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In Vivo Evaluation of A-56619 (Difloxacin) and A-56620: New Aryl-Fluoroquinolones

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A-56619 and A-56620 are two new aryl-fluoroquinolones which are as potent as or more potent than norfloxacin when administered orally and subcutaneously in mouse protection tests against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. A-56619 and A-56620 were more potent than norfloxacin when administered orally against *Escherichia coli*, *Proteus mirabilis*, *Serratia marcescens*, and *Pseudomonas aeruginosa*. A-56620 was as potent or two- to threefold more potent than norfloxacin when administered subcutaneously against members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Infection with *Salmonella typhimurium* was more effectively treated with A-56619 (50% effective dose [ED_{50}], 1.4 mg/kg per day) than with norfloxacin (ED_{50}, 62.8 mg/kg per day). *E. coli* or *Pseudomonas* pyelonephritis in mice was more effectively treated with A-56619 or A-56620 than with norfloxacin. After oral treatment, the ED_{50}s of A-56619 and A-56620 were <12.5 mg/kg per day against *E. coli* and 62.9 and 38 mg/kg per day against *P. aeruginosa* pyelonephritis, respectively. Norfloxacin was ineffective at 200 mg/kg per day against *E. coli* or *P. aeruginosa* pyelonephritis. A-56619 and A-56620 were also more potent than norfloxacin in treatment of mixed bacterial pyelonephritis caused by *E. coli* and *Streptococcus faecalis*. A-56619 was at least 30 times more potent than norfloxacin and A-56620 was 4 to 11 times more potent than norfloxacin when administered against *Klebsiella* pneumonia in mice. A-56619 and A-56620 were at least 2 to 10 times more potent than norfloxacin against *Staphylococcus aureus* infections in immunosuppressed mice. A-56619 was equally potent in all in vivo tests when administered orally or subcutaneously, whereas A-56620 was similar to norfloxacin in being more potent when administered subcutaneously. The peak serum levels after subcutaneous and oral administration of A-56619 and A-56620 were higher than that of norfloxacin. The serum half-lives of A-56619 and A-56620 after subcutaneous and oral administration were longer than the serum half-life of norfloxacin.

In recent years piperazinyl-substituted quinolones have been synthesized and shown to be very potent, broad-spectrum antibacterial agents. Norfloxacin, the first of the promising new fluoroquinolones, has broad-spectrum in vitro activity and excellent potency in vivo (2; H. H. Gadebusch, Proc. 13th Int. Congr. Chemother., p. 1/38–4/38, 1983). More recently, quinolones such as pefloxacin, ciprofloxacin, ofloxacin, enoxacin, and amifloxacin, which have significantly better activity in vivo than norfloxacin, have been described (R. N. Jones, Antimicrob. Newsl. 1:41, 1984; L. J. Piddock and R. Wise, Antimicrob. Newsl. 2:1–4, 1985). A-56620 is a new aryl-fluoroquinolone which is similar in structure to norfloxacin but differs in having a *p*-fluorophenyl substitution at position 1. A-56619 is structurally similar to A-56620 except that it has a methyl group in the piperazine ring. A-56619 and A-56620 have been found to be very potent in vitro against a wide variety of gram-positive and gram-negative bacteria (9).

We studied the in vivo potency and efficacy of A-56619 and A-56620 in experimental infections in mice, including mouse protection tests, *Escherichia coli* and *Pseudomonas aeruginosa* pyelonephritis, *E. coli* and *Streptococcus faecalis* mixed pyelonephritis, acute bacterial pneumonia caused by *Klebsiella pneumoniae*, and in *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* systemic infection in immunosuppressed mice. Norfloxacin was used as the reference quinolone for these studies. Selected β-lactams and aminoglycosides were also used for comparative purposes.

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MATERIALS AND METHODS

Antibacterial agents. A-56619, A-56620, and norfloxacin were synthesized at Abbott Laboratories (North Chicago, Ill.). The other reference antimicrobial agents were obtained from the following companies: tobramycin, moxalactam, and vancomycin, Eli Lilly & Co. (Indianapolis, Ind.); gentamicin sulfate, Schering Corp. (Kenilworth N.J.); cefotaxime, Hoechst-Roussel Pharmaceuticals, Inc. (Somerville, N.J.); cephradine, E. R. Squibb & Sons (Princeton, N.J.); piperacillin, Lederle Laboratories (Wayne, N.J.); and ampicillin and amoxicillin, Parke, Davis & Co. (Morris Plains, N.J.). All antibiotic doses are expressed as milligrams of base per kilogram.

Bacterial strains. The bacteria used in these studies were unselected clinical isolates which were stored frozen or lyophilized in the Abbott culture collection. The following organisms were used for mouse protection tests: *E. coli* Juhl, *Staphylococcus aureus* 10649, *Pseudomonas aeruginosa* 5005 and 5007, *Streptococcus pyogenes* C203, *Streptococcus pneumoniae* 6303, *Serratia marcescens* A5038, *Proteus*

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vulgaris JJ, *Proteus mirabilis* Fin9, *Providencia stuartii* 677b, *Salmonella typhimurium* LT2-Pr, and *Klebsiella pneumoniae* 4508. *E. coli* 3100, *Pseudomonas aeruginosa* 5007, and *Streptococcus faecalis* 663f were used in the pyelonephritis model. *K. pneumoniae* 4508 was used in the acute pneumonia model and *E. coli* Juhl, *Staphylococcus aureus* 10649, and *Pseudomonas aeruginosa* 5007 were used in the model for infection in the immunosuppressed host.

