

## In Vitro Activity of a New 8-Methoxyquinolone, BAY 12-8039, against *Chlamydia pneumoniae*

PATRICIA M. ROBLIN AND MARGARET R. HAMMERSCHLAG\*

Division of Infectious Diseases, Department of Pediatrics, State University of New York, Health Science Center at Brooklyn, Brooklyn, New York 11203

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**The in vitro susceptibilities of 10 strains of *Chlamydia pneumoniae* to a new 8-methoxyquinolone, BAY 12-8039, and to ofloxacin, doxycycline, and erythromycin were determined. The activity of BAY 12-8039 was similar to that of ofloxacin, with a MIC at which 90% of the isolates had no inclusions and a minimal chlamydicidal concentration at which 90% of the isolates had no inclusions after passage of 1.0 µg/ml, but this activity was less than those of doxycycline and erythromycin.**

*Chlamydia pneumoniae* is a frequent cause of community-acquired respiratory tract infections, including pneumonia and bronchitis, in adults and children (1, 6). Quinolones have attracted interest as potential therapies for community-acquired respiratory tract infections because they are active against a wide range of pathogens responsible for these infections, including *Mycoplasma pneumoniae*, *Streptococcus pneumoniae* (both non-penicillin-resistant and penicillin-resistant strains), and *C. pneumoniae* (2, 4, 12, 17). We previously reported that several quinolones, including ofloxacin, levofloxacin, grepafloxacin, sparfloxacin, and trovafloxacin, have significant activities against *C. pneumoniae* in vitro (3, 7, 8, 14, 15). We tested a new 8-methoxyquinolone, BAY 12-8039, for activity against *C. pneumoniae* in comparison with ofloxacin, doxycycline, and erythromycin.

BAY 12-3089 (Bayer Corp., West Haven, Conn.), ofloxacin (Ortho Pharmaceuticals, Raritan, N.J.), doxycycline, and erythromycin were supplied as powders and solubilized according to instructions from the manufacturers. Ten strains of *C. pneumoniae* were tested: TW-183 and AR39 (Washington Research Foundation, Seattle, Wash.); five clinical isolates from Brooklyn, namely, T2023 (ATCC VR1356), T2043 (ATCC VR1355), T2337, BAL15, and BAL16; a clinical isolate from Japan, J-21 (ATCC VR1435); CDC8 from Atlanta, Ga.; and W6805 from Wisconsin.

Susceptibility testing of *C. pneumoniae* in cell culture was performed with HEp-2 cells grown in 96-well microtiter plates (7). 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, and plates were 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 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, passaged 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 of BAY 12-8039 for *C. pneumoniae* are given in Table 1. As concentrations of the antibiotics increased, there was a clear point at which the morphology of the inclusions changed, becoming irregular and progressively smaller or frequently abruptly becoming fine dust-like particles. The extent of this phenomenon varied from antibiotic to antibiotic. It was most prominent with ofloxacin. These abnormal forms were not viable when they were passaged onto antibiotic-free cells. The activity of BAY 12-8039 was similar to that of ofloxacin, with a MIC at which 90% of the isolates had no inclusions and an MCC at which 90% of the isolates had no inclusions after passage of 1.0 µg/ml. Both quinolones were less active than doxycycline and erythromycin.

The MICs of BAY 12-8039 were very consistent from strain to strain, especially in view of the wide geographic distribution of the isolates tested. Data on the activity of BAY 12-8039 against *Chlamydia* species are limited. Woodcock et al. (17) tested BAY 12-8039 against three strains of *Chlamydia trachomatis* and one strain of *C. pneumoniae*, TW 183. The MICs for *C. trachomatis* were 0.06 to 0.12 µg/ml, and that for *C. pneumoniae* TW 183 was also 0.06 µg/ml, which indicates 10-fold more activity than our results. This discrepancy may be due in part to differences in methods. Woodcock et al. used McCoy cells, which are significantly less susceptible to infection with *C. pneumoniae* than HEp-2 cells (13). However, they reported that the MICs of another quinolone, ciprofloxacin, for chlamydiae were 1 to 2 µg/ml, which are similar to results we have obtained using HeLa cells (3). Felmingham et al. also assessed the activities of BAY 12-8039 against *C. trachomatis* and *C. pneumoniae*, although the number and identities of the strains tested were not specified (4). Using iododeoxyuridine-treated McCoy cells, they reported the MICs for both species to be 0.012 µg/ml.

