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In Vitro Activity of Azithromycin (CP-62,993) against *Chlamydia trachomatis* and *Chlamydia pneumoniae*

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The in vitro susceptibilities of 49 strains of *Chlamydia trachomatis* and 3 strains of *Chlamydia pneumoniae* to azithromycin and tetracycline or doxycycline were determined. The MIC of azithromycin ranged from ≤0.06 to 1.0 μg/ml, the MIC of tetracycline ranged from 0.03 to 0.12 μg/ml, and the MIC of doxycycline ranged from 0.015 to 0.06 μg/ml against *C. trachomatis*. The MIC ranges for *C. pneumoniae* were 0.12 to 0.25 μg/ml for azithromycin and 0.06 to 0.12 μg/ml for tetracycline. All minimal chlamydicidal concentrations were either equal to the MIC or one or two dilutions higher. No strains resistant to these antibiotics were detected. In vitro activity shows that azithromycin is highly active against *C. trachomatis* and *C. pneumoniae*.

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen (12), with *Chlamydia pneumoniae* being another important human respiratory pathogen (5). The antibiotic drug of choice against chlamydial infections is tetracycline. The alternative drug is erythromycin (2). Both drugs have their limitations, and an effective single-drug regimen would be useful. Azithromycin has such potential. In vivo studies of this drug found that a single 1-g oral dose is comparable to a 1-week-long course of doxycycline for genital chlamydial infections (7, 9). In addition, in vitro susceptibility tests show azithromycin to be an active drug against *C. trachomatis* and *C. pneumoniae* (1, 3, 6, 14, 15). These preliminary studies are based on a limited number of chlamydia isolates. Anticipating that azithromycin may have widespread use against chlamydial infections, we thought it would be of interest to test the susceptibilities of more chlamydia strains from different geographic areas and disease conditions. In our study, the MIC and minimal chlamydicidal concentration (MCC) activities of azithromycin were determined against several *C. trachomatis* strains (strains from patients with sexually transmitted disease [STD] and trachoma) and three *C. pneumoniae* strains.

MATERIALS AND METHODS

Source of isolates. Fifty-two chlamydia strains were propagated to high titers in antibiotic-free medium and were frozen at −70°C.

Strains from patients with recent STD. *C. trachomatis* isolates from 47 patients were obtained in the United States (31 strains from the University of California, San Francisco, Medical Center, San Francisco; 3 strains from Z. A. Dalu, St. Louis, Mo.; 3 strains from Adolescent Medicine, Oklahoma City, Okla.; 3 strains from Smith Kline Bioscience, Norristown, Pa.; 2 strains from C. Alford, Birmingham, Ala.; 2 strains from CMRG, Inc., Fresno, Calif.; 2 strains from P. Rice, Boston, Mass.; and 1 strain from Kings County Hospital Center, Brooklyn, N.Y.). These isolates were from the following clinical locations: 17 from male urethras, 27 from cervixes; 2 from endometriums, and 1 from the nasopharynx of a newborn. Twenty-one *C. trachomatis* strains were isolated from patients prior to treatment with azithromycin. These included 7 male urethral and 14 cervical isolates. The chlamydial infections in all of these patients were successfully treated with a 1-g dose of azithromycin.

(ii) Trachoma strains. Tunis 77 and Tunis 864 were isolated from patients with trachoma seen in Tunisia.

***C. pneumoniae* strains.** ATCC VR-1310 (Washington Research Foundation, Seattle) and ATCC VR-1356 and ATCC VR-1355 (M. R. Hammerschlag, Brooklyn, N.Y.) isolates were tested.

Serotyping of isolates. Forty-three of 47 *C. trachomatis* isolates from patients with STDs and 2 strains from patients with trachoma were serotyped by using the microimmunofluorescence (Micro-IF) test kit (Washington Research Foundation) with a combination of selected monoclonal antibodies.

Antimicrobial agents. Azithromycin (Pfizer Central Research, Groton, Conn.), tetracycline (Lederle Laboratories, Pearl River, N.Y.), and doxycycline (ESI Pharmaceuticals, Cherry Hill, N.J.) were the antibiotics tested.

Determination of antimicrobial susceptibilities. Susceptibility tests were performed as described previously, (10), with slight modifications. Most strains were tested in vials, 12 of the strains from patients with STDs (5 from urethras and 7 from cervixes) and the 2 strains from patients with trachoma were tested in microtiter plates.