Culture medium. Tryptic soy broth (Difco Laboratories, Detroit, Mich.) was used as the growth medium for *Pseudomonas aeruginosa*. Brain heart infusion broth supplemented with 5% sheep blood was used for *Streptococcus pneumoniae* and *Streptococcus pyogenes*; brain heart infusion broth was used for all other organisms.

Mouse protection tests. CF-1 female mice weighing 20 g were obtained from Charles River Breeding Laboratories, Inc. (North Wilmington, Mass.) The median lethal dose of each test organism was determined first in the following manner.

(i) LD_{50} determination. After 18 h of incubation, the cultures were serially diluted by using 10-fold dilutions in 5% (wt/vol) hog gastric mucin (American Laboratory Inc., Omaha, Nebr.). A 0.5-ml sample of each culture, diluted from 10^{-1} to 10^{-8}, was injected intraperitoneally into mice. Cultures of three organisms, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Salmonella typhimurium*, were diluted in brain heart infusion broth alone; these organisms are sufficiently virulent for mice and do not require mucin to induce disease. The 50% lethal dose (LD_{50}) for each test organism was calculated from the cumulative mortalities on day 6 by the Reed and Muench procedure (8).

(ii) Protection test (ED_{50} determination). The 18-h-old cultures of the test organisms were diluted in 5% (wt/vol) hog gastric mucin to obtain 10, 100, 1,000, etc., times the LD_{50}s as determined by preliminary experiments for each organism, and 0.5 ml was injected intraperitoneally into mice. Mucin was not used when *Streptococcus pyogenes*, *Streptococcus pneumoniae*, or *Salmonella typhimurium* was the test organism. The mice were treated subcutaneously at 1 and 5 h after infection except when the mice were infected with *Salmonella typhimurium* in which case the mice were treated 24 and 28 h after infection. Ten mice were left untreated as the infection control. These untreated mice died by day 2 after infection. The 50% effective doses (ED_{50}s) were calculated from the cumulative mortalities on day 6 after infection for all organisms except *Salmonella typhimurium*, using the trimmed version of the Logit method (4). The ED_{50}s for *Salmonella typhimurium* were calculated on day 22 after infection.

Norfloxacin was selected as the reference quinolone because it is the first of the new potent fluoroquinolones and because of its availability. Cefotaxime or moxalactam was selected as the reference β-lactam to be administered subcutaneously against all organisms except *Pseudomonas aeruginosa*. Piperacillin was chosen as the reference β-lactam to be used against *Pseudomonas aeruginosa*. Since there was no orally absorbed broad-spectrum cephalosporin available, cephradine was tested orally when cefotaxime was tested subcutaneously. Ampicillin or amoxicillin was also tested against *E. coli*. Gentamicin was tested as the reference aminoglycoside against all organisms except *Pseudomonas aeruginosa*. Tobramycin was used as the reference aminoglycoside against *Pseudomonas aeruginosa* because it is more active than gentamicin against this organism. Other new quinolones and β-lactams could not be tested in vivo because they were not available in sufficient quantity. All reference drugs were tested at the same time as A-56619 and A-56620.

Murine pyelonephritis. Mice were administered 10 mg of carrageenan (FMC Corp., Marine Colloids Div., Springfield, N.J.) per kg in 0.2 ml of water intravenously into the tail vein. Seven days after injection of carrageenan, 0.2 ml of an overnight culture diluted to contain approximately 10^5 CFU of *E. coli* or approximately 10^6 CFU of *Pseudomonas aeruginosa* was injected intravenously via the tail vein. This model for pyelonephritis has been described previously (O. Zak, F. Kradolfer, E. A. Konopka, S. Kunz, and A. Batt, Proc. 19th Int. Congr. Chemother., p. 846–848, 1978). The mixed bacterial pyelonephritis infection was obtained by intravenous injection of 0.2 ml of culture containing approximately 10^5 CFU of *E. coli* and 10^6 CFU of *Streptococcus faecalis*.

(i) Quantitation of infection level in the kidneys. Five mice were killed 24 h after infection; their kidneys were homogenized, serially diluted in phosphate-buffered saline, and cultured in duplicate on MacConkey agar plates. The plates were incubated at 37°C for 24 to 48 h, and the viable bacterial counts (CFU) in the two kidneys of each animal were calculated. The geometric mean of the CFU and the standard error of the mean in the five mice were calculated and accepted as the level of infection before treatment.

(ii) Treatment of mice with pyelonephritis. Groups of 10 mice were treated twice daily for 2 days beginning at 24 h postinfection. The daily dose was administered orally in two gavages spaced 4 h apart on each day. Three dose levels of each drug were tested. Ten untreated mice served as controls for the infection. Amoxicillin and norfloxacin were used as orally administered reference drugs against *E. coli*, and gentamicin was selected as the subcutaneously administered reference drug. Norfloxacin was the only reference drug used against *Pseudomonas aeruginosa* and against *E. coli* and *Streptococcus faecalis* in the mixed bacterial pyelonephritis model.

