ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1986, p. 948–950
0066-4804/86/120948-03$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 30, No. 6

In Vitro Activity of A-56619 (Difloxacin), A-56620, and Other New Quinolone Antimicrobial Agents against Genital Pathogens

LYNNE D. LIEBOWITZ,* JEAN SAUNDERS, GLENDA FEHLER, RONALD C. BALLARD, AND HENDRIK J. KOORNHOF

Emergent Pathogen Research Unit of the South African Medical Research Council, School of Pathology of the University of the Witwatersrand, and South African Institute for Medical Research, Johannesburg, South Africa*

Received 5 March 1986/Accepted 12 September 1986

The in vitro activities of two new carboxyquinolones, A-56619 (difloxacin) and A-56620, were compared with those of ciprofloxacin, norfloxacin, and ofloxacin against genital tract pathogens. All the quinolones were highly active against *Neisseria gonorrhoeae*. A-56619 had the lowest MICs against *Chlamydia trachomatis* (MIC range, 0.125 to 0.25 μg/ml) and *Haemophilus ducreyi* (MIC for 90% of isolates tested, 0.1 μg/ml).

The newer quinolone antimicrobial agents have been shown to have excellent activity in vitro against gonococci, including β-lactamase-producing strains (3, 11, 13, 16, 18), and moderate activity in vitro against *Chlamydia trachomatis* (2, 10, 14).

We compared the activities of A-56619 (difloxacin), A-56620, ciprofloxacin, norfloxacin, ofloxacin, and antimicrobial agents now used for the treatment of genital infections against clinical isolates of *Neisseria gonorrhoeae*, *Haemophilus ducreyi*, *Gardnerella vaginalis*, *Ureaplasma urealyticum*, and *C. trachomatis*.

Antimicrobial reference standards and sources were as follows: A-56619, A-56620, and erythromycin, Abbott Laboratories; ciprofloxacin, Bayer Pharmaceuticals; norfloxacin, Merck Sharp & Dohme; ofloxacin, Hoechst Ibérica; ampicillin, Beecham Laboratories; piperacillin, Lederle Laboratories; cefotaxime and penicillin, Glaxo Pharmaceuticals, Ltd.; spectinomycin and tetracycline, The Upjohn Co.; and metronidazole, Maybaker.

MIC tests with *N. gonorrhoeae*, *H. ducreyi*, and *G. vaginalis* were performed by an agar dilution technique using chocolate agar (Mueller-Hinton base; BBL Microbiology Systems) for *N. gonorrhoeae*, chocolate agar supplemented with 1% IsoVitaleX (BBL) for *H. ducreyi*, and 5% blood agar for *G. vaginalis*. For *N. gonorrhoeae* and *G. vaginalis*, overnight cultures in Mueller-Hinton broth (Difco Laboratories) supplemented with 1% IsoVitaleX were diluted with one-quarter-strength Ringer lactate solution and inoculated onto plates with a Steers multipoint replicator, delivering approximately 5×10^5 CFU. *H. ducreyi* isolates were processed as described previously (4). The isolates were incubated for 48 h at 37°C in 10% CO_2 on supplemented chocolate agar plates. Colonies were suspended in 1% IsoVitaleX-supplemented Mueller-Hinton broth, agitated on a vortex mixer, and allowed to sediment. The supernatant was diluted with one-quarter-strength Ringer lactate, and inocula of approximately 5×10^5 CFU were used. The plates were incubated at 37°C in 5 to 10% CO_2 overnight.

MIC testing of *Mycoplasma hominis* and *U. urealyticum* was performed as described previously (14, 20). Antimicrobial agents were prepared in doubling dilutions in Hayflick medium without penicillin G and thallium acetate for *M. hominis* and in Shepard medium without penicillin G for *U. urealyticum* (5). Samples (100 μl) of 3-day-old broth cultures of *M. hominis* and 2-day-old broth cultures of *U. urealyticum* were inoculated into 100 μl of twice the required final concentration of each of the antibiotic dilutions in U-well microtiter plates. Controls containing medium only, medium plus antimicrobial agent, and medium plus *M. hominis* or *U. urealyticum* were included. The microtiter plates were sealed and incubated at 37°C until the indicator in the *Mycoplasma-Ureaplasma* control well changed color. The MIC was defined as the lowest concentration of antimicrobial agent which prevented color change.

