ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1990, p. 2223–2227
0066-4804/90/112223-05$02.00/0
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Vol. 34, No. 11

In Vitro Activity of Tosufloxacin, a New Quinolone, against Respiratory Pathogens Derived from Cystic Fibrosis Sputum

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By using broth microdilution methods, the in vitro activity of tosufloxacin (A-64730), a new quinolone, was compared with those of other agents, including five quinolones, against geographically diverse cystic fibrosis sputum isolates obtained from 26 cystic fibrosis centers in the United States. These included *Pseudomonas aeruginosa*, conventional as well as especially resistant (ceftazidime, aztreonam, gentamicin, and/or tobramycin) isolates; *Escherichia coli*; *Pseudomonas cepacia*; *Staphylococcus aureus*; and *Haemophilus influenzae*. Tosufloxacin MICs for 50 and 90% of isolates of standard *P. aeruginosa* were 0.5 and 2.0 mg/liter, for resistant *P. aeruginosa* they were 4.0 and >16.0 mg/liter, for *E. coli* they were ≤0.016 and ≤0.016 mg/liter, for *P. cepacia* they were 4.0 and 8.0 mg/liter, for *S. aureus* they were 0.063 and 0.063 mg/liter, and for *H. influenzae* they were ≤0.016 and 0.032 mg/liter, respectively. Tosufloxacin activities against standard and resistant strains of *P. aeruginosa* were similar to those of comparative quinolones. Against *E. coli*, tosufloxacin activity was similar to those of other quinolones. Against *S. aureus*, tosufloxacin activity was similar to those of trimethoprim-sulfamethoxazole and cephalexin, but tosufloxacin was more active than other agents. Against *H. influenzae*, tosufloxacin activity was similar to those of other quinolones. There was minor diminution of activity at pH 8.2 but major diminution of activity at pH 5.2 and at inoculum sizes of ≥10^7 CFU/ml. Activity was unaffected by sputum but was enhanced by serum and by the omission of cation supplementation. Tosufloxacin has consistent activity against common cystic fibrosis pathogens. Its high degree of activity against *S. aureus* with activity maintained against *P. aeruginosa* and other gram-negative bacteria of interest suggests that further in vitro studies and assessment of activity in in vivo models of cystic fibrosis pulmonary infections are warranted.

Tosufloxacin, A-64730, is a new broad-spectrum fluoroquinolone agent with in vitro activity against a variety of microorganisms. These include gram-positive and gram-negative aerobic bacteria (P. Weber, G. Leturnier, A. Fremaux, P. Geslin, and Y. Boussougant, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 105, 1989; J. Segreti, L. J. Goodman, E. J. Glick, and G. M. Thewholme, 29th ICAAC, abstr. no. 99, 1989), anaerobic bacteria (9), and *Chlamydia trachomatis* (S. C. Lafredo, T. Fekete, and K. R. Cundy, 28th ICAAC, abstr. no. 1478, 1988). Its mechanism of action, like those of other fluoroquinolones, involves inhibition of bacterial DNA gyrase (17). At present, there are no data available regarding the pharmacokinetic profile of this new compound in humans; however, studies in a murine model have shown that after oral administration of 100 mg/kg, peak concentrations in serum are similar to those of ciprofloxacin (2.3 μg/ml), with a longer elimination half-life for tosufloxacin (3.9 versus 1.2 h, respectively) (9).

Because of their spectra of activity and oral bioavailabilities, agents from the fluoroquinolone family may play an important role in the treatment of infections in patients with cystic fibrosis (CF) (3, 4, 7, 14, 16). This study was designed to evaluate the in vitro activity of tosufloxacin against respiratory isolates obtained from the sputum of patients with CF. We measured tosufloxacin activity under conditions that reflect those found at the sites of infection in patients with CF.

(This study was presented in part at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy [A. Arguedas, J. C. Akaniro, H. R. Stutman, and M. I. Marks, 29th ICAAC, abstr. no. 98, 1989].)