(iii) Determination of ED_{50}. At 24 h after the last treatment, the kidneys of treated and untreated mice were cultured quantitatively as described above. The geometric mean of the viable counts in each treatment group was obtained and compared with the geometric mean of the viable counts in the kidneys of untreated mice. The lowest number of organisms detectable by this method was 50 CFU (1.7 log). The ED_{50}s were calculated by using inverse regression analysis. The ED_{50} was defined as the dose required to reduce bacterial counts in the kidneys of 50% of the infected mice to less than 50 CFU.

Development and treatment of acute bacterial pneumonia. Pneumonias caused by gram-negative bacteria are difficult to treat with currently available antibiotics. Potency and lung penetration are two important factors determining the effectiveness of an agent against such infections. To determine whether A-56619 or A-56620 was effective in treating such infections, we used a model for *Klebsiella* pneumonia in mice. Ether-anesthetized mice were infected by instilling $\sim 10^3$ CFU intranasally. The viable bacterial count in the lungs of five mice was determined at 4 h after infection by homogenizing the lungs and culturing serial 10-fold dilutions of the homogenate. The remaining mice were separated into groups of 10 and were treated with three dose levels of the test drug or reference compounds either subcutaneously or orally. Ten untreated mice served as controls for the infection. Cumulative mortalities in each group of mice were used to calculate the ED_{50}s at the end of 21 days, using the trimmed version of the Logit method (4). The untreated

control mice died within 7 days. Norfloxacin was used as the reference drug.

Infection in immunosuppressed mice. Mice were immunosuppressed by administering 150 mg of cyclophosphamide per kg on day 0 and 110 mg/kg on day 4. Mice treated in this manner had no detectable neutrophils on day 5. The cyclophosphamide-treated mice were infected with *E. coli*, *Staphylococcus aureus*, or *Pseudomonas aeruginosa* on day 5 in the same manner as described for the mouse protection test. Three groups of 10 mice were treated with each test compound at different dose levels of the drug. Ten infected mice were untreated and served as controls for the infection. The control mice died within 24 h after infection. The ED_{50}s were calculated on the basis of cumulative mortalities on day 6 after infection by the trimmed version of the Logit method (4). The choice of reference antimicrobial agents was the same as in the mouse protection tests except that vancomycin was included against *Staphylococcus aureus*.

Pharmacokinetic studies. Mice were administered 100 mg/kg as a single dose either orally or subcutaneously. At 30 min and 1, 2, 3, 6, and 24 h, blood was collected from groups of five mice. All samples were assayed for A-56619, A-56620, or norfloxacin by a disk agar diffusion bioassay procedure. *Bacillus subtilis* 6633 was used as the assay organism and seed agar medium no. 1 (BBL Microbiology Systems; Cockeysville, Md.) was the growth medium. The plates were incubated at 32°C for 18 h and read with an image analyzer (Optomax Inc.).

The serum samples of one experiment were also analyzed by high-pressure liquid chromatography with a Waters Associates liquid chromatograph system (M-6000A chromatography pump for solvent delivery system and a μBondapak C18 column). Sera and urine were injected directly with no pretreatment. A guard column (Waters Associates) was placed between the injector and the analytical column. CH_3CN–0.1% H_3PO_4 (20:80, vol/vol) was used as eluent. Detection was either by UV (280 nm) with a Schoeffel 6M 770 UV monochromator or fluorometry (281/418) with a Kratos FS 970 LC fluorometer.

RESULTS

Mouse protection tests. The potencies of A-56619, A-56620, norfloxacin, and other reference compounds in several mouse protection tests are shown in Table 1.

In general, A-56619 and A-56620 were as potent or 5- to 10-fold more potent than norfloxacin when administered orally or subcutaneously against *Staphylococcus aureus* and streptococci. A-56619 was as active as A-56620 against *Staphylococcus aureus* and the streptococci. Gentamicin, cefotaxime, and cephradine were equal to or more potent than the quinolones against *Staphylococcus aureus* and streptococci in vivo. Potencies of A-56620 and A-56619 were equal to or 2- to 10-fold greater than norfloxacin against members of the family *Enterobacteriaceae* when administered orally. The potency of A-56620 was generally similar to that of norfloxacin when administered subcutaneously against the enterics. A-56619 was less effective than both A-56620 and norfloxacin when administered subcutaneously against *Proteus vulgaris*, *Proteus mirabilis*, and *E. coli*. Potencies of A-56619 and A-56620 were equal to or greater than that of norfloxacin against *Pseudomonas aeruginosa* when administered subcutaneously; A-56619 and A-56620 were more potent than norfloxacin when administered orally against *Pseudomonas aeruginosa*. The potencies of A-56619 and A-56620 were generally equal to or greater than those of other reference compounds. A-56619 was at least 50 times more active than norfloxacin against *S. typhimurium* when administered subcutaneously or orally. A-56619 was 10 times more potent than cefotaxime or gentamicin when used subcutaneously and 10 and 30 times more active than cephradine and ampicillin, respectively, when administered orally. A-56620 was less potent than A-56619 but more potent than ampicillin and as effective as cephradine, cefotaxime, or gentamicin against *Salmonella typhimurium*. The potency of A-56620 against *Salmonella typhimurium* was equal to that of norfloxacin when administered orally and greater when administered via the subcutaneous route.

