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In Vitro Activity of Sparfloxacin Compared with Those of Five Other Quinolones

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The in vitro activity of sparfloxacin, a new difluorinated quinolone, was evaluated against 857 gram-positive and gram-negative clinical isolates and compared with those of ciprofloxacin, norfloxacin, ofloxacin, fleroxacin, and lomefloxacin. The MIC of sparfloxacin for 90% of the members of the family *Enterobacteriaceae* tested was 0.5 μg/ml (range, 0.06 to 4.0 μg/ml); only for members of the genera *Serratia*, *Citrobacter*, and *Providencia* were MICs above 1 μg/ml. Some 90% of *Pseudomonas aeruginosa* isolates were inhibited by 8 μg of the drug per ml. The MICs for 90% of *Staphylococcus* spp. and *Enterococcus faecalis* were 0.12 and 2 μg/ml, respectively. All (100%) *Streptococcus pneumoniae* strains were inhibited by 0.5 μg/ml. The inoculum size had little effect on either the MIC or the MBC of sparfloxacin. An increase in the magnesium concentration from 1.1 to 8.4 mM increased the MIC between 2 and 10 times, depending on the genus tested. Sparfloxacin was less active at pH 5. The antibacterial activity of sparfloxacin against gram-positive bacteria was generally higher than those of the quinolones with which it was compared; against *Streptococcus pneumoniae*, sparfloxacin was four- and eightfold more active than ofloxacin and ciprofloxacin, respectively. The activity of sparfloxacin against gram-negative rods was generally comparable to that of ciprofloxacin except against *Enterobacter* and *Acinetobacter* spp., *Pseudomonas cepacia*, *Xanthomonas maltophilia*, and *Alcaligenes* and *Flavobacterium* spp., against which sparfloxacin was the most active quinolone.

In 1962 Lesher et al. (9) synthesized nalidixic acid, the first urinary tract antiseptic of the quinolone group to be used clinically. In the last decade, more than 200 quinolone derivatives have been synthesized, some with a broad spectrum and improved pharmacology. Nevertheless, some important pathogens such as enterococci, streptococci, *Mycobacterium* spp., *Chlamydia* spp., and *Mycoplasma* spp. are not sufficiently susceptible to some quinolones. Sparfloxacin (AT-4140; Rp 64206), 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid, is a new difluorinated quinolone. Its structure is similar to that of ciprofloxacin; however, it has two methyl groups in the piperazinyl ring and an additional fluorine atom at position 8, which, according to Schentag and Domagala (17), enhances its activity against gram-positive organisms. Nakamura (13) and others (4, 18, 19) reported that sparfloxacin has a broad antibacterial spectrum that includes the pathogens mentioned above, which are not sufficiently susceptible to other quinolones. The present in vitro study was designed to establish (i) the antibacterial activity of sparfloxacin compared with those of other quinolones, (ii) the influence of changes in the pH of the culture medium, inoculum size, and Mg^{2+} concentration on the activity, (iii) the postantibiotic effect (PAE), and (iv) the in vitro mutational frequency rate to sparfloxacin resistance in a number of key pathogens.

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MATERIALS AND METHODS

Organisms. A total of 857 gram-positive and gram-negative bacterial strains (see Table 1) were tested. Isolates were obtained from different biological specimens (i.e., urine, blood, sputum, and cerebrospinal fluid) from patients admitted to different hospital units at La Fe Hospital, Valencia, Spain, between 1989 and 1990. The isolates were identified by standard microbiologic methods. Some multiply resistant isolates of *Enterobacter* and *Citrobacter* spp. and *Pseudomonas aeruginosa* which were collected over the previous 2 years were also used. Only one isolate from each patient was used to avoid testing of multiple copies of the same strain. The effect of inoculum, pH, and magnesium ion concentration on the MICs and MBCs were determined for 50 strains belonging to 10 different genera (see Table 2). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control strains.

Antimicrobial agents. Sparfloxacin in powder form was provided by Rhône-Poulenc, Antony, France. Lomefloxacin, fleroxacin, norfloxacin, ciprofloxacin, and ofloxacin were provided by Searle & Co., Roche S.A., Merck Sharp & Dohme, Quimica Farmaceutica Bayer, and Roussel Ibérica S.A., respectively. Dilutions of the compounds were prepared on the day of use by following the specifications of the manufacturers.

