Telavancin: in vitro activity against staphylococci in a biofilm model

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Objectives: To assess the in vitro activity of the novel lipoglycopeptide telavancin against staphylococcal biofilms using an in vitro pharmacokinetic model.

Methods: Using the Sorbarod model, biofilms were established. The strains tested included methicillin-susceptible and -resistant strains of Staphylococcus aureus and coagulase-negative staphylococci, as well as glycopeptide-intermediate S. aureus (GISA). The biofilms were exposed to exponentially decreasing concentrations of telavancin and four comparator antibiotics, vancomycin, teicoplanin, linezolid and moxifloxacin and the bactericidal activity of the antibiotics was assessed. The concentrations of the antibiotics used in these experiments corresponded to peak serum levels achievable in humans and the rates at which drug concentrations were decreased corresponded to their elimination half-lives.

Results: All of the drugs tested produced a reduction in the number of bacteria eluted from the biofilms. Telavancin was more effective than the commercially available glycopeptides, vancomycin and teicoplanin, and of the three, was the most active agent against both the non-GISA and GISA strains. Of all the antibiotics tested, moxifloxacin produced the greatest reduction in biofilm cells, but only against the non-GISA strains.

Conclusions: Telavancin exhibited substantial antimicrobial activity against staphylococcal biofilms, including GISA strains. This study supports the case for the evaluation of telavancin in the treatment of staphylococcal biofilm-associated infections.

Keywords: pharmacokinetic models, glycopeptides, GISA, indwelling medical devices

Introduction

Staphylococci are among the most common causes of both community and hospital-acquired infection, and their incidence has been steadily increasing. More specifically, methicillin-resistant Staphylococcus aureus (MRSA) infections have emerged over the past three decades as a worldwide problem with a consequent increase in the use of the glycopeptide antibiotics. A particular concern has been the isolation of clinical strains of MRSA exhibiting reduced susceptibility to the glycopeptides.

Coagulase-negative staphylococci (CoNS), although an uncommon cause of community-acquired infections, are a well established cause of nosocomial bacteraemia and indwelling medical device (IMD) associated infection. Such infections are frequently associated with adherent biofilms and are difficult to manage. Bacteria found in biofilms are often poorly controlled by antibiotics, which in particular may reflect a low growth rate and in some instances, a failure of the agent to penetrate the biofilm.

A particular problem of IMD-associated infections is that conventional in vitro assessment of growth inhibition in liquid medium often fails to predict performance in vivo. In this study, we have investigated the effects of antibiotics on bacteria grown as biofilms, in a manner that more closely reflects the in vivo situation.

Furthermore, to address the problem of multidrug-resistant staphylococcal infections, a novel lipoglycopeptide, telavancin (TD-6424), which possesses greater bactericidal activity against staphylococci, has been investigated in a pharmacodynamic manner to evaluate its efficiency in controlling staphylococcal biofilms and has been compared with a selection of conventional antibiotics in a biofilm model. This permits the quantification of the effect of antibiotics on bacterial biofilms using exponentially decreasing concentrations of a drug.

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Materials and methods

Organisms

The eight staphylococci studied included: a fully susceptible reference strain of *Staphylococcus aureus* (ATCC 29213); a methicillin-resistant *S. aureus* (MRSA, ATCC 33591); a methicillin-susceptible *S. aureus* (MSSA, MS 01); a methicillin-resistant *Staphylococcus epidermidis* (MRSE, RP 62A); a methicillin-susceptible *S. epidermidis* (MSSE, MS 501) and three glycopeptide intermediate *S. aureus* (GISA) strains (Mu 50 and Mu 3 isolated in Japan and HIP-5836 supplied by Theravance, Inc. (South San Francisco, CA, USA)). Strains MS 01 and MS 501 were clinical isolates from blood cultures collected at the City Hospital, Nottingham, UK.

The following antibiotics were obtained as reference powders: telavancin (Theravance, South San Francisco, CA, USA), linezolid (Pharmacia, Kalamazoo, MI, USA), vancomycin (Sigma, Dorset, UK), teicoplanin (Aventis Pharma, Strasbourg, France) and moxifloxacin (Bayer, Wuppertal, Germany). Stock solutions were prepared and stored according to the recommendations of the British Society for Antimicrobial Chemotherapy (BSAC).

