In Vitro and In Vivo Antibacterial Activities of DK-507k, a Novel Fluoroquinolone

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The antibacterial activities of DK-507k, a novel quinolone, were compared with those of other quinolones: ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, sitafloxacin, and garenoxacin (BMS284756). DK-507k was as active as sitafloxacin and was as active as or up to eightfold more active than gatifloxacin, moxifloxacin, and garenoxacin against *Streptococcus pneumoniae*, methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*, and coagulase-negative staphylococci. DK-507k was as active as or 4-fold more active than garenoxacin and 2- to 16-fold more active than gatifloxacin and moxifloxacin against ciprofloxacin-resistant strains of *S. pneumoniae*, including clinical isolates and in vitro-selected mutants with known mutations. DK-507k inhibited all ciprofloxacin-resistant strains of *S. pneumoniae* at 1 µg/ml. A time-kill assay with *S. pneumoniae* showed that DK-507k was more bactericidal than gatifloxacin and moxifloxacin. The activities of DK-507k against most members of the family *Enterobacteriaceae* were comparable to those of ciprofloxacin and equal to or up to 32-fold higher than those of gatifloxacin, levofloxacin, moxifloxacin, and garenoxacin. DK-507k was fourfold less active than sitafloxacin and ciprofloxacin against *Pseudomonas aeruginosa*, while it was two to four times more potent than levofloxacin, gatifloxacin, moxifloxacin, and garenoxacin against *P. aeruginosa*. In vivo, intravenous treatment with DK-507k was more effective than that with gatifloxacin and moxifloxacin against systemic infections caused by *S. aureus*, *S. pneumoniae*, and *P. aeruginosa* in mice. In a mouse model of pneumonia due to penicillin-resistant *S. pneumoniae*, DK-507k administered subcutaneously showed dose-dependent efficacy and eliminated the bacteria from the lungs, whereas gatifloxacin and moxifloxacin had no significant efficacy. Oral treatment with DK-507k was slightly more effective than that with ciprofloxacin in a rat model of foreign body-associated urinary tract infection caused by a *P. aeruginosa* isolate for which the MIC of DK-507k was fourfold higher than that of ciprofloxacin. Oral administration of DK-507k to rats achieved higher peak concentrations in serum and higher concentrations in cumulative urine than those achieved with ciprofloxacin. These data indicate the potential advantages of DK-507k over other quinolones for the treatment of a wide range of community-acquired infections.

As community-acquired pathogens are exhibiting increasing levels of resistance to β -lactams and macrolides, quinolones have emerged as one of the first-line drugs for therapy for respiratory infections and urinary tract infections in the community (3, 4, 24).

The application of newer quinolones, such as levofloxacin, gatifloxacin, and moxifloxacin, with enhanced potencies against gram-positive bacteria for the treatment of community-acquired respiratory infections has become important because of the worldwide prevalence of penicillin- and multidrug-resistant *Streptococcus pneumoniae* strains (1, 10, 14, 21, 22). However, even these newer quinolones have relatively high MICs that may limit their therapeutic value against some strains of ciprofloxacin-resistant pneumococci for which the MICs are close to or above the breakpoint (6, 11, 19, 26). On the other hand, community-acquired urinary tract infections are among the most commonly observed infections in clinical practice. Quinolones have primarily been used for therapy for patients with urinary tract infections, in which gram-negative bacteria are the most common causative agents (8, 16). Recent trends,

however, of the application of quinolones for the treatment of respiratory tract infections by improving their antibacterial activities against gram-positive bacteria are likely associated with a relative decrease in activity against gram-negative bacteria, even if the activity still has therapeutic value. Therefore, the development of quinolones with potential utility for the treatment of infections caused by both gram-positive and gram-negative bacteria would represent significant progress.

DK-507k is a novel fluoroquinolone with the chemical structure shown in Fig. 1 (K. Kawakami et al., Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-546, 2001). DK-507k possessed improved activity against gram-positive bacteria, including some strains resistant to the available quinolones, while the activity of the compound against gram-negative bacteria was maintained. To investigate the potency of DK-507k against a broad spectrum of bacterial pathogens, we studied its in vitro antibacterial activities against a variety of recent clinical isolates and its in vivo efficacy in mouse models of septicemia caused by *S. pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*; a mouse model of pneumococcal pneumonia; and a rat model of foreign body-associated urinary tract infection caused by *P. aeruginosa*. The comparator agents were ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, sitafloxacin, and garenoxacin (BMS284756).

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FIG. 1. Chemical structure of DK-507k.

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MATERIALS AND METHODS

Antimicrobial agents and bacterial strains. DK-507k, ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, norfloxacin, sitafloxacin, sparfloxacin, and garenoxacin were synthesized at Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan. Ampicillin, benzylpenicillin, cefaclor, and oxacillin were purchased from Sigma Aldrich Japan (Tokyo, Japan). All drugs were prepared just before use and were used as pure free-acid equivalents. Most bacterial strains were isolated from clinical specimens in 1998 in Japan and were collected by the Levofloxacin Surveillance Group (25). Penicillin-resistant *S. pneumoniae* (PRSP) 033890 and 033806 were isolated in 1996 in Japan and were collected by the Levofloxacin Surveillance Group. *P. aeruginosa* 910735 was isolated from a patient with a urinary tract infection in 1991 in Japan. Five strains of ciprofloxacin-resistant *S. pneumoniae* were isolated in 1997 and 1998 in Asia and Europe (22). *E. coli* KL-16 was obtained from Guma University, Maebashi, Japan. *Haemophilus influenzae* ATCC 49766 and *S. pneumoniae* ATCC 49619 were obtained from the American Type Culture Collection, Manassas, Va.

MIC determination. The MICs were determined by the standard agar dilution method with Mueller-Hinton agar (MHA; Difco Laboratories, Detroit, Mich.) (15). MHA supplemented with 2% NaCl was used for staphylococci, MHA supplemented with 5% sheep blood was used for streptococci and *Moraxella catarrhalis*, and MHA supplemented with 3% Fildes enrichment was used for *H. influenzae*. GC agar (Difco) supplemented with 1% hemoglobin and 1% IsoVital-X was used for *Neisseria gonorrhoeae*, buffered starch-yeast extract agar without charcoal was used for *Legionella pneumophila*, and PPLO broth (Difco) was used for *Mycoplasma pneumoniae*. Drug-containing agar plates were incubated with one loopful (5 μ l) of an inoculum corresponding to about 10^4 CFU per spot and were incubated at 35°C for 18 h. *N. gonorrhoeae* was incubated under 10% CO₂. *L. pneumophila* and *M. pneumoniae* were cultivated for 96 h and 14 days, respectively. The MIC was defined as the lowest drug concentration that prevented visible growth of the bacteria. The reference strains were included as internal controls throughout the study.

