

In Vitro Activities of Ciprofloxacin, Norfloxacin, Pipemidic Acid, Cinoxacin, and Nalidixic Acid Against *Chlamydia trachomatis*

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The in vitro activities of five quinolonecarboxylic acids against two laboratory strains of *Chlamydia trachomatis* were compared. The minimal inhibitory concentrations of nalidixic acid, cinoxacin, and pipemidic acid were all ≥ 50 $\mu\text{g/ml}$; the activity of norfloxacin was intermediate (minimal inhibitory concentration, 8 to 16 $\mu\text{g/ml}$). Ciprofloxacin was the most active of these drugs (minimal inhibitory concentration, 0.5 to 1 $\mu\text{g/ml}$).

The in vitro activities of some newly developed quinolonecarboxylic acids like norfloxacin and ciprofloxacin against gram-positive and gram-negative bacteria are superior to those of nalidixic acid (6). Little is known about the possibility of eradicating *Chlamydia trachomatis* with these drugs (4). Therefore, we compared the in vitro activities of five quinolonecarboxylic acids against two laboratory strains of *C. trachomatis*.

MATERIALS AND METHODS

The antibiotics used were ciprofloxacin (Bayer Nederland B.V., Mijdrecht, The Netherlands), norfloxacin (Merck Sharp & Dohme, Research Laboratories, Rahway, N.J.), cinoxacin (Lilly Research Centre Ltd., Windlesham, Surrey, England), pipemidic acid (Laboratoire Roger Bellon S.A., Neuilly-sur-Seine, France), nalidixic acid (Sterling Winthrop B.V., Haarlem, The Netherlands), and doxycycline (Pfizer Corp., Brussels, Belgium). The *C. trachomatis* strains used in this study were laboratory strains, serotypes E and L2, and were obtained and grown essentially as described before (5).

C. trachomatis was grown in HeLa 229 cells. Antibiotic-free chlamydial suspensions were prepared by two cycles of centrifugation and suspension in antibiotic-free maintenance medium. Maintenance medium was Eagle minimal essential medium with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.5), 6 g of glucose per liter, and 10% fetal bovine serum.

Monolayers of antibiotic-free HeLa 229 cells in flat-bottomed tubes provided with circular cover glasses were inoculated with 100 to 1,000 inclusion-forming units of E or L2 organisms in 1 ml of maintenance medium. In the case of type E, monolayers were exposed to DEAE-dextran at 30 $\mu\text{g/ml}$ for 30 min before inoculation and were centrifuged for 60 min at $2,200 \times g$ and 35°C after inoculation. A 1-ml amount of maintenance medium with a dilution of one of the antibiotics tested or of doxycycline, which was used as a control, was added to each of four monolayers 60 min after inoculation. Doubling concentrations of 1 to 16 $\mu\text{g/ml}$ were used. If necessary, this range was extended or changed to 100 to 1,600 $\mu\text{g/ml}$ or to 0.006 to 0.1 $\mu\text{g/ml}$. After 72 h of incubation at 36°C , two monolayers of each antibiotic dilution were stained with Giemsa and evaluated for the pres-

ence of inclusions by dark-ground illumination. The lowest concentration of each antibiotic which completely inhibited inclusion formation (minimal inhibitory concentration [MIC]) was determined. The medium was removed from the two other monolayers of each antibiotic dilution and replaced by 2SP transport medium with antibiotics (12).

Cultures were frozen at -60°C and thawed, and 1 ml was inoculated onto each of four new monolayers of HeLa 229 cells. Adsorption and incubation were performed as before except that the maintenance medium contained gentamicin, vancomycin, and amphotericin B and 0.5 μg of cycloheximide was added per ml of maintenance medium 3 h after inoculation. After 72 h, monolayers were fixed and stained or passaged further for determination of the lowest concentration of the antibiotic which completely inhibited any further multiplication after removal of the drug (minimal lethal concentration [MLC]). As cultures can become positive several passages after removal of a drug, multiple passages have to be made before an endpoint for a MLC determination can be found (7).

RESULTS AND DISCUSSION

Table 1 lists the MICs for the five antibiotics tested and for doxycycline. After doxycycline, for which an MIC of 0.03 $\mu\text{g/ml}$ was stated before (5), ciprofloxacin was the most active antichlamydial agent, followed by norfloxacin and pipemidic acid. Cinoxacin and nalidixic acid showed high MICs.

