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In Vitro and In Vivo Antibacterial Activities of CS-940, a New 6-Fluoro-8-Difluoromethoxy Quinolone

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The in vitro and in vivo activities of CS-940, a new 6-fluoro-8-difluoromethoxy quinolone, were compared with those of ciprofloxacin, tosufloxacin, sparfloxacin, and levofloxacin. The in vitro activity of CS-940 against gram-positive bacteria was nearly equal to or greater than those of the other quinolones tested. In particular, CS-940 was two to eight times more active against methicillin-resistant *Staphylococcus aureus* than the other quinolones, at the MIC at which 90% of the clinical isolates are inhibited. Against gram-negative bacteria, the activity of CS-940 was comparable to or greater than those of tosufloxacin, sparfloxacin, and levofloxacin, while it was lower than that of ciprofloxacin. The activity of CS-940 was largely unaffected by medium, inoculum size, or the addition of horse serum, but it was decreased under acidic conditions, as was also seen with the other quinolones tested. CS-940 showed potent bactericidal activity against *S. aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. In oral treatment of mouse systemic infections caused by *S. aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *E. coli*, *K. pneumoniae*, *Serratia marcescens*, and *P. aeruginosa*, CS-940 was more effective than ciprofloxacin, sparfloxacin, and levofloxacin against all strains tested. Against experimental pneumonia with *K. pneumoniae* in mice, CS-940 was the most effective of all the quinolones tested. These results suggest that CS-940 may be effective in the therapy of various bacterial infections.

A number of fluoroquinolones, such as norfloxacin (8), ofloxacin (13), and ciprofloxacin (17), show a broad spectrum of activities against gram-positive and gram-negative bacteria. These fluoroquinolones have been introduced into clinical practice and have proved to be potent antibacterial agents. Nevertheless, they show only moderate activities against gram-positive and anaerobic bacteria. Moreover, quinolone-resistant staphylococci and *Pseudomonas aeruginosa* have appeared (18). There is continued interest in the development of new quinolones in order to improve antibacterial activity, overcome bacterial resistance, or diminish toxicity.

CS-940, 1-cyclopropyl-6-fluoro-8-difluoromethoxy-1,4-dihydro-7-[(3*S*)-methyl-1-piperazinyl]-4-oxo-3-quinolinecarboxylic acid hydrochloride, is a new quinolone, first synthesized by Ube Industries, Ltd., Ube, Japan, and developed by both Sankyo Co. Ltd., Tokyo, Japan, and Ube Industries, Ltd. (Fig. 1). In this study, we compared the in vitro and in vivo antibacterial activities of CS-940 with those of ciprofloxacin, tosufloxacin (3, 14), sparfloxacin (9), and levofloxacin (4, 15).

MATERIALS AND METHODS

Antimicrobial agents. The antimicrobial agents used in this study were obtained as follows: CS-940, Sankyo Co. Ltd.; ciprofloxacin, Bayer Yakuhin Ltd., Osaka, Japan; tosufloxacin, Toyama Chemical Co. Ltd., Tokyo, Japan; sparfloxacin, Dainippon Pharmaceutical Co. Ltd., Osaka, Japan; and levofloxacin, Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan.

Bacterial strains. The bacterial strains used in this study were maintained in our laboratory.

Susceptibility testing. MICs were determined by the usual twofold agar dilution technique recommended by the Japan Society of Chemotherapy (2). As the test medium, sensitivity test agar (STA; Eiken Chemical Co. Ltd., Tokyo, Japan) was used for staphylococci, enterococci, and gram-negative enteric bacteria; STA supplemented with 10% defibrinated horse blood was used for streptococci; STA supplemented with 5% Fildes enrichment (Difco Laboratories, Detroit, Mich.) was used for *Haemophilus influenzae*; chocolate STA was used for *Moraxella catarrhalis*; chocolate GC medium base (Difco) was used for *Neisseria* spp.; and GAM agar (Nissui Seiyaku Co. Ltd., Tokyo, Japan) was used for obligate anaerobic bacteria. The final inoculum size was 5×10^3 CFU per spot (5 μl). The inoculated plates were incubated at 37°C for 18 h, except for *Neisseria* spp., which were incubated in a candle jar for 48 h. Obligately anaerobic bacteria were incubated in an anaerobic chamber (Forma Scientific, Marietta, Ohio).

Effects of various conditions on antibacterial activity. The effects of various factors on the antibacterial activities of CS-940, ciprofloxacin, sparfloxacin, and levofloxacin were determined with one isolate each of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *P. aeruginosa*. The effect of medium on the MICs was determined with STA, heart infusion agar (HIA; Nissui), trypto-soy agar (Nissui), and nutrient agar (Nissui). The effect of pH on the MICs was determined with STA adjusted to each of pH 6, 7, and 8. The effect of serum on the MICs was determined with STA supplemented with heat-inactivated horse serum to final concentrations of 10, 25, and 50%. The effect of inoculum size on the MICs was determined by inoculating 5×10^2 to 5×10^5 CFU per spot onto STA plates.

