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In Vitro Activity of Tosufloxacin (A-61827; T-3262) against Selected Genital Pathogens

JOHN SEGRETI,[1]* DANIEL J. HIRSCH,[1] ALAN A. HARRIS,[1] KATHI S. KAPELL,[1] HY ORBACH,[2] AND HAROLD A. KESSLER[1]

Section of Infectious Disease, Department of Medicine, Rush-Presbyterian-St. Luke's Medical Center, 1653 West Congress Parkway, Chicago, Illinois 60612,[1] and City of Chicago Department of Health, Daley Center, Chicago, Illinois 60601[2]

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The in vitro activity of tosufloxacin (A-61827; T-3262) against 15 clinical isolates of *Chlamydia trachomatis* and 31 recent clinical isolates of *Neisseria gonorrhoeae*, including 15 spectinomycin-resistant *N. gonorrhoeae* and 16 penicillinase-producing *N. gonorrhoeae*, was determined. Tosufloxacin was compared with doxycycline, ciprofloxacin, and temafloxacin against *C. trachomatis*. Susceptibility testing was performed by using McCoy cell monolayers. The in vitro activity of tosufloxacin against *N. gonorrhoeae* was compared with that of ciprofloxacin, temafloxacin, doxycycline, ceftriaxone, and spectinomycin by using an agar dilution method. Tosufloxacin was as active as temafloxacin against *C. trachomatis* (MIC for 90% of strains tested [MIC_{90}], 0.25 μg/ml; MBC for 90% of strains tested [MBC_{90}], 0.25 μg/ml) and was almost as active as doxycycline (MIC_{90}, 0.06 μg/ml; MBC_{90}, 0.125 μg/ml). Ciprofloxacin, temafloxacin, and tosufloxacin were extremely active against *N. gonorrhoeae*, including spectinomycin-resistant *N. gonorrhoeae* and penicillinase-producing *N. gonorrhoeae*, with MIC_{90}s of 0.004, 0.015, and 0.008 μg/ml, respectively. Ceftriaxone was slightly less active (MIC_{90}, 0.03 μg/ml), and doxycycline was the least active drug tested (MIC_{90}, 4.0 μg/ml). Tosufloxacin and temafloxacin had excellent activity against the *C. trachomatis* and *N. gonorrhoeae* strains tested.

Tosufloxacin is a new aryl-fluoronaphthyridine antimicrobial agent with potent in vitro activity against aerobic and anaerobic bacteria (1, 4–6). Other quinolones have been shown to have in vitro and in vivo activity against *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (6, 8). Since there are limited data on the activity of this drug against *C. trachomatis* and spectinomycin-resistant *N. gonorrhoeae* (SRNG), we tested the activity of tosufloxacin against 15 clinical isolates of *C. trachomatis* and 31 recent clinical isolates of *N. gonorrhoeae*, including 16 penicillinase-producing *N. gonorrhoeae* (PPNG) and 15 SRNG. The in vitro activity of tosufloxacin against *C. trachomatis* and *N. gonorrhoeae* was compared with that of ciprofloxacin, temafloxacin, and doxycycline. Additionally, the activity of tosufloxacin against *N. gonorrhoeae* was compared with that of ceftriaxone and spectinomycin.

MATERIALS AND METHODS

Tosufloxacin and temafloxacin (Abbott Laboratories, North Chicago, Ill.), ceftriaxone (Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.), ciprofloxacin (Miles Pharmaceuticals, West Haven, Conn.), and doxycycline and spectinomycin (Sigma Chemical Co., St. Louis, Mo.) were prepared as stock solutions in concentrations of 1,280 μg/ml according to their stated potency. Stock solutions were stored at −70°C for a maximum of 2 weeks. MICs for control strains of *C. trachomatis* run concomitantly with the test strains did not change over the 2-week period, indicating that the potencies of the drugs were maintained in solution over this period of time. Drugs were diluted to the appropriate concentration with medium containing Hanks balanced salt solution, amino acids, vitamins, 1% glutamine, 10% inactivated fetal calf serum, 5.4 g of glucose per liter, and 1 μg of cycloheximide per ml. Dilutions were made on the day of use. A total of 15 strains of *C. trachomatis* were

TABLE 1. Activity of tosufloxacin against 15 strains of *C. trachomatis*

Drug	MIC (μg/ml)[a]			MBC (μg/ml)[a]		
	Range	50%	90%	Range	50%	90%
Tosufloxacin	0.06–0.5	0.125	0.25	0.06–0.25	0.125	0.25
Ciprofloxacin	0.5–2.0	0.5	1.0	0.5–2.0	1.0	2.0
Temafloxacin	0.125–0.25	0.125	0.25	0.125–0.25	0.25	0.25
Doxycycline	0.008–0.125	0.06	0.06	0.015–2.0	0.06	0.125

[a] 50% and 90%, MIC and MBC for 50 and 90% of isolates, respectively.

* Corresponding author.

tested, including 1 D strain (ATCC VR-885), 1 LGV-2 strain (ATCC V9-902-B), and 13 clinical isolates. Ten isolates were of genital origin, and three were of conjunctival origin. The clinical strains had been passed fewer than 10 times in the laboratory. All isolates were passed an additional two times in antibiotic-free medium before susceptibility testing and were stored at −70°C until the time of use. The *N. gonorrhoeae* strains were recent isolates of genital origin from the clinical laboratory at Rush-Presbyterian-St. Luke's Medical Center and the Chicago Department of Health. These included 16 PPNG isolates and 15 SRNG isolates. Fourteen of the SRNG were also PPNG.

