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In Vitro Activity of AZD0914, a Novel DNA Gyrase Inhibitor, against *Chlamydia trachomatis* and *Chlamydia pneumoniae*

Stephan A. Kohlhoff,[a] Michael D. Huband,[b] Margaret R. Hammerschlag[a]

Division of Infectious Diseases, Department of Pediatrics, State University of New York Downstate Medical Center, Brooklyn, New York, USA[a]; Infection iMed, AstraZeneca Pharmaceuticals LP, Waltham, Massachusetts, USA[b]

The *in vitro* activities of AZD0914, levofloxacin, azithromycin, and doxycycline against 10 isolates each of *Chlamydia trachomatis* and *Chlamydia pneumoniae* were tested. For AZD0914, the MIC_{90}s for *C. trachomatis* and *C. pneumoniae* were 0.25 μg/ml (range, 0.06 to 0.5 μg/ml) and 1 μg/ml (range, 0.25 to 1 μg/ml), respectively, and the minimal bactericidal concentrations at which 90% of the isolates were killed (MBC_{90}s) were 0.5 μg/ml for *C. trachomatis* (range, 0.125 to 1 μg/ml) and 2 μg/ml for *C. pneumoniae* (range, 0.5 to 2 μg/ml).

Chlamydia trachomatis infection is the most common sexually transmitted infection in the United States, causing more than 1.4 million cases of cervicitis and urethritis each year (1). *Chlamydia pneumoniae* is a frequent cause of community-acquired respiratory infections, including pneumonia and bronchitis, in adults and children (2). Quinolones have excellent activity against a wide range of bacteria, including *Chlamydia* spp. (3). Antimicrobial activity of quinolones is mediated through inhibition of bacterial DNA gyrase and topoisomerase IV activities, which then inhibit bacterial DNA synthesis (3). AZD0914 is a member of a new class of antibacterials which incorporates a novel spiropyrimidinetrione that also targets DNA gyrase and topoisomerase IV through a novel mode of inhibition (4). AZD0914 has potent *in vitro* antibacterial activity against fluoroquinolone-resistant and multidrug-resistant methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae*, and *Neisseria gonorrhoeae* (4, 5).

We compared the *in vitro* activity of AZD0914 to those of levofloxacin, azithromycin, and doxycycline against 10 isolates each of *C. trachomatis* and *C. pneumoniae*.

The isolates of *C. trachomatis* tested included seven standard isolates from the ATCC (Manassas, VA, USA) [D-UW-57Cx (VR-878), E-BOUR (VR-348B), F-IC-CAL3 (VR-346), H-UW-43Cx (VR-879), I-UW-12Ur (VR-880), J-UW-36Cx (VR-886), and L2-434 (VR-902B)] and four clinical isolates [N18 (cervical), N19 (cervical), and 7015 (infant eye)]. The isolates of *C. pneumoniae* tested included four standard isolates from the ATCC [TW 183 (VR-2282), AR 39 (53592), CM-1 (VR-1360), and T 2043 (VR1355)] and six isolates from patients with community-acquired pneumonia, including isolates from bronchoalveolar lavage specimens from patients with human immunodeficiency virus infection and pneumonia from the United States (BAY 1, BAY13, BAL 18, BAL 19, BAL 37, and BAL 62).

AZD0914 (AstraZeneca), azithromycin (Sigma-Aldrich, MO, USA), levofloxacin (Sigma-Aldrich, MO, USA), and doxycycline (Sigma-Aldrich, MO, USA) were supplied as powders and solubilized according to the manufacturers' instructions. Drug suspensions were made fresh each time the assay was run. Susceptibility testing of *C. trachomatis* and *C. pneumoniae* was performed with HEp-2 cells grown in 96-well microtiter plates (6). Each well was inoculated with 0.2 ml of the test strain diluted to yield 10^4 inclusion-forming units per ml; the plates were centrifuged at 1,700 × *g* for 1 h and incubated at 35°C for 1 h. Wells were then aspirated and overlaid with medium containing 1 μg/ml of cycloheximide and serial 2-fold dilutions of the test drugs. After incubation at 35°C for 72 h, cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the chlamydial lipopolysaccharide genus-specific antigen (Pathfinder; Bio-Rad, Redmond, WA). The MIC was the lowest antimicrobial concentration at which no inclusions were seen. The minimal bactericidal concentration (MBC) was determined by aspirating the antibiotic-containing medium, washing the wells twice with phosphate-buffered saline, and adding antibiotic-free medium. The infected cells were frozen at −70°C, thawed, passed onto new cells, incubated for 72 h, and then fixed and stained as described above. The MBC was the lowest antimicrobial concentration that resulted in no inclusions after passage. All tests were run in duplicate.