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MATERIALS AND METHODS

Bacterial strains. Bacterial isolates from CF sputum were obtained from 26 CF centers in the United States. These included 30 conventional mucoid and nonmucoid *Pseudomonas aeruginosa* isolates and 15 mucoid and nonmucoid isolates resistant to ceftazidime, aztreonam, gentamicin, and/or tobramycin, *Escherichia coli* (n = 20), *Pseudomonas cepacia* (n = 14), *Staphylococcus aureus* (n = 30), and type b and nontypeable *Haemophilus influenzae* (n = 20). Prior to use, frozen isolates were thawed and subcultured onto appropriate solid media. *Pseudomonas* species were subcultured onto mucoid maintenance agar (11, 13). *H. influenzae* was subcultured onto chocolate agar plates and then transferred to chocolate agar slants. Initial subcultures of *E. coli* and *S. aureus* were on blood agar plates, and subsequent maintenance was on Trypticase agar slants.

Antimicrobial agents. Tosufloxacin (A-64730) powder was obtained from Abbott Laboratories, Abbott Park, Ill., and the other quinolones were obtained from their manufacturers: ciprofloxacin, Miles Pharmaceuticals, West Haven, Conn.; enoxacin, Warner-Lambert, Ann Arbor, Mich.; ofloxacin, Ortho Pharmaceutical Corp., Raritan, N.J.; and norfloxacin, Merck Institute, Rahway, N.J. All other antimicrobial agents were obtained from their respective manufacturers. All antimicrobial agents were initially dissolved in appropriate solvents as recommended by the manufacturer and initially diluted to a stock concentration of 2,560 mg/

TABLE 1. Antimicrobial activity of tosufloxacin against pathogens from CF sputum

Organism and antimicrobial agent	MIC (mg/liter)[a]		
	Range	50%	90%
P. aeruginosa			
Excluding resistant strains (n = 30)			
Tosufloxacin	0.063->16	0.50	2.0
Ciprofloxacin	0.032-2.0	0.25	0.5
Ofloxacin	0.063->16	2.0	4.0
Norfloxacin	0.125->16	1.0	2.0
Enoxacin	0.125->16	1.0	4.0
Gentamicin	0.25->64	4.0	>64
Tobramycin	0.25->64	2.0	64
Ceftazidime	0.25-64	2.0	4.0
Aztreonam	≤0.032-64	4.0	8.0
Imipenem	0.063-8.0	1.0	2.0
Ciprofloxacin- and gentamicin-resistant strains (n = 15)			
Tosufloxacin	0.50->16	4.0	>16
Ciprofloxacin	0.125->16	1.0	>16
Ofloxacin	1.0->16	>16	>16
Norfloxacin	0.5->16	4.0	>8.0
Enoxacin	1.0->16	4.0	>16
Gentamicin	4.0->64	8.0	>64
Tobramycin	1.0->64	2.0	>64
Ceftazidime	2.0->64	4.0	>64
Aztreonam	0.5->64	8.0	>64
Imipenem	1.0-4.0	2.0	4.0
P. cepacia (n = 14)			
Tosufloxacin	0.25-16	4.0	8.0
Ciprofloxacin	0.25->16	1.0	4.0
Ofloxacin	1.0->16	4.0	16
Norfloxacin	4.0->16	16	>16
Enoxacin	2.0->16	4.0	16
TMP-SMX[b]	0.063/1.19->16/304	1/19	>16/304
Gentamicin	4-64	32	64
Piperacillin	0.5-16	8.0	16
Chloramphenicol	8->128	16	128
S. aureus (n = 30)			
Tosufloxacin	0.032-0.125	0.063	0.063
Ciprofloxacin	0.125-0.5	0.5	0.5
Ofloxacin	0.125-0.5	0.5	0.5
Norfloxacin	0.25-2.0	1.0	2.0
Enoxacin	0.25-2.0	1.0	2.0
TMP-SMX	0.032/0.060-0.125/2.38	0.063/1.19	0.063/1.19
Gentamicin	0.063-32	0.25	0.50
Nafcillin	0.25-32	0.25	0.50
Cephalexin	0.125-16	0.125	0.125
Cefuroxime	2->128	4.0	8.0
H. influenzae (n = 20)			
Tosufloxacin	≤0.016-0.032	≤0.016	0.032
Ciprofloxacin	≤0.008-0.016	≤0.008	0.016
Ofloxacin	0.016-0.063	0.032	0.063
Norfloxacin	0.032-0.125	0.063	0.063
Enoxacin	0.063-0.25	0.125	0.25
TMP-SMX	0.063/1.29-0.25/4.75	0.125/2.38	0.125/2.38
Ampicillin	0.25-64	0.5	32.0
Chloramphenicol	0.25-8.0	1.0	4.0
Cefotaxime	≤0.016-0.125	≤0.016	0.125
Cefuroxime	0.25-8.0	1.0	8.0
E. coli (n = 20)			
Tosufloxacin	≤0.016	≤0.016	≤0.016
Ciprofloxacin	≤0.008	≤0.008	≤0.008