**MIC and MBC determinations**

MICs and MBCs were determined in a 96-well microtitre plate using the BSAC standard microdilution method except Mueller–Hinton broth (MHB; Oxoid, Basingstoke, UK) was used with an inoculum of $10^8$ cfu/mL.

**Biofilm studies**

The biofilm model used in this study was a modification of the Sorbarod model (Figure 1) described previously. Sorbarods (Iacon Ltd, Kent, UK) consist of a compacted concertina of cellulose fibres encased within a cylindrical paper sleeve. The Sorbarod filter is contained within a length of PVC tubing and has bacteria loaded on to it from a syringe. A biofilm is established by inoculating the Sorbarod and incubating overnight at 37°C. Cells attach to the cellulose fibre plug and are perfused with medium (Mueller–Hinton broth) from one side; cells shed from the opposite side are collected and counted by plating out. After the initial loss of loosely attached cells into the eluted medium, a steady state is established in which the adherent biomass and the rate of cell release from the fibres becomes constant. The number of cells eluted from the biofilm reflects the number of actively dividing cells within the biofilm. The model can then be used to quantify the effects of antibiotics on the biofilm cells.

**Pharmacokinetic/pharmacodynamic modelling.** The Sorbarod model (Figure 1) can be used to assess microbial growth and inhibition of cells exposed to antibiotics in a manner that reflects the human pharmacokinetic profiles. Such exposure of biofilms to exponentially decreasing concentrations of antibiotic was achieved by perfusing them with media via a dilution vessel (Figure 2, B) to which drugs had been added. Biofilms were established as described above, with the exception that the tubing carrying the medium from the reservoir to the biofilm went via the dilution vessel (B). Once the biofilms reached a steady state, following overnight incubation, the antibiotic was added directly to the dilution vessel. The biofilm cells were therefore exposed to an exponentially decreasing concentration of the drug. In this study, the rate of decrease was matched to the serum half-life of the antibiotics and the initial drug concentration matched to the maximum serum concentration following the recommended dose, thus mimicking the *in vivo* kinetics of drug administration in humans as closely as possible (see Table 1 for details). The volume of medium required in the dilution vessel was calculated from the equation $t_{1/2} = 0.6931 V/r$, based on first-order decay kinetics (where $V$ = the volume of medium and $r$ = the rate of flow of medium).

Drug exposure can be repeated in accordance with the known half-life of the drug by the addition of the antibiotic to the dilution vessel. The Sorbarods were set up in duplicate and the experiments were repeated three times. Control Sorbarod biofilms were established which were perfused solely with medium and no antibiotic.
Calculation of bactericidal index

The bactericidal index of each drug/bacteria combination was calculated using FigP software. This calculates the AUC for each curve when log₁₀ reduction in viable count is plotted against log₁₀ time (h).12

Results

The susceptibilities (MICs and MBCs) to antibiotics of the bacterial strains tested are shown in Table 2. The results from the exponentially decreasing drug concentration experiments are summarized in Table 3. These show the maximum log₁₀ reduction in bacterial numbers eluted from the biofilms after each dose of antibiotic administered, the sample time at which the maximum reduction occurred and the impact on the number of bacteria seen at the end of the experiment (36 h for vancomycin, teicoplanin and linezolid and 48 h for telavancin and moxifloxacin).

All antibiotics produced a reduction to some degree, in the number of bacteria eluted from the biofilms, however in one case, strain HIP-5836 (GISA) demonstrated no reduction at all after exposure to moxifloxacin. However, there were clear differences between the antibiotics tested, which again varied according to the strain of staphylococci.

