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# In Vitro Activities of Sparfloxacin, Tosufloxacin, Ciprofloxacin, and Fleroxacin

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**The in vitro activity of sparfloxacin was compared with those of tosufloxacin, ciprofloxacin, and fleroxacin against 730 bacterial isolates representing 49 different species. Sparfloxacin and ciprofloxacin had similar spectra of activity, but sparfloxacin was less active against *Pseudomonas aeruginosa* and more active against many gram-positive cocci and anaerobic bacteria. Tosufloxacin MICs were generally 8- to 16-fold lower than those for sparfloxacin or ciprofloxacin. All four fluoroquinolones were active against nalidixic acid-susceptible strains of the family *Enterobacteriaceae* (MIC for 90% of the isolates [$MIC_{90}$], ≤0.25 μg/ml) but nalidixic acid-resistant strains were less susceptible ($MIC_{90}$, ≥4.0 μg/ml). Against *Pseudomonas aeruginosa* isolates, $MIC_{90}$s were 1.0 μg/ml for tosufloxacin, 2.0 μg/ml for ciprofloxacin, and 4.0 μg/ml for sparfloxacin. Against *Enterococcus faecalis*, sparfloxacin and ciprofloxacin $MIC_{90}$s were 1.0 and 2.0 μg/ml, respectively. $MIC_{90}$s for ciprofloxacin-susceptible *Staphylococcus aureus* were 0.016 μg/ml for tosufloxacin, 0.06 μg/ml for sparfloxacin, and 0.5 μg/ml for both ciprofloxacin and fleroxacin. With four species of gram-negative bacilli, mutants resistant to two to four times the sparfloxacin MIC occurred spontaneously at frequencies of $10^{-7}$ to $10^{-9}$: single-step high-level resistance was not observed. In vitro-selected sparfloxacin-resistant mutants displayed cross-resistance to other quinolones, as did clinical isolates of ciprofloxacin-resistant *S. aureus*. Tosufloxacin MICs with broth microdilution methods were four- to eightfold greater than those obtained with agar dilution methods. The two procedures gave comparable results when sparfloxacin or ciprofloxacin was being tested.**

The fluoroquinolone class of compounds includes many extremely potent, broad-spectrum antimicrobial agents (2). Sparfloxacin (AT-4140; CI-978; PD 131501) is one such compound that is currently being considered for chemotherapy (4–7, 10, 11, 15, 17). Tosufloxacin (A-61827; A-60969) is another fluoroquinolone with an extended spectrum of in vitro activity (1, 3, 8, 9). In this report we describe the results of in vitro studies that compare sparfloxacin with tosufloxacin and two other fluoroquinolones. Additional studies were undertaken to compare susceptibility testing procedures and to evaluate the in vitro development of resistant mutants.

## MATERIALS AND METHODS

**Antimicrobial agents.** Sparfloxacin standard powder was provided by Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co. (Ann Arbor, Mich.). Tosufloxacin was provided by Abbott Laboratories (North Chicago, Ill.). Ciprofloxacin was obtained from Miles Laboratories (West Haven, Conn.), and fleroxacin was obtained from Hoffmann-La Roche, Inc. (Nutley, N.J.).

**Antimicrobial susceptibility tests.** Agar dilution and broth microdilution susceptibility tests were performed as outlined by the National Committee for Clinical Laboratory Standards (12, 13). Mueller-Hinton broth was adjusted to contain 20 mg of calcium and 10 mg of magnesium per liter. The effect of cation supplementation on sparfloxacin and ciprofloxacin MICs was documented by replicate tests with standard control strains. For testing fastidious species, the microdilution trays were supplemented with lysed horse blood (2 to 3%). For testing anaerobic bacteria, broth microdilution trays were prepared with Anaerobic Broth MIC (Difco Laboratories, Detroit, Mich.). The inocula were adjusted to give approximately $5 \times 10^5$ CFU/ml ($1 \times 10^6$ CFU/ml for anaerobes), and MICs were recorded after 16 to 18 h of incubation (48 h for anaerobes). Agar dilution tests were performed with inocula of $5 \times 10^4$ CFU per spot ($1 \times 10^5$ CFU per spot for anaerobes). Anaerobic broth microdilution tests were compared with agar dilution tests with two agar media, i.e., Wilkins-Chalgren agar and brucella agar with 5% sheep blood (12). Haemophilus test medium was used for agar dilution tests with *Haemophilus influenzae* isolates (13). For testing *Neisseria gonorrhoeae*, GC agar with a cysteine-free XV supplement was utilized (13). The latter two species were tested only against sparfloxacin.

