ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1986, p. 590–593
0066-4804/86/100590-04$02.00/0

Vol. 30, No. 4

In Vitro Activity of Difloxacin Hydrochloride (A-56619), A-56620, and Cefixime (CL 284,635; FK 027) against Selected Genital Pathogens

WILLIAM R. BOWIE,[1]* CAROL E. SHAW,[2] DAVID G. W. CHAN,[2] JANET BOYD,[1] AND WILLIAM A. BLACK[2,3]

Provincial Health Laboratory, Ministry of Health, British Columbia,[2] and Divisions of Infectious Diseases[1] and Medical Microbiology,[3] Faculty of Medicine, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada

Received 16 January 1986/Accepted 30 June 1986

Management of sexually transmitted diseases is facilitated by having antimicrobial agents with activity against all of the major genital pathogens. Newer quinolones show promise of being active against *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Two quinolones, difloxacin (A-56619) and A-56620, and an oral cephalosporin, cefixime (CL 284,635; FK 027), were evaluated in vitro. All three were highly active against 400 isolates of *N. gonorrhoeae*, including penicillinase-producing *N. gonorrhoeae*, *N. gonorrhoeae* with chromosomally mediated resistance, and isolates with penicillin MICs of <1 μg/ml. Susceptibilities to one antimicrobial agent were usually strongly correlated with susceptibilities to the other antimicrobial agents evaluated, but isolates with increasing resistance to beta-lactams were least likely to show increasing resistance to quinolones. Difloxacin and, to a lesser extent, A-56620 were active against all 10 strains of *C. trachomatis*, and both had moderate activity against over 200 strains of *Gardnerella vaginalis*. Based on in vitro activity, difloxacin and A-56620 merit in vivo assessment for management of both *C. trachomatis* and *N. gonorrhoeae* infections, and cefixime shows considerable promise for treatment of *N. gonorrhoeae* infections.

Management of sexually transmitted syndromes is facilitated by having antimicrobial agents active against all of the major genital pathogens. Newer quinolones, especially ciprofloxacin and ofloxacin, show promise of having broad in vitro activity against many genital bacterial pathogens, including activity against penicillin-susceptible and penicillin-resistant strains of *Neisseria gonorrhoeae* (1, 9, 10) and *Chlamydia trachomatis* (1, 2, 6, 7; R. J. Van Roosbroeck, D. R. Provinciael, and D. L. Van Caekenberghe, Letter, Br. J. Vener. Dis. **60**:350, 1984). Data on the activity of newer quinolones against *Gardnerella vaginalis* is limited (8). In this study, two new quinolones, difloxacin hydrochloride (A-56619) and A-56620, were evaluated for in vitro activity against *N. gonorrhoeae*, *C. trachomatis*, and *G. vaginalis*, and a new oral cephalosporin, cefixime (CL 284,635; FK 027), was evaluated for in vitro activity against *N. gonorrhoeae* and *C. trachomatis*.

MATERIALS AND METHODS

Most of the isolates of *N. gonorrhoeae* used were obtained from men and women who were patients at the Sexually Transmitted Disease Clinic in the Vancouver Provincial Health Building from June 1982 to June 1984. Also studied were additional isolates of *N. gonorrhoeae* from all over British Columbia that were penicillin producing or showed zones of inhibition of 19 mm or less around a 10-U penicillin disk applied to chocolate agar (GC medium base, 1% hemoglobin, 1% defined GC supplement) (Alpkem-Western Ltd.). Clinical specimens were directly inoculated on Thayer-Martin medium (chocolate agar containing colistin [7.5 μg/ml], vancomycin [3.0 μg/ml], and nystatin [12.5 μg/ml]) and incubated in 5% CO_2 at 35°C within 30 min. Oxidase-positive colonies showing typical colony morphology and containing gram-negative diplococci were identified as *N. gonorrhoeae* by fluorescent antibody staining, sugar fermentation, or both. Ten or more colonies were subcultured, and a modification of the iodometric paper strip was used to screen for beta-lactamase activity (8). The presence of beta-lactamase activity in isolates positive by the iodometric test was confirmed with chromogenic cephalosporin (nitrocefin; Glaxo Pharmaceuticals, Ltd., Greenford, United Kingdom) (4). Isolates were stored at −70°C in glycerol citrate. In total, 250 isolates with penicillin MICs of <1 μg/ml, 50 isolates with penicillin MICs of 1 to 4 μg/ml that were not beta-lactamase producing (having chromosomally mediated resistance to *N. gonorrhoeae* [CMRNG]), and 100 beta-lactamase-producing strains of *N. gonorrhoeae* (PPNG) were studied. Isolates were tested by standard agar dilution techniques (4) for in vitro susceptibility to penicillin G, tetracycline, cefaclor (Eli Lilly & Co., Indianapolis, Ind.), ceftriaxone (Hoffmann-La Roche Inc., Nutley, N.J.), cefixime (Cyanamid Canada Inc.), and difloxacin and A-56620 (Abbott Laboratories, North Chicago, Ill.). The susceptibility test medium was Difco GC agar base with 1% Kellogg defined supplement. The inoculum was suspended in glycerol-lactate-salt suspending medium (4) and adjusted to a 0.5 McFarland standard to obtain an inoculum which delivered approximately 10^5 CFU with a Steer replicator. All tests were performed at least in duplicate.