Telavancin gave the most consistent and extensive bactericidal effects of the three glycopeptides tested. Furthermore, telavancin showed the greatest reductions at the end of the experiments, although some re-growth was observed. Telavancin was again the most effective glycopeptide against the GISA strains in reducing the number of bacteria eluted from the biofilms. Following the first dose of telavancin, the number of eluted bacteria of the GISA strains HIP-5836, Mu 50 and Mu 3 fell by 3, 3 and 2 log₁₀, respectively. This contrasts with the lesser effects produced by vancomycin (1.0, 0.4 and 0.5 log₁₀) and teicoplanin (0.8 and 0.5 log₁₀). The reductions observed after the second dose (24 h) of telavancin were smaller than those following the first dose. The second (12 h) and third (24 h) doses of vancomycin and teicoplanin produced no effect on any of the strains with the exception of HIP-5836 following exposure to vancomycin where a reduction of 0.8 log₁₀ was observed. At the end of the experiments, all three GISA strains exposed to vancomycin and teicoplanin had recovered to the extent that the biofilms were eluting the same number of bacteria as at the beginning of the experiments. Mu 3 also recovered after exposure to telavancin. However, the other two GISA strains, HIP-5836 and Mu 50, showed reductions of 1.5 and 1.0 log₁₀.

Similar results were obtained with the non-GISA strains after exposure to the glycopeptide drugs to those seen with the GISA strains. Telavancin again demonstrated the most consistent and extensive reductions in the number of bacteria eluted, especially following the first dose. Of the three glycopeptide drugs, telavancin was the only one to inhibit the growth of all non-GISA strains at the end of the experiments.

Of all the antibiotics tested, moxifloxacin produced the greatest reduction in biofilm bacteria, but only against the non-GISA strains (range of 0–6.0 log₁₀) and following the first dose (GISA range 0–1.0 log₁₀). All GISA strains had recovered fully by the end of the experiments. Exposure to moxifloxacin however, resulted in a reduction of the number of bacteria eluted from the biofilms of all of the non-GISA strains except MSSE strain MS 501; at the end of the experiments, these reductions were greater than seen with any of the other antibiotics tested. The reductions obtained with linezolid, following the first dose, were more uniform with a range of 1.1–2.5 log₁₀ and was equally effective against both the GISA and non-GISA strains. All GISA strains had recovered fully by the end of the experiments.

Table 1. Human pharmacokinetic details of the antibiotics studied

<table>
<thead>
<tr>
<th>Antibiotic&lt;sup&gt;a&lt;/sup&gt;</th>
<th>t&lt;sub&gt;½&lt;/sub&gt; (h)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</th>
<th>Dosing interval (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telavancin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0</td>
<td>150</td>
<td>24</td>
</tr>
<tr>
<td>Vancomycin&lt;sup&gt;13&lt;/sup&gt;</td>
<td>8.0</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>Teicoplanin&lt;sup&gt;13&lt;/sup&gt;</td>
<td>50</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>Moxifloxacin&lt;sup&gt;31&lt;/sup&gt;</td>
<td>12.5</td>
<td>2.5</td>
<td>24</td>
</tr>
<tr>
<td>Linezolid&lt;sup&gt;32&lt;/sup&gt;</td>
<td>4.5</td>
<td>16</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup>Source of pharmacokinetic information.  
<sup>b</sup>Data provided by Theravance.

Table 2. MICs and (MBCs) (mg/L) for the bacterial strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (MBC) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>telavancin</td>
</tr>
<tr>
<td>MS 01 S. aureus (MSSA)</td>
<td>0.25 (1)</td>
</tr>
<tr>
<td>ATCC 29213 S. aureus (MSSA)</td>
<td>0.5 (2)</td>
</tr>
<tr>
<td>ATCC 33591 S. aureus (MRSA)</td>
<td>0.5 (1)</td>
</tr>
<tr>
<td>HIP-5836 S. aureus (GISA)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Mu 50 S. aureus (GISA)</td>
<td>0.5 (1)</td>
</tr>
<tr>
<td>Mu 3 S. aureus (GISA)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>MS 501 S. epidermidis (MSSE)</td>
<td>0.03 (0.06)</td>
</tr>
<tr>
<td>RP62A S. epidermidis (MRSE)</td>
<td>0.25 (1)</td>
</tr>
</tbody>
</table>
5 and 4 strains, respectively, which failed to show any reduction after the final doses of vancomycin and teicoplanin.

Table 4 shows the bactericidal indices (BI) of the antibiotics against the bacterial strains used. The greater the BI, the greater the bactericidal activity of the antibiotic. Generally, moxifloxacin gave the highest BI values, but only against susceptible strains; the resistant strains resulted in some of the lowest values. Telavancin gave the next highest range of BI values followed by linezolid and then vancomycin and teicoplanin. Overall, the resistant strains resulted in the lower BIs.

