Telithromycin post-antibiotic and post-antibiotic sub-MIC effects for 10 Gram-positive cocci

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Post-antibiotic effects (PAE) and post-antibiotic sub-MIC effects (PA-SME) of the ketolide telithromycin (HMR 3647) were determined for 10 Gram-positive cocci with various macrolide resistance mechanisms, including inducible and constitutive ribosomal methylase and macrolide efflux resistance genes. Strains tested included four Streptococcus pneumoniae, three Streptococcus pyogenes and three Staphylococcus aureus. Telithromycin MICs were 0.008–0.015 mg/L for pneumococci, 0.015–4.0 mg/L for S. pyogenes and 0.03 mg/L for staphylococci. PAE were determined after exposure of strains at 10 × MIC for 1 h. PA-SME were determined in the presence of 0.12x, 0.25x and 0.5x MIC of the agent after the initial 1 h exposure period. The PAE of telithromycin varied from 0.3 to 3.8 h; the PA-SME varied from 0.8 to 4.6 h, with maximal PA-SME varying from 1.5 to 4.6 h. PAE tended to be shortest for S. pyogenes (0.4–2.7 h) and S. aureus (0.3–2.4 h), compared to 1.5–3.8 h for S. pneumoniae. The duration of the PA-SME was similar for the three species tested. The low MICs for many strains as well as the PAE and PA-SME demonstrated in this study for telithromycin show promise for increasing the dosing interval of this ketolide, but will need verification by pharmacokinetic/pharmacodynamic and clinical studies.

Keywords: post-antibiotic effects, PAEs, ketolides, streptococci, staphylococci

Introduction

Whereas macrolides have been in use for several decades for a wide variety of infections, their use has become increasingly limited by the development of resistance to these and the related agents of the macrolide–lincosamide–streptogramin B (MLS) group.1–3 Macrolide resistance in staphylococci and streptococci is usually a result of ribosomal methylases coded for by ermA(A) or ermA(B) determinants and results in resistance to macrolides and lincosamides, but not to streptogramins.4 This resistance mechanism is constitutively expressed against macrolides, but may be either constitutively or inducibly expressed against lincosamides. A second macrolide common resistance mechanism is mediated by a macrolide efflux pump coded for by mef(E) determinants in streptococci and msr(A) determinants in staphylococci.5,6 This latter resistance mechanism has no effect on susceptibility of strains to lincosamides or streptogramins. Many other macrolide resistance mechanisms have also been described, including 23S rRNA and L4 and L22 ribosomal protein mutations in pneumococci.6 However, isolates with these resistance mechanisms are rarely detected.

Telithromycin is a recently developed member of the ketolide family, a new class of semi-synthetic 14-membered ring macrolides derived from erythromycin A, with human pharmacokinetic properties similar to erythromycin A.5–12 Previous studies have documented in vitro activity of ketolides against Streptococcus pneumoniae regardless of macrolide or lincosamide susceptibility.1,7,8,10 Ketolides are active against macrolide-susceptible staphylococci and strains with inducible macrolide resistance and do not induce resistance in the latter group.1 However, these agents are inactive against staphylococci and Streptococcus pyogenes with constitutive macrolide resistance.10

Post-antibiotic effect (PAE) is the term used to describe suppression of bacterial growth that persists after short exposure of organisms to antibacterials.13–15 This effect is the result of the prior antibacterial exposure rather than to persisting subinhibitory concentrations of the compound. PAE is one of the factors that has a clinical impact on antibacterial dosing regimens, allowing less frequent dosing than with agents with no PAE.13 The post-antibiotic subinhibitory effect (PA-SME), on the other hand, is used to determine the additional effect of subinhibitory concentrations of the agent, which will again increase the effect of the agent and further decrease the dosing frequency.13,16–19

This study examined the PAE and PA-SME of the new ketolide, telithromycin, against selected strains of Gram-positive cocci,
including strains with no macrolide resistance, as well as macrolide-resistant strains with constitutively and inducibly expressed \textit{erm}(A) or \textit{erm}(B) determinants, and streptococci with macrolide efflux mediated resistance associated with \textit{mef}(A) or \textit{mef}(E) determinants.