**Sparfloxacin-resistant mutants.** The frequency of spontaneously occurring mutants resistant to two, four, and eight times the MIC was documented for different microorganisms. Each strain was inoculated into 150 ml of Mueller-Hinton broth and incubated for 24 to 28 h in a shaking water bath. The broth culture was concentrated by centrifugation to provide a suspension with $2 \times 10^{10}$ to $1 \times 10^{11}$ CFU/ml. Mueller-Hinton agar plates containing a fluoroquinolone (2×, 4×, or 8× the MIC) were inoculated with 0.1 ml of the suspension. After 48 h of incubation, the number of viable CFU was recorded.

Colonies that were recovered from plates containing sparfloxacin (4× the MIC) were suspended in broth and immediately adjusted to match the turbidity of a McFarland 0.5 standard. Those direct suspensions were then used to inoculate broth microdilution trays containing dilutions of each study drug plus ofloxacin and nalidixic acid. Colonies from control plates without antibiotics were also directly tested to represent the susceptible parent strain.

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TABLE 1. In vitro activities of four fluoroquinolone compounds against 725 bacterial isolates

| Microorganism (no. tested) | Antimicrobial agent[a] | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Enterobacteriaceae*[b] | | | | |
| Nalidixic acid suceptible (219) | Sparfloxacin | ≤0.008–4.0 | 0.03 | 0.25 |
| | Tosufloxacin | ≤0.008–0.5 | ≤0.008 | 0.03 |
| | Ciprofloxacin | ≤0.008–2.0 | 0.03 | 0.06 |
| | Fleroxacin | ≤0.03–2.0 | 0.06 | 0.25 |
| Nalidixic acid resistant (36) | Sparfloxacin | 0.12–>16 | 2.0 | 16 |
| | Tosufloxacin | 0.03–>4.0 | 0.25 | 4.0 |
| | Ciprofloxacin | 0.12–>4.0 | 2.0 | >4.0 |
| | Fleroxacin | 0.25–>16 | 2.0 | 16 |
| *Acinetobacter calcoaceticus* subsp. *anitratus* (10) | Sparfloxacin | ≤0.008–0.06 | 0.016 | 0.06 |
| | Tosufloxacin | ≤0.008–0.03 | ≤0.008 | 0.03 |
| | Ciprofloxacin | 0.12–2.0 | 0.5 | 1.0 |
| | Fleroxacin | 0.12–1.0 | 0.25 | 1.0 |
| *Pseudomonas aeruginosa* (20) | Sparfloxacin | 0.5–8.0 | 1.0 | 4.0 |