In vitro time-kill study. PRSP 033806 was cultured overnight at 37°C on tryptic soy agar (Eiken Chemical Co., Tokyo, Japan) plates supplemented with 5% defibrinated horse blood. The colonies were seeded into Todd-Hewitt broth (Difco) and incubated with shaking at 37°C until the turbidity of the broth reached a 0.5 McFarland standard. The logarithmic-growth-phase broth culture was diluted with fresh Todd-Hewitt broth to adjust the cell density to approximately 10^6 CFU/ml. DK-507k, moxifloxacin, and gatifloxacin were then added to final concentrations of the MIC and four times the MIC; namely, the concentrations of DK-507k were 0.25 and 1 μ g/ml, respectively, and those of moxifloxacin and gatifloxacin were 0.5 and 2 μ g/ml, respectively. Viable bacterial counts were performed at 0.08, 0.25, 0.5, 1, 2, and 4 h of incubation at 37°C with shaking by removing an aliquot and preparing 100-fold dilutions in 0.033 M phosphate buffer (PB; pH 7.0). Each sample, including the initial culture, was inoculated onto a 5% blood tryptic soy agar plate at a volume of 0.1 ml/plate and incubated overnight at 37°C.

In vitro selection of fluoroquinolone-resistant *S. pneumoniae*. *S. pneumoniae* ATCC 49619 was plated onto MHA supplemented with 5% horse blood containing levofloxacin, ciprofloxacin, gatifloxacin, and sparfloxacin at the MICs for this strain. After incubation for 48 h, the colonies that grew on the plates were stored at -80°C for subsequent analyses. Levofloxacin, ciprofloxacin, gatifloxacin, and sparfloxacin were used for the selection of second-, third-, and fourth-step mutants. To determine the mutations in the DNA gyrase and topoisomerase IV genes, the quinolone resistance-determining regions in *parC* and *gyrA* were amplified by the PCR method. Primer SPgyrA1 (5'-CTGTTCACCGTCGCAT

TCTC-3') corresponds to nucleotides 381 to 400 in *gyrA*, and primer SPgyrA2 (5'-GGTTCCTTCATTGGC-3') is complementary to nucleotides 691 to 720. Primer SPparC1, which corresponds to nucleotides 2203 to 2221 (5'-CGGT TCAACGCGTATTCC-3') in *parC*, and complementary primer SPparC2, which corresponds to nucleotides 2483 to 2504 (5'-AACTGTCTTTTCTCGA TATCC-3'), were chosen (2, 18). The amplified fragments were purified with a QIA quick PCR purification kit (Qiagen, Hilden, Germany), as recommended by the manufacturer, and sequenced with a Thermo sequenase fluorescence-labeled primer cycle sequencing kit (Amersham Pharmacia Biotech, Uppsala, Sweden) with a ROB DNA Processor II instrument (Amersham) and a Pharmacia LKB ALFred DNA sequencer. Expression of the efflux pump was assessed by determination of the norfloxacin MIC with or without reserpine (5).

In vivo efficacy against experimental infections. Five-week-old male Slc:ddY mice (Japan SLC Inc., Shizuoka, Japan), 4-week-old male CBA/JNCrj mice (Charles River Japan, Inc., Kanagawa, Japan), and 7-week-old female Crj:CD(S-D)IGS rats (Charles River Japan) were used for the septicemia models, the pneumococcal pneumonia model, and the foreign body-associated urinary tract infection model, respectively. They were maintained in animal rooms maintained at 23 $\pm$ 2°C with 55% $\pm$ 20% relative humidity. All experimental procedures for the animals were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Daiichi Pharmaceutical Co. Ltd.

(i) Septicemia models in mice. Methicillin-susceptible *S. aureus* (MSSA) 037114, methicillin-resistant *S. aureus* (MRSA) 037004, penicillin-susceptible *S. pneumoniae* (PSSP) 037288, PRSP 033890, *E. coli* 037042, and *P. aeruginosa* 037096 were used as challenge organisms. For use as inocula, all bacterial strains except *S. pneumoniae* 037288 and *E. coli* 037042 were suspended in PB containing 3% mucin (Difco); *S. pneumoniae* 037288 and *E. coli* 037042 were suspended in PB. Mice were used in groups of 10 each and were challenged intraperitoneally with a single 0.2-ml portion of the bacterial suspension. Five doses of drug were obtained by using serial 1.414-fold (the square root of 2) to 1.732-fold (the square root of 3) dilutions, and these were used for each 50% effective dose (ED₅₀) determination, with 100 mg of drug per kg of body weight taken as the highest dose. DK-507k, levofloxacin, gatifloxacin, or moxifloxacin was administered into the tail vein immediately after infection. The ED₅₀s and 95% confidence intervals were obtained by using a probit method based on the survival rate on day 7 after infection. For pharmacokinetic analysis of the drugs tested, drug was injected into the tail veins of the mice at a dose of 10 mg/kg. Blood samples were obtained from three mice each at various time intervals (0.08, 0.25, 0.5, 1, 2, 4, and 6 h) after drug administration.

(ii) Pneumococcal pneumonia in mice. Experimental pneumonia was produced in CBA/JNCrj mice by a slight modification of the method reported by Tateda et al. (23). *S. pneumoniae* 033806 suspended in PB was intranasally inoculated into the mice at a volume of 50 μ l/mouse with an autopipette (10 to 100 μ l; Degift A; Shibata Chemical Co., Ltd., Tokyo, Japan). One day after the inoculation, the animals (in groups of four mice each) were treated with DK-507k, moxifloxacin, or gatifloxacin subcutaneously twice a day at a dose of 7.5, 15, or 30 mg/kg/day for 3 consecutive days. The number of bacteria in the lungs was examined on the day following the final administration of the test drugs, namely, 4 days after inoculation. The lungs were removed aseptically and weighed, and then the viable bacterial counts were determined. The detection limit was ≥ 2.30 log₁₀ CFU/g of lung tissue. For statistical comparisons, culture-negative samples were considered to contain 2.30 log₁₀ CFU/g of lung tissue. For the pharmacokinetic analysis, DK-507k, moxifloxacin, or gatifloxacin at a dose of 15 mg/kg each was subcutaneously injected into the infected mice. Blood and lung tissue samples were obtained from three mice each at various time intervals (0.08, 0.25, 0.5, 1, 2, 4, and 6 h) after administration of the drugs tested.

(iii) Rat model of foreign body-associated urinary tract infection caused by *P. aeruginosa*. A foreign body-associated urinary tract infection was induced in rats as described in detail elsewhere (13). A spiral polyethylene tube (PT; Intermedic Polyethylene Tubing PE-50; Becton Dickinson, Sparks, Md.) was placed transurethraly into the bladder of the rat by using a flexible metal stylet without surgical manipulation. Four days after placement of the PT, 0.5 ml of an inoculum with *P. aeruginosa* 910735 was introduced into the bladders of the animals transurethraly, and then the urethra was clamped for 4 h to prevent urine flow. Two days after inoculation, groups of six animals each were treated orally with DK-507k or ciprofloxacin at a dose of 10 or 20 mg/kg/day once a day for 3 consecutive days. The numbers of bacteria in the kidneys, bladder, and PT were examined on the day following the final administration of the test drugs, namely, 5 days after inoculation. The kidneys and bladder were removed aseptically and weighed; they were then homogenized in 2- and 19-fold PB (vol/wt), respectively. The PT sample was put into a vial with 2 ml of PB, and the vial was vortexed vigorously to remove the bacteria adhering to the surface of the PT. The detection limit of viable bacterial counts was ≥ 1.48 log₁₀ CFU/g of kidneys, ≥ 2.30

$\log_{10}$ CFU/g of bladder, or $\geq 1.30 \log_{10}$ CFU/PT of PT. For statistical comparisons, culture-negative samples were considered to contain $1.48 \log_{10}$ CFU/g for the kidneys, $2.30 \log_{10}$ CFU/g for the bladder, and $1.30 \log_{10}$ CFU/PT for PT. To examine the pharmacokinetics of the drugs tested in serum and excreted urine, DK-507k or ciprofloxacin at a dose of 20 mg/kg was each administered orally to groups of three infected rats. Blood samples were obtained from the tail veins of individual rats at 0.25, 0.5, 1, 2, 4, and 6 h after dosing. Urine samples were accumulated in individual metabolic cages from 0 to 4 h after drug administration and were then assayed for drug concentrations.

