in the lungs was more rapid with OPC-17116 than with tosufloxacin.

Pharmacokinetic evaluation. The serum drug concentrations in mice of OPC-17116 after a single oral dose of 50 mg/kg were significantly lower at each sampling than those of ofloxacin and enoxacin, but the half-life of OPC-17116 was longer than those of other drugs (in hours: OPC-17116, 3.96 ± 0.57; ofloxacin, 1.47 ± 0.08; enoxacin, 1.49 ± 0.09; ciprofloxacin, 0.90 ± 0.22; tosufloxacin, 0.78 ± 0.18). In addition, the drug was well distributed to tissue. In the lungs and kidneys (Table 5), OPC-17116 demonstrated peak concentrations of 20.67 and 26.89 μg/g, respectively, and the concentration of this drug was higher than those of the other drugs, even at 6 h after dosing.

DISCUSSION

New quinolones are known to be active against gram-positive and gram-negative bacteria. Ciprofloxacin has the highest activity against gram-negative bacteria among commercialized quinolones. The in vitro activity of OPC-17116 against gram-negative bacteria was higher than that of ofloxacin and enoxacin, comparable to that of tosufloxacin, but slightly lower than that of ciprofloxacin. Against gram-positive bacteria, OPC-17116 showed somewhat better activity compared with that of tosufloxacin and was better than the other compounds tested. The chemical structure of OPC-17116 is similar to that of ciprofloxacin, with the exceptions of a methyl group at the 5-position and a methyl side chain at the 7-position. These structural differences are evidently sufficient to account for the increased gram-positive activity observed compared with ciprofloxacin.

OPC-17116, like other new quinolones, showed potent bactericidal activity at concentrations close to the MIC against gram-positive and gram-negative bacteria, as described previously (11). In systemic infections in mice with methicillin-susceptible and -resistant *S. aureus* or *S. pneumoniae*, the therapeutic effect of OPC-17116 was slightly less than that of tosufloxacin and greater than those of ciprofloxacin, ofloxacin, and enoxacin. In systemic infections with *E. coli*, *K. pneumoniae* and *P. aeruginosa*, the in vivo activity with OPC-17116 was higher than those of ciprofloxacin, ofloxacin, and tosufloxacin. OPC-17116 also demonstrated a protective effect against localized infections such as acute ascending urinary tract and respiratory tract infections.

In particular, in pneumonia in normal mice with *K. pneumoniae* and in neutropenic mice infected with *S. aureus* or *S. pneumoniae*, the number of organisms in the lungs of OPC-17116-treated mice decreased more rapidly than in tosufloxacin-treated mice. The concentration of OPC-17116 in the lungs was significantly higher than those of tosufloxacin and the other reference quinolones after oral administration of the same dose, 50 mg/kg.

The phase I study indicated that the peak levels of OPC-17116 in plasma were 0.44 to 1.79 μg/ml in human volunteers given a single oral dose of 100 to 400 mg and its half-life in plasma was about 11 h (9a). If the concentration in lung tissue in humans will be 10 times more than serum level in mice, OPC-17116 may have a marked antibacterial activity against frequently encountered organisms, such as *H. influenzae*, *S. pneumoniae*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae*, in respiratory infections.

These results warrant more extensive testing of OPC-17116.

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