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In Vitro Activities of the Quinolone Antimicrobial Agents A-56619 and A-56620

GEORGE M. ELIOPOULOS,[1,2]* ANNE E. MOELLERING,[1] EDINA REISZNER,[1] AND ROBERT C. MOELLERING, JR.[1,2]

Department of Medicine, New England Deaconess Hospital,[1] and Harvard Medical School,[2] Boston, Massachusetts 02215*

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The in vitro activities of two new quinolone antimicrobial agents, A-56619 and A-56620, were compared with those of norfloxacin, ciprofloxacin, and other antimicrobial agents. The activity of A-56620 was comparable to that of ciprofloxacin against *Escherichia coli*, *Enterobacter cloacae*, and *Aeromonas hydrophila* (MICs for 90% of the strains were ≤0.06 μg/ml); *Acinetobacter anitratus* and *Staphylococcus aureus* (MIC for 90% of the strains was 0.5 μg/ml); and most streptococci. Against other gram-negative strains, A-56620 demonstrated activity comparable to that of norfloxacin, but the new drug was two to eight times more active than norfloxacin against gram-positive isolates. A-56620 was more active than A-56619 against most gram-negative organisms tested. Of the members of the family *Enterobacteriaceae* examined, 88% were inhibited by A-56619 and 99% by A-56620 at concentrations of ≤1.0 μg/ml. By time-kill methods, the new quinolones were bactericidal against gram-negative bacilli during the first 6 h of incubation, but against *S. aureus* and enterococci the drugs were primarily bacteriostatic during this period. The frequency of spontaneous resistance to 10 μg of these drugs per ml was $< 10^{-8}$ for all species tested except *E. cloacae*, but by serial passage through incremental concentrations of the antimicrobial agents, colonies many-fold more resistant than the initial isolate could be selected. However, resistance to concentrations of the drug greater than 100 μg/ml remained stable after passage on antibiotic-free media in only 1 of 35 strains tested.

Recent advances in the understanding of structure-activity relationships among substituted quinoline and naphthyridine antimicrobial agents has led to the development of several promising antibacterial compounds, including norfloxacin (9), ciprofloxacin (6), enoxacin (8), amifloxacin (7), and others (11, 14). Two new fluoroquinolones, A-56619 and A-56620, are representative of a novel series of compounds containing an aryl substitution at the 1-position of the quinolone ring (D. T. W. Chu, P. B. Fernandes, A. K. Claiborn, T. J. O'Donnell, E. Pihuleac, C. Nordeen, and A. Pernet, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 72, 1984). Preliminary evidence suggests that these drugs are active against a wide variety of gram-negative and gram-positive bacteria (C. Hanson, R. Bailer, E. Gade, D. Chu, P. E. Fernandes, and A. Pernet, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 75, 1984).

The present study examined the in vitro activity of A-56619 and A-56620 in comparison with those of ciprofloxacin, norfloxacin, piperacillin, cefotaxime, and tobramycin. The early bactericidal activity of these drugs against representative routine clinical bacterial isolates was evaluated by time-kill studies. In view of earlier concerns about the development of resistance during therapy with nalidixic acid (10), the frequency of spontaneous resistance to the newer agents and the extent to which resistance could be selected by serial exposure of clinical isolates to incremental concentrations of the drugs were also investigated.

MATERIALS AND METHODS

Bacterial strains. Gram-negative bacilli used in this study were routine clinical isolates recently collected at our hospital. Gram-positive bacteria had been previously collected at the Massachusetts General Hospital or obtained from other sources as reported elsewhere (5).

Antimicrobial agents. Antimicrobial reference standard powders were obtained from the following sources: A56619 and A56620, Abbott Laboratories, North Chicago, Ill.; ciprofloxacin, Miles Pharmaceuticals, West Haven, Conn.; norfloxacin, Merck Sharp & Dohme Research Laboratories, Rahway, N.J.; piperacillin, Lederle Piperacillin Inc., Carolina, P.R.; and cefotaxime, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J. Tobramycin sulfate was obtained from Eli Lilly & Co., Indianapolis, Ind.