Measurement of drug concentrations and in vitro protein binding. The concentrations of the drugs tested in sera and tissues were determined by an agar diffusion method. *Bacillus subtilis* ATCC 6051 (DK-507k, 0.02 to 2.5 μ g/ml; levofloxacin, 0.2 to 6.25 μ g/ml; gatifloxacin, 0.08 to 10 μ g/ml), *B. subtilis* ATCC 6633 (DK-507k, 0.02 to 2.5 μ g/ml; moxifloxacin, 0.04 to 2.5 μ g/ml; gatifloxacin, 0.08 to 10 μ g/ml), *E. coli* MK 3804c (gatifloxacin, 0.04 to 0.625 μ g/ml), and *E. coli* NIHJ (ciprofloxacin, 0.04 to 0.625 μ g/ml) were used as indicator organisms. The calibration curve was prepared by spiking the respective blank matrix with seven or eight different concentrations. The concentrations of levofloxacin in the sera of mice 4 and 6 h after intravenous administration were determined by high-performance liquid chromatography (HPLC)–fluorescence detection (17) because the concentrations were below the detection limit of the microbiological assay. HPLC analysis was performed with a Waters (Milford, Mass.) Alliance system. A Waters 474 scanning fluorescence detector was used for detection. The detection limit of this HPLC method was 0.02 μ g/ml. The areas under the concentration-time curves (AUCs) for the drugs tested were calculated by the trapezoidal method, and the half-lives ($t_{1/2}$ s) were calculated by the least-squares regression method with PSAG-CP (Pharmacokinetic, Statistic, Analysis and Graphics for Clinical Pharmacology) software (ASMedica Inc., Osaka, Japan).

The levels of binding of DK-507k, moxifloxacin, and gatifloxacin to serum proteins in mice were determined in vitro with an ultrafiltration device (Centrifree 4104; Millipore, Billerica, Mass.). The test compounds were added to about 1 ml of serum at 4 μ g/ml and centrifuged at $1,800 \times g$ for 10 min. The binding ratio (in percent) was calculated by the formula $[1 - (C_u/C)] \times 100$, where C_u is the concentration unbound and C is the total concentration.

Statistical analysis. The dose-response relationships of the efficacies of the test drugs, as assessed from the number of bacteria in the tissues, were evaluated by linear least-squares regression analysis. The difference in bacterial numbers between the tissues of the nontreated control group and those of the treated groups was analyzed statistically by Dunnett's multiple-comparison test. Tukey's multiple-comparison test was performed to determine the dose at which a significant difference was observed compared with the response of the nontreated control group to determine the difference between the treatment groups (pneumonia model, groups treated with DK-507k, moxifloxacin, and gatifloxacin; urinary tract infection model, groups treated with DK-507k and ciprofloxacin). A P value of ≤ 0.05 was considered significant.

RESULTS

Antibacterial activities against clinical isolates. Tables 1 and 2 compare the antibacterial activities of DK-507k against gram-positive and -negative bacteria to those of the reference drugs. Against MSSA strains, DK-507k showed activities comparable to those of sitafloxacin and moxifloxacin, twofold lower than those of garenoxacin, and two- to fourfold higher than those of the other quinolones tested at the MICs at which 90% of isolates are inhibited (MIC_{90} s). Against methicillin-susceptible coagulase-negative staphylococci, DK-507k and sitafloxacin were the most potent among the quinolones tested. Against ofloxacin-susceptible MRSA strains, DK-507k showed activities comparable to those of sitafloxacin; 2-fold lower than those of garenoxacin; and 2- to 16-fold higher than those of levofloxacin, ciprofloxacin, gatifloxacin, and moxifloxacin. Furthermore, against ofloxacin-resistant MRSA strains the activities of DK-507k and sitafloxacin were the highest among those of the quinolones tested. Against methicillin-resistant coagulase-negative staphylococci, the activities of DK-507k were comparable to those of sitafloxacin and at least eightfold higher than those of the other quinolones tested at the MIC_{90} s.

The MIC_{90} s of DK-507k for *S. pneumoniae*, including penicillin-resistant strains, and *Streptococcus pyogenes* were 0.12 μ g/ml, which were comparable to those of sitafloxacin and garenoxacin and four times lower than those of gatifloxacin and moxifloxacin. Five *S. pneumoniae* strains among 1,879 strains collected by the Asia and Europe surveillance group (19) were shown to be ciprofloxacin resistant (ciprofloxacin MIC range, 8 to 64 μ g/ml). The DK-507k MIC range for ciprofloxacin-resistant *S. pneumoniae* strains was 0.25 to 1 μ g/ml; DK-507k and sitafloxacin were the most active quinolones among the drugs tested. Against *Enterococcus faecalis* and *Enterococcus faecium*, DK-507k showed activities equivalent to those of sitafloxacin and garenoxacin.

Against most members of the family *Enterobacteriaceae*, the activities of DK-507k were up to 4 times higher than those of gatifloxacin and moxifloxacin and up to 32 times higher than those of garenoxacin at the MIC_{90} s. DK-507k inhibited 90% of isolates of *E. coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter* spp., *Proteus mirabilis*, and indole-positive *Proteus* at 0.25, 1, 1, 1, 0.5, and 1 μ g/ml, respectively. At the MIC_{90} s, DK-507k was four times less active than sitafloxacin and ciprofloxacin against ofloxacin-susceptible *P. aeruginosa* strains, while it was two to four times more potent than the other quinolones tested. Against *Acinetobacter* spp., the activities of DK-507k were roughly comparable to those of the other quinolones tested, with an MIC_{90} of 0.5 μ g/ml. DK-507k was highly active against *H. influenzae*, *M. catarrhalis*, and ofloxacin-susceptible *N. gonorrhoeae*. DK-507k was highly active against ofloxacin-resistant *N. gonorrhoeae* strains, with an MIC_{90} of 1 μ g/ml. The interpretive MICs of the drugs tested for the reference strains used for quality control were reproducible throughout the study.