Murine pyelonephritis models. Three models for pyelonephritis were used to evaluate the potency of A-56619 and A-56620. These model infections are difficult to treat because organisms multiply in kidney tissues damaged by the injection of carageenan.

(i) *E. coli* pyelonephritis. The kidneys of *E. coli*-infected mice contained approximately 10^4 CFU just before treatment. The effect of oral treatment for 2 days on bacteria in the kidneys is shown in Table 2. A-56619 and A-56620 were significantly superior to norfloxacin or amoxicillin in their ability to clear the kidneys of *E. coli*. After 2 days of oral treatment the ED_{50}s of A-56619 and A-56620 were <12.5 (0 to 12.5) mg/kg per day. Orally administered norfloxacin (ED_{50} = 138.4 [65 to 293.7] mg/kg per day) or amoxicillin (ED_{50} = 247 [125 to 486] mg/kg per day) was significantly less effective than A-56619 or A-56620 in treating *E. coli* pyelonephritis.

When mice with *E. coli* pyelonephritis were treated subcutaneously, A-56619 (ED_{50} = 2.8 [1.7 to 4.5] mg/kg per day) was significantly more effective than A-56620 (ED_{50} = 26.2 [11.7 to 58.5] mg/kg per day), norfloxacin (ED_{50} = 295.3 [25 to >999] mg/kg per day), and gentamicin (ED_{50} = 47.3 [16.8 to 133.5] mg/kg per day) in clearing the kidneys of infection (Table 2).

(ii) *Pseudomonas aeruginosa* pyelonephritis. The kidneys of infected mice contained approximately 10^6 CFU before treatment. Orally administered A-56619 or A-56620 was effective in treating *Pseudomonas aeruginosa* pyelonephritis. The results are shown in Table 2. The infection in the kidneys of 50% of the mice was cleared by A-56619 or A-56620 at doses of 62.9 (46.5 to 85) mg/kg per day or 38 (25 to 57) mg/kg per day, respectively, whereas norfloxacin was ineffective in clearing the infection even at a dose as high as 200 mg/kg per day (ED_{50} = 544.8 [297 to 998] mg/kg per day).

(iii) Mixed *E. coli* and *Streptococcus faecalis* pyelonephritis. To determine whether A-56619 or A-56620 was useful in treating urinary tract infections induced by a combination of *E. coli* and *Streptococcus faecalis*, mice with mixed bacterial pyelonephritis were treated orally for 3 days with A-56619, A-56620, or norfloxacin. The kidneys of infected mice contained approximately 10^3 CFU of *E. coli* and 10^3 CFU of *Streptococcus faecalis* before treatment (Table 2). A-56619 or A-56620 was significantly more effective than norfloxacin in eradicating both *E. coli* and *Streptococcus faecalis* in the kidneys of treated mice.

Efficacy of A-56619 and A-56620 against *K. pneumoniae* pneumonia. The lungs of mice infected intranasally with 10 LD_{50}s of *K. pneumoniae* contained approximately 10^5 CFU in the lungs just before treatment. The results are shown in Table 3. Administered subcutaneously, A-56619 or A-56620 was as effective as norfloxacin. However, when used orally, A-56620 was significantly more effective than norfloxacin.

Efficacy of A-56619 and A-56620 against infections in immunosuppressed mice. The protective effect of A-56619,