**Materials and methods**

Bacterial strains selected for the study included four \textit{S. pneumoniae} (macrolide-susceptible; macrolide-resistant-lincosamide-susceptible; and inducible and constitutive macrolide-lincosamide-resistant strains), three \textit{S. pyogenes} (macrolide-resistant-lincosamide-susceptible; and inducible and constitutive macrolide-lincosamide-resistant strains), and three \textit{Streptococcus aureus} (one macrolide susceptible and two inducible macrolide-lincosamide-resistant strains). Resistance phenotypes were identified by disc diffusion methodology, determined with erythromycin \textit{S. pneumoniae} (C) and \textit{S. pyogenes} (B), \textit{S. aureus} (C) as shown in Table 1.3,5,6

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{erm}(B)</td>
<td>\textit{S. pneumoniae}</td>
<td>5′-GAAAAGGTACTCAACCAATA-3′</td>
</tr>
<tr>
<td>\textit{erm}(A) subclass \textit{erm}(TR)</td>
<td>\textit{S. pyogenes}</td>
<td>5′-AGTAACGGTACTTAATTTTGGTAC-3′</td>
</tr>
<tr>
<td>\textit{erm}(A)</td>
<td>\textit{S. aureus}</td>
<td>5′-ACAGAAAAACCCGAAATACG-3′</td>
</tr>
<tr>
<td>\textit{erm}(C)</td>
<td>\textit{S. aureus}</td>
<td>5′-TTGGATATTATTCAGATCAG-3′</td>
</tr>
<tr>
<td>\textit{mef}(A)</td>
<td>\textit{S. pneumoniae}</td>
<td>5′-TCTCAATGTATTATTAATTAGT-3′</td>
</tr>
<tr>
<td>\textit{mef}(C)</td>
<td>\textit{S. pyogenes}</td>
<td>5′-TCAGACATAATATGATAAA-3′</td>
</tr>
</tbody>
</table>

PAE was determined, according to Craig & Gudmundsson, as \( T - C \), where \( T \) = time required for viability counts of an antibiotic-exposed culture to increase by 1 log base 10 above the counts observed immediately after dilution, and \( C \) = corresponding time for the antibiotic-free growth control. Viability counts, expressed as log base 10 cfu/mL, were plotted against time.

For PA-SME determination, the PAE was induced as described above.13,16-19 Following the 1:1000 dilution in broth to remove the antibiotic, three additional tubes were prepared containing subinhibitory concentrations of the compound at 0.125, 0.250 and 0.500 \textmu g/mL; the MIC of the compound for that strain. Tubes were then handled as for PAE determination above. The PA-SME was defined according to Odenholt-Tornqvist et al.17,18 as \( T_{PA} - T_{PAE} - C \), where \( T_{PA} \) = time for cultures previously exposed to antibiotic and then re-exposed to different subinhibitory concentrations to increase by 1 log base 10 above the counts observed immediately after dilution, and \( C \) = corresponding time for the antibiotic-free control.

**Results**

Strains selected for their susceptibility phenotypes and their genetics are shown in Table 2. Ketolide activity was related to macrolide susceptibility, with telithromycin being highly active against the erythromycin A-resistant, clindamycin-susceptible isolates of \textit{S. pneumoniae} and \textit{S. pyogenes} (MICs 0.015–0.125 mg/L) and the erythromycin A-resistant, inducibly clindamycin-resistant isolates (MICs 0.008–0.015 mg/L), but variably active against the erythromycin A-resistant and constitutively clindamycin-resistant isolates (MICs 0.008–4 mg/L). Telithromycin was active against the erythromycin A-susceptible strain of \textit{S. aureus}, as well as against the two erythromycin A-resistant isolates with inducible clindamycin resistance (MICs 0.03 mg/L).

Results of the PAE and PA-SME experiments are shown in Table 2. Regrowth rates of controls exposed to 0.01 \times MICs were identical to those of antimicrobial-free controls, indicating that the 1:1000 dilution step removed all detectable antimicrobial activity and that any residual antimicrobial did not interfere with PAE or PA-SME measurements. The length of the PAE varied from 0.3 to 3.8 h, and was not related to resistance phenotype or species. PAE for the \textit{S. pneumoniae}
Table 2. Results of MIC, PAE and PA-SME experiments with telithromycin against 10 Gram-positive cocci