Bactericidal activity. The bactericidal activity of DK-507k against PRSP 033806 was compared with those of moxifloxacin and gatifloxacin (Fig. 2). The dose dependency and rapid killing typical of fluoroquinolones were demonstrated at the MICs and four times the MICs of all compounds. DK-507k showed rapid killing even at the MIC, which reduced the number of viable bacteria below the detection limit within 2 h. At the concentration of four times the MIC, DK-507k reduced the number of viable bacteria below the detection limit within 1 h, whereas moxifloxacin and gatifloxacin required 4 h. The experiments were repeated thrice for each drug; the results were reproducible.

Selection of fluoroquinolone-resistant *S. pneumoniae* strains and activities of fluoroquinolones against the strains. Fluoroquinolone-resistant mutants were selected by using levofloxacin, ciprofloxacin, gatifloxacin, and sparflaxacin; and the single-step mutation rates with compounds at the MIC were found to be 6.5×10^{-6} , 3.1×10^{-4} , 4.4×10^{-5} , and 6.1×10^{-5} , respectively. For the second step, we obtained ciprofloxacin-resistant colonies from a ciprofloxacin-resistant first-step mutant (mutant CPFX-CPFX); gatifloxacin-resistant colonies from a levofloxacin-resistant first-step mutant (mutant LVFX-GFLX); and levofloxacin-, ciprofloxacin-, and sparflaxacin-resistant colonies from a sparflaxacin-resistant first step mutant (mutants SPFX-LVFX, SPFX-CPFX, and SPFX-SPFX, respectively). No resistant strain was selected by the other drug combinations. For the third step, only ciprofloxacin-resistant colonies (mutants CPFX-CPFX-CPFX1 and CPFX-CPFX-

TABLE 1. Antibacterial activities of DK-507k against gram-positive bacteria

Organism ^a (no. of strains) and compound	MIC (μg/ml)			Organism ^a (no. of strains) and compound	MIC (μg/ml)		
	Range	50%	90%		Range	50%	90%
MSSA (25)				Ciprofloxacin	1–8	2	4
DK-507k	0.03–0.25	0.06	0.12	Gatifloxacin	0.25–1	0.5	0.5
Sitafloxacin	0.008–0.12	0.03	0.12	Moxifloxacin	0.12–0.5	0.25	0.5
Levofloxacin	0.12–1	0.25	0.5	Garenoxacin	0.03–0.12	0.12	0.12
Ciprofloxacin	0.25–4	0.5	1	Benzylpenicillin	0.015–0.06	0.06	0.06
Gatifloxacin	0.06–0.5	0.12	0.25				
Moxifloxacin	0.06–0.25	0.06	0.12	PISP (3) and PRSP (14)			
Garenoxacin	≤0.004–0.12	0.03	0.06	DK-507k	0.06–0.12	0.12	0.12
Oxacillin	0.25–1	0.5	1	Sitafloxacin	0.06–0.25	0.06	0.12
				Levofloxacin	1–4	2	2
MRSA (24), ofloxacin susceptible				Ciprofloxacin	2–8	2	4
DK-507k	0.015–0.06	0.03	0.06	Gatifloxacin	0.25–1	0.5	0.5
Sitafloxacin	0.008–0.06	0.03	0.06	Moxifloxacin	0.25–0.5	0.25	0.5
Levofloxacin	0.12–1	0.25	0.5	Garenoxacin	0.06–0.12	0.12	0.12
Ciprofloxacin	0.25–2	0.5	1	Benzylpenicillin	2–4	4	4
Gatifloxacin	0.06–0.25	0.12	0.25				
Moxifloxacin	0.03–0.25	0.12	0.12	<i>S. pneumoniae</i> (5), ciprofloxacin resistant			
Garenoxacin	0.008–0.06	0.03	0.03	DK-507k	0.25–1	— ^b	—
Oxacillin	16–>128	64	>128	Sitafloxacin	0.25–1	—	—
				Levofloxacin	8–32	—	—
MRSA (25), ofloxacin resistant				Ciprofloxacin	8–64	—	—
DK-507k	0.25–32	1	4	Gatifloxacin	4–8	—	—
Sitafloxacin	0.25–32	1	4	Moxifloxacin	2–4	—	—
Levofloxacin	4–>128	16	64	Garenoxacin	0.5–2	—	—
Ciprofloxacin	16–>128	128	>128	Benzylpenicillin	0.03–8	—	—
Gatifloxacin	2–>128	8	16				
Moxifloxacin	1–64	4	8	<i>S. pyogenes</i> (25)			
Garenoxacin	0.25–64	2	8	DK-507k	0.03–0.12	0.06	0.12
Oxacillin	64–>128	>128	>128	Sitafloxacin	0.015–0.06	0.03	0.06
				Levofloxacin	0.06–1	0.25	0.5
MSCNS (27)				Ciprofloxacin	0.12–1	0.25	1
DK-507k	0.015–0.5	0.06	0.25	Gatifloxacin	0.12–0.5	0.25	0.5
Sitafloxacin	0.008–0.25	0.03	0.25	Moxifloxacin	0.12–0.5	0.12	0.5
Levofloxacin	0.12–8	0.25	4	Garenoxacin	0.03–0.25	0.06	0.25
Ciprofloxacin	0.06–64	0.25	8	Ampicillin	0.015–0.12	0.015	0.03
Gatifloxacin	0.06–4	0.25	2				
Moxifloxacin	0.03–4	0.12	1	<i>E. faecalis</i> (25)			
Garenoxacin	0.015–4	0.06	1	DK-507k	0.06–2	0.12	2
Oxacillin	0.06–0.25	0.25	0.25	Sitafloxacin	0.06–2	0.12	2
				Levofloxacin	0.5–64	1	32
MRCNS (36)				Ciprofloxacin	0.25–64	1	32
DK-507k	0.03–2	0.25	0.5	Gatifloxacin	0.25–16	0.5	16
Sitafloxacin	0.008–0.5	0.12	0.5	Moxifloxacin	0.25–16	0.25	8
Levofloxacin	0.12–32	4	16	Garenoxacin	0.12–4	0.25	2
Ciprofloxacin	0.12–128	8	64	Ampicillin	0.5–4	1	2
Gatifloxacin	0.12–4	2	4				
Moxifloxacin	0.06–8	1	4	<i>E. faecium</i> (23)			
Garenoxacin	0.03–4	1	4	DK-507k	0.25–16	2	8
Oxacillin	1–>128	128	>128	Sitafloxacin	0.12–8	1	8
				Levofloxacin	2–>128	32	128
PSSP (25)				Ciprofloxacin	1–>128	32	64
DK-507k	0.06–0.25	0.12	0.12	Gatifloxacin	1–64	8	32
Sitafloxacin	0.03–0.12	0.12	0.12	Moxifloxacin	0.5–64	4	16
Levofloxacin	1–4	2	2	Garenoxacin	0.25–64	4	16
				Ampicillin	2–128	128	128

^a MSCNS and MRCNS, methicillin-susceptible and -resistant coagulase-negative staphylococci, respectively; PISP, penicillin-intermediate *S. pneumoniae*.^b —, not calculated.