Killing-curve tests. The bactericidal activities of CS-940, ciprofloxacin, and levofloxacin were determined with one isolate each of *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. An overnight culture in trypto-soy broth (Nissui) was diluted with fresh trypto-soy broth and incubated at 37°C, with shaking, to yield approximately 10^6 CFU/ml; then quinolone was added to a final concentration of 1/4 to four times the MIC. After incubation for 1, 2, or 4 h at 37°C, with shaking, an aliquot of the sample was withdrawn and serially diluted with saline. One hundred microliters of the diluent was mixed with 10 ml of molten HIA, and the level of the surviving cells was determined by the pour plate method. Drug

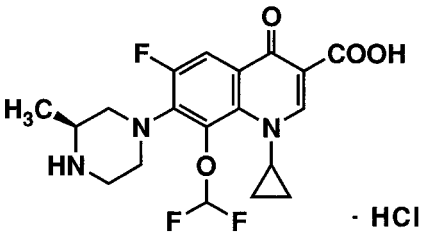

FIG. 1. Chemical structure of CS-940.

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TABLE 1. In vitro activities of CS-940 and other fluoroquinolones against clinical isolates

Organism (no. of strains)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Methicillin-susceptible[b] *S. aureus* (38)	CS-940	0.05–12.5	0.10	6.25
	Ciprofloxacin	0.20–100	0.78	6.25
	Tosufloxacin	0.025–>100	0.05	1.56
	Sparfloxacin	0.025–25	0.10	12.5
	Levofloxacin	0.10–12.5	0.20	12.5
Methicillin-resistant[c] *S. aureus* (33)	CS-940	0.05–25	1.56	12.5
	Ciprofloxacin	0.20–>100	12.5	100
	Tosufloxacin	0.025–>100	3.13	>100
	Sparfloxacin	0.012–50	3.13	25
	Levofloxacin	0.10–50	6.25	25
Quinolone-resistant[d] *S. aureus* (19)	CS-940	0.78–25	3.13	25
	Ciprofloxacin	12.5–>100	100	>100
	Tosufloxacin	1.56–>100	3.13	>100
	Sparfloxacin	1.56–25	3.13	25
	Levofloxacin	3.13–100	12.5	100
S. epidermidis (30)	CS-940	0.05–>100	3.13	>100
	Ciprofloxacin	0.20–100	6.25	100
	Tosufloxacin	0.05–>100	6.25	>100
	Sparfloxacin	0.05–100	3.13	100
	Levofloxacin	0.10–>100	3.13	100
Penicillin-susceptible[e] *S. pneumoniae* (28)	CS-940	0.05–0.78	0.20	0.39
	Ciprofloxacin	0.39–12.5	1.56	3.13
	Tosufloxacin	0.05–1.56	0.10	0.20
	Sparfloxacin	0.05–0.78	0.20	0.39
	Levofloxacin	0.39–3.13	0.78	1.56
Penicillin-resistant[f] *S. pneumoniae* (21)	CS-940	0.05–0.39	0.10	0.39
	Ciprofloxacin	0.39–3.13	1.56	3.13
	Tosufloxacin	0.05–0.20	0.20	0.20
	Sparfloxacin	0.05–0.39	0.10	0.20
	Levofloxacin	0.20–1.56	0.78	1.56
S. pyogenes (31)	CS-940	0.10–0.78	0.20	0.39
	Ciprofloxacin	0.39–1.56	0.78	0.78
	Tosufloxacin	0.05–0.39	0.20	0.39
	Sparfloxacin	0.20–0.78	0.39	0.78
	Levofloxacin	0.39–1.56	0.78	1.56
E. faecalis (30)	CS-940	0.20–1.56	0.39	0.78
	Ciprofloxacin	0.39–3.13	1.56	3.13
	Tosufloxacin	0.10–1.56	0.20	0.78
	Sparfloxacin	0.20–1.56	0.39	0.78
	Levofloxacin	0.39–3.13	0.78	1.56
E. faecium (19)	CS-940	0.05–25	0.78	6.25
	Ciprofloxacin	0.10–25	1.56	3.13
	Tosufloxacin	0.05–12.5	0.39	3.13
	Sparfloxacin	0.10–25	0.78	3.13
	Levofloxacin	0.39–12.5	1.56	3.13
E. avium (10)	CS-940	0.05–0.78	0.39	0.39
	Ciprofloxacin	0.20–1.56	0.39	1.56
	Tosufloxacin	0.05–0.78	0.20	0.39
	Sparfloxacin	0.10–0.78	0.39	0.78
	Levofloxacin	0.39–1.56	0.78	1.56
E. coli (42)	CS-940	0.012–1.56	0.05	0.10
	Ciprofloxacin	0.012–1.56	0.025	0.05