Susceptibility tests. MICs were determined by a standard twofold dilution technique in Mueller-Hinton agar (MHA; Difco) by following the specifications of the National Committee for Clinical Laboratory Standards (14). The standard conditions were modified as follows. MICs for *Proteus* strains were tested in MHA by adding sufficient agar to obtain a 2% concentration. *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *Streptococcus pyogenes* were tested in MHA supplemented with 5% sheep blood, and *Haemophilus* spp. were tested on chocolate agar medium; all of these organisms were incubated in a 10% CO_2 atmosphere. Inocula were grown overnight in Mueller-Hin-

ton broth (MHB) and were diluted in Ringer 1/4 (Oxoid) to give a final inoculum of approximately 10^6 CFU/ml. Strains of *Streptococcus pneumoniae* were grown in Todd-Hewitt medium (Oxoid). *Haemophilus* spp. were cultured from colonies grown on chocolate agar medium incubated at 10% CO_2 and were then suspended in Ringer 1/4. With a multipoint inoculator (Titertek; Denley, Sussex, England), 2 μl of the dilutions was inoculated onto agar plates containing graded concentrations of the drug being tested. The final inoculum size was approximately 5×10^3 CFU per spot. Inoculated plates were incubated at 35°C for 18 h. The MIC was defined as the concentration of drug at which the original inoculum was reduced to no more than two colonies.

The effects of pH, inoculum size, and magnesium ion concentration were assayed in microdilution trays containing 0.1 ml of MHB per well with a twofold dilution of antibiotic. Appropriate volumes of NaOH (1 N) and HCl (0.1 N) were added in order to obtain the desired pH. Broth medium was supplemented with $MgSO_4 \cdot 2H_2O$ to achieve final magnesium concentrations of 1.1, 4.4, and 8.4 mM. The MIC in broth was defined as the lowest concentration of drug that inhibited visible growth in 18 h. The MBC in broth was determined by subculturing 10 μl from all clear MIC wells onto MHA. The MBC was defined as the lowest concentration of drug that inhibited ≥99.9% of growth.

Mutational frequency. The frequency of selection of spontaneous mutants with decreased susceptibility to sparfloxacin was evaluated for seven strains by plating more than 10^{11} CFU in a 0.1-ml volume onto MHA plates containing drug at concentrations of 4×, 8×, and 16× the MIC for each isolate. The number of CFU at each drug concentration after 48 h of incubation was compared with the number of CFU on antibioticfree medium after appropriate dilution of the inoculum. The susceptibilities of the spontaneous mutants were tested and compared with that of the wild-type strain from which it was derived.

Determination of PAE. *Staphylococcus aureus* Sa-1 was used to test for the presence and duration of a PAE, after exposure to the drug at a concentration of 2 μg/ml, over time. Cell suspensions in the late logarithmic phase of growth (10^5 to 10^6 CFU/ml in MHB) were exposed to sparfloxacin and ciprofloxacin. After predetermined times, the antibiotics were removed by repeated centrifugation and suspension of cells in fresh drugfree medium. The number of viable organisms per milliliter was determined at time zero and at the end of each period of incubation in order to determine bactericidal activity. The tubes were then centrifuged at 5,200 × *g* for 10 min. After the final wash, the suspended organisms were poured into sterile glass tubes. The number of CFU per milliliter for each sample was determined at the end of the wash procedures and again at each hour until visible growth was observed. The antibioticfree bacterial suspension was reincubated at 37°C. The concentration of antimicrobial agents used was chosen to mimic the expected peak drug concentration in vivo (1, 11, 12). The PAE was evaluated by calculating the difference in time required for the number of drug-exposed and untreated control organisms to increase 10-fold above the number present immediately after removal of antibiotics.

RESULTS

In vitro activity. Table 1 compares the in vitro activity of sparfloxacin with those of other fluoroquinolones. Of the 463 members of the family *Enterobacteriaceae* tested, 93.3% were inhibited by 0.5 μg of sparfloxacin per ml; values above this were observed for strains of *Serratia*, *Citrobacter*, and *Proteus* spp., *Providencia stuartii*, and *Providencia rettgeri*. For only three strains of *Serratia* spp., four strains of *Citrobacter* spp., and one strain of *Providencia* sp. were the MICs above 1 μg/ml. The highest MIC of sparfloxacin obtained was 16 μg/ml for one strain of *Serratia marcescens*. The MICs for 50% (MIC_{50}) and 90% (MIC_{90}) of the strains tested for the various taxonomic groups of the family *Enterobacteriaceae* ranged from 0.007 to 0.5 μg/ml and 0.06 to 4 μg/ml, respectively. When it was compared with other fluoroquinolones, sparfloxacin had activity comparable to that of ciprofloxacin and generally greater activity than those of the other quinolones; the MIC_{90}s for the *Enterobacteriaceae* were 0.25 μg of ciprofloxacin per ml; 0.5 μg of sparfloxacin, norfloxacin, or fleroxacin per ml; and 1 μg of ofloxacin or lomefloxacin per ml. Sparfloxacin was less active against *Pseudomonas aeruginosa*; 80% of the strains were inhibited by 2 μg/ml (the mode MIC). By comparison, sparfloxacin was less active than ciprofloxacin and norfloxacin against *Pseudomonas aeruginosa* and had activity similar to those of lomefloxacin and fleroxacin. Sparfloxacin was the most active drug against *Pseudomonas cepacia*, with 2 μg/ml inhibiting 100% of strains. Against *Xanthomonas maltophilia*, sparfloxacin proved to be the most active drug, with 1 μg/ml inhibiting 100% of the strains, whereas for the other drugs, concentrations from 4 to 32 μg/ml were required to inhibit 90% of the strains. Sparfloxacin was the most active compound tested against *Acinetobacter calcoaceticus*, with 0.12 μg/ml inhibiting 95% of the strains and 0.25 μg/ml inhibiting 100% of the strains; it was significantly more active than the other fluoroquinolones assayed, which had MIC_{90}s ranging from 0.5 to 8 μg/ml. Against *Alcaligenes* and *Flavobacterium* spp., sparfloxacin and ciprofloxacin proved to be the most active drugs, although there were few strains assayed. None of the drugs showed good activity against members of the genera *Achromobacter*.