CPF2) were obtained from CPF2-CPF2 mutants at the MIC and two times the MIC, and no resistant strain was selected by the other drug combinations. For the fourth step, levofloxacin-, gatifloxacin-, ciprofloxacin-, and sparfloracin-resistant colonies were selected from the third-step mutants. Table 3 shows the amino acid substitutions in GyrA and ParC, the expression

states of the efflux pumps in the in vitro-selected mutants, and the activities of DK-507k and the comparator drugs against these bacterial strains. The substitutions and their positions in GyrA and ParC were consistent with those reported previously (7, 9). The MICs of DK-507k for the first-, second-, third-, and fourth-step mutants were one to four, two to four, four to

TABLE 2. Antibacterial activities of DK-507k against gram-negative bacteria and *Mycoplasma*

Organism (no. of strains) and compound	MIC ($\mu\text{g/ml}$)			Organism (no. of strains) and compound	MIC ($\mu\text{g/ml}$)		
	Range	50%	90%		Range	50%	90%
<i>E. coli</i> (22)				Ciprofloxacin	≤ 0.004 –0.06	0.008	0.015
DK-507k	≤ 0.004 –2	0.03	0.25	Gatifloxacin	≤ 0.004 –0.03	0.008	0.008
Sitafloxacin	≤ 0.004 –0.5	0.008	0.06	Moxifloxacin	0.008–0.06	0.03	0.03
Levofloxacin	≤ 0.004 –2	0.015	0.5	Garenoxacin	≤ 0.004 –0.015	≤ 0.004	0.008
Ciprofloxacin	≤ 0.004 –2	0.008	0.5	Cefaclor	1–64	2	32
Gatifloxacin	≤ 0.004 –2	0.03	0.5	Ampicillin	0.12–1	0.25	1
Moxifloxacin	≤ 0.004 –8	0.06	1	<i>H. influenzae</i> (14), β -lactamase negative, ampicillin-resistant			
Garenoxacin	≤ 0.004 –16	0.06	1	DK-507k	≤ 0.004 –0.008	≤ 0.004	0.008
<i>K. pneumoniae</i> (25)				Sitafloxacin	≤ 0.004 – ≤ 0.004	≤ 0.004	≤ 0.004
DK-507k	0.06–2	0.06	1	Levofloxacin	0.008–0.015	0.015	0.015
Sitafloxacin	0.015–1	0.03	0.25	Ciprofloxacin	≤ 0.004 –0.015	0.008	0.015
Levofloxacin	0.06–8	0.06	1	Gatifloxacin	≤ 0.004 –0.008	0.008	0.008
Ciprofloxacin	0.015–4	0.03	0.5	Moxifloxacin	0.008–0.03	0.015	0.03
Gatifloxacin	0.03–4	0.06	1	Garenoxacin	≤ 0.004 –0.015	0.008	0.008
Moxifloxacin	0.12–4	0.25	1	Cefaclor	32–128	62	128
Garenoxacin	0.06–4	0.12	2	Ampicillin	2–8	2	4
<i>S. marcescens</i> (22)				<i>H. influenzae</i> (21), β -lactamase positive			
DK-507k	0.25–2	0.5	1	DK-507k	≤ 0.004 –0.015	≤ 0.004	0.008
Sitafloxacin	0.06–1	0.12	0.25	Sitafloxacin	≤ 0.004 – ≤ 0.004	≤ 0.004	≤ 0.004
Levofloxacin	0.12–8	0.25	2	Levofloxacin	0.008–0.015	0.008	0.015
Ciprofloxacin	0.06–8	0.12	1	Ciprofloxacin	≤ 0.004 –0.015	0.008	0.008
Gatifloxacin	0.25–4	0.5	2	Gatifloxacin	≤ 0.004 –0.015	0.008	0.008
Moxifloxacin	0.25–8	1	2	Moxifloxacin	0.015–0.06	0.015	0.03
Garenoxacin	1–16	2	8	Garenoxacin	≤ 0.004 –0.03	≤ 0.004	0.008
<i>Enterobacter</i> spp. (23)				Cefaclor	1–64	8	32
DK-507k	0.03–4	0.12	1	Ampicillin	4–128	16	128
Sitafloxacin	0.008–1	0.03	0.25	<i>M. catarrhalis</i> (25)			
Levofloxacin	0.03–8	0.06	2	DK-507k	≤ 0.004 –0.06	0.06	0.06
Ciprofloxacin	0.008–16	0.03	2	Sitafloxacin	≤ 0.004 –0.03	0.015	0.015
Gatifloxacin	0.03–8	0.12	1	Levofloxacin	0.03–0.06	0.06	0.06
Moxifloxacin	0.06–4	0.25	2	Ciprofloxacin	0.015–0.06	0.06	0.06
Garenoxacin	0.06–16	0.25	2	Gatifloxacin	0.015–0.06	0.03	0.06
<i>P. mirabilis</i> (25)				Moxifloxacin	0.03–0.12	0.12	0.12
DK-507k	0.03–4	0.06	0.5	Garenoxacin	≤ 0.004 –0.06	0.03	0.03
Sitafloxacin	0.015–0.5	0.03	0.25	<i>N. gonorrhoeae</i> (12), ofloxacin susceptible			
Levofloxacin	0.03–4	0.06	1	DK-507k	≤ 0.004 –0.12	0.06	0.12
Ciprofloxacin	0.015–16	0.03	0.5	Sitafloxacin	≤ 0.004 –0.03	0.008	0.03
Gatifloxacin	0.12–8	0.12	2	Levofloxacin	0.015–0.5	0.25	0.5
Moxifloxacin	0.12–16	0.25	2	Ciprofloxacin	0.008–0.5	0.12	0.5
Garenoxacin	0.25–32	0.5	16	Gatifloxacin	0.008–0.12	0.06	0.12
Indole-positive <i>Proteus</i> (25)				Moxifloxacin	≤ 0.004 –0.25	0.06	0.25
DK-507k	0.06–1	0.12	1	Garenoxacin	≤ 0.004 –0.25	0.06	0.12
Sitafloxacin	0.008–0.5	0.03	0.25	<i>N. gonorrhoeae</i> (23), ofloxacin resistant			
Levofloxacin	0.03–2	0.06	1	DK-507k	0.25–2	0.5	1
Ciprofloxacin	0.008–4	0.03	1	Sitafloxacin	0.06–0.5	0.25	0.25
Gatifloxacin	0.03–2	0.12	2	Levofloxacin	2–16	8	16
Moxifloxacin	0.12–4	0.25	4	Ciprofloxacin	2–32	8	32
Garenoxacin	0.25–8	0.5	4	Gatifloxacin	0.5–4	2	4
<i>P. aeruginosa</i> (24), ofloxacin susceptible				Moxifloxacin	1–8	4	8
DK-507k	0.12–2	0.5	1	Garenoxacin	0.06–8	1	4
Sitafloxacin	0.03–0.5	0.12	0.25	<i>L. pneumophila</i> (17)			
Levofloxacin	0.25–2	1	2	DK-507k	0.008–0.015	0.008	0.015
Ciprofloxacin	0.03–0.5	0.12	0.25	Sitafloxacin	≤ 0.004	≤ 0.004	≤ 0.004
Gatifloxacin	0.25–4	1	2	Levofloxacin	0.008–0.015	0.015	0.015
Moxifloxacin	0.5–8	2	4	Ciprofloxacin	0.03–0.06	0.03	0.06
Garenoxacin	0.5–4	1	4	Gatifloxacin	0.008–0.015	0.008	0.015
<i>Acinetobacter</i> spp. (24)				Moxifloxacin	0.008–0.03	0.015	0.03
DK-507k	0.06–2	0.12	0.5	Garenoxacin	≤ 0.004 –0.008	0.008	0.008
Sitafloxacin	0.015–1	0.03	0.25	<i>M. pneumoniae</i> (5)			
Levofloxacin	0.12–4	0.25	1	DK-507k	0.06	— ^a	—
Ciprofloxacin	0.12–4	0.25	2	Sitafloxacin	0.06	—	—
Gatifloxacin	0.03–2	0.12	0.5	Levofloxacin	0.5–1	—	—
Moxifloxacin	0.06–4	0.12	1	Ciprofloxacin	1–2	—	—
Garenoxacin	0.015–4	0.06	1	Gatifloxacin	0.12	—	—
<i>H. influenzae</i> (38), ampicillin susceptible				Moxifloxacin	0.12–0.25	—	—
DK-507k	≤ 0.004 –0.015	≤ 0.004	0.008	Garenoxacin	0.06–0.12	—	—
Sitafloxacin	≤ 0.004 –0.008	≤ 0.004	≤ 0.004				
Levofloxacin	≤ 0.004 –0.03	0.015	0.015				