MIC testing of *C. trachomatis* was performed by a modification of a previously described method (15). Inocula of a 1-ml suspension of *C. trachomatis* in Eagle minimum essential medium supplemented with fetal bovine serum (10% vol/vol), glutamine (1% [vol/vol] of stock solution of 30 mg/ml), and vitamins (1% vol/vol), containing approximately 1.6×10^8 inclusion-forming units per ml, were added to flat-bottomed plastic tubes containing a cover slip monolayer of cycloheximide-treated McCoy cells. After centrifugation at 3,500 × *g* at 35°C for 60 min, the medium was replaced with 1 ml of supplemental Eagle medium containing the antimicrobial agents tested. The tubes were incubated at 35°C for 48 h. The cover slips were removed, fixed with methanol, stained with iodine, and examined by bright-field illumination for the presence of inclusions. The MIC was defined as the lowest concentration of antimicrobial agent which prevented the appearance of any inclusion bodies.

The MICs of the antimicrobial agents tested against isolates of *N. gonorrhoeae*, *H. ducreyi*, *U. urealyticum*, *G. vaginalis*, and *C. trachomatis* are shown in Tables 1 and 2. The quinolones, cefotaxime, and ceftazidime were highly active against gonococci, including β-lactamase-producing isolates. A-56619 and A-56620 were the most active quinolones against *H. ducreyi*. A-56619 was the most active quinolone against both the lymphogranuloma venereum and occulogenital strains of *C. trachomatis* tested, having MICs eightfold lower than those of ciprofloxacin. The isolates were fully resistant to norfloxacin, as previously reported (1, 9, 10, 14, 17). The agents showed poor activity against *G. vaginalis* and *U. urealyticum*. The MIC ranges of the antimicrobial agents against three isolates of *M. hominis* were as follows: ciprofloxacin, 0.045 to 0.09 μg/ml; A-56619 and A-56620, 0.09 to 0.78 μg/ml; tetracycline, 0.18 to 0.78 μg/ml; ofloxacin, 0.35 to 0.78 μg/ml; and norfloxacin, 0.35 to 1.56 μg/ml.

* Corresponding author.

TABLE 1. In vitro susceptibility of genital pathogens

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]			
		Geometric mean	50%	90%	Range
Neisseria gonorrhoeae (25)	A-56619	0.010	0.007	0.03	0.003–0.06
	A-56620	0.009	0.003	0.015	0.0015–0.03
	Ciprofloxacin	0.005	0.003	0.03	0.015–0.12
	Norfloxacin	0.024	0.03	0.12	≦0.007–0.25
	Ofloxacin	0.004	0.003	0.007	0.0015–0.03
	Penicillin	0.035	0.03	2	0.0015–4
	Piperacillin	0.033	0.03	0.25	0.007–2
	Cefotaxime	0.005	0.003	0.03	0.003–0.06
	Ceftazidime	0.019	0.015	0.12	0.003–0.12
	Spectinomycin	12.99	16	32	4–32
Haemophilus ducreyi (10)	A-56619	0.045	≦0.03	0.12	≦0.03–0.12
	A-56620	0.043	≦0.03	0.12	≦0.03–0.25
	Ciprofloxacin	0.099	≦0.03	0.5	≦0.03–2
	Norfloxacin	0.463	0.5	1	≦0.03–4
	Ofloxacin	0.461	0.5	2	≦0.03–8
	Erythromycin	0.075	≦0.015	4	≦0.015–4
Ureaplasma urealyticum (11)	A-56619	4.496	12.5	25	0.2–25
	A-56620	9.714	12.5	12.5	3.1–12.5
	Ciprofloxacin	4.365	3.1	6.3	1.6–6.3
	Norfloxacin	10.35	12.5	25	3.1–25
	Ofloxacin	9.712	12.5	25	3.1–25
	Tetracycline	0.511	0.4	0.8	0.4–1.6
	Erythromycin	0.787	0.8	1.6	0.4–1.6
Gardnerella vaginalis (20)	A-56619	4.438	4	8	2–8
	A-56620	5.278	4	8	4–8
	Ciprofloxacin	2.828	4	4	1–4
	Norfloxacin	8.000	8	8	8
	Ofloxacin	19.027	16	32	8–32
	Ampicillin	0.150	≦0.06	1	≦0.06–2
	Metronidazole	10.928	8	16	4–16

