

## NOTES

### In Vitro Activities of OPC-17116, a New Quinolone; Ofloxacin; and Sparfloxacin against *Chlamydia pneumoniae*

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Received 9 December 1993/Returned for modification 15 February 1994/Accepted 15 March 1994

**The in vitro susceptibilities of 12 strains of *Chlamydia pneumoniae* to a new quinolone, OPC-17116; ofloxacin; and sparfloxacin were determined. OPC-17116 was slightly less active than sparfloxacin but more active than ofloxacin, with a MIC for 90% of strains tested and a minimal chlamydiacidal concentration for 90% of strains tested of 0.5 µg/ml.**

*Chlamydia pneumoniae*, the newly described chlamydial species, is emerging as a frequent cause of community-acquired respiratory tract infections, including pneumonia and bronchitis (1, 2). Quinolones have attracted interest for their use as potential therapy for community-acquired respiratory tract infections because they are active against a wide range of pathogens responsible for these infections, including *Mycoplasma pneumoniae* and *C. pneumoniae* (4-9). We previously reported that ofloxacin, temafloxacin, and sparfloxacin had significant activities against *C. pneumoniae* in vitro (4). At present, only ofloxacin is available clinically; temafloxacin was withdrawn because of serious adverse effects, and sparfloxacin is still under investigation. OPC-17116, a new quinolone agent currently in phase 3 clinical trials, has excellent tissue penetration and appears to be safe and well tolerated (5). Therefore, we tested the activities of OPC-17116, ofloxacin, and sparfloxacin against 12 strains of *C. pneumoniae* in vitro.

Ofloxacin (Ortho Pharmaceuticals, Raritan, N.J.), sparfloxacin (Parke-Davis, Ann Arbor, Mich.), and OPC-17116 (Otsuka America Pharmaceuticals, Inc., Rockville, Md.) were supplied as powders and solubilized according to the instructions from the manufacturers. The following 12 strains of *C. pneumoniae* were tested: TW-183 (Washington Research Foundation, Seattle, Wash.) and 11 clinical strains, T2023 (ATCC VR1356), T2043 (ATCC VR1355), T2219, T2237, T2263, BAL14, BAL15, BAL16, BAL37, BAL48, and W6805.

Susceptibility testing of *C. pneumoniae* was performed in cell culture by using HEP-2 cells grown in 96-well microtiter plates (4). 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  $1,700 \times g$  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 being incubated 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 MICs and MCCs for *C. pneumoniae* are given in Table 1. As concentrations of antibiotics increased, there was a clear breakpoint at which the morphology of the inclusions changed, with the inclusions becoming irregular and progressively smaller or frequently and abruptly becoming fine dust-like particles. These abnormal forms were not viable when passed onto antibiotic-free cells. OPC-17116 gave sharp endpoints compared with those of the other drugs.

Sparfloxacin was the most active compound tested, with a MIC for 90% of strains tested (MIC<sub>90</sub>) and an MCC<sub>90</sub> of 0.25 µg/ml (Table 1). OPC-17116 had similar activity, with a MIC<sub>90</sub> and an MCC<sub>90</sub> of 0.5 µg/ml. Ofloxacin was less active, with a MIC<sub>90</sub> and an MCC<sub>90</sub> of 1.0 µg/ml.

Data on the in vitro susceptibility of *C. pneumoniae* are limited, in part because of the relatively small number of clinical isolates that have been available for testing. There are only two other reports of the activity of OPC-17116 against *C. pneumoniae*, and in both only one strain, TW-183, was tested (7, 10). The MICs and MCCs were 0.06 and 0.03 µg/ml, respectively, which is lower than the range we found, 0.25 to 0.5 µg/ml. Although results of in vitro testing have been largely consistent, we have noted some interstrain variation in susceptibilities among our isolates in previous studies with other compounds (1). The results of this study found the MICs and MCCs to be fairly tightly clustered. The activity of OPC-17116 against *C. pneumoniae* was almost 10-fold less than that reported for *C. trachomatis* (11). One may not be able to extrapolate from one chlamydial species to another.

How the results of in vitro susceptibility testing translate to in vivo efficacy is another issue. Few published data exist on the efficacy of any treatment regimen for eliminating *C. pneu-*

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TABLE 1. Activities of OPC-17116, sparfloxacin, and ofloxacin against 12 isolates of *C. pneumoniae*

| Drug         | MIC ( $\mu\text{g/ml}$ ) <sup>a</sup> |      |      | MCC ( $\mu\text{g/ml}$ ) <sup>b</sup> |      |
|--------------|---------------------------------------|------|------|---------------------------------------|------|
|              | Range                                 | 50%  | 90%  | Range                                 | 90%  |
| OPC-17116    | 0.25–0.5                              | 0.25 | 0.5  | 0.25–0.5                              | 0.5  |
| Sparfloxacin | 0.06–0.25                             | 0.25 | 0.25 | 0.06–0.25                             | 0.25 |
| Ofloxacin    | 0.5–2.0                               | 0.5  | 1.0  | 0.5–2.0                               | 1.0  |

<sup>a</sup> 50% and 90%, MICs for 50 and 90% of strains tested, respectively.

<sup>b</sup> 90%, MCCs for 90% of strains tested.

*moniae* from the respiratory tract. We have observed several patients who have remained persistently culture positive and clinically symptomatic despite 7- to 30-day courses of doxycycline and tetracycline (3). 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. (8) described four patients with bronchitis and pneumonia 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, as cultures were not done, microbiological efficacy could not be assessed. We have treated three patients with culture-documented *C. pneumoniae* infections (bronchitis and pneumonia) with OPC-17116, under a compassionate-release protocol. All three had either failed treatment with other antibiotics or could not be treated with macrolides or tetracyclines because of a history of hypersensitivity to these agents. One of the three patients responded to two 10-day courses of OPC-17116 with clinical improvement and eradication of *C. pneumoniae* from the nasopharynx. Two patients remained culture positive and symptomatic despite 2 weeks of treatment. OPC-17116 was well tolerated in all three patients. This anecdotal experience suggests that OPC-17116 may have a role in the treatment of *C. pneumoniae* infections, but prospective clinical studies utilizing culture would be needed to confirm its efficacy in vivo.

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