^a —, not calculated.

FIG. 2. Bactericidal activities at the MIC (A) and four times the MIC (B) against penicillin-resistant strain *S. pneumoniae* 033806. Symbols: ×, growth control; ○, DK-507k; Δ, gatifloxacin; □, moxifloxacin.

eight, and four to eight times higher than those for the parent strain, respectively. DK-507k inhibited all strains at 0.5 $\mu\text{g}/\text{ml}$. The MICs of sitafloxacin, levofloxacin, ciprofloxacin, gatifloxacin, and moxifloxacin for the fourth-step mutants were 4 to 8, 16 to 32, 32 to 64, 8 to 32, and 8 to 16 times higher than those for the parent strain, respectively. The activity of DK-507k against 13 of 14 resistant strains was not affected by reserpine, an efflux pump inhibitor, while the activity of norfloxacin

against all mutants was strengthened by the addition of reserpine (data not shown). Thus, we considered that all 14 mutants tested expressed efflux pumps, and the effect of the expression of efflux pumps on the activity of DK-507k was less than that on the activity of norfloxacin.

Therapeutic efficacy and pharmacokinetics in septicemia models in mice. Table 4 shows the ED_{50} s of DK-507k, levofloxacin, moxifloxacin, and gatifloxacin against septicemia in mice. DK-507k was the most effective drug tested against infections caused by gram-positive organisms, including MSSA, levofloxacin-resistant MRSA, PSSP, and PRSP strains, for which the ED_{50} s were 1.07, 9.23, 3.63, and 1.49 mg/kg, respectively. Against infections caused by *S. pneumoniae*, DK-507k was 3.8 to 17.5 times, 4.4 to 11.6 times, and 9.5 to >27.5 times more potent than gatifloxacin, moxifloxacin, and levofloxacin, respectively. DK-507k was shown to be highly effective against infections caused by gram-negative organisms. DK-507k was more potent than levofloxacin, gatifloxacin, and moxifloxacin against infection caused by *P. aeruginosa*. Against infection caused by *E. coli*, the ED_{50} of DK-507k (4.19 mg/kg) was similar to those of gatifloxacin (4.07 mg/kg) and levofloxacin (5.14 mg/kg) but was lower than that of moxifloxacin (12.30 mg/kg).

After administration of a single intravenous dose of 10 mg/kg, the concentration of DK-507k in serum reached 4.50 $\mu\text{g}/\text{ml}$ 5 min after administration, the concentration decreased with a $t_{1/2}$ of 0.83 h, and the AUC for the drug was 2.93 $\mu\text{g} \cdot \text{h}/\text{ml}$

TABLE 3. Antibacterial activities of DK-507k against in vitro-selected mutants of *S. pneumoniae*

Organism	Compound(s) ^a used for selection	Concn. ($\mu\text{g}/\text{ml}$)	Amino acid change		Efflux ^b	MIC ($\mu\text{g}/\text{ml}$)						
			GyrA	ParC		DK-507k	Sita-floxacin	Levo-floxacin	Cipro-floxacin	Gati-floxacin	Moxi-floxacin	Spar-floxacin
ATCC 49619					—	0.06	0.06	0.5	0.5	0.25	0.12	0.25
First-step mutant	LVFX	0.78	— ^c	—	+	0.25	0.12	2	4	2	0.5	4
	GFLX	0.20	—	—	+	0.12	0.12	2	2	1	0.5	4
	CPFX	0.39	—	83D→N	+	0.06	0.06	1	2	0.5	0.25	0.5
	SPFX	0.20	—	—	+	0.25	0.12	2	2	1	0.25	2
Second-step mutant	LVFX-GFLX	0.5	—	—	+	0.12	0.12	2	2	0.5	0.25	1
	CPFX-CPFX	2	—	83D→N	++	0.25	0.12	2	4	1	0.5	2
	SPFX-LVFX	2	—	79S→F	+	0.12	0.12	4	4	1	0.5	4
	SPFX-CPFX	2	—	—	+	0.25	0.25	4	8	2	0.5	4
	SPFX-SPFX	1	102D→H	—	+	0.12	0.12	2	1	0.5	0.5	2
Third-step mutant	CPFX-CPFX-CPFX	4	81S→F	83D→N	++	0.25	0.25	8	8	2	1	8
	CPFX-CPFX-CPFX	8	81S→Y	83D→N	ND	0.5	0.25	8	16	2	1	16
Fourth-step mutant	CPFX-CPFX-CPFX1-LVFX	8	81S→F	83D→N	++	0.5	0.5	16	32	8	1	16
	CPFX-CPFX-CPFX1-GFLX	2	81S→F	83D→N	++	0.25	0.25	8	16	2	1	8
	CPFX-CPFX-CPFX1-CPFX	16	81S→F	83D→N	++	0.5	0.5	16	32	4	2	16
	CPFX-CPFX-CPFX2-CPFX	16	81S→Y	83D→N	ND	0.5	0.5	16	32	8	2	32
	CPFX-CPFX-CPFX2-SPFX	16	81S→Y	83D→N	++	0.5	0.5	16	32	8	2	32

^a LVFX, levofloxacin; GFLX, gatifloxacin; CPFX, ciprofloxacin; SPFX, sparfloxacin. The compounds were used in the indicated order in each step of mutant selection.

^b —, no expression; +, expression; ++, overexpression; ND, not determined.

^c —, no amino acid change.