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

With the increasing incidence of infections caused by penicillinase-producing strains of *N. gonorrhoeae* and gonococci with chromosomal resistance to penicillin and other antimicrobial agents and of mixed infections involving *C. trachomatis*, it has become necessary to look for new antimicrobial agents for the treatment of sexually transmitted diseases. The carboxyquinolones might fulfill such a role. They are very active in vitro against gonococci, and clinical studies of some of these agents have been undertaken. Ciprofloxacin, given as a single 250-mg oral dose, cured 57 cases of gonorrhea, including 7 rectal and 3 pharyngeal infections (12). Norfloxacin given orally in two 600-mg doses 4 h apart cured all of 59 cases of gonococcal urethritis, eliminating the nasopharyngeal colonization encountered in two patients (6). Very promising are the low MICs of A-56619 against *C. trachomatis*. Poor results were observed in a clinical trial performed by one of us (R.C.B.) using 500 mg of ciprofloxacin twice daily for 2 days for the treatment of chlamydial urethritis. A 2-day treatment for chlamydial infections may be unrealistically short in view of the relatively long generation time of this organism. However, the eightfold-lower MICs of A-56619 should make this agent more suitable for treatment of these infections. In another clinical trial performed by one of us (R.C.B.), 47 of 49 chancroid infections were cured by a single 500-mg dose and all of 48 were cured by a single 1,000-mg dose of ciprofloxacin. In this in vitro study, A-56619 and A-56620 were more active than ciprofloxacin against *H. ducreyi*.

Pharmacokinetic studies in humans have been performed with some of the quinolones. Ciprofloxacin produces peak levels in serum ranging from 0.42 to 4.2 mg/liter after single oral doses of 250 and 1,000 mg, respectively (19). In animal studies, A-56619 produced higher levels in serum (7). After oral administration of 100 mg of A-56619, A-56620, and norfloxacin per kg to mice, levels in serum of 22.4, 4.5, and 1.2 μg/ml, areas under the curve of 207.3, 165.3, and 41.37 μg · h/ml, and half-lives of 10.9, 2.6, and 1.8 h, respectively, were demonstrated (7).

The quinolones demonstrated poor activity against *G. vaginalis*. However, *G. vaginalis* infections respond well to treatment with metronidazole as a result of its activity on anaerobes, and because A-56619 and A-56620 demonstrate

TABLE 2. In vitro susceptibility of *C. trachomatis* strains

Antimicrobial agent	MIC (μg/ml) for:				
	Occulogenital strains			LGV[a] strains	
	D/IC-Cal8	E/DK-20	F/MRC301	L3/404	L2/434B
A-56619		0.25	0.25	0.125	
A-56620		1	1	1	
Ciprofloxacin	2	2		1	
Norfloxacin		32	32		32
Ofloxacin		1	1		1
Tetracycline	0.125	0.125	0.125	0.125	0.125

[a] LGV, Lymphogranuloma venereum.

reasonable activity against anaerobic bacteria they may be appropriate for the treatment of *Gardnerella*-associated vaginosis (2).

We found most *U. urealyticum* strains tested to be resistant to the quinolones. Although lower MICs of ciprofloxacin against *U. urealyticum* have been reported (14), similar MIC ranges have also been documented (1, 8, 17).

A-56619 was very active in vitro against gonococci, *C. trachomatis*, *M. hominis*, and *H. ducreyi*. With its good pharmacokinetic profile, A-56619 should prove useful in the treatment of sexually transmitted diseases, provided it proves to be nontoxic in humans.