All of the fluoroquinolones had high levels of activity against strains of *Haemophilus influenzae*; sparfloxacin MIC_{90}s were 0.12 μg/ml, with no differences observed between β-lactamase-producing and -nonproducing strains.

Sparfloxacin had comparatively good activity against gram-positive microorganisms, inhibiting 97.6% of the ciprofloxacin-susceptible staphylococci at a concentration of 0.25 μg/ml. Sparfloxacin was also active against *Streptococcus* spp., although there were some strains of *Streptococcus pyogenes* and *Streptococcus agalactiae* for which the MICs were above 4 μg/ml. No differences in susceptibility were found between penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae* strains. Comparatively, strain by strain, ciprofloxacin MICs were two- or fourfold greater than those of sparfloxacin. Sparfloxacin also proved to be the most active drug against the other gram-positive microorganisms tested.

Effects of pH, inoculum size, and Mg^{2+} concentration. The effects of pH, inoculum size, and Mg^{2+} concentration on the MICs and MBCs are given in Tables 2 to 4, respectively. Sparfloxacin showed bactericidal activity, with MBCs being equal to or twice the MICs. Sparfloxacin was less active at pH 5; members of the genera *Escherichia*, *Enterobacter*, *Klebsiella*, and *Salmonella* were the most susceptible to pH changes. MICs were three or four dilution steps higher at pH 5 than they were at pH 8; the genera *Proteus* and *Morganella* were less affected by pH. For gram-positive microorganisms, no differences in MICs were found between pH 6 and 8, but there were twofold dilution differences between pH 5 and 6.