	Tosufloxacin	0.025–3.13	0.05	0.10
	Sparfloxacin	0.012–1.56	0.05	0.10
	Levofloxacin	0.025–1.56	0.05	0.10
C. freundii (36)	CS-940	0.05–100	0.78	3.13
	Ciprofloxacin	≤0.006–100	0.10	0.78
	Tosufloxacin	0.025–>100	0.39	3.13
	Sparfloxacin	0.05–>100	1.56	6.25
	Levofloxacin	0.05–100	0.39	3.13
K. pneumoniae (44)	CS-940	0.025–3.13	0.10	0.10
	Ciprofloxacin	0.012–3.13	0.05	0.10
	Tosufloxacin	0.012–3.13	0.05	0.10
	Sparfloxacin	0.012–3.13	0.05	0.10
	Levofloxacin	0.05–3.13	0.10	0.20
E. cloacae (39)	CS-940	≤0.006–0.39	0.05	0.20
	Ciprofloxacin	≤0.006–0.78	0.025	0.10
	Tosufloxacin	≤0.006–0.39	0.05	0.10
	Sparfloxacin	≤0.006–3.13	0.05	0.20
	Levofloxacin	≤0.006–0.78	0.10	0.10
E. aerogenes (39)	CS-940	0.025–0.20	0.10	0.20
	Ciprofloxacin	0.012–0.05	0.05	0.05
	Tosufloxacin	0.012–0.10	0.05	0.10
	Sparfloxacin	0.025–0.20	0.10	0.20
	Levofloxacin	0.05–0.20	0.10	0.20
S. marcescens (44)	CS-940	0.10–50	3.13	25
	Ciprofloxacin	0.05–50	3.13	25
	Tosufloxacin	0.05–>100	3.13	>100
	Sparfloxacin	0.10–100	3.13	50
	Levofloxacin	0.10–50	3.13	25
P. vulgaris (39)	CS-940	0.05–0.78	0.20	0.39
	Ciprofloxacin	0.012–0.10	0.025	0.05
	Tosufloxacin	0.05–0.20	0.10	0.20
	Sparfloxacin	0.10–0.78	0.20	0.39
	Levofloxacin	0.025–0.20	0.05	0.20
P. mirabilis (40)	CS-940	0.05–25	0.10	0.39
	Ciprofloxacin	0.012–25	0.025	0.20
	Tosufloxacin	0.05–>100	0.10	0.39
	Sparfloxacin	0.10–50	0.20	0.39
	Levofloxacin	0.05–25	0.10	0.20
M. morganii (37)	CS-940	≤0.006–1.56	0.20	0.39
	Ciprofloxacin	≤0.006–3.13	0.025	0.025
	Tosufloxacin	≤0.006–1.56	0.10	0.20
	Sparfloxacin	≤0.006–1.56	0.20	0.39
	Levofloxacin	≤0.006–1.56	0.05	0.20
P. rettgeri (33)	CS-940	0.025–25	0.78	6.25
	Ciprofloxacin	0.025–>100	0.20	12.5
	Tosufloxacin	0.025–>100	0.39	6.25
	Sparfloxacin	0.05–50	1.56	12.5
	Levofloxacin	0.05–100	1.56	12.5
P. aeruginosa (93)	CS-940	0.20–>100	1.56	25
	Ciprofloxacin	0.10–>100	0.39	6.25
	Tosufloxacin	0.10–>100	0.78	>100
	Sparfloxacin	0.39–>100	1.56	50
	Levofloxacin	0.20–>100	1.56	25
A. calcoaceticus (28)	CS-940	≤0.006–0.20	0.025	0.10
	Ciprofloxacin	0.025–1.56	0.20	0.39
	Tosufloxacin	≤0.006–0.20	0.025	0.05
	Sparfloxacin	≤0.006–0.20	0.012	0.05
	Levofloxacin	0.025–0.78	0.10	0.20
H. influenzae (25)	CS-940	≤0.006–0.012	≤0.006	0.012
	Ciprofloxacin	≤0.006–0.012	0.012	0.012
	Tosufloxacin	≤0.006–0.025	≤0.006	0.012
	Sparfloxacin	≤0.006–0.012	≤0.006	0.012
	Levofloxacin	0.012–0.025	0.012	0.025
M. catarrhalis (23)	CS-940	0.012–0.025	0.025	0.025
	Ciprofloxacin	0.025–0.05	0.05	0.05
	Tosufloxacin	0.012–0.025	0.025	0.025
	Sparfloxacin	0.012–0.025	0.012	0.025
	Levofloxacin	0.05	0.05	0.05

[a] 50% and 90%, MICs at which 50 and 90% of the isolates are inhibited, respectively.
[b] MIC of methicillin was below 6.25 μg/ml.
[c] MIC of methicillin was above 12.5 μg/ml.
[d] MIC of ciprofloxacin was above 12.5 μg/ml.
[e] MIC of benzylpenicillin was below 0.05 μg/ml.
[f] MIC of benzylpenicillin was above 0.10 μg/ml.

TABLE 2. Antibacterial activities of CS-940, ciprofloxacin, tosufloxacin, sparfloxacin, and levofloxacin against obligatory anaerobes and *Salmonella*, *Shigella*, and *Neisseria* spp.