Agar dilution susceptibility studies. Susceptibility testing was performed by a standard agar dilution technique (15) with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.). The medium was supplemented with 5% defibrinated sheep blood when testing nonenterococcal streptococci or with agar to a concentration of 4% (Difco Laboratories, Detroit, Mich.) when testing *Proteus* species to prevent swarming. Brucella agar (Difco) supplemented with 10% sheep blood was used for *Campylobacter jejuni*. Inocula were prepared by suspending several colonies from fresh cultures of the test organism in normal saline and diluting in Mueller-Hinton broth to ca. 10^7 CFU/ml. Final inocula of ca. 10^4 CFU were applied to plates by means of a 32-prong inoculator. Plates were examined for growth after 20 h of incubation at 37°C in room air or, for *C. jejuni*, in a microaerophilic atmosphere (Campy-Pak; BBL).

Time-kill curve studies. Studies of early bactericidal activity of the new quinolones were performed as previously described (6). Tests were carried out in 20-ml volumes of Mueller-Hinton broth, containing desired concentrations of the antimicrobial agent and inoculated with overnight broth cultures of the test strains to yield a final inoculum of ca. 10^6 CFU/ml. Samples (2 ml) were withdrawn immediately at the start of an experiment and at 3 and 6 h of incubation at 37°C

* Corresponding author.

without agitation. Bacteria in the samples were collected on 0.45-μm filters (Millipore Corp., Bedford, Mass.), washed with 10 ml of sterile normal saline to prevent antibiotic carry-over, and resuspended in 2 ml of saline by vigorous agitation on a vortex mixer. Serial 10-fold dilutions of this suspension were prepared for colony counts, which were performed in duplicate. Preliminary studies indicated that this method resulted in the complete removal of all elutable antimicrobial agent which may have been adsorbed to the filters and, thus, satisfactorily prevented carry-over of detectable concentrations of the drugs.

Selection of resistant organisms. To determine the ease with which resistance to the new quinolones developed in vitro, five representative isolates of each of several bacterial species were exposed to incremental concentrations of antimicrobial agents (13). Each isolate was applied to an agar plate containing A-56619, A-56620, or ciprofloxacin at a concentration approximately one-half the MIC. Colonies arising after 24 h of incubation were then serially transferred to plates containing twofold incremental concentrations of the drug to a maximum concentration of 128 μg/ml or until a concentration was reached which prevented further growth. To determine whether resistance to each agent selected for in this manner was stable and whether it extended to other quinolones, colonies from plates containing the highest concentration of antimicrobial agent which permitted growth were transferred three times sequentially on antibiotic-free blood agar plates. Resulting colonies were tested for susceptibility to each of the drugs by using the standard agar dilution method described above.

Determination of frequency of spontaneous resistance. Representative isolates from each of several species were examined for the spontaneous occurrence of resistance to the quinolones. Duplicate pour plates were prepared, one set containing the antimicrobial agents at a concentration of eight times the MIC required to inhibit the test strain and the other a concentration of 10 μg/ml. One milliliter of an overnight culture of the test strain was added to 19 ml of molten antibiotic-containing Mueller-Hinton agar. Plates were allowed to solidify at room temperature and were then incubated at 37°C. Colonies arising by 24 h of incubation were counted, and the frequency of spontaneous resistance was determined for each concentration of antimicrobial agent.

RESULTS

Susceptibility studies. The results of agar dilution susceptibility studies are shown in Table 1. Ciprofloxacin was the most active quinolone tested against both gram-positive and gram-negative bacteria. A-56620, like ciprofloxacin, was highly active against *Escherichia coli*, *Enterobacter cloacae*, and *Aeromonas hydrophila* (MICs for 90% of the strains were ≤0.06 μg/ml). The two drugs were also similar in activity against *Staphylococcus aureus* (including methicillin-resistant strains) and most streptococci, with the exception of *Streptococcus faecalis* and group B streptococci. The new agent A-56620 demonstrated activity comparable to that of norfloxacin against most other gram-negative bacteria tested except *Proteus* species, against which it was somewhat less active, but was two to eight times more active than norfloxacin against gram-positive isolates. A-56619 was found to be two to four times less active than A-56620 against most gram-negative bacilli and streptococci, but was comparable to or slightly more active than the latter against *Pseudomonas maltophilia*, *C. jejuni*, *Listeria monocytogenes*, *S. faecalis*, and staphylococci.