TABLE 1. Comparative in vitro activity of sparfloxacin

Microorganism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]			
		Range	50%	90%	Mode
Escherichia coli (36)	Sparfloxacin	0.007–0.25	0.015	0.06	0.015
	Ciprofloxacin	0.007–1	0.007	0.06	0.007
	Norfloxacin	0.007–2	0.06	0.12	0.06
	Ofloxacin	0.03–1	0.03	0.06	0.03
	Fleroxacin	0.03–1	0.03	0.12	0.03
	Lomefloxacin	0.06–4	0.06	0.25	0.06
Enterobacter spp.[b] (40)	Sparfloxacin	0.007–0.015	0.06	0.12	0.06
	Ciprofloxacin	0.015–1	0.03	0.5	0.015
	Norfloxacin	0.007–16	0.06	0.12	0.25
	Ofloxacin	0.06–0.25	0.12	0.25	0.12
	Fleroxacin	0.06–0.25	0.06	0.25	0.06
	Lomefloxacin	0.03–2	0.12	1	0.12
Klebsiella spp.[c] (70)	Sparfloxacin	0.015–1	0.06	0.06	0.06
	Ciprofloxacin	0.015–0.25	0.03	0.06	0.015
	Norfloxacin	0.03–4	0.12	1	0.12
	Ofloxacin	0.06–1	0.12	1	0.12
	Fleroxacin	0.03–0.25	0.12	0.12	0.12
	Lomefloxacin	0.06–8	0.12	0.25	0.12
Serratia spp.[d] (33)	Sparfloxacin	0.12–16	0.5	2	0.5
	Ciprofloxacin	0.007–4	0.06	0.5	0.06
	Norfloxacin	0.007–4	0.12	4	0.12
	Ofloxacin	0.06–8	0.25	1	0.25
	Fleroxacin	0.015–4	0.06	2	0.06
	Lomefloxacin	0.03–16	0.25	4	0.25
Citrobacter freundii (30)	Sparfloxacin	0.03–8	0.06	4	0.06
	Ciprofloxacin	0.007–1	0.007	0.03	0.007
	Norfloxacin	0.03–1	0.03	0.12	0.03
	Ofloxacin	0.03–0.25	0.03	0.25	0.06
	Fleroxacin	0.03–0.25	0.06	0.12	0.06
	Lomefloxacin	0.06–4	0.12	0.5	0.12
Providencia spp.[e] (29)	Sparfloxacin	0.06–8	0.25	1	0.12
	Ciprofloxacin	0.015–1	0.03	0.5	0.015
	Norfloxacin	0.015–4	0.06	1	0.03
	Ofloxacin	0.03–4	0.12	2	0.5; 2[f]
	Fleroxacin	0.03–4	0.12	4	0.06
	Lomefloxacin	0.12–8	0.25	4	0.12
Proteus vulgaris (40)	Sparfloxacin	0.12–1	0.5	0.5	0.5
	Ciprofloxacin	0.007–0.12	0.03	0.06	0.03
	Norfloxacin	0.03–0.12	0.03	0.06	0.06
	Ofloxacin	0.06–2	0.06	0.12	0.06
	Fleroxacin	0.03–0.12	0.06	0.12	0.06
	Lomefloxacin	0.12–0.5	0.25	0.25	0.25
Proteus mirabilis (40)	Sparfloxacin	0.12–1	0.5	0.5	0.5
	Ciprofloxacin	0.007–0.015	0.007	0.015	0.015
	Norfloxacin	0.03–0.12	0.06	0.06	0.06
	Ofloxacin	0.015–0.5	0.12	0.5	0.12
	Fleroxacin	0.12–0.25	0.12	0.12	0.12
	Lomefloxacin	0.12–8	0.5	1	1
Morganella morganii (58)	Sparfloxacin	0.06–0.5	0.12	0.5	0.25
	Ciprofloxacin	0.007–0.015	0.015	0.015	0.015
	Norfloxacin	0.03–0.25	0.03	0.06	0.03
	Ofloxacin	0.06–0.12	0.06	0.12	0.12
	Fleroxacin	0.015–2	0.03	0.06	0.03
	Lomefloxacin	0.06–8	0.06	0.5	0.12
Salmonella spp. (19)	Sparfloxacin	0.03–0.06	0.03	0.06	0.03
	Ciprofloxacin	0.007–0.03	0.007	0.007	0.007
	Norfloxacin	0.015–0.12	0.03	0.06	0.06
	Ofloxacin	0.015–0.06	0.03	0.06	0.03
	Fleroxacin	0.12–1	0.5	0.5	0.5
	Lomefloxacin	0.06–2	0.12	0.5	0.12

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TABLE 1—*Continued*

Microorganism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]			
		Range	50%	90%	Mode
Shigella spp.[g] (40)	Sparfloxacin	0.007–0.5	0.007	0.015	0.007
	Ciprofloxacin	0.007–0.007	0.007	0.007	0.007
	Norfloxacin	0.007–0.03	0.007	0.015	0.015
	Ofloxacin	0.015–0.03	0.03	0.03	0.03
	Fleroxacin	0.06–2	0.06	0.12	0.06
	Lomefloxacin	0.06–4	0.06	0.12	0.06
Yersinia enterocolitica (17)	Sparfloxacin	0.25–0.25	0.25	0.25	0.25
	Ciprofloxacin	0.007–0.06	0.03	0.03	0.03
	Norfloxacin	0.015–0.06	0.03	0.06	0.06
	Ofloxacin	0.06–0.06	0.06	0.06	0.06
	Fleroxacin	0.12–0.12	0.12	0.12	0.12
	Lomefloxacin	0.12–0.12	0.12	0.12	0.12
Aeromonas spp. (44)	Sparfloxacin	0.015–0.25	0.03	0.12	0.03
	Ciprofloxacin	0.007–0.06	0.007	0.06	0.007
	Norfloxacin	0.015–0.06	0.015	0.06	0.06
	Ofloxacin	0.007–0.5	0.015	0.5	0.5
	Fleroxacin	0.007–2	0.06	0.12	0.12
	Lomefloxacin	0.007–2	0.015	0.12	0.12
Acinetobacter calcoaceticus (20)	Sparfloxacin	0.015–0.25	0.06	0.12	0.06
	Ciprofloxacin	0.06–4	0.5	1	0.5
	Norfloxacin	0.05–16	4	8	8
	Ofloxacin	0.25–1	0.25	0.5	0.25
	Fleroxacin	0.25–2	1	2	1
	Lomefloxacin	0.12–8	1	2	1
Pseudomonas aeruginosa (64)	Sparfloxacin	0.25–16	2	8	2
	Ciprofloxacin	0.03–1	0.12	0.5	0.12
	Norfloxacin	0.25–2	0.5	1	0.5
	Ofloxacin	1–8	2	4	1
	Fleroxacin	1–8	2	8	2
	Lomefloxacin	1–128	4	8	2
Pseudomonas cepacia (10)	Sparfloxacin	0.015–2	0.12	2	0.12
	Ciprofloxacin	0.03–8	0.12	4	0.12
	Norfloxacin	0.5–32	8	32	4
	Ofloxacin	0.5–32	8	16	0.5
	Fleroxacin	0.5–4	1	4	0.5
	Lomefloxacin	0.25–4	2	4	0.5
Xanthomonas maltophilia (28)	Sparfloxacin	0.06–1	0.25	1	0.25
	Ciprofloxacin	0.5–8	4	8	4
	Norfloxacin	1–32	8	32	8
	Ofloxacin	0.5–8	4	8	4
	Fleroxacin	1–4	1	4	1
	Lomefloxacin	0.12–8	2	4	2; 4
Achromobacter spp. (18)	Sparfloxacin	1–16	2	4	4
	Ciprofloxacin	1–4	1	4	1
	Norfloxacin	8–32	16	32	16
	Ofloxacin	2–16	4	16	16
	Fleroxacin	1–8	2	4	4
	Lomefloxacin	16–128	16	32	16
Alcaligenes spp. (6)	Sparfloxacin	2–2	2	2	2
	Ciprofloxacin	0.015–32	2	16	16
	Norfloxacin	0.5–128	16	32	32
	Ofloxacin	0.5–128	4	16	16
	Fleroxacin	0.5–32	4	16	
	Lomefloxacin	2–8	4	8	4
Flavobacterium spp. (7)	Sparfloxacin	0.015–0.25	0.25	0.25	0.25
	Ciprofloxacin	0.007–0.5	0.12	0.5	0.5
	Norfloxacin	0.015–8	2	8	8
	Ofloxacin	0.06–1	0.25	1	1
	Fleroxacin	0.06–1	0.12	1	1
	Lomefloxacin	0.06–128	1	128	2