Organism	MIC (μg/ml)				
	CS-940	Ciprofloxacin	Tosufloxacin	Sparfloxacin	Levofloxacin
Peptostreptococcus asaccharolyticus ATCC 14953	0.20	0.39	0.20	0.20	0.20
Peptostreptococcus magnus ATCC 14952	0.39	1.56	0.39	0.39	3.13
Clostridium tetani KC-1	0.20	0.20	0.10	0.20	0.20
Clostridium sporogenes KC-1	0.05	0.10	0.05	0.05	0.10
Clostridium sporogenes GAI 0005	0.78	25	0.39	1.56	6.25
Clostridium perfringens KC-1	0.39	0.39	0.20	0.39	0.39
Bacteroides fragilis GM 7000	0.78	6.25	3.13	0.78	3.13
Bacteroides fragilis V-283	0.78	6.25	0.78	0.78	1.56
Bacteroides fragilis ATCC 25285	0.78	6.25	0.78	0.78	1.56
Bacteroides thetaiotaomicron 5600	0.78	50	1.56	1.56	3.13
Bacteroides distasonis clin-99-3	0.78	12.5	0.78	1.56	6.25
Bacteroides vulgatus Es-14	0.78	50	1.56	0.78	1.56
Bacteroides ovatus Ju-6-1	6.25	>100	50	25	100
Salmonella typhi T-287	0.012	≤0.006	0.012	≤0.006	0.025
Salmonella typhi O-901	≤0.006	≤0.006	≤0.006	≤0.006	0.012
Salmonella paratyphi A	0.025	≤0.006	0.025	≤0.006	0.025
Salmonella paratyphi B	0.025	≤0.006	0.05	0.012	0.025
Salmonella enteritidis KC-1	0.025	≤0.006	0.05	≤0.006	0.05
Shigella dysenteriae EW-7	0.025	0.025	0.012	≤0.006	0.05
Shigella flexneri 2a EW-10	0.05	0.025	0.025	≤0.006	0.05
Shigella flexneri Komagome	0.05	0.012	0.025	≤0.006	0.05
Shigella boydii EW-28	0.025	≤0.006	0.025	≤0.006	0.05
Shigella sonnei EW-33	0.012	≤0.006	0.012	≤0.006	0.025
Neisseria gonorrhoeae KC-1	0.012	≤0.006	0.012	≤0.006	0.012
Neisseria meningitidis KC-1	0.10	0.20	0.05	0.20	0.20

carryover did not affect colony formation, since samples were diluted 100-fold with HIA.

Systemic infections in mice. Male ddY mice weighing about 20 g (SLC Japan Inc., Shizuoka, Japan) were infected intraperitoneally with 0.5 ml of bacterial suspension. The strains used for systemic infections (challenge dose per mouse) were as follows: *S. aureus* Smith (1.9×10^6 CFU, 32 times the 50% lethal dose [LD_{50}]), *S. aureus* QR-51 (1.1×10^8 CFU, 5.5 times the LD_{50}), *Streptococcus pneumoniae* type III (1.1×10^2 CFU, 85 times the LD_{50}), *Streptococcus pyogenes* C-203 (1.8×10^2 CFU, 120 times the LD_{50}), *E. coli* KC-14 (7.4×10^5 CFU, 460 times the LD_{50}), *K. pneumoniae* KC-1 (1.7×10^3 CFU, 550 times the LD_{50}), *Serratia marcescens* T-55 (2.9×10^6 CFU, 15 times the LD_{50}), and *P. aeruginosa* E-2 (9.8×10^5 CFU, 65 times the LD_{50}). *S. pneumoniae* and *S. pyogenes* were injected as suspensions in heart infusion broth (Nissui). The other organisms were injected as suspensions in 3% gastric mucin (Wako Chemicals, Osaka, Japan). CS-940, ciprofloxacin, sparfloxacin, and levofloxacin were administered orally in a volume of 0.2 ml of 5% Arabic gum (Wako Chemicals), 2 h after infection. Six levels (serial twofold doses) of the test quinolones were employed, using 10 mice at each level. Results were calculated as the 50% effective dose (ED_{50}), including 95% confidence limits, by the probit method (1) from the survival rates on day 7 after challenge.

Experimental pneumonia in mice. Experimental pneumonia in mice was induced by *K. pneumoniae* B-54 as described previously (11). Male ddY mice weighing about 20 g (SLC Japan Inc.) were placed in an exposure chamber (Ikemoto Rika, Tokyo, Japan) and infected by exposure to an aerosol, which was produced by nebulization of 10 ml of the bacterial suspension (1.8×10^9 CFU/