| | Tosufloxacin | 0.03–1.0 | 0.12 | 1.0 |
| | Ciprofloxacin | 0.12–4.0 | 0.25 | 2.0 |
| | Fleroxacin | 0.5–16 | 2.0 | 8.0 |
| *Pseudomonas* species (20)[c] | Sparfloxacin | 0.06–4.0 | 0.5 | 2.0 |
| | Tosufloxacin | ≤0.008–2.0 | 0.12 | 0.5 |
| | Ciprofloxacin | 0.016–>4.0 | 0.25 | 2.0 |
| | Fleroxacin | 0.06–8.0 | 1.0 | 4.0 |
| *Xanthamonas maltophilia* (10) | Sparfloxacin | 0.12–4.0 | 0.5 | 2.0 |
| | Tosufloxacin | 0.06–1.0 | 0.25 | 1.0 |
| | Ciprofloxacin | 2.0–>4.0 | 4.0 | >4.0 |
| | Fleroxacin | 2.0–8.0 | 2.0 | 4.0 |
| *Moraxella* (*Branhamella*) *catarrhalis* (30) | Sparfloxacin | 0.015–0.03 | 0.015 | 0.03 |
| | Tosufloxacin | ≤0.008 | ≤0.008 | ≤0.008 |
| | Ciprofloxacin | 0.03–0.06 | 0.06 | 0.06 |
| | Fleroxacin | 0.12–0.25 | 0.25 | 0.25 |
| *Neisseria meningitidis* (10) | Sparfloxacin | ≤0.008 | ≤0.008 | ≤0.008 |
| | Tosufloxacin | ≤0.008 | ≤0.008 | ≤0.008 |
| | Ciprofloxacin | ≤0.008 | ≤0.008 | ≤0.008 |
| | Fleroxacin | ≤0.03 | ≤0.03 | ≤0.03 |
| *Neisseria gonorrhoeae* (31) | Sparfloxacin | ≤0.002–0.03 | 0.008 | 0.015 |
| *Haemophilus influenzae* (31) | Sparfloxacin | 0.004–0.06 | 0.016 | 0.06 |
| *Bacillus* species (10) | Sparfloxacin | 0.03–0.25 | 0.12 | 0.25 |
| | Tosufloxacin | ≤0.008–0.03 | ≤0.008 | 0.016 |
| | Ciprofloxacin | 0.06–0.5 | 0.06 | 0.5 |
| | Fleroxacin | 0.12–0.5 | 0.25 | 0.5 |
| *Listeria monocytogenes* (10) | Sparfloxacin | 0.5–2.0 | 1.0 | 1.0 |
| | Tosufloxacin | 0.03–0.25 | 0.06 | 0.12 |
| | Ciprofloxacin | 0.5–2.0 | 1.0 | 2.0 |
| | Fleroxacin | 2.0–4.0 | 2.0 | 4.0 |
| *Enterococcus faecalis* (15) | Sparfloxacin | 0.12–2.0 | 0.5 | 1.0 |
| | Tosufloxacin | 0.03–0.5 | 0.12 | 0.5 |
| | Ciprofloxacin | 0.5–4.0 | 1.0 | 2.0 |
| | Fleroxacin | 2.0–8.0 | 4.0 | 8.0 |
| *Enterococcus faecium* (10) | Sparfloxacin | 0.5–1.0 | 1.0 | 1.0 |
| | Tosufloxacin | 0.12–1.0 | 0.5 | 1.0 |
| | Ciprofloxacin | 1.0–4.0 | 4.0 | 4.0 |
| | Fleroxacin | 8.0–16 | 8.0 | 8.0 |
| *Streptococcus agalactiae* (20) | Sparfloxacin | 0.12–0.5 | 0.25 | 0.5 |
| | Tosufloxacin | 0.06–0.12 | 0.06 | 0.12 |
| | Ciprofloxacin | 0.5–1.0 | 1.0 | 1.0 |
| | Fleroxacin | 4.0–16 | 8.0 | 8.0 |

