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 chlamydial 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-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 chlamydial 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% CO₂ 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.
Table 1. In vitro activity ranges of azithromycin, tetracycline, and doxycycline against Chlamydia strains

<table>
<thead>
<tr>
<th>Strain and site of isolation</th>
<th>No. of isolates</th>
<th>Azithromycin</th>
<th>Tetracycline</th>
<th>Doxycycline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC (µg/ml)</td>
<td>MCC (µg/ml)</td>
<td>MIC (µg/ml)</td>
</tr>
<tr>
<td>C. trachomatis from patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with recent cases of STD</td>
<td>17</td>
<td>≤0.06–1.0</td>
<td>0.12–1.0</td>
<td>0.03–0.06</td>
</tr>
<tr>
<td>Cervical</td>
<td>27</td>
<td>≤0.06–1.0</td>
<td>0.12–2.0</td>
<td>0.03–0.12</td>
</tr>
<tr>
<td>Endometrium</td>
<td>2</td>
<td>0.12–0.25</td>
<td>0.25–0.5</td>
<td>0.03–0.06</td>
</tr>
<tr>
<td>Nasopharyngeal</td>
<td>1</td>
<td>0.12</td>
<td>0.25</td>
<td>0.03</td>
</tr>
</tbody>
</table>

C. pneumoniae

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg/ml)</th>
<th>MCC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VR-1310</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>VR-1356</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>VR-1355</td>
<td>0.12</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* Tetracycline susceptibility was determined for 10 isolates.
+ Doxycycline susceptibility was determined for 7 isolates.
< Tetracycline susceptibility was determined for 12 isolates.
$ Doxycycline susceptibility was determined for 15 isolates.
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% CO2 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 MCC 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 MIC ranges for C. trachomatis and C. pneumoniae were equal to or less than 0.5 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 N. 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

<table>
<thead>
<tr>
<th>Azithromycin concn (µg/ml)</th>
<th>No. (%) of urethral isolates (n = 17)</th>
<th>No. (%) of cervical isolates (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MCC</td>
</tr>
<tr>
<td>≤0.06</td>
<td>2 (11.8)</td>
<td>0</td>
</tr>
<tr>
<td>0.12</td>
<td>4 (23.5)</td>
<td>3 (17.7)</td>
</tr>
<tr>
<td>0.25</td>
<td>5 (29.4)</td>
<td>7 (41.2)</td>
</tr>
<tr>
<td>0.5</td>
<td>5 (29.4)</td>
<td>4 (23.5)</td>
</tr>
<tr>
<td>1.0</td>
<td>1 (5.9)</td>
<td>3 (17.7)</td>
</tr>
<tr>
<td>2.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
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 Chlamydia trachomatis.

Forty-three of 47 Chlamydia 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 Chlamydia 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 Chlamydia 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 Chlamydia trachomatis isolates from diverse origins. The in vitro results support the need for further clinical studies to determine the usefulness of azithromycin against Chlamydia pneumoniae and also against trachoma infections.

ACKNOWLEDGMENTS

We thank Gina Marie Gomez for excellent secretarial assistance and the staff of the Chlamydia Research Laboratory for technical assistance.

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

<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. of isolates</th>
<th>Azithromycin MIC (μg/ml)</th>
<th>Azithromycin MCC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>1</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>0.12-0.5</td>
<td>0.25-0.5</td>
</tr>
<tr>
<td>E</td>
<td>17</td>
<td>≤0.06-0.5</td>
<td>0.12-1.0</td>
</tr>
<tr>
<td>F</td>
<td>6</td>
<td>0.12-0.5</td>
<td>0.12-0.5</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>≤0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>J</td>
<td>9</td>
<td>0.12-1.0</td>
<td>0.25-1.0</td>
</tr>
<tr>
<td>K</td>
<td>4</td>
<td>0.12-0.5</td>
<td>0.25-0.5</td>
</tr>
</tbody>
</table>

REFERENCES