Continued

TABLE 1—*Continued*

Organism and antimicrobial agent	MIC (mg/liter)[a]		
	Range	50%	90%
Ofloxacin	≤0.008–0.032	≤0.008	0.016
Norfloxacin	≤0.008–0.032	≤0.008	0.016
Enoxacin	≤0.008–0.063	0.016	0.032
TMP-SMX	≤0.063/1.19–0.5/9.5	0.125/2.38	0.25/4.75
Gentamicin	0.25–4	0.5	1.0
Tobramycin	0.5–16	0.5	1.0
Piperacillin	0.5–>256	1.0	256
Ceftazidime	0.062–0.25	0.125	0.25
Aztreonam	≤0.032–0.5	0.062	0.125
Imipenem	0.125–0.5	0.25	0.25

[a] 50% and 90%, MICs for 50 and 90% of strains tested, respectively.
[b] TMP-SMX, Trimethoprim-sulfamethoxazole.

liter. All working antimicrobial solutions were stored at −70°C and used within 4 weeks of preparation.

Antimicrobial susceptibility tests. Determinations of antimicrobial susceptibilities were performed by a microdilution technique by using Mueller-Hinton broth adjusted to pH 7.2 to 7.4 with 1 M HCl or 1 M NaOH and supplemented with 50 mg of calcium and 25 mg of magnesium per liter. For testing of *H. influenzae*, the broth was additionally supplemented with hematin, β-NAD, and 2 to 3% lysed horse blood to yield *Haemophilus* test medium (12). Using the MIC-2000 Plus System (Dynatech Laboratories, Inc., Chantilly, Va.), 0.1 ml of the medium-antimicrobial broth was dispensed into wells of microdilution trays, forming a concentration gradient for each antimicrobial agent. All filled microdilution trays were sealed and stored in plastic bags at −70°C until needed. The plates were used within 4 weeks of preparation.

From 24-h-old subcultures, inocula were grown in Trypticase soy broth (*Haemophilus* test medium for *H. influenzae*) and incubated for 3 h at 37°C (or until visible turbidity occurred). By using a nephelometer, the bacterial density was adjusted to obtain a turbidity comparable to that of a 0.5 McFarland standard. Further dilution yielded a final concentration of 2.0×10^6 CFU/ml after the microtiter wells were seeded with the bacterial suspension with the MIC-2000 Plus automatic inoculator. Verification of the desired inoculum concentration was done by performing colony counts on solid antibiotic-free media. The MIC was the lowest concentration that showed no visible turbidity after an incubation period of 18 to 20 h at 37°C in ambient air. To determine the MBC in the serum, sputum, and Mueller-Hinton broth for the isolates used for comparative purposes, 0.01 ml from each microdilution well was transferred to antibiotic-free agar. Following a reincubation period of 18 to 24 h (in 10% CO_2 for *H. influenzae*), the MBC was defined as the lowest antibiotic concentration that yielded a 99.9% kill of the initial inoculum.

In these evaluations, MIC determinations of all antimicrobial agents were controlled by inclusion of reference strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 29213. In all cases, these results were within one twofold dilution of the established MICs.

Variation of growth conditions. Sputa obtained by voluntary expectoration by patients with CF were pooled, homogenized in sputolysin (Calbiochem-Behring Corp., La Jolla, Calif.), sonicated, and centrifuged, and the supernatant was sterilized by membrane filtration. Sterile pooled sputum was then aseptically added in a 1:1 dilution to appropriately supplemented Mueller-Hinton broth. By using the sputum–Mueller-Hinton broth medium as a diluent, various antimicrobial agent test panels were prepared, and MICs and MBCs were determined by using selected isolates. Similarly, pooled human sera, which were obtained from patients with CF, were used in a 1:1 ratio with Mueller-Hinton broth for susceptibility testing.