Of the members of the family *Enterobacteriaceae* tested, 88% were inhibited by A-56619 at concentrations of ≤1.0 μg/ml, whereas 99% of those strains were inhibited by A-56620 at similar concentrations. Of the gram-negative bacilli, *P. maltophilia* demonstrated the greatest degree of resistance to the new drugs (MICs for 90% of the strains of 4 to 8 μg/ml). In general, streptococci exhibited greater resistance to the quinolones than did gram-negative bacilli, with MICs for 90% of the strains ≥2 μg/ml. The activities of the new agents against methicillin-resistant *S. aureus* and *Staphylococcus epidermidis* were not influenced by whether plates were examined for growth after 24 or 48 h of incubation.

Bactericidal activity. The bactericidal activities of A-56619 and A-56620 after 3 and 6 h of incubation were determined at several drug concentrations, employing a method designed to prevent carry-over of these potent antimicrobial agents. The magnitude of killing measured at 6 h is shown in Table 2. Over this time period, both quinolones were bactericidal against the gram-negative isolates tested but were primarily bacteriostatic against the gram-positive cocci. However, when killing curves were carried out to 24 h, cultures of both strains of *S. aureus* tested were sterilized by the quinolones (10 μg/ml). After only 3 h of incubation, the 10-μg/ml concentration of each antibiotic resulted in a two to five $\log_{10}$ reduction in viable bacteria for each of the gram-negative bacilli tested, reaching levels of killing identical to those observed at 6 h against one-half of the isolates.

A paradoxical effect, wherein higher concentrations of the quinolones resulted in lesser degrees of killing than seen with lower concentrations of the drugs, was noted with each of the species tested except *S. faecalis* and *Pseudomonas aeruginosa*. This phenomenon is illustrated in Fig. 1.

Stepwise selection of resistance. Five strains each of several bacterial species were serially transferred on agar plates containing incremental concentrations of A-56619 or A-56620, to a final concentration of 128 μg/ml or until no further growth occurred. With all species tested, colonies resistant to drug concentrations many-fold higher than the initial MICs were obtained (Table 3). Resistance to the quinolones at concentrations >128 μg/ml was noted with one strain of *Enterobacter aerogenes* and two strains of *Serratia marcescens*. In most cases, MICs of the quinolones against these resistant strains diminished after serial transfer on antibiotic-free media, but in no case did a strain regain full susceptibility to either drug.

For purposes of comparison, five strains each of *E. coli* and *P. aeruginosa* were passed on incremental concentrations of ciprofloxacin in a similar fashion. Modal MICs of ciprofloxacin against the original isolates of *E. coli* (≤0.06 μg/ml) and *P. aeruginosa* (0.125 μg/ml) and against the strains recovered after serial passage through increasing concentrations of ciprofloxacin (0.25 and 16 μg/ml, respectively) were comparable to those of A-56620. Colonies selected for resistance to A-56619, A-56620, and ciprofloxacin were passed three times on antibiotic-free plates and retested for susceptibility to each of these agents. Selection of resistance to each agent resulted in enhanced resistance to each of the other quinolones as well (Table 4).

