In Vitro Activity of Dirithromycin against *Chlamydia trachomatis*

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Dirithromycin is a new macroide antibiotic with an active metabolite, erythromycylamine. We evaluated the in vitro activities of both drugs against 16 isolates of *Chlamydia trachomatis* and compared them with that of doxycycline. In vitro susceptibility testing was performed with McCoy cell monolayers. The MIC was defined as the lowest concentration of antibiotic without inclusions. The MBC was defined as the lowest concentration of antibiotic yielding no inclusions after passage onto 24-h-old antibiotic-free McCoy cell monolayers. Dirithromycin and erythromycylamine appeared to be equally effective against these 16 strains of *C. trachomatis* (MIC for 90% of strains tested, 1 mg/ml; MBC for 90% of strains tested, 2 μg/ml). Both were less active than doxycycline (MIC for 90% of strains tested, 0.06 μg/ml; MBC for 90% of strains tested, 0.12 μg/ml). The combination of dirithromycin and erythromycylamine appeared to be additive.

Dirithromycin is a new macroide antibiotic which is metabolized to an active compound, erythromycylamine (2). It has in vitro activity comparable to that of erythromycin (9). Like erythromycin, dirithromycin is concentrated intracellularly (3). Erythromycin and other macrolides have been shown to have both in vitro and in vivo activity against *Chlamydia trachomatis* (1, 4, 5, 7). However, there is no published information on the activity of dirithromycin against *C. trachomatis*. Therefore, we tested the in vitro activities of dirithromycin and erythromycylamine against clinical isolates of *C. trachomatis* and compared them with that of doxycycline, an antibiotic commonly used to treat *C. trachomatis* infections. We also tested dirithromycin and erythromycylamine for synergy.

Dirithromycin and erythromycylamine were obtained from Lilly Pharmaceuticals (Indianapolis, Ind.), and doxycycline was obtained from Sigma Chemicals (St. Louis, Mo.). These drugs were prepared as stock dilutions in concentrations of 1,280 μg/ml according to their stated potency. Stock solutions were stored at −70°C for a maximum of 2 weeks. Drugs were diluted to the appropriate concentration with medium containing Hank's balanced salt solution, amino acids, vitamins, 1% glutamine, 10% inactivated fetal calf serum, 5.4 g of glucose per liter, and 1 μg of cycloheximide per ml. Dilutions were made on the day of use. A total of 16 strains of *C. trachomatis*, including one serotype D strain (ATCC VR-885), one lymphogranuloma venereum type 2 strain (ATCC VR-902b), and 14 clinical isolates, were tested. All clinical isolates were of genital origin and had been passed fewer than 10 times in the laboratory. All isolates were passed an additional two times in antibiotic-free medium before susceptibility testing and were stored at −70°C until the time of use.

The antimicrobial susceptibility of *C. trachomatis* was determined by using 96-well dilution plates as previously described (6). McCoy cell monolayers, 24-h old and grown in antibiotic-free medium, were inoculated with a dilution of *C. trachomatis* test strain known to yield 500 to 1,000 inclusions per well. Plates were centrifuged at 1,000 × g at 24°C for 60 min and then overlaid with 0.1 ml of each drug solution to yield appropriate twofold dilutions. Each solution was tested in duplicate. Antibiotic-free controls were included on each plate. Cultures were incubated for 48 h at 37°C in 5% CO₂, fixed with absolute ethanol, and stained with fluorescein-conjugated mouse monoclonal antibody to *C. trachomatis* (Ortho Diagnostics, Raritan, N.J.) according to the directions of the manufacturer. The MIC was defined as the lowest concentration of antibiotic without inclusions. The MBC was defined as the lowest concentration of antibiotic yielding no inclusions after passage onto 24-h-old McCoy cell monolayers grown on antibiotic-free medium.

Synergy was determined by a checkerboard method and was defined as a fractional inhibitory concentration index of 0.5 or less. Antagonism was defined as a fractional inhibitory concentration index of 2.0 or greater.

Dirithromycin and erythromycylamine demonstrated equivalent activities against the *C. trachomatis* strains tested (Table 1). Both of these drugs were significantly less active than doxycycline. The fractional inhibitory concentration index of dirithromycin plus erythromycylamine for all 16 *C. trachomatis* strains tested was 1.

Infections caused by *C. trachomatis* are the most common bacterial sexually transmitted infections in the United States. It is estimated that over 4.5 million cases occur annually (8). Unlike other sexually transmitted diseases, the incidence of *Chlamydia* infections remains fairly constant (8). Therefore, it is necessary to evaluate new drugs for the treatment of *C. trachomatis* infections. In this study, dirithromycin and erythromycylamine were equally active against the recent clinical isolates tested and the combination of dirithromycin and erythromycylamine was additive for all of the strains tested. However, doxycycline was more active than dirithromycin or erythromycylamine.

Dirithromycin has been shown to achieve high concentrations in tissue despite low concentrations in serum (3). It has a long terminal half-life of 20 to 30 h, and concentrations in tissue reach a maximum between 5 to 10 h postdose (3). Therefore, dirithromycin can be administered once daily. These properties make dirithromycin very attractive for the potential treatment of *C. trachomatis* infections. However, since dirithromycin is less active than doxycycline, any potential advantage of dirithromycin over doxycycline in the treat-

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TABLE 1. In vitro susceptibilities of 16 *C. trachomatis* strains

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (µg/ml)*</th>
<th>MBC (µg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td>Dirithromycin</td>
<td>0.03–4.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.5–2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>&lt;0.03–0.125</td>
<td>0.125</td>
</tr>
</tbody>
</table>

* 50% and 90%, MICS for 50 and 90% of strains tested.
* 50% and 90%, MBCs for 50 and 90% of strains tested.

...ment of *C. trachomatis* would be its improved pharmacokinetic properties.

REFERENCES