*Continued on following page*

TABLE 1—*Continued*

| Microorganism (no. tested) | Antimicrobial agent[a] | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Streptococcus pyogenes* (20) | Sparfloxacin | 0.12–1.0 | 0.25 | 0.5 |
| | Tosufloxacin | 0.016–0.25 | 0.03 | 0.06 |
| | Ciprofloxacin | 0.25–2.0 | 0.5 | 0.5 |
| | Fleroxacin | 2.0–>16 | 4.0 | 4.0 |
| *Streptococcus* serogroups C and G (20) | Sparfloxacin | 0.25–0.5 | 0.5 | 0.5 |
| | Tosufloxacin | 0.03–0.12 | 0.03 | 0.06 |
| | Ciprofloxacin | 0.25–1.0 | 0.5 | 1.0 |
| | Fleroxacin | 2.0–8.0 | 4.0 | 4.0 |
| *Streptococcus pneumoniae* (30) | Sparfloxacin | 0.12–1.0 | 0.25 | 0.5 |
| | Tosufloxacin | 0.03–0.12 | 0.03 | 0.06 |
| | Ciprofloxacin | 0.5–4.0 | 1.0 | 1.0 |
| | Fleroxacin | 4.0–16 | 4.0 | 8.0 |
| *Staphylococcus aureus* (35)[d] | Sparfloxacin | 0.016–0.06 | 0.06 | 0.06 |
| | Tosufloxacin | ≤0.008–0.03 | ≤0.008 | 0.016 |
| | Ciprofloxacin | 0.12–0.5 | 0.25 | 0.5 |
| | Fleroxacin | 0.12–1.0 | 0.5 | 0.5 |
| *Staphylococcus epidermidis* (40)[e] | Sparfloxacin | 0.016–0.12 | 0.06 | 0.12 |
| | Tosufloxacin | ≤0.008–0.12 | 0.016 | 0.03 |
| | Ciprofloxacin | 0.12–0.5 | 0.25 | 0.5 |
| | Fleroxacin | 0.25–2.0 | 0.5 | 1.0 |
| *Bacteroides fragilis* (30) | Sparfloxacin | 1.0–2.0 | 2.0 | 2.0 |
| | Tosufloxacin | 0.25–1.0 | 0.5 | 1.0 |
| | Ciprofloxacin | 8.0–>16 | 8.0 | 16 |
| *Bacteroides fragilis* group[f] (38) | Sparfloxacin | 0.5–16 | 2.0 | 4.0 |
| | Tosufloxacin | 0.25–8.0 | 1.0 | 2.0 |
| | Ciprofloxacin | 4.0–>16 | 16 | >16 |
| *Clostridium* species (11) | Sparfloxacin | ≤0.12–8.0 | 1.0 | 4.0 |
| | Tosufloxacin | ≤0.12–1.0 | 0.25 | 0.5 |
| | Ciprofloxacin | ≤0.12–16 | 1.0 | 8.0 |
| *Peptostreptococcus* species (15) | Sparfloxacin | ≤0.12–2.0 | 0.5 | 1.0 |
| | Tosufloxacin | ≤0.12–4.0 | 0.5 | 2.0 |
| | Ciprofloxacin | ≤0.12–4.0 | 0.5 | 4.0 |

[a] Four fluoroquinolones were evaluated, except that sparfloxacin was the only drug tested against *N. gonorrhoeae* and *H. influenzae* isolates and fleroxacin was not tested against anaerobic bacteria.

[b] Includes 46 *E. coli*, 10 *C. diversus*, 20 *C. freundii*, 10 *E. agglomerans*, 20 *E. aerogenes*, 20 *E. cloacae*, 20 *K. pneumoniae*, 20 *S. marcescens*, 10 *P. mirabilis*, 10 *P. vulgaris*, 10 *M. morganii*, 10 *P. stuartii*, 9 *P. rettgeri*, 10 *S. enteritidis*, 20 *Shigella* spp. (5 of each species), and 10 *Y. enterocolitica* isolates.

[c] Includes five *P. fluorescens*, five *P. putida*, five *P. cepacia*, and five *P. stutzeri* isolates.

[d] Includes 20 methicillin-susceptible and 15 methicillin-resistant strains; all were ciprofloxacin susceptible. Data with five ciprofloxacin-resistant strains are not shown (see text).

[e] Includes 20 methicillin-susceptible and 20 methicillin-resistant strains; all were ciprofloxacin susceptible.

[f] Includes 10 *B. thetaiotaomicron*, 10 *B. distasonis*, 9 *B. ovatus*, and 9 *B. vulgatus* isolates.