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TABLE 1—*Continued*

Microorganism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]			
		Range	50%	90%	Mode
Haemophilus spp. (19)	Sparfloxacin	0.007–0.5	0.007	0.12	0.007
	Ciprofloxacin	0.015–0.03	0.015	0.015	0.015
	Norfloxacin	0.03–4	0.5	2	0.12
	Ofloxacin	0.03–2	0.5	2	0.12
	Fleroxacin	0.03–8	0.03	1	0.03
	Lomefloxacin	0.007–1	0.007	0.06	0.007
Listeria monocytogenes (11)	Sparfloxacin	0.25–1	1	1	1
	Ciprofloxacin	0.5–2	0.5	1	1
	Norfloxacin	2–16	8	16	8
	Ofloxacin	4–16	4	16	4
	Fleroxacin	2–8	4	4	4
	Lomefloxacin	2–8	4	4	4
Corynebacterium spp. (18)	Sparfloxacin	0.007–16	0.5	2	0.5
	Ciprofloxacin	0.25–1	0.5	1	0.5
	Norfloxacin	0.015–64	64	64	64
	Ofloxacin	0.25–8	4	8	8
	Fleroxacin	1–4	4	4	4
	Lomefloxacin	0.25–8	4	8	4
Staphylococcus aureus (65)	Sparfloxacin	0.03–0.5	0.12	0.25	0.12
	Ciprofloxacin	0.12–0.5	0.25	0.5	0.25
	Norfloxacin	0.25–2	0.5	1	0.5
	Ofloxacin	0.25–0.5	0.25	0.25	0.25
	Fleroxacin	0.25–0.5	0.25	0.25	0.25
	Lomefloxacin	0.5–8	0.5	1	0.5
Coagulase-negative staphylococci (20)	Sparfloxacin	0.06–1	0.12	0.25	0.12
	Ciprofloxacin	0.06–0.5	0.12	0.25	0.25
	Norfloxacin	0.12–1	0.25	0.5	0.25
	Ofloxacin	0.5–1	0.5	1	0.5
	Fleroxacin	0.25–1	0.5	1	0.5
	Lomefloxacin	0.06–8	1	8	1; 8
Enterococcus faecalis (20)	Sparfloxacin	0.06–2	0.5	2	0.015; 0.5[f]
	Ciprofloxacin	0.5–2	1	2	2
	Norfloxacin	1–8	2	4	4
	Ofloxacin	0.5–4	2	2	2
	Fleroxacin	4–4	4	4	4
	Lomefloxacin	4–16	4	8	4
Streptococcus pneumoniae (18)	Sparfloxacin	0.25–0.5	0.25	0.5	0.5
	Ciprofloxacin	1–8	4	4	4
	Norfloxacin	4–32	8	16	
	Ofloxacin	2–2	2	2	2
	Fleroxacin	16–128	16	64	16
	Lomefloxacin	4–32	8	32	8
	Penicillin	0.001–4	0.12	1	0.001
Streptococcus agalactiae (21)	Sparfloxacin	0.25–8	0.25	1	0.25
	Ciprofloxacin	0.5–16	1	4	0.5
	Norfloxacin	4–32	4	16	4
	Ofloxacin	1–32	2	8	1
	Fleroxacin	4–32	8	16	8
	Lomefloxacin	4–32	8	32	8
Streptococcus pyogenes (16)	Sparfloxacin	0.25–16	0.5	8	0.5
	Ciprofloxacin	0.5–16	8	16	8
	Norfloxacin	1–4	4	4	4
	Ofloxacin	1–64	2	16	2
	Fleroxacin	8–32	8	16	8
	Lomefloxacin	4–8	8	8	8