As control strains, three strains of *N. gonorrhoeae* recommended by the World Health Organization (WHO III, WHO V, and WHO VII) as reference strains for penicillin susceptibility testing were obtained from the Antimicrobials and Molecular Biology Division, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada. An additional two isolates from our own laboratory, including a beta-lactamase producer and an isolate showing intermediate resistance to penicillin (MIC = 4 μg/ml), were also selected as controls. The MIC range and MICs for 50 and 90% of the organisms tested (MIC_{50} and MIC_{90}, respectively) with individual antimicrobial agents were calculated by standard techniques. Pearson correlation coefficients were calculated

* Corresponding author.

TABLE 1. In vitro activity against 400 isolates of *N. gonorrhoeae*

Antimicrobial agent	Activity (μg/ml) against:						MIC range
	PPNG (n = 100)		CMRNG (n = 50)		Others (n = 250)[a]		
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	
Penicillin	>8.0	>8.0	2.0	4.0	0.25	0.50	≤0.008–>8.0
Tetracycline	4.0	4.0	2.0	4.0	0.25	1.0	0.063–8.0
Cefaclor	32.0	64.0	8.0	32.0	4.0	8.0	0.25–64.0
Ceftriaxone	0.008	0.016	0.016	0.063	0.004	0.008	≤0.001–0.063
Cefixime	0.008	0.016	0.016	0.032	0.004	0.008	≤0.001–0.063
Difloxacin	0.016	0.032	0.032	0.032	0.016	0.016	0.002–0.063
A-56620	0.008	0.016	0.016	0.032	0.004	0.008	0.002–0.063

[a] Penicillin MIC of <1.0 μg/ml.

to assess the relationship between patterns of susceptibility of different antimicrobial agents against *N. gonorrhoeae* by using SPSS-10.

In vitro susceptibility testing was performed on 10 clinical isolates of *C. trachomatis* by our previously described methodology (3). MICs and MBCs were determined for each strain in triplicate for tetracycline, difloxacin, A-56620, ofloxacin (Ortho Diagnostics, Inc., Raritan, N.J.), and cefixime.

In vitro susceptibility testing was performed on 214 strains of *G. vaginalis* by using an agar dilution technique similar to that used with *N. gonorrhoeae*. The test medium was Columbia agar base with 5% defibrinated horse blood and 1% defined GC supplement (Alpkem-Western Ltd.). Defibrinated horse blood produced growth similar to that produced by human blood and was used because the supply of human blood was curtailed. Suspensions of isolates were adjusted to a 0.5 McFarland standard, which delivered an inoculum of approximately 10^4 CFU with a Steer replicator. All tests were performed at least in triplicate. Control isolates had previously been obtained from the Neisseria Reference Laboratory, Division of Infectious Diseases, U.S. Public Health Service Hospital, Seattle, Wash., and included VI (ATCC 14018), V1092, V1096, V1098, and V1106 (Centers for Disease Control, Atlanta, Ga., type strain).

RESULTS

Table 1 shows the MIC ranges, MIC_{50}s, and MIC_{90}s for isolates of *N. gonorrhoeae* according to categories of PPNG, CMRNG, and other isolates with penicillin MICs of <1.0 μg/ml. The results for penicillin, tetracycline, and cefaclor were highly dependent on the distribution of the isolates studied, with MICs being highest for PPNG, intermediate for CMRNG, and least for isolates having penicillin MICs of <1.0 μg/ml. In vitro activities of ceftriaxone, cefixime, and A-56620 were all much higher but similar, whereas difloxacin was approximately 1 dilution less active than A-56620. In contrast to the three less active compounds, the MICs for the four more active compounds were highest for CMRNG strains, intermediate for PPNG strains, and least for those with penicillin MICs of <1 μg/ml. These differences were statistically significant for each of the four active compounds ($P < 0.001$ by Student's t test).