## RESULTS

**Gram-negative species.** Table 1 presents the results of tests with 255 members of the family *Enterobacteriaceae*, combining data with nalidixic acid-susceptible strains (MIC, ≤16 μg/ml) and with nalidixic acid-resistant strains (MIC, ≥32 μg/ml). The latter group of isolates showed decreased susceptibility to the fluoroquinolones, and the fluoroquinolone MICs were great enough so that some isolates might be considered clinically resistant. All four fluoroquinolones were much more active against nalidixic acid-susceptible strains, i.e., MICs for 90% of the isolates ($MIC_{90}$s) were ≤0.25 μg/ml compared with ≥4.0 μg/ml for nalidixic acid-resistant strains.

Against *Pseudomonas aeruginosa* isolates, the median MIC of ciprofloxacin was 0.25 μg/ml: tosufloxacin was twice as active (MIC for 50% of the isolates [$MIC_{50}$], 0.12 μg/ml), sparfloxacin and fleroxacin were less active ($MIC_{50}$s, 1.0 and 2.0 μg/ml, respectively). *Xanthomonas maltophilia* was relatively resistant to all four study drugs ($MIC_{90}$s, from 1.0 to >4.0 μg/ml). *H. influenzae* and *N. gonorrhoeae* isolates were very susceptible to sparfloxacin (MICs, ≤0.06 μg/ml).

**Gram-positive species.** Among the gram-positive bacilli, *Listeria monocytogenes* isolates were particularly susceptible to tosufloxacin: sparfloxacin and ciprofloxacin were at least 10-fold less active. Against the enterococci, the $MIC_{90}$s of the four fluoroquinolones ranged from 0.5 to 8.0 μg/ml: sparfloxacin and tosufloxacin were generally more potent than ciprofloxacin against *Enterococcus faecium* and, to a lesser extent, against *Enterococcus faecalis*. Against nonenterococcal streptococci, sparfloxacin and ciprofloxacin were

TABLE 2. In vitro activities of six quinolones against four gram-negative bacilli before and after a single passage on agar plates with sparfloxacin (4× MIC)

| Species | Microdilution MIC (μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Sparfloxacin | Tosufloxacin | Ciprofloxacin | Ofloxacin | Fleroxacin | Nalidixic acid |
| *Escherichia coli* | | | | | | |
| Parent | 0.016 | <0.008 | 0.03 | 0.12 | 0.06 | <8.0 |
| Mutant | 1.0 | 0.25 | 1.0 | 2.0 | 1.0 | >32 |
| *Enterobacter cloacae* | | | | | | |
| Parent | 0.06 | 0.016 | 0.03 | 0.12 | 0.25 | ≤8.0 |
| Mutant | 1.0 | 0.25 | 0.5 | 2.0 | 1.0 | 32 |
| *Klebsiella pneumoniae* | | | | | | |
| Parent | 0.06 | 0.016 | 0.06 | 0.25 | 0.12 | ≤8.0 |
| Mutant | 1.0 | 0.25 | 1.0 | 2.0 | 1.0 | 32 |
| *Pseudomonas aeruginosa* | | | | | | |
| Parent | 4.0 | 0.25 | 1.0 | 4.0 | 4.0 | >32 |
| Mutant | >16 | 4.0 | >4.0 | >16 | >16 | >32 |

similar in potency, but tosufloxacin was 8- to 16-fold more active.

*Staphylococcus aureus* and *Staphylococcus epidermidis* isolates (methicillin susceptible or resistant) were susceptible to all four fluoroquinolones: tosufloxacin was the most potent ($MIC_{90}$s, 0.016 and 0.03 μg/ml, respectively), whereas sparfloxacin $MIC_{90}$s were 0.06 and 0.12 μg/ml, respectively. In contrast, ciprofloxacin $MIC_{90}$s were 0.5 μg/ml for both species. Five ciprofloxacin-resistant (MIC, ≥4.0 μg/ml) strains of methicillin-resistant *S. aureus* were also evaluated (data not shown). Those five strains demonstrated cross-resistance to the other fluoroquinolones studied (MIC, ≥4.0 μg/ml). Three other strains with intermediate susceptibility to ciprofloxacin (MIC, 2.0 μg/ml) were susceptible to the other study drugs.