[a] 50% and 90%, MICs for 50 and 90% of isolates, respectively.
[b] *E. cloacae*, *n* = 23; *E. aerogenes*, *n* = 13; *E. agglomerans*, *n* = 2; *E. sakazakii*, *n* = 2.
[c] *K. pneumoniae*, *n* = 55; *K. oxytoca*, *n* = 15.
[d] *S. marcescens*, *n* = 20; *S. liquefaciens*, *n* = 13.
[e] *P. stuartii*, *n* = 18; *P. rettgeri*, *n* = 9; *P. alcalifaciens*, *n* = 2.
[f] Both values have the same frequency.
[g] *S. sonnei*, *n* = 35; *S. flexneri*, *n* = 5.

TABLE 2. Effect of pH on the in vitro activity of sparfloxacin

Microorganism[a]	Geometric mean MIC/MBC (μg/ml) in MHB at pH:			
	5	6	7	8
Escherichia coli	0.14/0.21	0.019/0.02	0.007/0.009	0.005/0.009
Enterobacter spp.	0.58/1.15	0.12/0.33	0.12/0.21	0.06/0.1
Klebsiella spp.	0.24/0.57	0.10/0.16	0.08/0.08	0.02/0.02
Morganella morganii	0.57/3.5	0.37/1	0.21/0.25	0.21/0.28
Proteus vulgaris	NG[b]	0.1/3.03	0.75/1.32	0.87/1.74
Proteus mirabilis	0.24/1.5	0.19/0.87	0.14/0.21	0.1/0.12
Salmonella spp.	0.07/0.08	0.03/0.04	0.008/0.01	0.006/0.009
Shigella spp.	0.023/0.04	0.007/0.017	0.002/0.006	0.012/0.03
Pseudomonas aeruginosa	1.32/3.42	1/2	0.32/1	0.5/0.87
Staphylococcus aureus	0.29/0.33	0.04/0.06	0.04/0.04	0.05/0.06
Enterococcus faecalis	1.3/2	0.33/0.5	0.33/0.43	0.37/0.43

[a] Five isolates of each microorganism were tested.
[b] NG, no growth.

TABLE 4. Effect of Mg^{2+} concentration on the in vitro activity of sparfloxacin

Microorganism[a]	Geometric mean MIC/MBC (μg/ml) in MHB with Mg^{2+} at a final concn (mM) of:			
	0.3	1.1	4.4	8.4
Escherichia coli	0.007/0.009	0.02/0.03	0.05/0.08	0.04/0.06
Enterobacter spp.	0.07/0.12	0.03/0.04	0.05/0.06	0.09/0.09
Klebsiella spp.	0.08/0.12	0.06/0.09	0.07/0.08	0.16/0.18
Morganella morganii	0.21/0.33	0.03/0.04	0.25/0.5	0.33/0.65
Proteus vulgaris	0.76/1.3	0.87/1.7	1.0/1.7	1.15/2.3
Proteus mirabilis	0.18/0.18	0.09/0.24	0.16/0.5	0.3/0.5
Salmonella spp.	0.009/0.014	0.02/0.03	0.04/0.06	0.04/0.08
Shigella spp.	0.003/0.004	0.006/0.009	0.015/0.016	0.02/0.04
Pseudomonas aeruginosa	0.32/1.15	0.75/1.14	2.0/3.5	4.6/7
Staphylococcus aureus	0.04/0.06	0.06/0.08	0.21/0.24	0.18/0.21
Enterococcus faecalis	0.21/0.43	0.29/0.43	0.58/1	1.3/1.7

[a] Five isolates of each microorganism were tested.

Slight inoculum effects on MICs and MBCs were seen in the range from 10^3 to 10^5 CFU/ml and between 10^5 and 10^7 CFU/ml, but the effect was considerably greater when the comparison was between inocula of 10^3 and 10^7 CFU/ml.