TABLE 1. Mouse protection tests with a variety of aerobic bacteria

Bacterial strain	Infection dose (CFU/mouse) ($\times LD_{50}$)	Test compound[a]	MIC (μg/ml)	ED_{50} (mg/kg per day)[b] (confidence limits)	
				Subcutaneous	Oral
Staphylococcus aureus 10649	2.5×10^5 (500)	A-56619	0.12	1.7 (1.0–2.9)	3.2 (1.9–5.5)
		A-56620	0.12	1.6 (0.9–2.6)	5.4 (2.9–10.3)
		Norfloxacin	1.0	3.2 (1.9–5.5)	49.7 (28.9–85.4)
		Gentamicin	0.12	0.3 (0.1–0.5)	
		Cefotaxime	2	6.6 (1.0–42)	
		Cephradine	2		1.8 (0.9–3.5)
Streptococcus pyogenes C203[c]	1.5×10^3 (100)	A-56619	1	5.2 (2.5–10.8)	8.7 (4.9–15.5)
		A-56620	0.5	15.6 (6.2–39.4)	14.7 (5.7–38.3)
		Norfloxacin	2	38.6 (20.5–72.2)	174.9 (4.8–6,413)
		Gentamicin	2	5.8 (0.5–64)	
		Cefotaxime	0.03	<0.4	
		Cephradine	0.06		0.2 (0.01–3.2)
Streptococcus pneumoniae 6303[c]	1.3×10^7 (500)	A-56619	2.0	14.9 (9.8–22.9)	19.6 (12.7–30.3)
		A-56620	1.0	17.5 (10.9–28.3)	40.0 (25.3–63.4)
		Norfloxacin	4.0	92.4 (51.5–165.8)	>200
		Gentamicin	4.0	32.6 (21.3–49.9)	
		Cefotaxime	0.03	12.6 (6.7–23.5)	
		Cephradine	0.06		15.7 (6.6–37.4)
Escherichia coli Juhl	2.5×10^6 (100)	A-56619	0.12	2.2 (1.3–3.6)	3.9 (2.3–6.5)
		A-56620	0.03	0.7 (0.4–1.1)	1.6 (0.9–3.0)
		Norfloxacin	0.06	0.7 (0.4–1.2)	12.6 (8.6–18.6)
		Ampicillin	2.0	16.5 (8.3–32.5)	68.2 (41.1–113.2)
		Gentamicin	1.0	2.3 (1.5–3.4)	
		Cefotaxime	0.06	2.1 (3.7–9.9)	
		Cephradine	4		61.7 (34.1–111.8)
Klebsiella pneumoniae 4508	5.0×10^4 (1,000)	A-56619	0.12	0.5 (0.3–0.8)	1.1 (0.5–2.3)
		A-56620	0.03	0.4 (0.2–0.7)	1.2 (0.6–2.6)
		Norfloxacin	0.06	0.4 (0.2–0.8)	4.4 (2.4–8.0)
		Gentamicin	0.12	0.4 (0.2–0.9)	
		Moxalactam	0.06	0.5 (0.2–1.0)	
		Cephradine	2		33.1 (15.4–71.3)
Salmonella typhimurium LT2-Pr	7.9×10^3 (10)	A-56619	0.25	1.6 (0.7–3.8)	1.4 (0.7–2.7)
		A-56620	0.06	20.9 (11.4–38.8)	10.4 (6.1–17.7)
		Norfloxacin	0.125	>50	62.8 (13.4–293.3)
		Gentamicin	1.0	13.8 (7.1–26.7)	
		Cefotaxime	0.06	15.3 (7.–32.40	
		Cephradine	2		16.1 (5–52)
		Ampicillin	2.0		51.3 (23–112)
Proteus mirabilis Fin9	1.5×10^6 (10)	A-56619	1.0	2.9 (1.9–4.5)	7.3 (4.8–11.2)
		A-56620	0.12	1.9 (1.3–2.8)	7.7 (5.4–11.0)
		Norfloxacin	0.12	1.1 (0.8–1.7)	16.2 (11.3–23.1)
		Gentamicin	1.0	2.6 (1.7–4.0)	
		Cefotaxime	0.12	0.6 (0.3–1.2)	
		Cephradine	16		>100
Proteus vulgaris JJ	1.2×10^6 (100)	A-56619	2.0	4.2 (2.5–6.9)	14.9 (8.8–25.3)
		A-56620	0.25	1.6 (0.6–4.2)	8.5 (5.1–14.1)
		Norfloxacin	0.03	1.1 (0.8–1.7)	11.4 (6.7–19.5)
		Gentamicin	1.0	1.2 (0.5–2.9)	
		Cefotaxime	0.06	2.6 (1.1–6.4)	
		Cephradine	2		>100
Serratia marcescens A5038	1.9×10^5 (500)	A-56619	1.0	1.5 (0.8–2.6)	1.9 (1.1–3.2)
		A56620	0.06	0.5 (0.2–0.9)	1.4 (0.9–2.3)
		Norfloxacin	0.25	0.9 (0.6–1.7)	9.7 (6.7–14.1)
		Gentamicin	1.0	0.9 (0.6–1.4)	
		Cefotaxime	0.25	36.7 (14.6–91.8)	
Providencia stuartii 677b	1.0×10^6 (100)	A-56619	0.5	3.7 (2.2–6.3)	4.9 (2.9–8.5)
		A-56620	0.06	0.5 (0.2–0.9)	3.5 (1.6–7.6)
		Norfloxacin	0.06	0.4 (0.01–19.7)	6.2 (3.6–10.8)

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TABLE 1.—*Continued*

Bacterial strain	Infection dose (CFU/mouse) (×LD_{50})	Test compound[a]	MIC (μg/ml)	ED_{50} (mg/kg per day)[b] (confidence limits)	
				Subcutaneous	Oral
		Gentamicin	0.25	0.5 (0.2–0.9)	
		Cefotaxime	0.06	0.04 (0.001–3.1)	
		Cephradine	2		87.1 (51.5–147.4)
Pseudomonas aeruginosa 5005	7.9×10^5 (100)	A-56619	1.0	5.2 (3.1–8.9)	10.9 (6.2–19.3)
		A-56620	0.05	1.9 (0.9–4.0)	5.9 (3.4–10.2)
		Norfloxacin	0.5	6.4 (4.4–9.2)	43.6 (31.1–61.2)
		Tobramycin	1.0	3.2 (1.5–6.9)	
		Piperacillin	>16	42.1 (17.7–100)	

[a] Test compound administered 1 and 5 h after infection.
[b] ED_{50}, Median effective dose calculated on day 6.
[c] Mucin not used to induce infection.