For each of the five strains of *E. coli*, resistant colonies derived by serial passage through incremental concentrations of A-56620 and subsequently transferred on antibiotic-free medium required for inhibition MICs (4 μg/ml) that were substantially higher than the minimum concentration of drug which inhibited growth during serial passage (0.125 μg/ml) (Tables 3 and 4). This phenomenon was also seen with one

TABLE 1. Comparative in vitro activities of A56619 and A56620 against clinical isolates

Strain (no.)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Escherichia coli (30)	A56619	≤0.06–0.125	≤0.06	0.125
	A56620	≤0.06	≤0.06	≤0.06
	Norfloxacin	≤0.06–0.25	≤0.06	0.125
	Ciprofloxacin	≤0.06	≤0.06	≤0.06
	Piperacillin	2–≥256	2	≥256
	Cefotaxime	≤0.06–0.125	≤0.06	0.125
	Tobramycin	0.5–8	2	2
Klebsiella pneumoniae (30)	A56619	≤0.06–2	0.5	1.0
	A56620	≤0.06–0.5	≤0.06	0.25
	Norfloxacin	≤0.06–1.0	0.125	0.25
	Ciprofloxacin	≤0.06–0.25	≤0.06	≤0.06
	Piperacillin	4–≥256	16	32
	Cefotaxime	≤0.06–0.25	≤0.06	0.125
	Tobramycin	0.5–32	0.5	1.0
Proteus mirabilis (20)	A56619	1.0–2	1.0	2
	A56620	0.25–0.5	0.5	0.5
	Norfloxacin	0.125–0.25	0.125	0.25
	Ciprofloxacin	≤0.06	≤0.06	≤0.06
	Piperacillin	1.0–2	1.0	1.0
	Cefotaxime	≤0.06	≤0.06	≤0.06
	Tobramycin	0.5–2	1.0	1.0
Proteus vulgaris (10)	A56619	1.0–2	1.0	2
	A56620	0.25–0.5	0.25	0.5
	Norfloxacin	0.125	0.125	0.125
	Ciprofloxacin	≤0.06–0.125	≤0.06	≤0.06
	Piperacillin	2–128	4	16
	Cefotaxime	≤0.06–4	0.25	2
	Tobramycin	0.5–4	1.0	4
Morganella morganii (10)	A56619	0.125–0.5	0.5	0.5
	A56620	0.125–0.5	0.125	0.25
	Norfloxacin	≤0.06–1.0	0.125	0.125
	Ciprofloxacin	≤0.06–0.25	≤0.06	≤0.06
	Piperacillin	0.5–≥256	1.0	≥256
	Cefotaxime	≤0.06–16	≤0.06	8
	Tobramycin	0.25–0.5	0.5	0.5
Citrobacter freundii (20)	A56619	≤0.06–2	0.125	1.0
	A56620	≤0.06–0.5	≤0.06	0.25
	Norfloxacin	0.125–0.25	0.125	0.25
	Ciprofloxacin	≤0.06–0.125	≤0.06	≤0.06
	Piperacillin	4–≥256	8	128
	Cefotaxime	0.125–128	0.25	16
	Tobramycin	0.5–8	1.0	2
Enterobacter cloacae (30)	A56619	≤0.06–4	0.125	0.25
	A56620	≤0.06–0.5	≤0.06	≤0.06
	Norfloxacin	≤0.06–4	≤0.06	0.25
	Ciprofloxacin	≤0.06–0.5	≤0.06	≤0.06
	Piperacillin	2–≥256	8	64
	Cefotaxime	≤0.06–≥256	2	128
	Tobramycin	0.5–2	0.5	1.0
Enterobacter aerogenes (20)	A56619	≤0.06–0.25	0.125	0.125
	A56620	≤0.06–0.125	≤0.06	0.125
	Norfloxacin	0.125–0.5	0.125	0.25
	Ciprofloxacin	≤0.06	≤0.06	≤0.06
	Piperacillin	2–≥256	8	16
	Cefotaxime	≤0.06–32	0.25	0.5
	Tobramycin	0.25–1.0	0.5	1.0
Serratia marcescens (20)	A56619	0.5–32	0.5	2
	A56620	≤0.06–4	0.125	0.5
	Norfloxacin	0.125–16	0.25	0.5
	Ciprofloxacin	≤0.06–2	0.125	0.25
	Piperacillin	8–≥256	8	32