An increase in the Mg^{2+} concentration from 1.1 to 8.4 mM was accompanied by a slight loss of activity, depending on the genus and strain; MICs increased by one or two dilutions, with increases being greater in *Enterococcus faecalis*, *Morganella* spp., and *Pseudomonas aeruginosa*. For some strains of *Escherichia coli*, MICs were higher with 4.4 mM Mg^{2+} than they were with 8.4 mM Mg^{2+}, but an increase in the concentration of Mg^{2+} from 0.3 mM in unsupplemented MHB to 1.1 mM (the concentration of Mg^{2+} in serum) had a minimal effect on the sparfloxacin MICs, with MICs for only *Escherichia coli* and *Salmonella* and *Shigella* spp. being increased.

Generation of resistant mutants and PAE. Table 5 gives the frequencies for the generation of spontaneous single-step resistant mutants. Sparfloxacin selected resistant mutants only of *Escherichia coli* and *Klebsiella pneumoniae*; for *Klebsiella pneumoniae*, it was at a concentration of four times the MIC. The same number of spontaneous single-step resistant *Escherichia coli* mutants were selected with the three concentrations assayed. No differences in the frequencies of spontaneous single-step resistant mutants were found between 30 and 37°C (data not shown).

Table 6 compares the duration of the PAEs produced by sparfloxacin and ciprofloxacin. The PAE produced by sparfloxacin was greater than that produced by ciprofloxacin after 15 and 30 min of contact time; nevertheless, after 60 min, the PAE was greater for ciprofloxacin. For sparfloxacin, the PAE increased with an increase in contact time, whereas for ciprofloxacin, it was nearly the same between 15 and 30 min. A persistent growth suppression was observed with sparfloxacin after the drug was removed. This effect was not demonstrated with ciprofloxacin.

DISCUSSION

Sparfloxacin is a recently developed quinolone that has potent antibacterial activity. Results of this study indicate that sparfloxacin is active against the majority of aerobic clinical pathogens. Against the members of the family *En-*

TABLE 3. Effect of inoculum size on the in vitro activity of sparfloxacin

Microorganism[a]	Geometric mean MIC/MBC (μg/ml) in MHB with the following inoculum size (CFU/ml):		
	10^7	10^5	10^3
Escherichia coli	0.015/0.02	0.007/0.009	0.004/0.007
Enterobacter spp.	0.09/0.14	0.07/0.12	0.015/0.02
Klebsiella spp.	0.14/0.18	0.08/0.12	0.019/0.026
Morganella morganii	0.43/1.5	0.21/0.33	0.14/0.24
Proteus vulgaris	0.56/2	0.76/1.3	0.5/0.66
Proteus mirabilis	0.16/0.37	0.18/0.28	0.12/0.16
Salmonella spp.	0.03/0.08	0.009/0.014	0.004/0.007
Shigella spp.	0.01/0.02	0.003/0.004	0.0007/0.001
Pseudomonas aeruginosa	1.3/2.3	0.32/1.15	0.24/0.87
Staphylococcus aureus	0.1/0.57	0.04/0.06	0.03/0.04
Enterococcus faecalis	1.5/1.7	0.21/0.43	0.18/0.25

[a] Five isolates of each microorganism were tested.

TABLE 5. Spontaneous single-step mutants resistant to sparfloxacin

Microorganism	MIC (μg/ml)	Inoculum (10^{11})	No. of resistant colonies at the following selecting concn:		
			4× the MIC	8× the MIC	16× the MIC
Staphylococcus aureus Sa-1	0.06	1	0	0	0
Pseudomonas aeruginosa Psa-1	1	1	0	0	0
Enterobacter cloacae E-5	0.12	2.6	0	0	0
Escherichia coli C-100	0.015	1.2	59	59	59
Serratia marcescens S-80	0.5	8.2	0	0	0
Citrobacter freundii CT-46	0.06	0.81	0	0	0
Klebsiella pneumoniae K-118	0.03	0.10	65	0	0

TABLE 6. PAEs for *Staphylococcus aureus* Sa-1[a] following several time periods of exposure to each drug at 2 μg/ml

Inoculum	Exposure time (min)	Drug	% Lethality	PAE
3.03×10^6	15	Ciprofloxacin	39.14	50 min
3.03×10^6	15	Sparfloxacin	59.1	1 h, 50 min
6.72×10^5	30	Ciprofloxacin	78.32	50 min
6.72×10^5	30	Sparfloxacin	62.38	2 h, 45 min
4.46×10^6	60	Ciprofloxacin	83.1	>3 h, 45 min
4.46×10^6	60	Sparfloxacin	91.04	3 h, 38 min

[a] For *Staphylococcus aureus*, the MIC of sparfloxacin is 0.06 μg/ml and the MIC of ciprofloxacin is 0.12 μg/ml.

