Lefamulin was highly active In Vitro Against Multi-drug Resistant Mycoplasma genitalium Strains

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Abstract

M. genitalium strains. A collection of 41 M. genitalium isolates originating from 39 patients were tested. These included the M. genitalium G37 type-strain, an early passage of the M30 strain isolated by David Taylor-Robinson in 1980 (Tully et al., 1983), and one isolate kindly provided by Pat Totten, Seattle, USA. The remaining 38 strains were isolated in Copenhagen between 1996 and 2014. Twenty strains were macrolide-resistant, showed azithromycin MICs of 216 µg/ml, and had mutations in position 2058 (n=9) or 2059 (n=11, C to G numbering). Eight strains had combined resistance to azithromycin and moxifloxacin. The geographical origin of the strains is shown in Table 1.

Methods

Determination of minimum inhibitory concentration (MIC). MICs of lefamulin, azithromycin, doxycycline, and moxifloxacin were determined by inoculating 2500 genome equivalents of each strain into 96-well microplates containing two-fold dilutions of test antibiotics (Thomaus et al., 2005). After a three-week incubation period, cells and supernatants were harvested and growth of M. genitalium was determined by quantitative PCR. MIC was expressed as the minimal concentration of the test-antibiotic causing a 99% inhibition of growth when compared to the mean of the control cultures grown without antibiotic (Fig. 2).

Table 1. Distribution of M. genitalium strains according to country of origin and macrolide susceptibility.

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>Number of strains</th>
<th>Number of macrolide resistant</th>
<th>Number of macrolide susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Norway</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Denmark</td>
<td>6</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Sweden</td>
<td>12</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>France</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Japan</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Australia</td>
<td>7</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Control 1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Control 2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. MICs of lefamulin, azithromycin, doxycycline, and moxifloxacin against M. genitalium.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC range [µg/ml]</th>
<th>MIC50 [µg/ml]</th>
<th>MIC90 [µg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lefamulin (macrolide suscept.)</td>
<td>≤0.008</td>
<td>0.008</td>
<td>0.0005-0.08</td>
</tr>
<tr>
<td>Lefamulin (macrolide resistant)</td>
<td>0.016</td>
<td>0.02</td>
<td>0.002-0.06</td>
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<tr>
<td>Azithromycin</td>
<td>≥0.125</td>
<td>0.06</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.06</td>
<td>0.06</td>
<td>0.002-0.06</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.06</td>
<td>0.06</td>
<td>0.002-0.06</td>
</tr>
</tbody>
</table>

References


Results and Discussion

Lefamulin was the most active compound with an MIC50 of 0.008 µg/ml compared with azithromycin (MIC90 of >16 µg/ml), moxifloxacin (MIC90 of 8 µg/ml), and doxycycline (MIC90 of 1 µg/ml) (Table 2). Although the 20 macrolide resistant strains had a significantly higher MIC (MIC90, 0.06 µg/ml) than that of the 21 macrolide susceptible strains (MIC90, 0.0005-0.08 µg/ml) (p<0.001), lefamulin remained highly active against all macrolide resistant strains (Figure 2).

M. genitalium strains with macrolide resistance mediating mutations in 23S rRNA at position 2056 had lower lefamulin MIC (MIC90 0.008 µg/ml range 0.002-0.063 µg/ml) than those with mutations in position 2059 (MIC90 0.05 µg/ml range 0.008-0.035 µg/ml) (p<0.007). The higher lefamulin MIC found in strains with mutations in position 2059 compared to 2056 is different to what is seen for erythromycin against Isospora belli (Jensen et al., 2014), where macrolide resistance was equally distributed between the two groups (p<0.001).

MICs for the eight MDR strains was similar to those of the 12 macrolide resistant, macrolide susceptible strains (p>0.3 (MIC90 0.003 for both groups)). Thus, moxifloxacin resistance did not influence lefamulin MICs.

Conclusions

- Lefamulin was highly active against all M. genitalium strains tested, including fluoroquinolone- and macrolide-resistant strains. Despite an increase in the MICs to strains with macrolide resistance mediated via a mutation at position 2059 and 2059 of the 23S rRNA gene, lefamulin MICs remained extremely low.
- With the growing problems of MDR M. genitalium strains, particularly in the Asia-Pacific region, a clinical trial of lefamulin resistance was equally distributed between the two groups, p=0.3.
- Using lefamulin in combination with another antibiotic could potentially protect against the development of resistance to lefamulin, without interfering with its activity worth of exploration.

Introduction

Mycoplasma genitalium is an important cause of sexually transmitted infections (STIs) accounting for approximately 25% of non-chlamydial-non-gonococcal urethritis. First-line treatment is azithromycin, but rates of resistance are increasing globally. Moxifloxacin is the only effective second-line therapy but resistance is common, in particular in the Asia-Pacific region and dual resistance to macrolides and fluoroquinolones is observed. Thus, alternative therapies for which there is no cross-resistance are urgently needed.

Methods

The strains were screened using a real time PCR method as previously described. A total of 41 M. genitalium strains were tested regardless of their macrolide and fluoroquinolone resistance phenotypes. With the growing prevalence of MDR M. genitalium strains, further evaluation of lefamulin in a clinical trial is warranted.

Results

Lefamulin was highly active against all M. genitalium strains tested, including fluoroquinolone- and macrolide-resistant strains. Despite an increase in the MICs to strains with macrolide resistance mediated via a mutation at position 2059 and 2059 of the 23S rRNA gene, lefamulin MICs remained extremely low.

With the growing problems of MDR M. genitalium strains, particularly in the Asia-Pacific region, a clinical trial of lefamulin resistance was equally distributed between the two groups, p=0.3. Lefamulin resistance susceptibility was equally distributed between the two groups.

References


Background

Mycoplasma genitalium is the second most common bacterial sexually transmitted infection (STI) accounting for approximately 25% of non-chlamydial-non-gonococcal urethritis. First-line treatment is azithromycin, but rates of resistance are increasing globally. Moxifloxacin is the only effective second-line therapy but resistance is common, in particular in the Asia-Pacific region and dual resistance to macrolides and fluoroquinolones is observed. Thus, alternative therapies for which there is no cross-resistance are urgently needed.