A-56620, norfloxacin, and other reference compounds against systemic *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* infections in immunosuppressed mice is shown in Table 4. A-56619 and A-56620 were equally potent in all tests except when administered subcutaneously against *E. coli*. The potencies of A-56619 and A-56620 were equal to or better than that of norfloxacin when tested subcutaneously. When administered orally, A-56619 and A-56620 were approximately 10 times more active than norfloxacin against *E. coli* and *Staphylococcus aureus* and as active as norfloxacin against *Pseudomonas aeruginosa*.

Pharmacokinetic profile in mice. After oral administration of 100 mg of A-56619, A-56620, or norfloxacin per kg, the serum levels shown in Fig. 1 were obtained. The peak serum level of A-56619 (22.4 μg/ml) was higher than that of norfloxacin (1.2 μg/ml). The peak serum level of A-56620 (4.5 μg/ml) was also higher than that of norfloxacin. The areas under the curve were 222.4 μg · h/ml for A-56619, 23.9 μg · h/ml for A-56620, and 0.6 μg · h/ml for norfloxacin. The serum half-lives of A-56619, A-56620, and norfloxacin were 6.7, 2.7, and <0.5 h, respectively.

After subcutaneous administration of 100 mg of A-56619 per kg, the peak serum level (18.5 μg/ml) and the area under the serum curve (207.3 μg · h/ml) were similar to those obtained after oral administration (Fig. 2). This indicates that A-56619 is virtually completely absorbed after oral administration. The peak serum level of A-56620 after subcutaneous administration was 28.9 μg/ml compared with 9.2 μg/ml for

TABLE 2. Potency of A-56619, A-56620, norfloxacin, gentamicin, and amoxicillin against murine pyelonephritis

Infecting organism(s)	Drug[a]	Route of administration	Viable bacterial counts[b] (log_{10} CFU ± SEM) at doses of (mg/kg per day):						
			Control	12.5	50	200	1.6	6.3	25.0
Escherichia coli 3100	A-56619	Oral		1.8 ± 0.2	1.8 ± 0.2	1.7 ± 0.2			
	A-56620	Oral		2.8 ± 0.3	2.1 ± 0.2	1.9 ± 0.2			
	Norfloxacin	Oral		5.7 ± 0.4	4.7 ± 0.3	2.6 ± 0.2			
	Amoxicillin	Oral		6.9 ± 0.5	5.5 ± 0.4	3.8 ± 0.7			
			7.3 ± 0.7						
Pseudomonas aeruginosa 5007	A-56619	Oral		6.4 ± 04	4.8 ± 0.6	1.7 ± 0.2			
	A-56620	Oral		5.6 ± 0.4	3.1 ± 0.5	1.9 ± 0.3			
	Norfloxacin	Oral		7.0 ± 0.3	6.5 ± 0.4	4.6 ± 0.4			
			7.3 ± 0.7						
Escherichia coli 3100	A-56619	s.c.[c]					5.5 ± 0.8	2.2 ± 0.6	1.7 ± 0.3
	A-56620	s.c.					5.5 ± 0.5	3.3 ± 0.7	2.0 ± 0.4
	Norfloxacin	s.c.					6.8 ± 0.7	6.7 ± 0.7	4.3 ± 0.6
	Gentamicin	s.c.					7.3 ± 0.6	4.8 ± 0.9	3.1 ± 0.6
			6.8 ± 1.1						
Streptococcus faecalis 663f + *Escherichia coli* 3100	A-56619	Oral		5.1 ± 0.4 + 2.5 ± 0.1	4.0 ± 0.3 + 2.7 ± 0.1	2.5 ± 0.4 + 1.7 ± 0.0			
	A-56620	Oral		5.5 ± 0.2 + 1.7 ± 0.0	5.3 ± 0.4 + 1.8 ± 0.1	3.1 ± 0.6 + 1.8 ± 0.1			
	Norfloxacin	Oral		5.6 ± 0.5 + 5.5 ± 0.7	5.5 ± 0.4 + 3.8 ± 0.7	5.2 ± 0.4 + 2.1 ± 0.3			
			5.6 ± 0.4 + 8.0 ± 0.2						

[a] MICs against *E. coli* 3100: A-56619, 0.25 μg/ml; A-56620, 0.06 μg/ml; norfloxacin, 0.03 μg/ml; amoxicillin, 2 μg/ml; gentamicin, 1 μg/ml. MICs against *Pseudomonas aeruginosa* 5007: A-56619, 4 μg/ml; A-56620, 0.5 μg/ml; norfloxacin, 0.25 μg/ml. MICs against *Streptococcus faecalis* 663f; A-56619, 8 μg/ml; A-56620, 4 μg/ml; norfloxacin, 4 μg/ml.
[b] Geometric mean CFU in two kidneys.
[c] s.c. Subcutaneous.