terobacteriaceae tested, sparfloxacin showed good activity, inhibiting 90% of the strains at a concentration of 0.5 μg/ml; MICs were above 1 μg/ml for only three strains of *Serratia*, four strains of *Citrobacter*, and one strain of *Providencia*. Comparatively, sparfloxacin was slightly less active than ciprofloxacin against gram-negative bacteria, but its activity was greater than that of ciprofloxacin against *Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas cepacia*, *Xanthomonas maltophilia*, *Alcaligenes* spp., and *Flavobacterium* spp. Against the other gram-negative microorganisms tested, the MIC_{90}s of sparfloxacin were twice those of ciprofloxacin except for *Citrobacter*, *Serratia*, *Proteus*, *Morganella*, and *Yersinia* spp. and *Pseudomonas aeruginosa*, for which the differences were more than two times greater. Like the other quinolones, sparfloxacin inhibited microorganisms, such as *Enterobacter* spp., *Citrobacter* spp., and *Pseudomonas aeruginosa*, which were resistant to expanded-spectrum cephalosporins and/or aminoglycosides.

The major improvement in the activity of sparfloxacin was against gram-positive microorganisms, against which sparfloxacin proved to be the most active drug. Although the quinolones showed relatively poor activity against the streptococcal strains, including *Streptococcus pneumoniae* (the mode MICs were ≥2 μg/ml), sparfloxacin had a mode MIC of 0.5 μg/ml, being four- and eightfold more active than ofloxacin and ciprofloxacin, respectively.

As for the other quinolones, the effect of an increase in the inoculum size from 10^3 to 10^5 CFU/ml on the inhibitory activity of sparfloxacin was minor (2, 3, 10, 15); the activity decreased significantly with increasing Mg^{2+} concentration only for *Pseudomonas aeruginosa*, *Morganella morganii*, and *Enterococcus faecalis*. The acidification of the growth medium lessened the activity of sparfloxacin, as has been shown for other agents of this type (2, 3, 10, 15). There were no changes in the MICs for coagulase-negative staphylococci in the range from pH 6 to 8, as has also been reported by Chaudhry et al. (3).

Our results are in agreement with those reported by Cooper et al. (4) and Doebbeling et al. (5), with the exception that our *Klebsiella* spp. and *Acinetobacter* spp. were more susceptible; on the other hand, our *Streptococcus pyogenes* isolates were more resistant to all the fluoroquinolones than were those reported by other investigators (4, 7, 8, 16, 18). Chaudhry et al. (3) reported for coagulase-negative staphylococci an MIC_{90} that was twofold higher than ours. Our *Streptococcus pneumoniae* isolates were also more resistant to ciprofloxacin than were those reported previously, perhaps because of changes in the resistance patterns, as Fernandes and Ackerman (6) reported for *Streptococcus pneumoniae*; the MIC_{90} of ciprofloxacin in 1985 was 1 μg/ml, and now 33% of strains are unaffected by this concentration (6).

The frequency of spontaneous, single-step resistant mutants was low, as has been reported for other quinolones (7, 10). At 16× the MIC, only spontaneous resistant mutants of *Escherichia coli* were selected. No resistant mutants could be detected in *Staphylococcus aureus*, which is in agreement with the results of Kojima et al. (8).

The increase in the time of exposure to drug was associated with a prolongation of the sparfloxacin PAE, and this PAE was greater than the PAE observed with ciprofloxacin, when the drug contact time was between 15 and 30 min; nevertheless, after 60 min, the PAE of ciprofloxacin was greater.

Before interpreting the data obtained from the present study, one must take into account the fact that the in vitro efficiency of an antibacterial agent is not only related to the MICs but is also related to the levels of drug achievable in serum or tissues. After daily oral doses of 400 mg of sparfloxacin, levels of 1.18 μg/ml in serum have been obtained (12). By selecting a concentration of 1 μg/ml as a breakpoint for susceptibility, 97.6% of the members of the family *Enterobacteriaceae* tested, 94.2% of the gram-positive strains tested, 54.77% of the *Pseudomonas* spp. tested, and 100% of the *Xanthomonas maltophilia*, *Flavobacterium* spp., *Aeromonas* spp., *Haemophilus* spp., and *Acinetobacter* spp. tested would then be considered to be susceptible to this compound.

Our overall results showed that sparfloxacin produces antibacterial activity very similar to that of ciprofloxacin and some improved activity against gram-positive organisms. With its prolonged half-life in serum (16 h after oral doses of 400 mg [11]), sparfloxacin may be given as a once-daily dosage, and in suitable cases, it may be given as single-dose therapy for susceptible pathogens. Nevertheless, the efficacy of sparfloxacin must be assessed in vivo.

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