**Anaerobic bacteria.** Sparfloxacin, tosufloxacin, and ciprofloxacin were tested by the broth microdilution method. $MIC_{50}$s for sparfloxacin ranged from 0.5 to 2.0 μg/ml for different species, and $MIC_{90}$s ranged from 1.0 to 4.0 μg/ml (Table 1). Ciprofloxacin MICs were generally two- to fourfold greater than those for sparfloxacin, and tosufloxacin MICs tended to be two- to fourfold lower.

**Sparfloxacin-resistant mutants.** The frequency of spontaneously occurring mutants resistant to sparfloxacin at 2×, 4×, and 8× the MIC was determined with one strain of each of five bacterial species (*Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *P. aeruginosa*, and *E. faecalis*). Resistance to 2× the MIC occurred at frequency rates of $10^{-7}$ or $10^{-8}$ with the four gram-negative bacilli and resistance, to 4× the MIC occurred less frequently ($10^{-8}$ or $10^{-9}$). The *E. faecalis* culture contained cells resistant to 2× the MIC at a frequency of $7 \times 10^{-9}$, and resistance to greater concentrations could not be documented. For all five strains, resistance to 8× the MIC occurred very rarely ($<10^{-10}$).

Resistant mutants that were selected by a single passage on agar plates containing 4× MIC of sparfloxacin were further tested for their susceptibility to the four study drugs plus ofloxacin and nalidixic acid (Table 2). With the resistant mutants the MICs of sparfloxacin and of all other quinolone compounds tested, including nalidixic acid, were increased.

**Effects of cations and pH.** Five separate tests with each of four control strains (*E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, and *E. faecalis* ATCC 29212) were performed in Mueller-Hinton broth with three levels of cation supplementation, i.e., no supplements (5 mg of Ca and 5 mg of Mg per liter), currently recommended (13) cation adjustment (25 mg of Ca and 12.5 mg of Mg per liter), and previously recommended supplementation (50 mg of Ca and 25 mg of Mg per liter). Both ciprofloxacin and sparfloxacin were evaluated in this way. The addition of calcium and magnesium had minimal effects on the fluoroquinolone MICs, i.e., no more than one doubling dilution increase in MICs. Furthermore, broth was adjusted to pH 5.5, 7.3, and 8.0. Sparfloxacin was two- to eightfold less active in the acid medium, and no significant change was seen in the alkaline medium (data not shown).

**Broth microdilution versus agar dilution MICs.** Studies with 30 enteric bacilli and 30 staphylococci compared two standard dilution susceptibility testing procedures with sparfloxacin, tosufloxacin, and ciprofloxacin (Table 3). For reasons that remain unclear, tosufloxacin demonstrated much lower MICs with agar dilution methods than with the broth microdilution procedure. Tests with sparfloxacin and ciprofloxacin did not display the same methodologic differences. Anaerobic bacteria were also tested by agar dilution and broth microdilution methods with Wilkins-Chalgren agar or a broth version of the same medium (Anaerobic Broth MIC; Difco). The two types of anaerobic tests gave essentially identical results with all three study drugs. Additional studies compared agar dilution tests with two media (Wilkins-Chalgren agar and brucella blood agar). The two National Committee for Clinical Laboratory Standards recommended agar media (12) gave essentially identical MICs with 60 different anaerobes tested against the three fluoroquinolones (data not shown).

## DISCUSSION

Sparfloxacin has proven to be a very potent broad-spectrum antibacterial agent. Its spectrum of activity and in vitro potency were quite similar to those of ciprofloxacin, except that sparfloxacin was less active than ciprofloxacin against *Pseudomonas* spp. and more active against the gram-positive cocci and against anaerobic bacteria. Others have reported similar conclusions (5–7, 15, 17). Tosufloxacin MICs are method dependent, since broth microdilution MICs were four to eight times greater than agar dilution MICs. Such disparities were not seen when sparfloxacin or