(i) Vial procedure. The titer of each chlamydia strain was adjusted so that it yielded approximately 100 to 300 inclusions per coverslip. Vials containing McCoy cell monolayers on coverslips were inoculated with 1.0 ml of the isolate and centrifuged at 2,500 × *g* for 1 h. The medium was aspirated, and twofold dilutions of azithromycin (0.06 to 2.0 μg/ml), tetracycline (0.015 to 0.5 μg/ml), or doxycycline (0.007 to 0.12 μg/ml) in Eagle's minimal essential medium containing 10% fetal bovine serum, 1% L-glutamine (200 nM solution), and 0.003 mM glucose per ml with 1 μg of cycloheximide per ml was added. Vials were incubated at 35°C in 5% CO_2 for 72 h. Two vials containing each drug dilution were fixed with methanol and stained with iodine, the coverslips were examined microscopically, and the number of inclusions was counted. For *C. pneumoniae* strains, the monolayers were fixed with ethanol and stained with a fluorescein-conjugated monoclonal antibody to *Chlamydia* genus-specific antigen

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TABLE 1. In vitro activity ranges of azithromycin, tetracycline, and doxycycline against *Chlamydia* strains

Strain and site of isolation	No. of isolates	Azithromycin		Tetracycline		Doxycycline	
		MIC (μg/ml)	MCC (μg/ml)	MIC (μg/ml)	MCC (μg/ml)	MIC (μg/ml)	MCC (μg/ml)
C. trachomatis from patients with recent cases of STD							
Urethral	17	≤0.06–1.0	0.12–1.0	0.03–0.06[a]	0.06–0.12[a]	0.015–0.06[b]	0.015–0.06[b]
Cervical	27	≤0.06–1.0	0.12–2.0	0.03–0.12[c]	0.06–0.12[c]	0.015–0.06[d]	0.015–0.06[d]
Endometrium	2	0.12–0.25	0.25–0.5	0.03–0.06	0.06	ND[e]	ND
Nasopharyngeal	1	0.12	0.25	0.03	0.06	ND	ND
C. trachomatis from patients with trachoma							
Tunis 77		0.25	0.5	0.06	0.06	ND	ND
Tunis 864		0.25	0.5	0.06	0.12	ND	ND
C. pneumoniae							
VR-1310		0.25	0.25	0.06	0.12	ND	ND
VR-1356		0.25	0.5	0.12	0.12	ND	ND
VR-1355		0.12	0.25	0.12	0.12	ND	ND

[a] Tetracycline susceptibility was determined for 10 isolates.
[b] Doxycycline susceptibility was determined for 7 isolates.
[c] Tetracycline susceptibility was determined for 12 isolates.
[d] Doxycycline susceptibility was determined for 15 isolates.
[e] ND, not done.

(Ortho Diagnostic Systems, Raritan, N.J.). To determine the MCC, the remaining vials were passed into a new monolayer of McCoy cells in antibiotic-free medium and were processed as described above. Antibiotics were not added to the medium on the second passage.

(ii) Microtiter procedure. Chlamydiae were inoculated into 96-well microtiter plates (Costar, Cambridge, Mass.) seeded with McCoy cells. The plates were centrifuged at 1,500 × *g* for 1 h. The wells were aspirated, and twofold dilutions of azithromycin (0.06 to 2.0 μg/ml) and tetracycline (0.015 to 0.5 μg/ml) in Eagle's minimal essential medium containing 10% fetal bovine serum, 1% L-glutamine (200 nM solution), and 0.003 mM of glucose per ml with 1 μg of cycloheximide per ml were added. After incubation at 35°C in 5% CO_2 for 72 h, the wells were fixed with methanol and stained with iodine. Wells were examined and inclusions were counted to determine the MIC. A second passage with antibiotic-free medium was then done to determine the MCC.

The MIC for chlamydiae was defined as the concentration of antibiotic that allowed no inclusions on the first passage. The MCC was defined as the lowest concentration that allowed no inclusions in a further passage in the absence of antibiotics.

RESULTS

The MIC and MCC results for *C. trachomatis* and *C. pneumoniae* strains are summarized in Table 1. All strains were susceptible to azithromycin and tetracycline or doxycycline. The MICs of azithromycin for *C. trachomatis* ranged from ≤0.06 to 1.0 μg/ml. The MICs of azithromycin for *C. pneumoniae* were 0.12 to 0.25 μg/ml. All MCC ranges for *C. trachomatis* and *C. pneumoniae* were equal to the MIC ranges or were one or two dilutions higher. Table 2 shows that the MICs and MCCs of azithromycin for *C. trachomatis* urethral and cervical isolates were similar. The azithromycin MICs for three isolates were ≤0.06 μg/ml.