### Discussion

The experiments, performed with exponentially decreasing concentrations of antibiotics, were designed to simulate the parenteral...
administration of the antibiotics to humans; the rate of decrease was calculated to reflect the half-lives of the various drugs tested.

The experiments demonstrated considerable variation in the effects of the various antibiotics on the maximum log_{10} reductions in the number of bacteria eluted from the biofilms. The glycopeptide antibiotics generally elicited a more rapid effect than either linezolid or moxifloxacin following the initial dose. This may be related to the fact that vancomycin and teicoplanin are generally more rapidly bactericidal against staphylococci,14 however, moxifloxacin is also normally recognized to be rapidly bactericidal against susceptible strains.14 Linezolid, on the other hand is considered bacteriostatic against staphylococci.15 The same patterns of response were seen with the GISA and non-GISA strains in the times to maximum reductions and were not related to the MICs.

Some of the observations made will reflect the fact that the drugs were being challenged by bacteria grown as biofilms. It is known that biofilm cells do not behave in a similar fashion to planktonically grown bacteria. Routine susceptibility tests, such as the determination of the MIC, often fail to predict therapeutic success where biofilm-associated infections are involved. For example König et al.16 found that although clinical isolates of coagulase-negative staphylococci were highly susceptible to vancomycin when tested in vitro as planktonic cells, the same organisms were resistant or tolerant to the antibiotic when grown as biofilms. The recalcitrant nature of biofilm infections is well recognized clinically.17

It is important to note that none of the drugs came close to eliminating the bacteria, even though the dosage regimen, which was selected to reflect that used in humans, meant that for much of the experimental period, the concentrations were above the MICs of the bacteria tested. The exceptions were GISA strain Mu 50 and linezolid where the drug was above the MIC (4.0 mg/L) for approximately 9 h of the 48 h experiment and moxifloxacin which failed to exceed the MIC for all GISA strains and MSSE strain MS 501. These findings underscore the challenge of eradicating bacteria growing as biofilms.

The pharmacodynamic properties of the antibiotics tested may provide some explanation of the observed results. Antibiotics frequently demonstrate bactericidal activity that is either concentration- or time-dependent.18,19 In the case of concentration-dependent antibiotics, the parameters that best correlate with bactericidal action are the ratio of area under the concentration curve at 24 h (AUC_{24}) to MIC (AUC_{24}/MIC) and the ratio of maximum serum concentration (C_{max}) to MIC (C_{max}/MIC). In contrast, the pharmacodynamic parameters which best predict bactericidal effect of time-dependent agents varies20 between those antibiotics which exhibit only mild or moderate persistent effects where the important parameter is the time (T) above the MIC (T > MIC), compared with those exhibiting a prolonged persistent effect where the important parameter is AUC_{24}/MIC.18

Among the glycopeptide antibiotics, vancomycin is generally considered to exhibit time-dependent inhibition; however, as it exhibits prolonged persistence effects, AUC_{24}/MIC is the parameter which best correlates with efficacy.20,22–24 In our studies, AUC_{24}/MIC ranged from 0.5 (strain HIP-5836, MIC 16 mg/L) to 7.9 (strains with an MIC of 1 mg/L). The results, i.e. the log_{10} reduction in bacteria eluted from the biofilms, do not correlate with the AUC_{24}/MIC values.

In the case of teicoplanin, the majority of studies have reported time-dependent bactericidal activity22,25–27 although, one recent study suggested a concentration-dependent bactericidal effect.28 In our experiment, the antibiotic concentration was above the MIC for all strains and there was no apparent correlation between the MICs and the maximum reductions observed emphasizing the clear differences between biofilm and planktonically growing cells.

Unlike other glycopeptide antibiotics, telavancin exhibits concentration-dependent activity and the pharmacodynamic parameter associated with efficacy is AUC_{24}/MIC.29 The results obtained demonstrated no distinction between GISA and non-GISA strains and were again unrelated to MIC, although the number of cells eluted from the biofilms was reduced for all strains following both doses of the drug, with the exception of GISA strain HIP-5836 where there was no reduction following the 24 h dose. Additionally, only one strain, GISA strain Mu 3, had recovered fully by the end of the experiment.