<table>
<thead>
<tr>
<th>Strain</th>
<th>Resistance phenotypea [genotypeb]</th>
<th>MIC (mg/L)</th>
<th>PAE (h)</th>
<th>0.12×MIC</th>
<th>0.25×MIC</th>
<th>0.5×MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae ATCC 49619</td>
<td>ery^R, cli^R</td>
<td>0.008</td>
<td>2.7</td>
<td>3.9</td>
<td>4.5</td>
<td>5.2</td>
</tr>
<tr>
<td>Streptococcus pneumoniae 5179936</td>
<td>ery^R, cli^R{mef(A)}</td>
<td>0.015</td>
<td>2.5</td>
<td>1.6</td>
<td>3.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Streptococcus pneumoniae 60607</td>
<td>ery^R, cli^R{erm(B)}</td>
<td>0.008</td>
<td>3.8</td>
<td>4.4</td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Streptococcus pneumoniae 95333</td>
<td>ery^R, cli^R{erm(A)} subclass erm(STR)</td>
<td>0.015</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Streptococcus pyogenes 20</td>
<td>ery^R, cli^R{erm(A)}</td>
<td>0.015</td>
<td>2.7</td>
<td>3.3</td>
<td>3.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Streptococcus pyogenes 73</td>
<td>ery^R, cli^R{mef(A)}</td>
<td>0.125</td>
<td>0.8</td>
<td>0.8</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Streptococcus pyogenes 27</td>
<td>ery^R, cli^R{erm(B)}</td>
<td>4.0</td>
<td>0.9</td>
<td>0.9</td>
<td>3.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Staphylococcus aureus 5175400</td>
<td>ery^R, cli^R{erm(A)}</td>
<td>0.03</td>
<td>1.9</td>
<td>2.2</td>
<td>2.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Staphylococcus aureus 5271903</td>
<td>ery^R, cli^R{erm(A)}</td>
<td>0.03</td>
<td>2.4</td>
<td>2.4</td>
<td>2.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Staphylococcus aureus 1A129</td>
<td>ery^R, cli^R{erm(A)}</td>
<td>0.03</td>
<td>0.3</td>
<td>1.9</td>
<td>1.9</td>
<td>2.6</td>
</tr>
</tbody>
</table>

aery^R, cli^R-susceptible to erythromycin A and clindamycin; ery^R, cli^R-resistant to erythromycin A, susceptible to clindamycin, with clindamycin resistance not able to be induced; ery^R, cli^R{erm(A)}, erythromycin A-resistant, inducibly clindamycin-resistant; ery^R, cli^R{erm(B)}, erythromycin A-resistant, constitutively clindamycin-resistant.
bMacrolide resistance gene present in strain.

Discussion

MIC results for the ketolide telithromycin against *S. pneumoniae*, *S. pyogenes* and *S. aureus* were comparable to those described previously for another ketolide, HMR 3004, as well as this compound. Activity against *S. pneumoniae* was not affected by the presence of either *erm(B)* or *mef(A)* genes. However, activity against *S. pyogenes* showed more variability, with the MIC for the strain containing the *mef(A)* gene measuring 0.125 mg/L, and that for one strain with an inducibly expressed *erm(B)* gene measuring 0.015 mg/L, whereas the other strain with a constitutively expressed *erm(B)* gene had an MIC of 4 mg/L. Activity was the same against the two *S. aureus* strains with inducibly expressed *erm(B)* genes as against the macrolide-susceptible strain (MICs 0.03 mg/L). However, telithromycin is generally inactive against strains of staphylococci with constitutively expressed *erm(B)* resistance genes (MICs > 32 mg/L). Ketolides are less active against *S. pyogenes* containing the *erm(B)* gene, either constitutively or inducibly expressed. Inducibly erythromycin A-resistant strains of this species containing *erm(A)* genes are susceptible to ketolides and 16-membered macrolides such as josamycin, although inducible resistance to both groups of agents occurs with some strains.

The PAE was short (0.3–0.9 h) for three of the strains, and longer (1.5–3.8 h) for the remaining strains, with the four *S. pneumoniae* strains all belonging to the latter group. PA-SME values were most consistent for the *S. aureus* strains, but were either the same as or slightly higher than PAE values for two of the strains. PA-SME values for *S. pyogenes* were generally one to five-fold longer than PAE values. For *S. pneumoniae*, PA-SME values were similar to the PAE values for macrolide-resistant strains, and were prolonged by up to two-fold only for the macrolide-susceptible strain.

These results indicate potential benefits of these PAE and PA-SME effects against all three species. For *S. pneumoniae*, the benefit was demonstrated in this study from both effects for macrolide-susceptible strains, and from the PAE for macrolide-resistant strains. Both effects were significant for *S. pyogenes* and *S. aureus* strains with all three common macrolide-resistance determinants, although they were more modest for the latter group. The concentrations used to document PAE and PAE-SME effects were within achievable concentrations of telithromycin for all experiments except for those using the constitutively macrolide-resistant strain of *S. pyogenes*, which had a telithromycin MIC of 4 mg/L.

These results indicate that the dosage regimen of telithromycin might be affected by the combination of its serum half-life and the length of the PAE and/or PA-SME, as these represent the total period of time during which regrowth does not occur. Additional pharmacokinetic/pharmacodynamic and clinical studies will be necessary to validate this hypothesis.

Acknowledgements

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References