Forty-three of 47 *C. trachomatis* strains from patients with recent STDs were serotyped. Seventeen were serovar E, nine were serovar J, eight were serovar F, four were serovar D, four were serovar K, and one was serovar H. Strain Tunis 867 from a patient with trachoma was serotyped as serovar B, but strain Tunis 77 was untypeable. Table 3 gives the MIC and MCC ranges of azithromycin for these chlamydia serovars.

DISCUSSION

C. trachomatis infections are generally treated with tetracycline or doxycycline (11). However, tetracyclines are contraindicated for use in pregnant women and infants. Erythromycin is the alternative drug (8). Gastrointestinal upset following oral administration of tetracyclines or erythromycin is common. Because the treatment regimen for both antibiotics is 7 days, patient noncompliance can be a problem.

Azithromycin is a new azalide antibiotic with a spectrum of activity similar to those of macrolides, but it has a greater level of tissue penetration and a longer elimination period (4). Clinical trials found that a single 1-g oral dose of azithromycin can successfully be used to treat genital chlamydial infections (7, 9). The same dosage appears to be 85 to 90% effective in the treatment of *Neisseria gonorrhoeae* (9). The results of our study with a relatively large number of

TABLE 2. MICs and MCCs of azithromycin for *C. trachomatis* strains from patients with recent cases of STD

Azithromycin concn (μg/ml)	No. (%) of urethral isolates (*n* = 17)		No. (%) of cervical isolates (*n* = 27)	
	MIC	MCC	MIC	MCC
≤0.06	2 (11.8)	0	1 (3.7)	0
0.12	4 (23.5)	3 (17.7)	7 (25.9)	1 (3.7)
0.25	5 (29.4)	7 (41.2)	10 (37.0)	8 (29.6)
0.5	5 (29.4)	4 (23.5)	8 (29.6)	14 (51.9)
1.0	1 (5.9)	3 (17.7)	1 (3.7)	3 (11.1)
2.0	0	0	0	1 (3.7)

TABLE 3. In vitro activity ranges of azithromycin against the determined serovars of *C. trachomatis*

Serovar	No. of isolates	Azithromycin MIC (μg/ml)	Azithromycin MCC (μg/ml)
B	1	0.25	0.5
D	4	0.12–0.5	0.25–0.5
E	17	≤0.06–0.5	0.12–1.0
F	8	0.12–0.5	0.12–0.5
H	1	≤0.06	0.12
J	9	0.12–1.0	0.25–1.0
K	4	0.12–0.5	0.25–0.5

isolates of diverse origins confirm other laboratory findings on the in vitro activities of azithromycin (MIC range, ≤0.06 to 1.0 μg/ml) against *C. trachomatis*.

Forty-three of 47 *C. trachomatis* strains from patients with STDs were serotyped. Serovar E was the most common serovar among the strains from patients with STDs. No differences between the MIC and MCC ranges of azithromycin and those of tetracycline or doxycycline were observed for strains of the different serovars. In contrast, Welsh et al. (15) detected a higher level of resistance to tetracycline and azithromycin among strains of serovars F and K (MCCs, 2.0 to 4.0 μg/ml). However, for the 12 isolates of serovar F or K that we tested, MCCs were ≤0.5 μg/ml.

Isolates from 21 patients (azithromycin MIC ranges, 0.12 to 1.0 μg/ml) were obtained before the patients were successfully treated with a single 1-g dose of azithromycin. Our in vitro results confirmed the susceptibilities of the infectious strains to azithromycin.

Azithromycin may also be useful in the treatment of trachoma and *C. pneumoniae* infections. The in vitro activity of azithromycin against two strains from patients with trachoma (Tunis 77 and Tunis 864) was similar to its activity against genital chlamydial isolates (MIC, 0.25 μg/ml). The MIC results for *C. pneumoniae* (0.12 to 0.25 μg/ml) are comparable to those in other published reports. Azithromycin also appears to be clinically effective in the treatment of atypical pneumonia caused by *Chlamydia* spp. (13). However, there are no data on its microbiological efficacy in vivo.

In conclusion, the in vitro activities of azithromycin show that it is active against 47 sexually transmitted *C. trachomatis* isolates from diverse origins. The in vitro results support the need for further clinical studies to determine the usefulness of azithromycin against *C. pneumoniae* and also against trachoma infections.

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