Linezolid is reported as showing time-dependent killing.20,30 In our experiments, the antibiotic was above the MIC for all strains (2.0 mg/L) with the exception of GISA strain Mu 50 (MIC of 4.0 mg/L), against which the first dose of antibiotic produced the smallest reduction (1.1 log_{10}) of all the strains tested. At the end of the experiment, three of the eight strains tested failed to recover fully (S. aureus strains MS 01, ATCC 29213 and ATCC 33591). This lack of concordance with published data for planktonically grown cells suggests that linezolid is unlikely to prove effective against biofilm-associated infections.

Moxifloxacin was the only antibiotic studied which is characterized by concentration-dependent killing.20 The pharmacodynamic parameters AUC_{24}/MIC and C_{max}/MIC ranged from

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**Telavancin: in vitro performance**

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Telavancin</th>
<th>Vancomycin</th>
<th>Teicoplanin</th>
<th>Linezolid</th>
<th>Moxifloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS 01</td>
<td>3.52</td>
<td>1.18</td>
<td>1.03</td>
<td>2.25</td>
<td>4.24</td>
</tr>
<tr>
<td>ATCC 29213</td>
<td>2.22</td>
<td>0.65</td>
<td>0.73</td>
<td>1.31</td>
<td>6.35</td>
</tr>
<tr>
<td>ATCC 33591</td>
<td>3.19</td>
<td>0.6</td>
<td>0.81</td>
<td>3.47</td>
<td>7.44</td>
</tr>
<tr>
<td>HIP-5836</td>
<td>3.35</td>
<td>1.06</td>
<td>0.16</td>
<td>1.09</td>
<td>0</td>
</tr>
<tr>
<td>Mu 50</td>
<td>3.32</td>
<td>0.05</td>
<td>0.26</td>
<td>0.92</td>
<td>0.61</td>
</tr>
<tr>
<td>Mu 3</td>
<td>1.92</td>
<td>0.32</td>
<td>0.07</td>
<td>0.14</td>
<td>0</td>
</tr>
<tr>
<td>MS 501</td>
<td>3.32</td>
<td>0.77</td>
<td>1.6</td>
<td>1.78</td>
<td>0.61</td>
</tr>
<tr>
<td>RP62A</td>
<td>3.87</td>
<td>1.28</td>
<td>1.16</td>
<td>2.52</td>
<td>7.15</td>
</tr>
</tbody>
</table>

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0.125 to 16.67 and 0.625 to 83.34, respectively. The findings did support a relationship to the MIC values. Strains with MICs of 4.0 mg/L (the three GISA strains and the MSSE strain MS 501) resulted in the smallest reductions in the number of bacteria eluted from the biofilms and they were also the only strains to show full recovery at the end of the experiment. In contrast, exposure to moxifloxacin demonstrated extensive reductions for all non-GISA strains and showed strong bactericidal activity even after 48 h.

In all the experiments regardless of the agent tested and bacterial strain exposed, the maximum log₁₀ reduction in the number of cells eluted from the biofilms occurred with the first dose. The most likely explanation for this is that the more susceptible cells are killed or inhibited by the first dose in comparison with subsequent doses. Alternatively, it may be that the first dose damages a proportion of the cells which are unable to recover fully before the second dose is administered. To test this theory, sequential MICs would need to be performed on both the biofilm and eluted cells at various times throughout the experiments.

The BI is an analytical tool developed to assess the bactericidal activity of antibiotics, and was calculated for all the drug/bacteria combinations investigated in this study. Using this method of analysis, the antibiotic showing the greatest bactericidal activity against the susceptible strains is moxifloxacin. The two glycopeptides vancomycin and teicoplanin generally had the lowest BIs. Interestingly, it appears that the values of BIs calculated for growth control of bacterial biofilms. Using exponentially decreasing drug concentrations, exposure to moxifloxacin demonstrated extensive reductions for all non-GISA strains and showed strong bactericidal activity after 48 h.

Telavancin: *in vitro* performance