TABLE 3. Effect of medium on antibacterial activity

Organism	Medium	MIC (μg/ml)[a]			
		CS-940	Ciprofloxacin	Sparfloxacin	Levofloxacin
S. aureus Smith	STA	0.05	0.20	0.05	0.20
	HIA	0.05	0.20	0.05	0.20
	TSA[b]	0.05	0.20	0.05	0.20
	NA[c]	0.05	0.20	0.05	0.20
E. coli KC-14	STA	0.025	≤0.006	0.025	0.025
	HIA	0.012	≤0.006	0.012	0.012
	TSA	0.05	0.012	0.05	0.025
	NA	0.05	0.025	0.05	0.025
K. pneumoniae KC-1	STA	0.05	0.012	0.05	0.05
	HIA	0.025	0.012	0.025	0.05
	TSA	0.05	0.05	0.05	0.10
	NA	0.025	0.05	0.05	0.05
P. aeruginosa E-2	STA	1.56	0.39	3.13	1.56
	HIA	0.78	0.20	0.78	0.78
	TSA	0.78	0.39	1.56	0.78
	NA	0.78	0.39	1.56	0.78

[a] Inoculum size, 5×10^3 CFU per spot (5 μl).
[b] TSA, trypto-soy agar.
[c] NA, nutrient agar.

TABLE 4. Effect of medium pH on antibacterial activity

Organism	pH	MIC (μg/ml)[a]			
		CS-940	Ciprofloxacin	Sparfloxacin	Levofloxacin
S. aureus Smith	6	0.39	0.78	0.39	0.78
	7	0.05	0.20	0.05	0.20
	8	0.05	0.20	0.05	0.20
E. coli KC-14	6	0.39	0.20	0.39	0.39
	7	0.025	≤0.006	0.025	0.025
	8	0.025	≤0.006	0.025	0.025
K. pneumoniae KC-1	6	0.78	0.78	0.78	1.56
	7	0.05	0.012	0.05	0.05
	8	0.025	≤0.006	0.025	0.05
P. aeruginosa E-2	6	6.25	3.13	12.5	6.25
	7	1.56	0.39	3.13	1.56
	8	0.78	0.20	0.78	1.56

[a] In STA; inoculum size, 5×10^3 CFU per spot (5 μl).

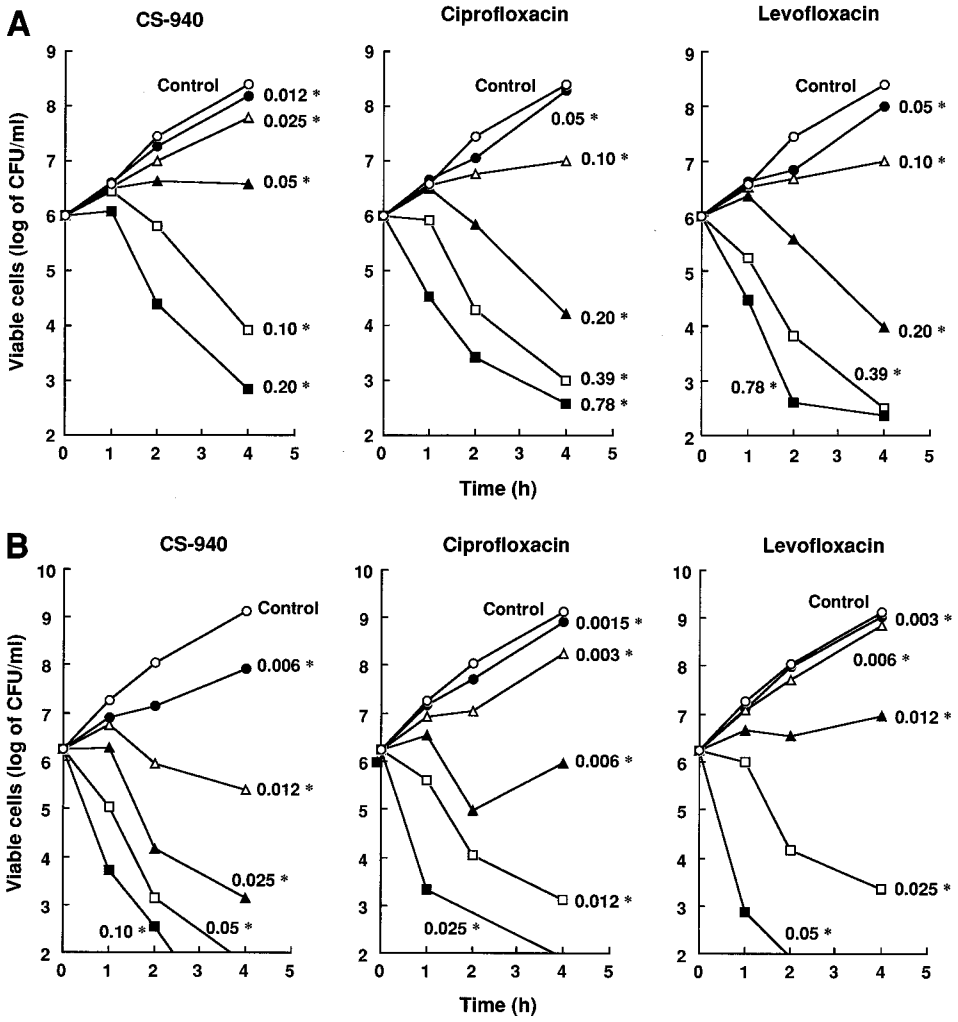

FIG. 2. Bactericidal activities of CS-940, ciprofloxacin, and levofloxacin against *S. aureus* Smith (A), *E. coli* KC-14 (B), *K. pneumoniae* KC-1 (C), and *P. aeruginosa* E-2 (D). The asterisk indicates micrograms per milliliter.

ml). Graded doses of CS-940, ciprofloxacin, sparfloxacin, and levofloxacin were administered orally to groups of seven mice each, at 15 and 39 h after infection. All mice that did not receive drug treatment died within 3 days. The ED_{50}s, including 95% confidence limits, were calculated by the Litchfield-Wilcoxon method (10) from the number of survivors at 6 days after infection.