Tables 2 and 3 show the Pearson correlation coefficients (r) when MICs of cefixime and A-56620 were compared with the MICs of the other antimicrobial agents according to the pattern of penicillin susceptibility of isolates. The Pearson correlation coefficients were highly statistically significantly related for all of the beta-lactams, but much less associated for the quinolones, and intermediate for tetracycline. Patterns were similar for ceftriaxone and the other compounds on the one hand and penicillin and the other compounds on the other, with the exception that penicillin MICs were well correlated with tetracycline MICs (data not shown). Table 3 indicates that MICs with A-56620 were least correlated with the beta-lactams but had similar correlations to tetracycline and difloxacin, with the exception that for isolates with penicillin MICs of <1 μg/ml the association was weak between A-56620 and tetracycline but strong between A-56620 and difloxacin.

The results against *C. trachomatis* are shown in Table 4. In general, the MIC and MBC results were virtually identical for each antimicrobial agent. Overall, tetracycline was the most active compound, being approximately 1 dilution more active than difloxacin. A-56620 and ofloxacin had similar activities but were less active than difloxacin. Cefixime had essentially no activity against *C. trachomatis*.

The results against *G. vaginalis* are shown in Table 5. Not all isolates survived for triplicate analyses against all antimicrobial agents. For isolates that survived, the most active compound on a weight basis was ampicillin. Difloxacin and A-56620 were next most active and produced virtually identical results. Metronidazole was considerably less active on a weight basis.

TABLE 2. Pearson correlation coefficients with cefixime

Isolates tested	Correlation coefficient between cefixime and:					
	Penicillin	Tetracycline	Cefaclor	Ceftriaxone	Difloxacin	A-56620
PPNG		0.317	0.484[a]	0.590	0.283	0.227
		$P = 0.001$			$P = 0.004$	$P = 0.023$
CMRNG	0.691	0.485	0.785	0.579	0.287	0.384
					$P = 0.043$	$P = 0.006$
Others[b]	0.336	0.408	0.381	0.729	0.098	0.186
					$P = 0.123$	$P = 0.003$
All	0.217	0.457	0.405	0.700	0.368	0.395

[a] P not shown indicates $P < 0.001$.
[b] Penicillin MIC of <1.0 μg/ml.

TABLE 3. Pearson correlation coefficients with A-56620

Isolates tested	Correlation of coefficient between A-56620 and:					
	Penicillin	Tetracycline	Cefaclor	Cefixime	Ceftriaxone	Difloxacin
PPNG		0.751[a]	0.095	0.227	0.381	0.737
			$P = 0.347$	$P = 0.023$		
CMRNG	0.438	0.588	0.321	0.384	0.115	0.607
	$P = 0.002$		$P = 0.023$	$P = 0.006$	$P = 0.427$	
Others[b]	0.103	0.131	0.100	0.186	0.131	0.716
	$P = 0.106$	$P = 0.039$	$P = 0.115$	$P = 0.003$	$P = 0.039$	
All	0.376	0.709	0.344	0.395	0.443	0.776

[a] P not shown indicates $P < 0.001$.
[b] Penicillin MIC of <1.0 μg/ml.

TABLE 4. In vitro activity of antimicrobial agents against 10 strains of *C. trachomatis*

Antimicrobial agent	Range (μg/ml) of activity	
	MIC[a]	MBC[b]
Tetracycline	0.063–0.125	0.063–0.125
Difloxacin	0.125–0.25	0.125–0.25
A-56620	1.0–2.0	0.5–1.0
Ofloxacin	1.0–2.0	0.5–2.0
Cefixime	512–>1,024	512–>1,024

[a] Lowest concentration at which no inclusions were detected when stained at 72 h.
[b] Lowest concentration at which no inclusions were detected when cultures were passed to new monolayers at 72 h, reincubated for 72 h, and stained.

DISCUSSION

Compared with other published studies, A-56620 appeared to have activity against *N. gonorrhoeae* similar to that of other very active new quinolones (1, 9, 10; P. J. Rettig, P. A. Haddad, R. K. Scribner, and M. I. Marks, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 576, 1985). For both difloxacin and A-56620, the level of activity indicated that they may be clinically useful. Cefixime was highly active against *N. gonorrhoeae* with activity similar to that of ceftriaxone. Depending on bioavailability and pharmacokinetics in vivo, it could be the oral equivalent of parenterally administered ceftriaxone. Preliminary results of using oral cefixime for treatment of uncomplicated urethral *N. gonorrhoeae* infection in men appear promising (W. R. Bowie, unpublished data).