Although there are no data on the use of BAY 12-8039 to treat chlamydial infections in humans, preliminary data from a mouse model of *C. pneumoniae* pneumonia found that the drug was effective in eliminating the organism from the lungs of infected animals (16). The lung/plasma ratios of the concentrations of BAY 12-8039 ranged from 3.75 to 6.8. After a single 400-mg oral dose in human male volunteers, the maximum concentration of the drug in serum was  $3.10 \pm 1.12$

\* Corresponding author. Mailing address: Department of Pediatrics, Box 49, SUNY Health Science Center at Brooklyn, 450 Clarkson Ave., Brooklyn, NY 11203-2098. Phone: (718) 245-4075. Fax: (718) 245-2118. E-mail: mhammerschlag@pol.net.

TABLE 1. Activities of BAY 12-8039 and other antibiotics against 10 isolates of *C. pneumoniae*<sup>a</sup>

Drug	MIC (μg/ml)			MCC (μg/ml)	
	Range	50%	90%	Range	90%
BAY 12-8039	0.5–1	1	1	1	1
Ofloxacin	0.5–2	0.5	1	0.5–2	1
Doxycycline	0.125–0.25	0.125	0.25	0.125–0.25	0.25
Erythromycin	0.015–0.25	0.06	0.125	0.03–0.25	0.25

<sup>a</sup> 50% and 90%, MIC (or MCC) at which 50 and 90% of strains had no inclusions after passage, respectively.

mg/liter, which is at least three times the MIC for *C. pneumoniae* (9). Assuming that BAY 12-8039 is also concentrated in lung tissue in humans, it should be very effective for the treatment of respiratory infections.

BAY 12-8039 has been shown to have excellent activity against gram-positive bacteria such as *S. pneumoniae*, including strains intermediately and highly resistant to penicillin, and its activity has been shown to exceed those of trovafloxacin, sparfloxacin, ofloxacin, and levofloxacin (2). It also has good activity against *Moraxella catarrhalis*, *Haemophilus influenzae*, and *M. pneumoniae* (2, 4, 12). This spectrum of activity suggests that BAY 12-8039 has a role in the treatment of community-acquired pneumonia. However, there are no published reports of studies that have used culture to assess the efficacy of any quinolone for the treatment of *C. pneumoniae* infection. Lipsky et al. (10) described four patients with bronchitis who had been treated with a 10-day course of ofloxacin and who were retrospectively identified as having had 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. Plouffe et al. (11), as part of the Community Acquired Pneumonia Study, retrospectively examined treatment with ofloxacin versus standard therapy (usually a beta-lactam with or without a macrolide) of patients with community-acquired pneumonia requiring hospitalization. Treatment with ofloxacin appeared to be equivalent to standard therapy (11). However, as the diagnosis of *C. pneumoniae* was made serologically, only clinical outcome was assessed. File et al. recently reported the results of a large, multicenter pneumonia treatment study comparing treatment with intravenous and/or oral levofloxacin to that with ceftriaxone and/or cefuroxime axetil (5). As in previous studies (10, 11), the diagnosis of *C. pneumoniae* infection was based on serology alone. They found no difference in the clinical responses of those patients who were treated with levofloxacin compared to those who were treated with a cephalosporin. We have treated three patients with culture-documented *C. pneumoniae* infection (bronchitis and pneumonia) with grepafloxacin, which is slightly more active than ofloxacin in vitro (14). One patient responded to a 10-day course of grepafloxacin with clinical improvement and eradication of *C. pneumoniae* from the nasopharynx, but the two other patients remained culture positive and symptomatic despite 2 weeks of treatment with the drug. Prospective studies of BAY 12-8039 for the treatment of community-acquired pneumonia which use cul-

ture of *C. pneumoniae* will determine the role of this drug in the treatment of these infections.

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