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TABLE 1—*Continued*

Strain (no.)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
	Cefotaxime	2–64	2	16
	Tobramycin	0.5–64	4	16
Acinetobacter anitratus (10)	A56619	≤0.06–0.5	0.125	0.5
	A56620	≤0.06–0.5	0.5	0.5
	Norfloxacin	2–8	8	8
	Ciprofloxacin	≤0.06–0.5	0.5	0.5
	Piperacillin	16–≥256	32	64
	Cefotaxime	2–32	16	32
	Tobramycin	0.5–1.0	0.5	1.0
Aeromonas sp. (6)	A55619	≤0.06–0.125		
	A55620	≤0.06		
	Norfloxacin	≤0.06		
	Ciprofloxacin	≤0.06		
	Piperacillin	1–64		
	Cefotaxime	≤0.06–0.5		
	Tobramycin	0.5–4		
Pseudomonas aeruginosa (20)	A56619	1.0–4	2	4
	A56620	0.25–2	0.5	1.0
	Norfloxacin	0.5–4	0.5	1.0
	Ciprofloxacin	0.125–0.5	0.25	0.5
	Piperacillin	4–32	8	16
	Cefotaxime	16–128	32	64
	Tobramycin	0.5–8	1.0	2
Pseudomonas maltophilia (10)	A56619	1.0–8	2	4
	A56620	1.0–16	2	8
	Norfloxacin	8–64	32	64
	Ciprofloxacin	1.0–16	2	8
	Piperacillin	64–≥256	≥256	≥256
	Cefotaxime	16–≥256	128	≥256
	Tobramycin	1.0–≥256	64	≥256
Campylobacter jejuni (10)	A56619	0.5–2	1.0	1.0
	A56620	0.5–1.0	1.0	1.0
	Norfloxacin	1.0–4	2	4
Staphylococcus aureus (methicillin susceptible) (14)	A56619	0.125–0.5	0.25	0.5
	A56620	0.125–0.5	0.25	0.5
	Norfloxacin	0.5–4	1.0	4
	Ciprofloxacin	0.125–0.5	0.25	0.5
	Piperacillin	2–64	8	64
	Cefotaxime	1.0–8	2	2
	Tobramycin	0.25–128	0.5	16
Staphylococcus aureus (methicillin resistant) (16)	A56619	0.25–0.5	0.25	0.25
	A56620	0.5	0.5	0.5
	Norfloxacin	1.0–4	2	2
	Ciprofloxacin	0.25–0.5	0.5	0.5
	Piperacillin	64–≥256	≥256	≥256
	Cefotaxime	16–≥256	≥256	≥256
	Tobramycin	≥256	≥256	≥256
Staphylococcus epidermidis (10)	A56619	0.25–1.0	0.5	0.5
	A56620	0.125–1.0	0.5	0.5
	Norfloxacin	0.5–2	1.0	1.0
	Ciprofloxacin	0.125–0.5	0.25	0.25
	Peperacillin	2–128	16	64
	Cefotaxime	0.5–128	16	64
	Tobramycin	0.125–64	0.5	64
Streptococci, groups A, C, G (20)	A56619	4–16	4	4
	A56620	1.0–8	1.0	2
	Norfloxacin	2–8	2	8
	Ciprofloxacin	1.0–2	1.0	2
	Piperacillin	0.125	0.125	0.125
	Cefotaxime	≤0.06	≤0.06	≤0.06