TABLE 4. Therapeutic efficacies of DK-507k, levofloxacin, moxifloxacin, and gatifloxacin on septicemia models in mice^a

Organism	Inoculum (no. of CFU/mouse ^c)	Drug	MIC ($\mu\text{g/ml}$)	ED ₅₀ (mg/kg)	95% CI ^b (mg/kg)
<i>S. aureus</i> 037114 (MSSA)	2.9×10^8 (1.3)	DK-507k	0.06	1.07	0.74–1.45
		Levofloxacin	0.25	16.20	12.02–19.57
		Moxifloxacin	0.06	5.48	3.34–9.82
		Gatifloxacin	0.12	4.66	3.84–5.43
<i>S. aureus</i> 037004 (MRSA)	4.0×10^7 (1.4)	DK-507k	1	9.23	5.14–16.94
		Levofloxacin	16	>100.00	
		Moxifloxacin	2	56.70	45.39–73.08
		Gatifloxacin	4	67.36	46.01–142.96
<i>S. pneumoniae</i> 037288 (PSSP)	1.8×10 (2.9)	DK-507k	0.06	3.63	2.98–4.48
		Levofloxacin	1	>100.00	
		Moxifloxacin	0.25	41.94	28.05–55.56
		Gatifloxacin	0.25	63.36	41.32–215.48
<i>S. pneumoniae</i> 033890 (PRSP)	5.6×10^6 (2.8)	DK-507k	0.12	1.49	0.89–2.17
		Levofloxacin	2	14.21	6.75–20.03
		Moxifloxacin	0.5	6.52	2.41–11.06
		Gatifloxacin	0.5	5.69	3.18–9.32
<i>E. coli</i> 037042	3.2×10^8 (4.8)	DK-507k	0.12	4.19	2.89–6.82
		Levofloxacin	0.25	5.14	4.34–6.02
		Moxifloxacin	0.25	12.30	10.43–14.73
		Gatifloxacin	0.12	4.07	3.01–5.77
<i>P. aeruginosa</i> 037096	4.2×10^6 (4.2)	DK-507k	0.25	3.23	2.07–4.05
		Levofloxacin	0.5	8.15	5.55–9.57
		Moxifloxacin	1	28.56	22.52–34.53
		Gatifloxacin	0.25	12.23	10.24–14.26

^a Groups of 10 mice were challenged intraperitoneally with the inoculum. The drugs were administered intravenously immediately after infection.^b 95% CI, confidence interval.^c Multiple of the minimum lethal dose.

(Table 5). The values of the pharmacokinetic parameters for DK-507k, moxifloxacin, and levofloxacin in serum were roughly comparable, whereas gatifloxacin showed a larger AUC and a longer $t_{1/2}$ than those of the other drugs tested.

Therapeutic efficacy and pharmacokinetics in pneumococcal pneumonia model in mice. The MICs of DK-507k, gatifloxacin, and moxifloxacin for PRSP 033806 were 0.25, 0.5, and 0.5 $\mu\text{g/ml}$, respectively. After infection with the strain at $4.34 \log_{10}$ CFU/mouse, the bacterial counts in the lungs increased to $6.96 \pm 0.18 \log_{10}$ CFU/g 4 days after infection. As shown in Fig. 3, DK-507k exhibited therapeutic efficacy against the model of pneumococcal pneumonia in a dose-dependent manner at doses ranging from 7.5 to 30 mg/kg/day, whereas gatifloxacin and moxifloxacin showed no such effects. DK-507k at doses of 15 and 30 mg/kg/day significantly reduced the bacterial counts in the lungs compared with the counts in the lungs of the

nontreated control group, the gatifloxacin-treated group, and the moxifloxacin-treated group.

The values of the pharmacokinetic parameters for the drugs in the sera and lungs of infected mice were determined. Following administration of a single subcutaneous dose of 15 mg/kg, the AUCs from 0 to 6 h for DK-507k, moxifloxacin, and gatifloxacin in sera were 3.60, 3.25, and 3.74 $\mu\text{g} \cdot \text{h/ml}$, respectively; and those in the lungs were 14.78, 11.68, and 11.19 $\mu\text{g} \cdot \text{h/ml}$, respectively. The $t_{1/2}$ s in the sera and lungs and the maximum concentrations of these drugs in the sera and lungs were roughly comparable (data not shown).

In vitro protein binding to mouse serum. DK-507k and the comparator drugs exhibited low levels of protein binding; the levels of protein binding for DK-507k, moxifloxacin, and gatifloxacin were $26.7\% \pm 3.2\%$, $19.1\% \pm 1.7\%$, and $20.1\% \pm 1.0\%$, respectively.

Therapeutic efficacies and pharmacokinetics in a rat model of foreign body-associated urinary tract infection caused by *P. aeruginosa*. The therapeutic effects of DK-507k and ciprofloxacin were assessed by measurement of the number of bacteria recovered from the kidneys, bladders, and PTs as a foreign body inside the bladders of the rats, each of which was infected with $6.46 \log_{10}$ CFU of *P. aeruginosa* 910735, for which the DK-507k and ciprofloxacin MICs were 0.5 and 0.125 $\mu\text{g/ml}$, respectively. Figure 4 indicates that treatment with DK-507k significantly reduced the bacterial counts in the kidneys, bladders, and PTs compared with those in the nontreated controls; and the efficacy of DK-507k tended to be greater than that of

TABLE 5. Pharmacokinetic parameters in serum after an administration of a single intravenous dose of DK-507k, levofloxacin, moxifloxacin, and gatifloxacin at 10 mg/kg to mice^a

Drug	C_5 ($\mu\text{g/ml}$)	AUC ($\mu\text{g} \cdot \text{h/ml}$)	$t_{1/2}$ (h)
DK-507k	4.50	2.93	0.83
Levofloxacin	5.34	3.17	1.02
Moxifloxacin	3.52	2.37	1.15
Gatifloxacin	5.48	4.35	1.94

^a Three mice each were tested at 0.08, 0.25, 0.5, 1, 2, 4, and 6 h after drug administration. C_5 , concentration 5 min after drug administration.

FIG. 3. Therapeutic efficacies of DK-507k, moxifloxacin, and gatifloxacin in a mouse model of pneumonia due to penicillin-resistant strain *S. pneumoniae* 033806. The drugs were administered subcutaneously twice a day for 3 consecutive days. The numbers of bacteria in the lungs were determined on the day after the final administration. Each bar represents the mean $\pm$ standard error of the mean number of bacteria from the lungs of four mice. *, $P < 0.05$ versus the control; ***, $P < 0.01$ versus the control; ###, $P < 0.01$ versus moxifloxacin at the corresponding doses; \$, $P < 0.05$ versus gatifloxacin at a dose of 15 mg/kg/day; \$\$\$, $P < 0.01$ versus gatifloxacin at a dose of 30 mg/kg/day.

ciprofloxacin. At the same doses ciprofloxacin significantly decreased the bacterial numbers in the kidneys and PTs. Ciprofloxacin at a dose of 20 mg/kg also reduced the bacterial burdens in the bladders, although they were not statistically different from the burdens for the nontreated controls. DK-507k at a dose of 20 mg/kg was highly active against the bacteria localized on the surfaces of the PTs, reducing the bacterial numbers by approximately 4.5 log₁₀ compared with those for the untreated controls (3.00 and 7.50 log₁₀ CFU/PT, respectively; $P < 0.05$).