RESULTS

Antibacterial activity. The antibacterial activities of CS-940, ciprofloxacin, tosufloxacin, sparfloxacin, and levofloxacin were determined for 821 clinical isolates. Table 1 summarizes the MIC ranges and the MICs at which 50 and 90% of the clinical isolates are inhibited (MIC_{50}s and MIC_{90}s, respectively). Although the activity of CS-940 against methicillin-susceptible *S. aureus* was comparable to those of ciprofloxacin, sparfloxacin, and levofloxacin and fourfold lower than that of tosufloxacin, at the MIC_{90} level the activity of CS-940 against methicillin-resistant *S. aureus* was two to eight times greater than those of all the other quinolones tested. The activity of CS-940 against quinolone-resistant *S. aureus* was greater by fourfold or more than those of ciprofloxacin, tosufloxacin, and levofloxacin and comparable to that of sparfloxacin. The MIC_{90}s of all quinolones tested for *Staphylococcus epidermidis* were 100 μg/ml or greater. Against streptococci and *Enterococcus avium*, CS-940 was two- to eightfold more active than ciprofloxacin and levofloxacin and as active as tosufloxacin and sparfloxacin. The activities of all quinolones tested against *Enterococcus faecium*, *E. coli*, *K. pneumoniae*, *Enterobacter cloacae*, *S. marcescens*, *Proteus mirabilis*, *Providencia rettgeri*, *H. influenzae*, and *M. catarrhalis* were comparable. The activity of CS-940 against *Citrobacter freundii*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Morganella morganii*, and *P. aeruginosa* was comparable to those of tosufloxacin, sparfloxacin, and levofloxacin and lower than that of ciprofloxacin. The activity of CS-940 against *Enterococcus faecalis* and *Acinetobacter calcoaceticus* was greater than that of ciprofloxacin and comparable to those of tosufloxacin, sparfloxacin, and levofloxacin.

Antibacterial activities of CS-940, ciprofloxacin, tosufloxacin, sparfloxacin, and levofloxacin against obligatory anaerobes and *Salmonella*, *Shigella*, and *Neisseria* spp. are given in Table 2. The antibacterial activity of CS-940 against obligatory anaerobes was greater than those of ciprofloxacin and levofloxacin and nearly equal to those of tosufloxacin and sparfloxacin. CS-940 inhibited *Salmonella* and *Shigella* spp. at lower concentrations than 0.05 μg/ml, although the antibacterial activity of CS-940 against these species was lower than those of cipro-

