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In Vitro Activities of Five Quinolones against *Chlamydia pneumoniae*

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The in vitro susceptibilities of 10 strains of *Chlamydia pneumoniae* were determined for five quinolones, including ciprofloxacin, ofloxacin, fleroxacin, temafloxacin, and sparfloxacin. Sparfloxacin was the most active compound tested, followed by ofloxacin and temafloxacin. Ciprofloxacin and fleroxacin were the least active. The use of HEp-2 cells for testing *C. pneumoniae* resulted in larger inclusions but essentially the same endpoints as were seen with use of HeLa 229 cells.

Chlamydia pneumoniae, the newly described chlamydial species, is emerging as a frequent cause of community-acquired respiratory tract infections, including pneumonia and bronchitis (1, 5). Quinolones have attracted interest as potential therapy for community-acquired respiratory tract infections because they are active against a wide range of pathogens responsible for these infections. Preliminary studies have demonstrated that ciprofloxacin and ofloxacin are active in vitro against *C. pneumoniae* (2, 7). We tested the activity of several quinolones, including ciprofloxacin, ofloxacin, fleroxacin, temafloxacin, and sparfloxacin (CI-978, AT4140), against clinical isolates of *C. pneumoniae*.

Ciprofloxacin (Miles Pharmaceuticals, West Haven, Conn.), ofloxacin (Ortho Pharmaceuticals, Raritan, N.J.), fleroxacin (Hoffmann-LaRoche Inc., Nutley, N.J.), temafloxacin (Abbott Laboratories, North Chicago, Ill.), and sparfloxacin (Parke-Davis, Ann Arbor, Mich.) were supplied as powders and solubilized according to instructions from the manufacturers.

We tested 10 strains of *C. pneumoniae*: TW-183 (Washington Research Foundation, Seattle); eight clinical isolates from Brooklyn, T2023 (ATCC VR1356), T2043 (ATCC VR1355), T2337, BAL15, BAL16, BAL62, BAL37, and BAL48; and one clinical isolate from Wisconsin, W6805.

Susceptibility testing of *C. pneumoniae* was performed in cell culture by using HeLa 229 and HEp-2 cells grown in 96-well microtiter plates. The HeLa cells were pretreated with 30 μg of DEAE-dextran per ml for 10 min. No dextran was used with the HEp-2 cells (10).

Each well was inoculated with 0.1 ml of the test strain diluted to yield 10^3 to 10^4 inclusion-forming units per ml, centrifuged at 1,700 × *g* for 1 h, and incubated at 35°C for 1 h. Wells were then aspirated and overlaid with 0.2 ml of medium containing 1 μg of cycloheximide per ml and serial twofold dilutions of the test drug. After incubation at 35°C for 72 h, cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the lipopolysaccharide genus antigen (Pathfinder, Kallestad Diagnostics, Chaska, Minn.). The MIC was the lowest antibiotic concentration at which no inclusions were seen. The minimal chlamydiacidal concentration (MCC) was determined by aspirating the antibiotic-containing medium, washing wells twice with phosphate-buffered saline, and adding antibiotic-free medium. Cultures were frozen at −70°C, thawed, passed onto new cells, incubated for 72 h, and then fixed and stained as described above. The MCC was the lowest antibiotic concentration which resulted in no inclusions after passage. All tests were run in triplicate.

The geometric mean MICs and MCCs for *C. pneumoniae* are given in Table 1. There were no differences in the endpoints achieved with the different cell lines. The MIC was sometimes difficult to define because of the absence of a clear-cut endpoint. As concentrations of antibiotics increased there was a clear breakpoint at which the inclusions changed, becoming irregular and progressively smaller or frequently abruptly becoming fine dustlike particles. These abnormal forms were not viable when passed onto antibiotic-free cells. One exception was temafloxacin, in which we saw sharp, well-defined endpoints with no abnormal inclusions. Inclusions were larger in HEp-2 cells, which made the determination of the endpoints easier than in HeLa 229 cells.

Sparfloxacin was the most active compound tested, with a geometric mean MIC and MCC of 0.09 μg/ml (range, 0.06 to 0.25) (Table 1). Ofloxacin and temafloxacin were the next most active drugs, with geometric mean MICs of 0.7 and 0.58 μg/ml and geometric mean MCCs of 0.76 and 0.66 μg/ml, respectively. Ciprofloxacin and fleroxacin were the least active compounds, with geometric mean MICs of 2.13 and 3.2 μg/ml, respectively. The geometric mean MCCs were 3 and 3.5 μg/ml, respectively.

Data on the in vitro susceptibility of *C. pneumoniae* are limited, a major reason being that relatively few clinical isolates have been available for testing. We have noted some interstrain variation in susceptibilities among our isolates. Our results are consistent with those reported by Lipsky et al. (7), who tested three isolates of *C. pneumoniae*, including TW-183, against ofloxacin and found the MICs and MCCs to be 1.0 to 2.0 μg/ml. Data concerning other quinolones are limited to the two reference strains, TW-183 and I0L 207. Orfila and colleagues (8, 9) have tested I0L 207 against sparfloxacin and temofloxacin, reporting MICs of <0.01 and 0.1 μg/ml, respectively. Wise et al. (12) reported MICs and MCCs of sparfloxacin and ciprofloxacin for TW-183 of 0.25 to 2 μg/ml. The activities of these drugs against *C. pneumoniae* appear to be similar to those reported for *C. trachomatis* (11).

Differences in results from different laboratories may also be due in part to variations in methods. In vitro susceptibility testing of *Chlamydia* species is not standardized (3). We have recently reported that HEp-2 cells appear to be more sensitive than HeLa 229 cells for the isolation and propagation of *C.*

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TABLE 1. Activity of five quinolones against *C. pneumoniae*

Drug (no. of strains tested)	MIC (μg/ml)		MCC (μg/ml)	
	Range	Mean (geometric)	Range	Mean (geometric)
Sparfloxacin (6)	0.06–0.25	0.09	0.06–0.25	0.09
Ofloxacin (10)	0.5–2	0.7	0.5–2	0.76
Temafloxacin (10)	0.125–1	0.68	0.125–2	0.66
Ciprofloxacin (10)	0.25–4	2.13	0.25–8	3.0
Fleroxacin (6)	2–8	3.2	2–8	3.5

pneumoniae (10). When these cells were used for antibiotic testing, we did not observe any difference in the endpoints achieved. However, since the inclusions tended to be larger in HEp-2 cells, the endpoints were easier to determine.

Few published data exist for describing the clinical response to *C. pneumoniae* infection or the efficacy of any treatment for eliminating the organism from the respiratory tract. We have observed several patients who have been persistently culture positive despite 7- to 30-day courses of doxycycline and tetracycline (6). Lipsky et al. (7) described four patients with lower respiratory tract infection treated with a 10-day course of ofloxacin who were retrospectively identified as having serologic evidence of acute *C. pneumoniae* infection. All reportedly demonstrated marked clinical improvement. However, no cultures were done; thus, microbiological efficacy could not be assessed. We have results of acute and follow-up cultures on three patients with bronchitis who also received 10-day courses of ofloxacin. Although all three demonstrated clinical improvement, one of the patients remained culture positive after therapy. The MICs of the most active quinolone tested, sparfloxacin, were similar to those reported for erythromycin and tetracycline (2). Although the MICs of the other drugs, including ofloxacin and temafloxacin, are almost 10-fold higher, they are well within achievable serum levels. Intracellular levels of quinolones may in fact, substantially exceed serum levels (4). Until prospective controlled studies utilizing culture are available, the best drug, as well as the optimum dose and duration of therapy, is uncertain.

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