TABLE 3. Efficacy of A-56619, A-56620, and reference compounds in *Klebsiella* pneumonia in mice[a]

Test compounds[b]	MIC (μg/ml)	ED_{50} (mg/kg per day) (confidence limits)[c]	
		Subcutaneous	Oral
A-56619	1.0	0.73 (0.2–2.5)	0.3 (0.007–17.1)
A-56620	0.03	0.5 (0.2–1.2)	2.6 (1.2–5.7)
Norfloxacin	0.12	0.8 (0.4–1.7)	18.6 (8.5–41.0)

[a] The strain used was *K. pneumoniae* 4508 at an infectious dose of 5.0×10^2 CFU per mouse (10 times the LD_{50}).
[b] Test compounds administered 4, 24, and 28 h after infection.
[c] ED_{50}, Median effective dose calculated at the end of 21 days.

norfloxacin. The areas under the curve for A-56619, A-56620, and norfloxacin were 207.3, 165.3, and 41.37 μg · h/ml, respectively. The half life was 10.9 h for A-56619, 2.6 h for A-56620, and 1.8 h for norfloxacin. The serum samples from one experiment after subcutaneous administration and one experiment after oral administration were analyzed by high-pressure liquid chromatography. The results were in general agreement with the results obtained by bioassay methods.

DISCUSSION

The ability of compounds to protect mice from lethal infection after an intraperitoneal challenge of virulent bacteria is a time-tested method of evaluating the efficacy of new compounds relative to reference compounds. The activity of A-56619, A-56620, and reference compounds after subcutaneous administration was in accord with in vitro MICs, i.e., A-56620 was generally as potent as A-56619 against the gram-positive cocci but was more potent than A-56619 when tested against members of the *Enterobacteriaceae* and *Pseudomonas aeruginosa*. When administered orally, however, the relative activity of A-56619 was much greater than would be expected from the in vitro MICs as it was more potent than norfloxacin against all organisms except *Proteus vulgaris* and *Providencia stuartii* in mouse protection tests. Pharmacokinetic studies showed that the oral absorption of A-56619 was significantly better than that of A-56620 or norfloxacin (3). It is possible that the methyl group on the piperazine ring could contribute to the excellent pharmacokinetic properties of A-56619. A similar modification, addition of a methyl group to the piperazine ring of norfloxacin, yields pefloxacin which has improved pharmacokinetic properties (7). Detailed pharmacokinetic and metabolic studies will be published elsewhere. The high and sustained serum levels achieved by A-56619 could explain the superior efficacy of this compound in experimental animal infections.

When orally administered, A-56619 and A-56620 were significantly more potent than norfloxacin, amoxicillin, or subcutaneously administered gentamicin in treating *E. coli* pyelonephritis. These new aryl-fluoroquinolones were also more potent than norfloxacin in treating *Pseudomonas aeruginosa* pyelonephritis. It was surprising that in this relatively severe infection of damaged kidneys, A-56619 or A-56620 could clear all the bacteria from the kidneys. There was no significant difference between the potency of A-56619 and A-56620 when administered orally, in this infection model.

Urinary tract infections caused by a combination of *E. coli* and *Streptococcus faecalis* are difficult to treat with the extended-spectrum cephalosporins; *Streptococcus faecalis* is not susceptible to these antibiotics (1, 6). In this study, we found that A-56619 and A-56620 have in vivo activity against both *Streptococcus faecalis* and *E. coli* in a mixed pyelonephritis model. Although norfloxacin was active against these organisms in vitro it was significantly less effective in eradicating both the organisms from the kidneys.

The older quinolones nalidixic acid and pipemidic acid and the new, more potent quinolone norfloxacin (5) do not achieve significant serum levels. Since high serum levels could be achieved with A-56619 and A-56620, the efficacy of these new quinolones in treatment of respiratory tract infections was determined with a mouse model for acute bacterial pneumonia. In this study, A-56619 and A-56620 were both very effective in mice when administered orally against

TABLE 4. In vivo efficacy of A-56619, A-56620, and reference compounds in treating infections in immunosuppressed mice

Organism (strain)	Infection dose (CFU./mouse) (×LD_{50})	Test compounds[a]	MIC (μg/ml)	ED_{50} (mg/kg per day) (confidence limits)[b]	
				Subcutaneous	Oral
Escherichia coli Juhl	1.2×10^6 (1,000)	A-56619	0.12	4.1 (2.6–6.6)	3.8 (2.2–6.7)
		A-56620	0.03	1.0 (0.5–2.3)	4.2 (2.3–7.4)
		Norfloxacin	0.06	5.7 (3.5–9.3)	31.9 (19.8–51.2)
		Gentamicin	1.0	2.1 (1.3–3.5)	
		Cefotaxime	0.06	13.2 (6.4–27.1)	
		Amoxicillin	2		87.5 (44.6–171)
Pseudomonas aeruginosa 5007	1.9×10^5 (1,000)	A-56619	2	20.9 (13.6–32.1)	53.1 (29–97)
		A-56620	0.5	16.0 (9.5–26.9)	72.4 (41.9–124.8)
		Norfloxacin	0.125	37.8 (12.8–111.9)	180.4 (72–451)
		Tobramycin	2	6.6 (3–14.2)	
		Piperacillin	8	>200	
Staphylococcus aureus 10649	6.3×10^5 (1,000)	A-56619	0.12	2.1 (1.3–3.5)	3.4 (2.2–5.2)
		A-56620	0.12	3.5 (2.1–5.8)	7.5 (4.9–11.2)
		Norfloxacin	1.0	10.0 (6–16.6)	57.6 (36.9–89.8)
		Gentamicin	0.12	2.7 (0.5–13.1)	
		Cefotaxime	2	5.9 (0.9–35)	
		Vancomycin	3.1	5.2 (0.9–28)	

[a] Test compounds administered 4, 24, and 28 h after infection.
[b] ED_{50}, Median effective dose calculated at the end of 21 days.