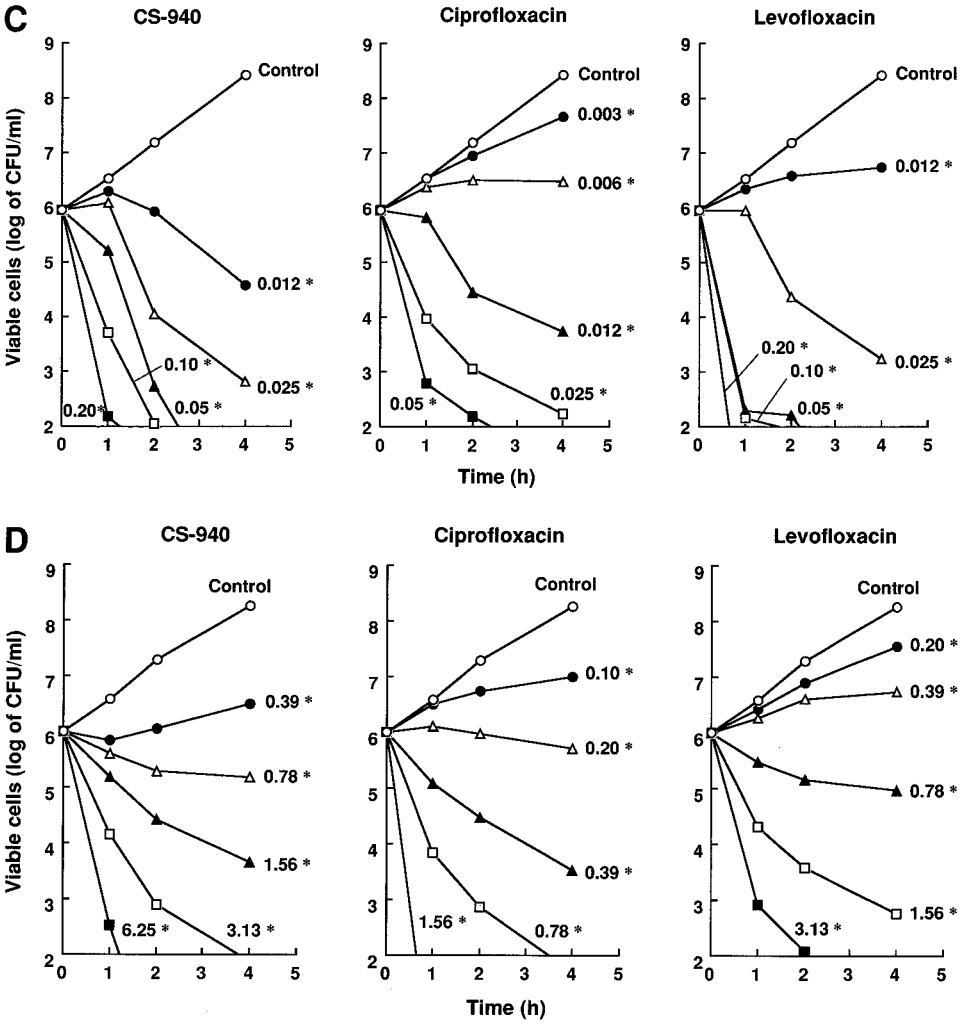

FIG. 2—*Continued.*

floxacin and sparfloxacin. CS-940 showed potent activity against *Neisseria* spp., as did the other quinolones, and it inhibited this species at lower concentrations than 0.10 μg/ml.

Effects of various conditions on antibacterial activity. The activities of CS-940, ciprofloxacin, sparfloxacin, and levofloxacin against *S. aureus* Smith, *E. coli* KC-14, *K. pneumoniae* KC-1, and *P. aeruginosa* E-2 determined in HIA, trypto-soy agar, and nutrient agar were nearly equal to those in STA (Table 3). Varying the pH of STA between 7 and 8 had no significant effect on the activity of CS-940 against each strain (Table 4). However, its activity in STA at pH 6 decreased by 4- to 16-fold, as did those of the other quinolones tested. Adding 10, 25, and 50% horse serum to STA or increasing the inoculum size from 5×10^2 to 5×10^5 CFU per spot had no significant effect on the activity of CS-940 against each strain (data not shown).

Bactericidal activity. The bactericidal activity of CS-940 was compared with those of ciprofloxacin and levofloxacin against *S. aureus* Smith, *E. coli* KC-14, *K. pneumoniae* KC-1, and *P. aeruginosa* E-2 (Fig. 2). The number of viable cells decreased rapidly during incubation with CS-940 at one- to fourfold the MICs, as was also seen with ciprofloxacin and levofloxacin.

Efficacy against systemic infections in mice. The protective effects of CS-940 against systemic infections with *S. aureus* Smith, *S. aureus* QR-51 (quinolone-resistant strain), *S. pneumoniae* type III, *S. pyogenes* C-203, *E. coli* KC-14, *K. pneumoniae* KC-1, *S. marcescens* T-55, and *P. aeruginosa* E-2 in mice were compared with those of ciprofloxacin, sparfloxacin, and levofloxacin (Table 5). CS-940 showed greater efficacy against systemic infections caused by *S. aureus* Smith, the quinolone-resistant *S. aureus* QR-51, *S. pneumoniae*, *E. coli*, and *P. aeruginosa* than did ciprofloxacin and levofloxacin. The efficacy of CS-940 against *S. pyogenes* and *K. pneumoniae* infections was better than that observed with ciprofloxacin and comparable to that observed with levofloxacin. The efficacy of CS-940 against *E. coli* infections was better than that of levofloxacin and comparable to that of ciprofloxacin. The efficacy of CS-940 against these bacteria was comparable to that of sparfloxacin, despite the fact that the ED_{50} of CS-940 against most of the strains tested was lower than that of sparfloxacin.

Efficacy against experimental pneumonia in mice. The efficacy of CS-940 against experimental pneumonia induced with *K. pneumoniae* was compared with those of ciprofloxacin, sparfloxacin, and levofloxacin (Table 6). The efficacy of CS-940 against experimental pneumonia was better than that of ciprofloxacin and comparable to those of sparfloxacin and levofloxacin, despite the fact that the ED_{50} of CS-940 was 2 to 15 times lower than those of the others.