TABLE 3. Direct comparison of agar dilution and broth microdilution susceptibility test results[a] with three fluoroquinolone compounds

| Antimicrobial agent and test strains (no. tested) | No. of times the following MIC ratio (AD/BMD)[b] was recorded: | | | | | | |
|---|---|---|---|---|---|---|---|
| | ≤0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 |
| Sparfloxacin | | | | | | | |
| Enteric bacilli (30) | | | [3 | 19 | 7] | 1 | |
| Staphylococci (30) | | 6 | [17 | 7 | 0] | | |
| Anaerobes (53) | | | [12 | 20 | 18] | 3 | |
| Tosufloxacin | | | | | | | |
| Enteric bacilli (30) | 3 | 19 | [6 | 2 | 0] | | |
| Staphylococci (30) | 16 | 14 | [0 | 0 | 0] | | |
| Anaerobes (53) | | | [2 | 20 | 25] | 2 | 4 |
| Ciprofloxacin | | | | | | | |
| Enteric bacilli (30) | | | [0 | 6 | 20] | 4 | |
| Staphylococci (30) | | 1 | [8 | 13 | 8] | | |
| Anaerobes (53) | | | [6 | 29 | 14] | 3 | 1 |

[a] Enteric bacilli and staphylococci were tested in Mueller-Hinton agar or in cation adjusted Mueller-Hinton broth. Wilkins-Chalgren agar and Anaerobic Broth MIC (Difco) were used for the anaerobes.

[b] Ratios of <1 designate pairs in which the agar dilution (AD) MIC was lower than the broth microdilution (BMD) MIC, and ratios of >1 designate pairs in which the agar dilution MIC was higher than the broth microdilution MIC. Brackets enclose MIC ratios that are considered essentially comparable (±1 dilution interval).

ciprofloxacin was being tested. With either method tosufloxacin was consistently more potent than sparfloxacin. The increased potency of tosufloxacin must be interpreted in view of the low levels in blood that have been observed in animals receiving tosufloxacin (8). The sparsity of human pharmacokinetic data for sparfloxacin or tosufloxacin makes it difficult to assign interpretive categories for the in vitro data that are included in this report. However, the relative potency of different compounds can be compared. In general, the in vitro potency of the study drugs can be ordered as follows: tosufloxacin > sparfloxacin > ciprofloxacin > fleroxacin.

The species of *Enterobacteriaceae* were inhibited by all four fluoroquinolones, but nalidixic acid-resistant strains required increased concentrations for inhibition. The same phenomenon has been described for other fluoroquinolones (2). Although staphylococci are generally susceptible to the fluoroquinolones (2), methicillin-resistant *S. aureus* strains with high-level resistance to ciprofloxacin are now appearing in some institutions (14, 16). Those strains also tend to be resistant to other fluoroquinolones (4).

Among gram-negative bacilli, spontaneously occurring mutants resistant to two to four times the MIC of sparfloxacin were demonstrated in vitro but at frequency rates of $10^{-7}$ to $10^{-9}$. Even lower mutation rates were observed as the concentration of the drug increased. Single-step, high-level resistance to sparfloxacin was not observed in our studies. Sparfloxacin-resistant mutants that were selected in vitro displayed cross-resistance to nalidixic acid, ofloxacin, and the three other study drugs.

Broth microdilution and agar dilution susceptibility tests generally gave comparable results when sparfloxacin or ciprofloxacin was tested. Tosufloxacin, on the other hand, displayed disparities that need to be confirmed and further studied. Cation supplementation of Mueller-Hinton broth had no significant effect on sparfloxacin MICs, but the pH of the medium was very important.

In summary, the in vitro activities of sparfloxacin and ciprofloxacin were similar, except that sparfloxacin had greater activity against many gram-positive cocci and against anaerobic bacteria. If satisfactory levels in blood can be safely maintained during therapy, sparfloxacin deserves to be further evaluated in clinical studies. Tosufloxacin displayed even better in vitro activity, but the importance of that increased potency may be diminished by the low levels in blood that have been observed in experimental animals (8). Human pharmacokinetic data for sparfloxacin and for tosufloxacin will be needed before the clinical relevance of these in vitro data can be estimated.

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