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TABLE 1—*Continued*

Strain (no.)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
	Tobramycin	4–16	16	16
Streptococci, group B (10)	A56619	4–8	4	4
	A56620	2–4	2	4
	Norfloxacin	8	8	8
	Ciprofloxacin	1.0	1.0	1.0
	Piperacillin	0.5–2	0.5	0.5
	Cefotaxime	≤0.06–0.125	≤0.06	0.125
	Tobramycin	32–64	32	64
Streptococcus pneumoniae (penicillin resistant) (10)	A56619	2–4	2	4
	A56620	0.5–2	0.5	2
	Norfloxacin	4–16	4	8
	Ciprofloxacin	1.0–2	1.0	2
	Piperacillin	0.5–32	1.0	32
	Cefotaxime	0.125–8	0.5	4
	Tobramycin	1.0–32	16	32
Viridans streptococci (penicillin susceptible) (10)	A56619	2–8	2	8
	A56620	0.5–4	2	2
	Norfloxacin	4–16	4	8
	Ciprofloxacin	1.0–2	1.0	2
	Piperacillin	0.25–2	0.25	2
	Cefotaxime	≤0.06–4	0.25	2
	Tobramycin	1.0–16	2	4
Viridans streptococci (penicillin resistant) (10)	A56619	2–8	2	4
	A56620	0.5–2	1.0	2
	Norfloxacin	4–32	8	16
	Ciprofloxacin	1.0–4	2	4
	Piperacillin	2–32	16	32
	Cefotaxime	0.5–8	2	8
	Tobramycin	4–64	16	32
Listeria monocytogenes (10)	A56619	2–4	2	2
	A56620	2	2	2
	Norfloxacin	4–8	8	8
	Ciprofloxacin	1.0–2	2	2
	Piperacillin	4–8	4	4
	Cefotaxime	128–≥256	128	≥256
	Tobramycin	0.5	0.5	0.5
Streptococcus faecalis (30)	A56619	2–8	4	8
	A56620	1.0–8	4	8
	Norfloxacin	2–16	8	16
	Ciprofloxacin	0.5–4	2	2
	Piperacillin	4–16	4	8
	Cefotaxime	128–≥256	≥256	≥256
	Tobramycin	16–128	64	64
Streptococcus faecium (30)	A56619	8–32	16	32
	A56620	4–32	16	16
	Norfloxacin	2–64	8	32
	Ciprofloxacin	1.0–16	4	16
	Piperacillin	4–128	64	128
	Cefotaxime	1–≥256	32	≥256
	Tobramycin	8–≥256	64	≥256

strain each of *K. pneumoniae* and *S. marcescens* and presumably reflects the recovery of normal growth characteristics of mutant colonies upon removal from continuous antimicrobial exposure.

Frequency of resistance. By using selection plates containing A-56619 or A-56620 at a concentration equal to eight times the MIC for each organism, resistant colonies were detected with frequencies ranging from 3×10^{-7} to $<1.7 \times$

TABLE 2. Early bactericidal activity of A-56619 and A-56620 against representative bacterial species

Organism	Magnitude of killing at drug concn (μg/ml) of[a]					
	A56619			A56620		
	10	100	500	10	100	500
E. coli	5.0	2.25	3.5	5.25	3.5	2.0
E. cloacae	5.25	5.25	2.0	5.25	2.75	1.75
P. aeruginosa	5.75	5.75	5.75	4.75	4.75	4.75
S. aureus	1.0	0	0	1.75	0.5	0.25
S. faecalis	1.0	1.0	1.0	1.0	1.0	1.25

[a] Log_{10} reduction in CFU per milliliter at 6 h relative to inoculum. Results represent mean values for two strains of each species.

TABLE 3. Stepwise selection of resistance to A-56619 and A-55620

Organism	MIC (μg/ml)[a]			
	A56619		A56620	
	Before passage	After passage	Before passage	After passage
E. coli	0.08	2.3	≤0.06	0.125
K. pneumoniae	0.43	7.0	≤0.06	1.1
E. aerogenes	0.14	9.2	0.08	9.2
S. marcescens	0.87	37	0.33	9.2
P. aeruginosa	1.5	42	0.5	37
S. aureus	0.29	3.0	0.33	9.2
S. faecalis	4.6	97	5.3	12

[a] Geometric mean MIC for five strains of each species.