The antimicrobial susceptibility of *C. trachomatis* was determined by using 96-well microdilution plates as previously described (9). Briefly, 24-h-old McCoy cell monolayers grown in antibiotic-free medium were inoculated with the dilution of the *C. trachomatis* test strain known to yield 100 to 1,000 inclusions per well. The plates were centrifuged at 1,000 × *g* at 24°C for 60 min and then overlaid with 0.1 ml of each drug solution to yield appropriate twofold dilutions. Each solution was tested in triplicate. Antibiotic-free controls were included on each plate. Cultures were incubated for 48 h at 37°C in 5% CO_2, fixed with absolute ethanol, and stained with fluorescein-conjugated mouse monoclonal antibody to *C. trachomatis* (Ortho Diagnostics, Inc., Raritan, N.J.) according to the directions of the manufacturer. The MIC was defined as the lowest concentration of antibiotic without inclusions. The MBC was defined as the lowest concentration of antibiotic yielding no inclusions after passage onto 24-h-old McCoy cell monolayers grown in antibiotic-free medium.

In vitro susceptibility testing of *N. gonorrhoeae* was performed by using an agar dilution method with a GC agar base (Difco Laboratories, Detroit, Mich.) supplemented with 1% hemoglobin and 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.). Plates were read after 24 h of incubation at 35°C in an increased CO_2 atmosphere. Penicillinase production was determined by the nitrocefin dish method according to the directions of the manufacturer (BBL).

RESULTS

Results of the *C. trachomatis* antimicrobial susceptibility testing are shown in Table 1. Tosufloxacin was as active as temafloxacin against *C. trachomatis* (MIC for 90% of strains tested [MIC_{90}], 0.25 μg/ml; MBC for 90% of strains tested [MBC_{90}], 0.25 μg/ml). Both of these quinolones were almost as active as doxycycline (MIC_{90}, 0.06 μg/ml; MBC_{90}, 0.25 μg/ml). Ciprofloxacin was the least active drug tested.

Each antibiotic caused morphological changes in the inclusions. At one to two dilutions below the MIC, inclusions became fewer, smaller, and pyknotic. Passage of cells containing abnormal inclusions into antibiotic-free cell monolayers resulted in inclusions with normal morphology. The in vitro susceptibility of the *N. gonorrhoeae* strains is presented in Tables 2 and 3. Ciprofloxacin, temafloxacin, and tosufloxacin were extremely active against both SRNG and PPNG. Ceftriaxone was slightly less active than the quinolones, with a MIC_{90} of 0.03 μg/ml for both SRNG and PPNG. Doxycycline displayed limited activity against PPNG and SRNG (MIC_{90}, 4.0 μg/ml).

TABLE 2. In vitro activity of tosufloxacin against 16 PPNG isolates

Drug	MIC (μg/ml)[a]		
	Range	50%	90%
Tosufloxacin	≤0.002–0.008	0.004	0.008
Ciprofloxacin	≤0.002–0.008	0.004	0.004
Temafloxacin	≤0.002–0.015	0.008	0.015
Ceftriaxone	≤0.008–0.03	≤0.008	0.03
Doxycycline	0.125–8.0	0.5	4.0
Spectinomycin	16–32	16	16

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.

TABLE 3. In vitro activity of tosufloxacin against 15 SRNG isolates

Drug	MIC (μg/ml)[a]		
	Range	50%	90%
Tosufloxacin	≤0.002–0.008	0.004	0.008
Ciprofloxacin	≤0.002–0.008	0.004	0.004
Temafloxacin	≤0.002–0.015	0.008	0.015
Ceftriaxone	≤0.008–0.03	≤0.008	0.03
Doxycycline	0.125–4.0	4.0	4.0
Spectinomycin	>64	>64	>64

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.

DISCUSSION

The in vitro activity of tosufloxacin against *C. trachomatis* was recently reported by Maeda et al. (7). Our results indicate that tosufloxacin is as active against *C. trachomatis* from our geographic area as it is against *C. trachomatis* from Japan. The MICs and MBCs of temafloxacin and ciprofloxacin that we report are comparable to those obtained by other investigators (7–9). There is no published information on the activity of tosufloxacin against SRNG. Although less common than PPNG, SRNG has become increasingly frequent in some parts of the world (2). Our results indicate that tosufloxacin is more active than ceftriaxone against this type of *N. gonorrhoeae* and also more active than ceftriaxone against PPNG.

While some studies of the clinical use of ciprofloxacin in genital chlamydial infection have been disappointing, other studies have been encouraging (3, 8). Since concentrations of tosufloxacin in serum vary from 0.4 to 0.8 μg/ml when a dose of 150 mg twice daily is administered, sufficient distribution of the drug to eradicate *C. trachomatis* would be expected and clinical trials are warranted (7). The excellent in vitro activity of tosufloxacin against *N. gonorrhoeae*, including SRNG and PPNG strains, indicates that this drug may be as active as other quinolones in the treatment of genital gonococcal infections. Since a significant number of people with cervicitis and urethritis are concomitantly infected with *C. trachomatis* and *N. gonorrhoeae*, tosufloxacin may have a role as monotherapy for these patients.

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