TABLE 5. Protective effects of CS-940 and other fluoroquinolones against experimental systemic infections in mice

Organism (challenge dose/mouse)	Drug	MIC (μg/ml)	ED_{50} (mg/mouse), 95% confidence limits
S. aureus Smith[a] (1.9×10^6 CFU; $32\times$ LD_{50})	CS-940	0.05	0.016, 0.009–0.026
	Ciprofloxacin	0.20	0.16, 0.13–0.21
	Sparfloxacin	0.05	0.020, 0.014–0.028
	Levofloxacin	0.20	0.057, 0.043–0.078
S. aureus QR-51[a] (1.1×10^8 CFU; $5.5\times$ LD_{50})	CS-940	0.20	0.18, 0.13–0.27
	Ciprofloxacin	3.13	>4
	Sparfloxacin	0.20	0.35, 0.26–0.47
	Levofloxacin	0.78	1.4, 0.9–2.1
S. pneumoniae type III (1.1×10^2 CFU; $85\times$ LD_{50})	CS-940	0.05	0.98, 0.65–1.80
	Ciprofloxacin	0.20	>4
	Sparfloxacin	0.10	1.4, 1.0–2.0
	Levofloxacin	0.20	2.5, 1.6–5.2
S. pyogenes C-203 (1.8×10^2 CFU; $120\times$ LD_{50})	CS-940	0.20	0.44, 0.27–0.63
	Ciprofloxacin	0.78	>4
	Sparfloxacin	0.39	0.71, 0.57–0.87
	Levofloxacin	0.78	0.53, 0.40–0.72
E. coli KC-14[a] (7.4×10^5 CFU; $460\times$ LD_{50})	CS-940	0.025	0.0035, 0.0025–0.0055
	Ciprofloxacin	0.006	0.010, 0.007–0.014
	Sparfloxacin	0.006	0.0035, 0.0025–0.0049
	Levofloxacin	0.012	0.0085, 0.0063–0.0116
K. pneumoniae KC-1[a] (1.7×10^3 CFU; $550\times$ LD_{50})	CS-940	0.05	0.012, 0.009–0.016
	Ciprofloxacin	0.012	0.027, 0.020–0.037
	Sparfloxacin	0.05	0.010, 0.006–0.015
	Levofloxacin	0.05	0.016, 0.012–0.023
S. marcescens T-55[a] (2.9×10^6 CFU; $15\times$ LD_{50})	CS-940	0.39	0.074, 0.048–0.109
	Ciprofloxacin	0.10	0.11, 0.07–0.16
	Sparfloxacin	0.39	0.12, 0.09–0.17
	Levofloxacin	0.20	0.046, 0.029–0.066
P. aeruginosa E-2[a] (9.8×10^5 CFU; $65\times$ LD_{50})	CS-940	1.56	0.076, 0.050–0.120
	Ciprofloxacin	0.39	0.18, 0.13–0.27
	Sparfloxacin	3.13	0.11, 0.08–0.15
	Levofloxacin	0.78	0.28, 0.18–0.52

[a] With 3% mucin.

DISCUSSION

In the past decade, a large number of quinolone derivatives have been synthesized, and most of them have been developed as orally administered antibacterial agents. However, strains of quinolone-resistant *S. aureus* and *P. aeruginosa* have increased in number, possibly because of the widespread use of quinolones. Therefore, a large number of the existing quinolones have insufficient activity against these strains.

CS-940 is a new quinolone, with a difluoromethoxy group at position 8 of the quinolone ring. CS-940 showed a broad spectrum of antibacterial activity against clinical isolates of gram-positive, gram-negative, and anaerobic bacteria. An interesting characteristic of CS-940 is its improved activity against quinolone-resistant *S. aureus*. The activity of CS-940 against quinolone-resistant *S. aureus* was fourfold or more greater than those of ciprofloxacin, tosufloxacin, and levofloxacin and comparable to that of sparfloxacin, although the activity of CS-940 against methicillin-susceptible *S. aureus* was comparable to those of ciprofloxacin, sparfloxacin, and levofloxacin and fourfold lower than that of tosufloxacin. We think one reason for this potent activity against quinolone-resistant *S. aureus* is that CS-940, as well as sparfloxacin, is more active against a NorA mutant than are other quinolones (16, 19).

TABLE 6. Protective effects of CS-940 and other fluoroquinolones against experimental pneumonia caused by *K. pneumoniae* B-54 in mice

Drug	MIC (μg/ml)	ED_{50} (mg/mouse)	95% confidence limits
CS-940	0.20	0.11	0.04–0.29
Ciprofloxacin	0.012	1.6	0.5–4.6
Sparfloxacin	0.20	0.24	0.10–0.55
Levofloxacin	0.10	0.49	0.11–2.13

In vivo activity of CS-940 against all the strains tested was greater than those of ciprofloxacin and levofloxacin, although in vitro activity of CS-940 against the gram-negative strains was lower than that of ciprofloxacin and comparable to that of levofloxacin. We think CS-940 has the best in vivo efficacy, because after oral administration to laboratory animals, it is well absorbed, rapidly distributed to various tissues, and primarily excreted in the urine as an unchanged drug (5). Ciprofloxacin showed insufficient efficacy against experimental pneumonia induced with *K. pneumoniae*, despite its potent in vitro activity against this species, as reported previously (6, 12). The reason for this discrepancy is probably that ciprofloxacin is distributed to the lungs to a lesser extent than are other quinolones (7).

These results indicate that CS-940 may be a useful quinolone for the treatment of various infections. Clinical studies on CS-940 are in progress.

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