10^{-9} (Table 5). For each species, the frequency of resistance was less than or equal to that noted with nalidixic acid. When plates containing the new quinolones at a concentration of 10 μg/ml were used, the frequencies of spontaneous resistance were below the limits of detection of our method (frequency of $<1.7 \times 10^{-9}$ to $<5.5 \times 10^{-9}$). The one exception was that resistance to A-56619 (10 μg/ml) in a strain of *E. cloacae* occurred at a frequency of 1.4×10^{-7}, which was comparable to that observed when this strain was tested against nalidixic acid.

Because of the dependence of A-56619 activity on pH of the test medium (E. St. Martin, J. Stamm, E. McDonald, C. Vojtko, and P. B. Fernandes, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 74, 1984), the frequency of resistance to this agent was also determined in Mueller-Hinton agar adjusted to pH 6.0. Under these conditions, frequencies of resistance (mean of three determinations) were: *E. coli*, $<3.3 \times 10^{-9}$; *K. pneumoniae*, $<2.5 \times 10^{-9}$; and *E. cloacae*, 1.3×10^{-8} at antimicrobial concentrations equal to eight times the MIC.

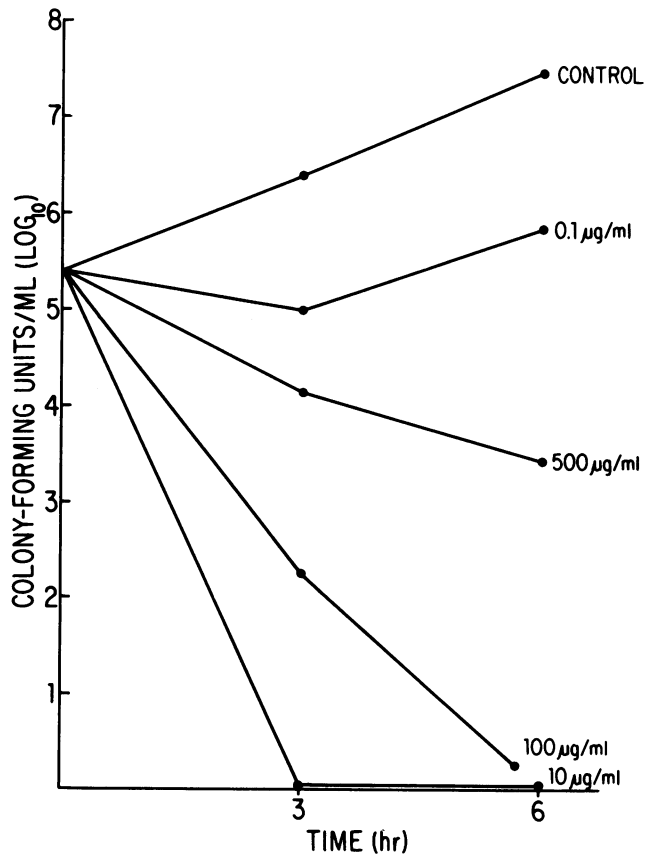

FIG. 1. Bactericidal activity of A-56619 against *E. cloacae* K-2 (MIC, 0.25 μg/ml).

DISCUSSION

Renewed interest in the quinolones as therapeutic agents has been kindled by the development of 6-fluoroquinolone and naphthyridine analogs which, in comparison with nalidixic acid, demonstrate activity against a broader range of bacteria, including *P. aeruginosa* and gram-positive cocci (6–9, 11, 14). In addition, the high potency of the newer quinolones against common pathogens may permit successful use of these agents in systemic infections beyond the urinary tract.

The present study demonstrated that A-56620, a novel 1-aryl-substituted fluoroquinolone, was comparable in activity to ciprofloxacin against several species, including *E. coli*, *E. cloacae*, *A. anitratus*, and *S. aureus*. Against a variety of other bacteria, the new quinolone exhibited activity comparable to that of norfloxacin. Although the related drug, A-56619, was two to four times less potent than A-56620 against most organisms, preliminary animal studies suggest

TABLE 4. Cross-resistance among quinolone antimicrobial agents after stepwise selection of resistance in strains of *E. coli* and *P. aeruginosa*

Organism	MIC (μg/ml)[a]		
	A-56619	A-56620	Ciprofloxacin
E. coli			
Before passage	≤0.06	≤0.06	≤0.06
After passage in:			
A-56619	2.6	1.7	0.4
A-56620	4.0	4.0	1.0
Ciprofloxacin	2.3	0.4	0.4
P. aeruginosa			
Before passage	1.5	0.5	0.2
After passage in:			
A-56619	11	5.3	0.9
A-56620	14	8.0	2.0
Ciprofloxacin	21	4.0	1.0

[a] Geometric mean MIC of five strains determined after passage on antibiotic-free plates.