LITERATURE CITED

1. **Aznar, J., M. C. Caballero, M. C. Lozano, C. de Miguel, J. C. Palomares, and E. J. Perea.** 1985. Activities of new quinolone derivatives against genital pathogens. Antimicrob. Agents Chemother. **27**:76–78.
2. **Bailey, J. M. G., C. Heppleston, and S. J. Richmond.** 1984. Comparison of the in vitro activities of ofloxacin and tetracycline against *Chlamydia trachomatis* as assessed by indirect immunofluorescence. Antimicrob. Agents Chemother. **26**: 13–16.
3. **Barry, A. L., C. Thornsberry, and R. N. Jones.** 1986. In vitro evaluation of A-56619 and A-56620, two new quinolones. Antimicrob. Agents Chemother. **29**:40–43.
4. **Bilgeri, Y. R., R. C. Ballard, M. O. Duncan, A. C. Mauff, and H. J. Koornhof.** 1982. Antimicrobial susceptibility of 103 strains of *Haemophilus ducreyi* isolated in Johannesburg. Antimicrob. Agents Chemother. **22**:686–688.
5. **Black, F. T.** 1973. Modifications of the growth inhibition test and its application to human T-mycoplasmas. Appl. Microbiol. **25**:528–533.
6. **Crider, S. R., S. D. Colby, L. K. Killer, W. O. Harrison, S. B. J. Kerbs, and S. W. Berg.** 1984. Treatment of penicillin-resistant *Neisseria gonorrhoeae* with oral norfloxacin. N. Engl. J. Med. **311**:137–140.
7. **Fernandes, P. B., D. T. W. Chu, R. R. Bower, K. P. Jarvis, N. R. Ramer, and N. Shipkowitz.** 1986. In vitro evaluation of A-56619 (difloxacin) and A-56620: new aryl-fluoroquinolones. Antimicrob. Agents Chemother. **29**:201–208.
8. **Gadebusch, H. H., D. L. Shungu, E. Weinberg, and S. K. Chung.** 1982. Comparison of the antibacterial activity of norfloxacin (MK0366, AM715), a new organic acid, with that of other orally absorbed chemotherapeutic agents. Infection **10**:41–44.
9. **Heessen, F. W. A., and H. L. Muytjens.** 1984. In vitro activities of ciprofloxacin, norfloxacin, pipemidic acid, cinoxacin, and nalidixic acid against *Chlamydia trachomatis*. Antimicrob. Agents Chemother. **25**:123–124.
10. **Heppleston, C., and S. Richmond.** 1985. Antichlamydial activity of quinolone carboxylic acids. J. Antimicrob. Chemother. **15**:645–646.
11. **Khan, M. Y., Y. Siddiqui, and R. P. Gruninger.** 1981. Comparative in vitro activity of Mk-0366 and other selected oral antimicrobial agents against *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **20**:265–266.
12. **Loo, P. S., G. L. Ridgway, and J. D. Oriel.** 1985. Single dose ciprofloxacin for treating gonococcal infections in men. Genitourin. Med. **61**:302–305.
13. **Peeters, M., E. Van Dyck, and P. Piot.** 1984. In vitro activities of spectinomycin analog U-63366 and four quinolone derivatives against *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **26**:608–609.
14. **Ridgway, G. L., G. Mumtaz, F. G. Gabriel, and J. D. Oriel.** 1984. The activity of ciprofloxacin and other 4-quinolones against *Chlamydia trachomatis* and *Mycoplasmas in vitro*. Eur. J. Clin. Microbiol. **3**:344–346.
15. **Ridgway, G. L., J. M. Owen, and J. D. Oriel.** 1978. The antimicrobial susceptibility of *Chlamydia trachomatis* in cell culture. Br. J. Vener. Dis. **54**:103–106.
16. **Rohlfing, S. R., J. E. Landmesser, J. F. Gerster, S. E. Pecore, and R. M. Stern.** 1985. Differentiation of fluorinated quinolone antibacterials with *Neisseria gonorrhoeae* isolates. J. Antimicrob. Chemother. **15**:539–544.
17. **Simon, C., and U. Lindner.** 1983. In vitro activity of norfloxacin against *Mycoplasma hominis* and *Ureaplasma urealyticum*. Eur. J. Clin. Microbiol. **2**:479–480.
18. **Stamm, J. M., C. W. Hanson, D. T. W. Chu, R. Bailer, C. Vojtko, and P. B. Fernandes.** 1986. In vitro evaluation of A-56619 (difloxacin) and A-56620: new aryl-fluoroquinolones. Antimicrob. Agents Chemother. **29**:193–200.
19. **Tartaglione, T. A., A. C. Raffalovich, W. J. Poynor, A. Espinel-Ingroff, and T. M. Kerkering.** 1986. Pharmacokinetics and tolerance of ciprofloxacin after sequential increasing oral doses. Antimicrob. Agents Chemother. **29**:62–66.
20. **Taylor-Robinson, D.** 1967. Mycoplasmas of various hosts and their antibiotic sensitivities. Postgrad. Med. J. **43**(Suppl.): 100–104.