The pattern of correlations among the in vivo activities of different antimicrobial agents against *N. gonorrhoeae* may have relevance in vivo. For the newer agents, MICs to CMRNG were the highest. Furthermore, increasing resistance to one beta-lactam correlated highly with increasing resistance to other beta-lactams. In the past, independent of plasmid-mediated resistance to penicillins, isolates of *N. gonorrhoeae* have become increasingly more resistant to penicillin (11). This resulted in the need for increasingly high doses of antimicrobial agents to treat gonorrhea (5). The correlations indicate that the newer antimicrobial agents already show a trend toward increased resistance in these isolates. This could predict that, with time, the active compounds will become less useful because of increasing development of chromosomally mediated resistance. This might especially be a problem if treatment regimens that only slightly exceed the minimal effective doses are used and isolates with increasing resistance to one antimicrobial agent, especially beta-lactams, continue to correlate with increased resistance to other antimicrobial agents. It might not be a problem if treatment regimens based on relatively high doses of antimicrobial agents are used. In the past, when the dose of penicillin used to treat gonorrhea was greatly increased, the trend toward increasing resistance to penicillin among isolates of *N. gonorrhoeae* was actually reversed (5). The lesser correlations between the quinolones and the beta-lactams might indicate that they will be less likely to demonstrate increased resistance on the basis of further spread or development of strains of *N. gonorrhoeae* demonstrating chromosomally mediated resistance to penicillin.

TABLE 5. In vitro activity against *G. vaginalis* incubated aerobically

Antimicrobial agent	No. of isolates	Activity (μg/ml)		
		MIC_{50}	MIC_{90}	MIC range
Ampicillin	206	0.085	0.363	0.016–>4.0
Difloxacin	211	1.433	1.945	0.025–16
A-56620	214	1.394	1.946	0.025–16
Metronidazole	205	38.86	188.8	1.0–>256

With respect to *C. trachomatis*, our results with ofloxacin are similar to those that have been reported in the literature (1, 2, 7; Van Roosbroeck et al., Letter). Difloxacin would appear more active than other quinolones against *C. trachomatis* (1, 6). For *G. vaginalis*, our results with difloxacin and A-56620 appear comparable to those of King et al. (9) for ofloxacin and ciprofloxacin.

This study showed that, like ofloxacin and ciprofloxacin, difloxacin and A-56620 had considerable in vitro activity against all strains of *N. gonorrhoeae*, *C. trachomatis*, and, to a lesser extent, *G. vaginalis*. They merit in vivo evaluation, especially for the syndromes of urethritis and cervicitis and their complications. On the basis of these data, difloxacin may be more clinically useful than A-56620 because of its greater activity against *C. trachomatis*. However, the ultimate decision will depend on pharmacokinetic and tolerance data. Cefixime lacks useful activity against *C. trachomatis* but could be a very effective single-dose oral treatment for *N. gonorrhoeae* infections.

ACKNOWLEDGMENTS

This research was funded by Abbott International Ltd. and Lederle International Clinical Research.

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 quinoline 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. **Bowie, W. R., C. K. Lee, and E. R. Alexander.** 1978. Prediction of efficacy of antimicrobial agents in treatment of infections due to *Chlamydia trachomatis*. J. Infect. Dis. **138**:655–659.
4. **Dillon, J. R.** 1983. Laboratory methods for *Neisseria gonorrhoeae* (MIC, auxotype, screening for PPNG). Department of Health and Welfare, Ottawa, Canada.
5. **Evans, A. J., G. D. Morrison, and D. J. Price.** 1980. Prolonged use of the Greenland method of treatment of gonorrhea. Br. J. Vener. Dis. **56**:88–91.
6. **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.
7. **Heppleston, C., S. Richmond, and J. Bailey.** 1985. Antichlamydial activity of quinolone carboxylic acids. J. Antimicrob. Chemother. **15**:645–647.
8. **Jorgensen, J. H., J. C. Lee, and G. A. Alexander.** 1977. Rapid penicillinase paper strip test for detection of beta-lactamase-producing *Haemophilus influenzae* and *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **11**:1087–1088.
9. **King, A., K. Shannon, and I. Phillips.** 1985. The in-vitro activities of enoxacin and ofloxacin compared with that of ciprofloxacin. J. Antimicrob. Chemother. **15**:551–558.
10. **Peeters, M., E. Van Dyck, and P. Piot.** 1984. In vitro activities of the spectinomycin analog U-63366 and four quinolone derivatives against *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **26**:608–609.
11. **Rice, R. J., J. W. Biddle, Y. JeanLouis, J. H. Blount, and S. A. Morse.** 1984. Changing trends in gonococcal antibiotic resistance in the United States, 1983–1984. CDC Surveillance Summaries, 1984. Morbid. Mortal. Weekly Rep. **33**(No. 4SS): 11SS–15SS.