Selected isolates, consisting of *P. aeruginosa* (n = 6), *P. cepacia* (n = 2), *E. coli* (n = 3), and *S. aureus* (n = 3) at a final inoculum density of 10^6 CFU/ml, were exposed to tosufloxacin at pHs 5.2, 6.2, 7.2, and 8.2. The pH was adjusted with 1 M NaOH or 1 M HCl. Similarly, these

TABLE 2. Effects of pH variations on in vitro activities of tosufloxacin and ciprofloxacin in Mueller-Hinton broth

Organism (no. tested)	Quinolone[a]	Median MIC (mg/liter) at pH:			
		5.2	6.2	7.2	8.2
P. cepacia (2)	T	12	4.5	4	1
	C	16	12	2	1.25
P. aeruginosa (6)	T	2.5	1.5	1.25	3
	C	1.5	2.5	1.25	1
S. aureus (3)	T	≤0.008	≤0.008	≤0.008	≤0.008
	C	0.125	0.03	0.12	0.5
E. coli (3)	T	0.25	≤0.008	≤0.008	≤0.008
	C	≤0.008	≤0.008	≤0.008	≤0.008

[a] T, Tosufloxacin; C, ciprofloxacin.

TABLE 3. Effects of inoculum size variations on in vitro activities of tosufloxacin and ciprofloxacin in Mueller-Hinton broth

Organism (no. tested)	Quinolone[a]	Median MIC (mg/liter) at the following inoculum (CFU/ml):			
		10^4	10^5	10^7	10^8
P. cepacia (2)	T	2.25	4.12	8.5	8
	C	4	5	6	>16
P. aeruginosa (6)	T	1	1	4	>16
	C	0.75	1	2	>16
S. aureus (2)	T	≤0.008	0.012	1.12	12
	C	0.024	0.012	>16	>16
E. coli (3)	T	≤0.008	≤0.008	0.25	>16
	C	≤0.008	≤0.008	>16	>16

[a] T, Tosufloxacin; C, ciprofloxacin.

TABLE 4. Effects of human serum and CF sputum on in vitro activity of tosufloxacin

Organism (no. tested)	Median MBC (mg/liter) in:		
	Mueller-Hinton broth	Human serum	CF sputum
P. cepacia (2)	2.03	<0.008	2.1
P. aeruginosa (6)	3	0.012	3
S. aureus (2)	0.016	<0.008	<0.008
E. coli (2)	0.012	<0.008	<0.008

isolates were tested at inoculum densities of 10^4, 10^5, 10^7, and 10^8 CFU/ml and with Mueller-Hinton broth for which cation (calcium and magnesium) supplementation was omitted. Microdilution plates containing a series of increasing concentrations of tosufloxacin and comparative antibiotics were then seeded with these organisms. Following incubation at 37°C for 18 to 20 h, the MICs were determined.

RESULTS

The antibacterial activities of tosufloxacin and the comparative quinolones and other antimicrobial agents that were evaluated are depicted in Table 1. Tosufloxacin activity against *P. aeruginosa* isolates was similar to that of norfloxacin, twofold greater than those of ofloxacin and enoxacin, and fourfold less than that of ciprofloxacin. Against the resistant isolates, tosufloxacin activity was similar to those of the other quinolones. Against *P. cepacia*, results were similar to those obtained for ciprofloxacin, ofloxacin, norfloxacin, and enoxacin. Against *H. influenzae*, tosufloxacin was eightfold more potent than enoxacin but had activity similar to those of the other comparative quinolones. Against *E. coli*, tosufloxacin activity was similar to those of the other quinolone agents.

Tosufloxacin activity against methicillin-susceptible *S. aureus* isolates was similar to that of the combination trimethoprim-sulfamethoxazole and to that of cephalexin but was 8- to 32-fold greater than those of the comparative quinolones and nafcillin.