Six passages were carried out after removal of norfloxacin and ciprofloxacin for determination of MLCs since these two antibiotics were the most promising (Table 2). After five passages, no further cultures became positive, and MLCs could be determined. MLCs were sometimes considerably higher than MICs, to a maximum of three doubling dilutions. For type E (six experiments), MLCs were repeatedly the same as MICs with the exception of one experiment with norfloxacin. For type L2 (eight experiments) MLCs were higher than MICs, again with the exception of a single experiment with norfloxacin. This caused the increased range of MLCs found with type L2. The number of passages required to see an increase in the inhibiting concentration varied between three and five. This caused the increased range of inhibiting concentrations found on passage with ciprofloxacin and type L2. Despite this observation, the MLCs of ciprofloxacin and norfloxacin remained in a useful range.

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TABLE 1. Activities of five quinolinecarboxylic acids against *C. trachomatis*

Antibiotic	MIC ($\mu\text{g/ml}$)	
	Type E	Type L2
Nalidixic acid	800–1,600	>1,600
Cinoxacin	400	400
Pipemidic acid	50–100	100
Norfloxacin	8–16	8
Ciprofloxacin	1	0.5–1
Doxycycline	0.012–0.025	0.025

The trend that ciprofloxacin was the most active drug against most bacterial species, followed by norfloxacin, whereas pipemidic acid had an intermediate position and nalidixic acid was least active was also observed in this study (1, 6, 11). Activity measurements can be limited with respect to serotype coverage, since there is little difference in the antibiotic susceptibility of different serotypes (10).

These observations may lead to the possibility of clinical application of ciprofloxacin and norfloxacin for the eradication of *C. trachomatis* from the urinary tract. A sufficient concentration in blood and tissues is needed for treatment of *C. trachomatis* infections. From what is known about concentrations attainable in serum and tissues with these new drugs ($\geq 1 \mu\text{g/ml}$; data on file at the medical departments of Merck Sharp & Dohme and Bayer A.G.), effective treatment with ciprofloxacin, and possibly norfloxacin, against genital and systemic *C. trachomatis* infections can be expected. The newer quinolinecarboxylic acids like norfloxacin and ciprofloxacin are active against a broad spectrum of bacteria (6). Norfloxacin and ciprofloxacin are also active in vitro against *Neisseria gonorrhoeae* (3, 11).

A drug regimen for the treatment of gonorrhea which is also effective against *C. trachomatis* might be taken into consideration, because these infections often coexist. Single-dose procaine penicillin G, ampicillin, or spectinomycin, which are often used for the treatment of gonorrhea, do not eradicate *C. trachomatis* (8). Only the tetracyclines, which are not considered as a first choice for the treatment of gonococcal urethritis, are also effective against *C. trachomatis*.

It is not known whether ciprofloxacin or norfloxacin is active against other sexually transmitted agents like *Mycoplasma hominis* and *Ureaplasma urealyticum*. Ofloxacin, a related compound, is active against *M. hominis* and other *Mycoplasma* species, but *U. urealyticum* was not tested (9).

Rosoxacin, another quinolinecarboxylic drug, is active

against *C. trachomatis*, *N. gonorrhoeae*, and *U. urealyticum* (2). Its MIC against 11 *C. trachomatis* strains was $5 \mu\text{g/ml}$. Its MLC was not determined.

If these studies can be extended and completed, the possibility of treating genital infections with one of these drugs as a single therapy is growing. The results of clinical trials with norfloxacin and especially ciprofloxacin against *C. trachomatis* infections are awaited with interest.

C. trachomatis infections may remain asymptomatic but communicable to sexual partners and can infect infants during the passage of the birth canal and bring about a neonatal inclusion conjunctivitis or pneumonia in this manner. The reservoir of infectious chlamydia is not likely to be reduced by most antibiotics (e.g., β -lactams) prescribed for other reasons (5, 8). It is possible that drugs like ciprofloxacin are able to make this reservoir smaller.

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TABLE 2. Effect of passage on growth of *C. trachomatis* after initial treatment with norfloxacin and ciprofloxacin

Antibiotic	<i>C. trachomatis</i> type	MIC ($\mu\text{g/ml}$) at passage no.: ^a					
		1	2	3	4	5	6
Norfloxacin	E	8–16	8–16	8–16	8–16	8–16	8–16
	L2	8	8	8–16	8–64	8–64	8–64
Ciprofloxacin	E	1	1	1–2	1–2	1–2	1–2
	L2	0.5–1	0.5–1	0.5–1	2–8	8	8

^a Drugs were only present at the first passage.