After administration of a single oral dose of 20 mg/kg, DK-507k achieved a higher concentration and a larger AUC in the sera of rats than ciprofloxacin. Absorption of DK-507k was rapid (time to the maximum concentration in serum, 0.67 ± 0.29 h), reaching a peak level in serum of 0.70 ± 0.22 µg/ml; in comparison, the peak level of ciprofloxacin in serum was 0.19 ± 0.05 µg/ml. The AUCs from 0 to 6 h for DK-507k and ciprofloxacin in sera were 1.91 ± 0.27 and 0.70 ± 0.16 µg · h/ml, respectively. The concentration of DK-507k in cumulative

urine from 0 to 4 h after drug administration was 58.59 ± 5.78 µg/ml, whereas that of ciprofloxacin was 41.44 ± 24.73 µg/ml.

DISCUSSION

DK-507k demonstrated in vitro and in vivo antibacterial activities against both gram-positive and gram-negative bacteria superior to those of the most active fluoroquinolones marketed to date, including moxifloxacin, gatifloxacin, and levofloxacin. This was true for both quinolone-susceptible and -resistant strains. The overall antibacterial activity of DK-507k against gram-positive bacteria was equivalent to that of sitafloxacin, and DK-507k was slightly more potent than garenoxacin. DK-507k was also highly active against most members of the family *Enterobacteriaceae*, with activities comparable to those of ciprofloxacin and activities consistently higher than those of garenoxacin but lower than those of sitafloxacin.

DK-507k had the lowest MICs for *S. pneumoniae* strains, including strains resistant to the quinolones available at

FIG. 4. Therapeutic efficacies of DK-507k and ciprofloxacin in a rat model of foreign body-associated urinary tract infection caused by *P. aeruginosa* 910735. The drugs were administered orally once a day for 3 consecutive days. The numbers of bacteria in the kidneys (A), bladder (B), and PT as a foreign body inside the bladder (C) were determined on the day after final drug administration. Each bar represents the mean $\pm$ standard error of the mean number of bacteria from specimens from six rats. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ versus the control.

present, irrespective of the quinolone resistance mechanism. This was the case for in vitro-selected mutants harboring mutations in both *gyrA* and *parC*. Although newer quinolones showed improved activities against in vitro-selected ciprofloxacin-resistant mutants, the MICs of these quinolones were the most highly elevated for high-level ciprofloxacin-resistant mutants with mutations in both *gyrA* and *parC* (ciprofloxacin MICs, ≥ 16 $\mu\text{g/ml}$). The high-level resistance to ciprofloxacin was associated with 4- to 8-fold increases in the MICs of DK-507k and 8- to 16-fold and 8- to 32-fold increases in the MICs of moxifloxacin and gatifloxacin, respectively. All high-level ciprofloxacin-resistant strains were inhibited by 0.5 μg of DK-507k per ml, whereas they were inhibited by 2 and 8 μg of moxifloxacin and gatifloxacin per ml, respectively. The high level of activity of DK-507k against clinical isolates of quinolone-resistant *S. pneumoniae* strains (22) was also confirmed in this study. In addition, the present data indicate that the efflux-mediated mechanism of resistance has no significant role on the activity of DK-507k. This finding may be consistent with that of a previous study (6), which reported that the newer quinolone gemifloxacin is highly active against quinolone-resistant pneumococci with an efflux-mediated mechanism of resistance. DK-507k, gatifloxacin, and moxifloxacin had good killing activities relative to their MICs for the *S. pneumoniae* strain that was the causative agent of pneumonia in a model used in the present study. Interestingly, DK-507k was more bactericidal than gatifloxacin and moxifloxacin, with significant and complete killing occurring earlier than the times of killing obtained with the other agents tested.

The therapeutic efficacy of DK-507k in the pneumococcal pneumonia model was far greater than those of gatifloxacin and moxifloxacin. The excellent in vivo activity of DK-507k is partly due to the fact that its MIC for the challenge organism is one-half those of gatifloxacin and moxifloxacin. However, this advantage of DK-507k is likely insufficient to explain its significant in vivo efficacy. The high in vivo efficacies of DK-507k against infections caused by *S. pneumoniae* were also demonstrated in models of septicemia in mice. The ED_{50} s of DK-507k were 4.4 to 11.6 and 3.8 to 17.5 times lower than those of moxifloxacin and gatifloxacin, respectively, against *S. pneumoniae* in models of septicemia. The MICs of DK-507k for these strains were four times lower than those of moxifloxacin and gatifloxacin. The pharmacokinetic parameters and levels of protein binding to mouse serum for DK-507k were roughly comparable to those for the comparator drugs. Taken together, our in vitro data suggest that the better killing activity of DK-507k, in combination with its lower MICs, seems to be one of the major determinants of its in vivo efficacy against *S. pneumoniae* infections. In septicemia models in mice, DK-507k was found to be the most effective compound against infections caused not only by gram-positive bacteria but also by gram-negative bacteria. The differences in the ED_{50} s between the compounds correlate well with the overall in vitro activities, with DK-507k having up to 16 times increased activity compared to those of the comparator drugs. The pharmacokinetic parameters for DK-507k administered intravenously were roughly similar to those for the comparator drugs in mice. Oral administration of DK-507k demonstrated its favorable pharmacokinetic characteristics in a rat model of a foreign body-associated urinary tract infection caused by *P. aeruginosa*. After

oral administration, DK-507k was absorbed rapidly and was excreted well into the urine. Therefore, even though the MIC of DK-507k for the challenge organism was higher than that of ciprofloxacin, DK-507k exhibited therapeutic effects comparable to or rather greater than those of ciprofloxacin, as assessed by the reductions in the bacterial burdens in the bladder and on the surface of the foreign object (PT) in the bladder. In this model, the bacteria enmeshed in biofilms formed on the surface of the PT in the bladder play an important role in the persistence of the infection in the bladder and kidneys (13). The antibacterial activity against the bacteria in a biofilm mode of growth might have clinical relevance (20), especially for urinary tract infections associated with foreign bodies, including urinary catheters and stents (12).

To be useful for the empirical treatment of community-acquired infections, a novel quinolone would have to exhibit higher levels of activity against both gram-positive and gram-negative pathogens with various resistance profiles and pharmacokinetic properties better than those of the quinolones available at present. DK-507k exhibits all of these characteristics and is shown to be effective in septicemia models in mice, a pneumococcal pneumonia model, and a model of foreign body-associated urinary tract infection caused by *P. aeruginosa*. In particular, the greater intrinsic potency of DK-507k for *S. pneumoniae* in vitro and in vivo has been confirmed in this study. A key area is that the greater intrinsic activity of DK-507k against *S. pneumoniae* can translate into sufficient activities against strains resistant to the available quinolones: the activity of DK-507k was affected to a lesser degree by the presence of mutations in the genes encoding subunits of DNA gyrase and topoisomerase IV. Clinical studies will be required to ascertain the role of DK-507k in the empirical treatment of community-acquired infections.

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