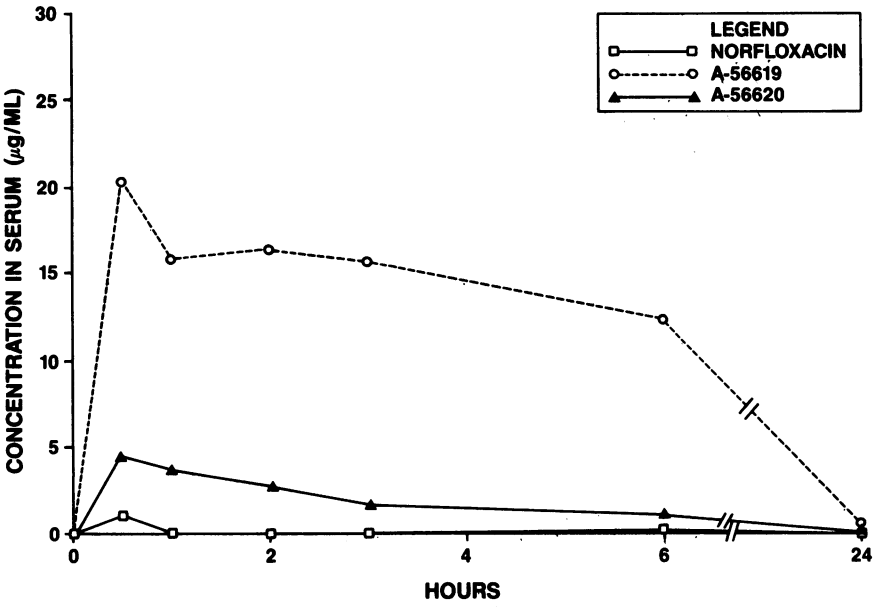

FIG. 1. Concentration of A-56619, A-56620, and norfloxacin in serum after oral administration of 100 mg/kg to mice.

Klebsiella pneumonia. A-56619 was more active than A-56620.

Bacterial infections in immunocompromised patients are difficult to treat because the antibacterial agent must be effective alone without the help of the immune system of the host. In such cases, a bactericidal agent is generally more effective than a bacteriostatic agent. In addition, infections in these patients may be caused by any aerobic or anaerobic organism or by a mixture of two or more organisms. The above factors favor broad-spectrum, rapidly bactericidal agents such as the new quinolones. A-56619 and A-56620 are broad-spectrum quinolones which kill 99.9% of bacteria in 2 h when added to a logarithmic-phase culture in vitro at four times the MIC (9). These agents were very effective in treating *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* systemic infections in mice immunosuppressed with cyclophosphamide. They were superior to norfloxacin when tested orally and, in general, were equal to or better than aminoglycosides and β-lactams.

In summary, A-56619 and A-56620 were more active than norfloxacin in most experimental animal infections. A-56620 had the advantage of lower MICs, longer half-life, and higher peak serum levels compared with norfloxacin. The almost complete absorption after oral administration, long half-life, and high peak serum levels of A-56619 accounted for its in vivo potency.

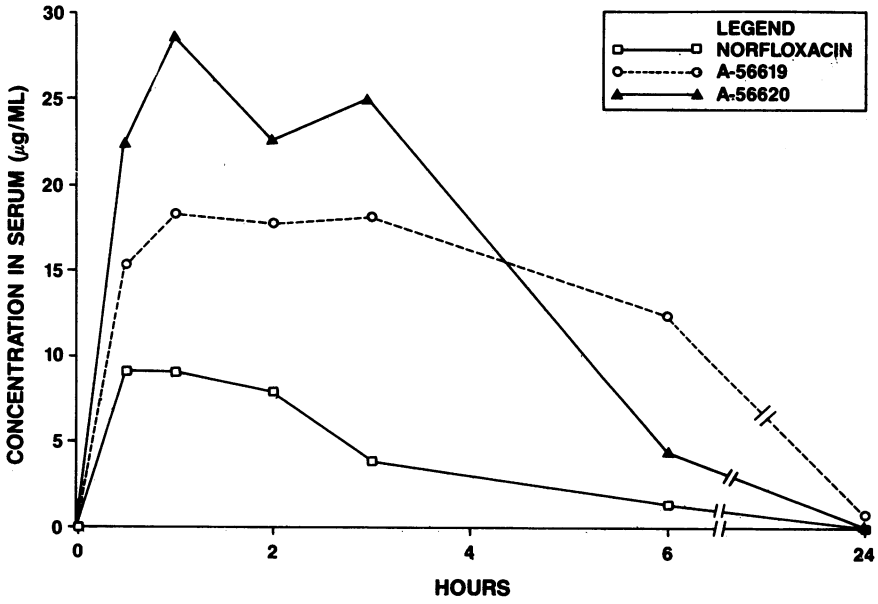

FIG. 2. Concentration of A-56619, A-56620, and norfloxacin in serum after subcutaneous administration of 100 mg/kg to mice.

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