Effects of various growth conditions on tosufloxacin activity. Table 2 shows the effect of changes (median) in the pH of the Mueller-Hinton broth on the MIC of tosufloxacin for *P. aeruginosa*. Reduction of the pH to 6.2 did not affect the MICs for *P. aeruginosa*, *P. cepacia*, *S. aureus*, *E. coli*, or *H. influenzae*. When the pH was reduced to 5.2, the MICs for 8 of 14 microorganisms tested increased substantially. When the pH was increased to 8.2, the MICs for *P. aeruginosa* and one isolate of *S. aureus* increased 4- to 32-fold but remained constant for *E. coli*, *P. cepacia*, and other *S. aureus* strains. Ciprofloxacin, which was used for comparative purposes, showed a similar trend under acidic and alkaline conditions.

Tosufloxacin activity was stable up to an inoculum size of 10^6 CFU/ml but was considerably reduced at an inoculum of $\geq 10^7$ CFU/ml (Table 3). Its activity was not affected when tested in sputum from patients with CF but was increased in human serum (Table 4). Tosufloxacin activity was increased 2- to 256-fold by omitting cation supplementation.

DISCUSSION

Progressive pulmonary infection is the primary cause of morbidity and mortality in patients with CF (3). Treatment of these infections is problematic because of characteristics of the disease process at the site of infection that often preclude effective antimicrobial action (3).

Tosufloxacin activity against gram-negative and gram-positive isolates that were obtained from patients without CF has been excellent (8–10). In our study, tosufloxacin activity against conventional *P. aeruginosa* isolates from patients with CF was similar to those of norfloxacin and imipenem; greater than those of ofloxacin, enoxacin, gentamicin, tobramycin, ceftazidime, and aztreonam; but two dilutions less than that of ciprofloxacin. No differences between mucoid and nonmucoid strains were noted. Against resistant *P. aeruginosa* isolates, tosufloxacin activity was similar to those of the comparative quinolones. These results are consistent with those of previous investigations in which cross resistance among fluoroquinolones, whether by alterations in the outer membrane proteins (5) or by changes in the configuration of bacterial DNA gyrase (4, 15), has been common. Against *P. cepacia*, *E. coli*, and *H. influenzae*, tosufloxacin activity was comparable to those of other quinolones. However, tosufloxacin activity against *S. aureus* was higher than those of the comparative quinolone agents and nafcillin. This result, which was similar to those of previous observations for strains from patients without CF (8–10, 18), is encouraging in that mixed *S. aureus-P. aeruginosa* pulmonary infection remains a common etiology in patients with CF, affecting more than 20% of patients with CF in the United States (Cystic Fibrosis Foundation, unpublished data).

Tosufloxacin bactericidal activity was not influenced by CF sputum but was enhanced in the presence of human serum. Results of previous studies (6, 16) with other quinolones support these findings with CF sputum but are different from the results obtained when human serum was used (4, 8, 9). Our findings could be the result of an interaction between tosufloxacin and nonspecific bactericidal factors in serum that are directed against the tested microorganisms obtained from the population with CF. Additional experiments are necessary to fully evaluate this observation.

The significant reduction in the activity of tosufloxacin when tested at pHs 5.2 and 8.2 and at an inoculum density of $\geq 10^7$ CFU/ml is similar to results obtained with other quinolone agents (1, 2, 4). Its clinical relevance must be evaluated in future therapeutic trials.

An antagonistic effect of Ca^{2+} and Mg^{2+} on the in vitro activity of tosufloxacin was noted. Although this observation is consistent with the reports of other investigators (1, 8, 9), its clinical significance is not clear and should be evaluated in specific clinical trials.

In view of its broad activity against gram-negative bacilli, and especially against *S. aureus*, and the minimal effect of sputum obtained from patients with CF, tosufloxacin appears to be a potentially useful agent against the pathogens that produce bronchopulmonary infections in patients with CF. Further kinetic and animal model studies are therefore warranted as a prelude to possible clinical trials in humans.

ACKNOWLEDGMENTS

We thank Joyce Bagan for editorial assistance.

This study was supported in part by grant MA 026 from the Cystic Fibrosis Foundation, Bethesda, Md., and by Public Health Service grant R01 24801 from the National Institute of Allergy and Infectious Disease.

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