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In Vitro Activity of Trovafloxacin against *Chlamydia pneumoniae*

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The in vitro susceptibilities of 12 strains of *Chlamydia pneumoniae* to a new quinolone, trovafloxacin, and ofloxacin, doxycycline, erythromycin, and azithromycin were determined. The activity of trovafloxacin was similar to that of ofloxacin, with a MIC at which 90% of the isolates are inhibited and a minimal concentration at which 90% of the isolates are killed of 1.0 μg/ml, but trovafloxacin was less active than doxycycline, erythromycin, and azithromycin.

Chlamydia pneumoniae is a frequent cause of community-acquired respiratory tract infection, including pneumonia and bronchitis in adults and children (2, 4). Quinolones have attracted interest as a potential therapy for community-acquired respiratory tract infections because they are active against a wide range of pathogens responsible for these infections. These pathogens include *Mycoplasma pneumoniae*, *Streptococcus pneumoniae* (including penicillin-resistant strains), and *C. pneumoniae* (3, 6, 8). We previously reported that several quinolones, including ofloxacin, levofloxacin, grepafloxacin (OPC 17116), and sparfloxacin, have significant activity against *C. pneumoniae* in vitro (6, 7, 12). As of this writing, ofloxacin, levofloxacin, and sparfloxacin are available clinically. Grepafloxacin is currently in phase 3 clinical trials. We tested a new quinolone, trovafloxacin (CP-99,219), with known high activity against gram-positive organisms for activity against *C. pneumoniae* and compared its activity with those of ofloxacin, doxycycline, erythromycin, and azithromycin.

Trovafloxacin and azithromycin (Pfizer, Inc., Groton, Conn.), ofloxacin (Ortho Pharmaceuticals, Raritan, N.J.), doxycycline, and erythromycin were supplied as powders and solubilized according to instructions from the manufacturers. Twelve strains of *C. pneumoniae* were tested: TW-183 and AR39 (Washington Research Foundation, Seattle); seven clinical isolates from Brooklyn, N.Y. (T2023 [ATCC VR1356], T2043 [ATCC VR1355], T2337, BAL15, BAL16, BAL37, and BAL62); a clinical isolate from Japan, J-21 (ATCC VR1435); CDC8 from Atlanta, Ga.; and W6805 from Wisconsin.

Susceptibility testing of *C. pneumoniae* was performed in cell culture using HEp-2 cells grown in 96-well microtiter plates (12). 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 chlamydicidal 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 MICs and MCCs for *C. pneumoniae* are given in Table 1. As the concentrations of the antibiotics increased there was a clear point at which the inclusions became irregular and progressively smaller or, more frequently, fine, dust-like particles. The extent of this phenomenon varied from antibiotic to antibiotic. It was most prominent with ofloxacin, whereas trovafloxacin had more definable endpoints. These abnormal forms were not viable when passed onto antibiotic-free cells. The MIC essentially was the lowest concentration at which no normal inclusions were seen. The activity of trovafloxacin was similar to that of ofloxacin, with a MIC at which 90% of the isolates are inhibited (MIC_{90}) and an MCC at which 90% of the isolates are killed (MCC_{90}) of 1.0 μg/ml, compared to a MIC_{90} and MCC_{90} of 1.0 and 2 μg/ml, respectively, for ofloxacin. Both quinolones were less active than doxycycline, erythromycin, and azithromycin.

Trovafloxacin may be a very attractive agent for the treatment of community-acquired respiratory infections. It has excellent activity against *S. pneumoniae*, including strains intermediately and highly resistant to penicillin, *Moraxella catarrhalis*, *Haemophilus influenzae*, and *M. pneumoniae* and *Legionella* spp. (3, 8, 14). The pharmacokinetics allow for once-a-day dosing and shorter courses of treatment (13). The mean maximum concentration of drug in serum after a 300-mg oral dose was 2.9 mg/liter, which is almost three times the MIC and MCC for *C. pneumoniae*.

The MICs of trovafloxacin obtained were very consistent from strain to strain, especially in view of the wide geographic distribution of the isolates tested. The activity of trovafloxacin against *C. pneumoniae* was less than reported by Van Der Pol and Jones for *Chlamydia trachomatis* (15). They found the MICs to range from 0.031 to 1 μg/ml depending on whether the *C. trachomatis* strains were resistant to tetracycline. Trovafloxacin had consistently lower MICs for *C. trachomatis* than doxycycline, erythromycin, or ofloxacin. Preliminary data show that trovafloxacin, taken in 200-mg doses daily for 5 days, was effective for the eradication of *C. trachomatis* from the endocervix in women and the urethra in men (10).

Few published data on the efficacy of any treatment regimen

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TABLE 1. Activities of trovafloxacin and other antibiotics against 12 isolates of *C. pneumoniae*

Drug	MIC (μg/ml)			MCC (μg/ml)	
	Range	50%	90%	Range	90%
Trovafloxacin	0.5–1	1	1	0.5–1	1
Ofloxacin	0.25–2	0.5	1	0.5–2	2
Doxycycline	0.125–0.5	0.25	0.25	0.125–0.5	0.25
Erythromycin	0.015–0.25	0.062	0.125	0.031–0.25	0.25
Azithromycin	0.015–0.25	0.125	0.25	0.015–0.25	0.25

for eliminating *C. pneumoniae* from the respiratory tract exist. The results of in vitro susceptibility testing may not always predict in vivo efficacy. We have observed several patients who have remained persistently culture positive and clinically symptomatic despite 7- to 30-day courses of doxycycline and tetracycline (5). A recent study comparing erythromycin to clarithromycin suspensions for the treatment of community-acquired pneumonia in children found that clarithromycin was not more effective than erythromycin in eliminating *C. pneumoniae* from the respiratory tract, even though clarithromycin is more active in vitro and has superior pharmacokinetics and tissue penetration (1). There are no published studies that have assessed the efficacy of quinolones for the treatment of *C. pneumoniae* infection that have utilized culture. Lipsky et al. (9) described four patients with bronchitis treated with 10-day courses of ofloxacin who were retrospectively identified as having serologic evidence of acute *C. pneumoniae* infection. All reportedly demonstrated marked clinical improvement. However, as no cultures were done, microbiological efficacy could not be assessed. A recent study examined ofloxacin versus standard therapy (usually a beta-lactam with or without a macrolide) in treatment of community-acquired pneumonia requiring hospitalization. Ofloxacin appeared to be equivalent to standard therapy (11). However, as the diagnosis of *C. pneumoniae* was made serologically, only the clinical outcome was assessed. We have treated three patients with culture-confirmed *C. pneumoniae* infection (bronchitis and pneumonia) with grepafloxacin, which is slightly more active than ofloxacin in vitro (12). One patient responded to a 10-day course of grepafloxacin with clinical improvement and eradication of *C. pneumoniae* from the nasopharynx. Two patients remained culture positive and symptomatic despite 2 weeks of treatment. The ultimate role of trovafloxacin in the treatment of *C. pneumoniae* infections will depend on the results of prospective clinical studies utilizing culture.

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