TABLE 5. Frequency of resistance to newer quinolone antimicrobial agents at concentrations of eights times the MIC

Organism	Frequency of resistance to:			
	A-56619[a]	A-56620	Ciprofloxacin	Nalidixic acid
E. coli	1.7×10^{-7}	$<2.3 \times 10^{-9}$	$<2.3 \times 10^{-9}$	1.5×10^{-7}
K. pneumoniae	2.6×10^{-8}	8.9×10^{-8}	7.0×10^{-9}	2.6×10^{-7}
E. cloacae	3.0×10^{-7}	$<1.7 \times 10^{-9}$	$<1.7 \times 10^{-9}$	1.5×10^{-7}
P. aeruginosa	$<3.8 \times 10^{-9}$	$<3.8 \times 10^{-9}$	$<3.8 \times 10^{-9}$	
S. aureus	1.1×10^{-8}	$<5.5 \times 10^{-9}$	$<5.5 \times 10^{-9}$	

[a] Data obtained at pH 6 provided in text.

that the former drug reaches higher peak concentrations in the serum after oral administration (P. B. Fernandes, N. Shipkowitz, D. Chu, L. Coen, N. Ramer, and G. R. Granneman, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 79, 1984).

Both new quinolones were rapidly bactericidal against the gram-negative bacteria tested but more slowly sterilized cultures of *S. aureus*. Similar results have been noted in time-kill studies with ciprofloxacin (3). Against several species, the new drugs were more effectively bactericidal at lower concentrations (10 μg/ml) than at higher concentrations. Such a paradoxical effect has been seen with nalidixic acid, which at low concentrations inhibits DNA synthesis (in *E. coli*) leading to cell death but at higher concentrations inhibits RNA and protein synthesis as well, exerting primarily a bacteriostatic effect (4).

Early clinical experience with nalidixic acid in the treatment of urinary tract infections gave rise to reports of the emergence of drug resistance during therapy (10). In spite of the fact that underdosage may, in part, have contributed to this problem (12), these observations remain a concern in the clinical application of quinolone antimicrobial agents since the stepwise selection of bacterial colonies with increased levels of resistance to various new quinolones can be readily accomplished in vitro by serial transfers on increasing concentrations of the drugs (2, 6, 13). In the present study, colonies many-fold more resistant than the parent isolates to A-56619 or A-56620 could be selected; however, MICs for resistant colonies exceeded 128 μg/ml for only 3 of 35 strains tested. As is the case with other quinolones (1), strains selected for resistance to A-56619, A-56620, or ciprofloxacin demonstrated cross-resistance to the other agents tested. Frequencies of spontaneous resistance to A-56620 were similar to rates of ciprofloxacin resistance and lower than those of resistance to nalidixic acid. Although data obtained with standard media suggested higher rates of resistance to A-56619, observations that activity of this compound is significantly lower at pH 8 than at pH 7.2 (C. W. Hanson, D. T. W. Chu, R. Baylor, C. Vojtko, and P. B. Fernandes, submitted for publication) prompted us to reexamine the development of resistance to this agent at pH 6.0. Under these conditions, rates of resistance to this agent were comparable to those of the other new quinolones.

Whether the development of resistance in vivo will limit the therapeutic usefulness of these or other quinolone antimicrobial agents remains to be determined by clinical experience. Based upon the results of this study of their in vitro properties, the novel 1-acyl-substituted quinolone antimicrobial agents A-56620 and A-56619 appear to merit further clinical investigation.

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