TABLE 1 Activities of AZD0914 and comparator antibacterials against 10 isolates of *C. trachomatis*

Drug	MIC (μg/ml)			MBC (μg/ml)	
	Range	50%	90%	Range	90%
AZD0914	0.06–0.5	0.125	0.25	0.125–1	0.5
Levofloxacin	0.125–0.5	0.25	0.25	0.125–1	0.5
Doxycycline	0.03–0.25	0.06	0.125	0.03–0.25	0.125
Azithromycin	0.004–0.03	0.008	0.015	0.008–0.03	0.015

The MICs and MBCs for *C. trachomatis* and *C. pneumoniae* are shown in Tables 1 and 2. For AZD0914, The MIC_{90}s for *C. trachomatis* and *C. pneumoniae* were 0.25 μg/ml (range, 0.06 to 0.5 μg/ml) and 1 μg/ml (range, 0.25 to 1 μg/ml), respectively, and the minimal bactericidal concentrations at which 90% of the isolates were killed (MBC_{90}s) were 0.5 μg/ml for *C. trachomatis* (range,

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Address correspondence to Margaret R. Hammerschlag, mhammerschlag@downstate.edu.

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TABLE 2 Activities of AZD0914 and comparator antibacterials against 10 isolates of *C. pneumoniae*

Drug	MIC (μg/ml)			MBC (μg/ml)	
	Range	50%	90%	Range	90%
AZD0914	0.25–1	0.5	1	0.5–2	2
Levofloxacin	0.5	0.5	0.5	0.125–2	2
Doxycycline	0.06–0.125	0.125	0.125	0.25–0.5	0.5
Azithromycin	0.03–0.06	0.06	0.06	0.06–0.25	0.25

0.125 to 1 μg/ml) and 2 μg/ml for *C. pneumoniae* (range, 0.5 to 2 μg/ml).

The *in vitro* activity of AZD0914 against *C. trachomatis* was comparable to those of levofloxacin and doxycycline and 16-fold less than that of azithromycin, based on MIC_{90} values. The *in vitro* activity of AZD0914 against *C. pneumoniae* was comparable with that of levofloxacin, 4-fold less than that of doxycycline, and 16-fold less than that of azithromycin, based on MIC_{90} values. However, *in vitro* activity may not necessarily predict microbiologic efficacy *in vivo* against *C. pneumoniae*. For example, clarithromycin is 10- to 100-fold more active than erythromycin but was not more effective in the eradication of *C. pneumoniae* (7).

The *in vitro* activity of AZD0914 is comparable to those of several antibiotics with proven clinical efficacy against chlamydial infections (8). The results presented here suggest that AZD0914 may be effective for the treatment of infections due to *C. trachomatis* and *C. pneumoniae*. Furthermore, the *in vitro* susceptibility testing protocol used for this study is the only one whose results have been shown to correlate with clinical outcome and microbiologic eradication for infections caused by *C. pneumoniae* (8, 9). Of special interest is the fact that AZD0914 has activity against both *C. trachomatis* and *N. gonorrhoeae* (including drug-resistant strains), which may allow treatment of infections with strains of both species with a single drug. The role of AZD0914 in the treatment of *C. trachomatis* and *C. pneumoniae* infections will ultimately depend on the results of clinical studies that assess microbiologic efficacy.

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