Comparative activity of the new lipoglycopeptide telavancin in the presence and absence of serum against 50 glycopeptide non-susceptible staphylococci and three vancomycin-resistant

*Staphylococcus aureus*

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**Background:** Telavancin, a new multifunctional lipoglycopeptide antibiotic, exhibits broad-spectrum Gram-positive activity against a variety of pathogens. We examined the effects of human serum and antimicrobial concentrations on the activity of telavancin against glycopeptide-intermediate staphylococcal species (GISS), heteroresistant GISS (hGISS) and three vancomycin-resistant *Staphylococcus aureus* (VRSA) compared with vancomycin, quinupristin/dalfopristin, linezolid and daptomycin.

**Methods:** MIC and MBCs were performed against all antimicrobials. Time–kill experiments were performed using two strains of GISS (Mu50; NJ992) and VRSA (VRSA1; VRSA2) at 1, 2, 4, 8, 16 and 32·MIC. Telavancin and daptomycin were evaluated in the presence and absence of serum.

**Results:** All GISS and hGISS were susceptible to the tested agents with telavancin and quinupristin/dalfopristin demonstrating the lowest MIC, followed by daptomycin, linezolid and vancomycin. Against VRSA, daptomycin and quinupristin/dalfopristin had the lowest MIC, followed by linezolid, telavancin and vancomycin. In the presence of serum, telavancin and daptomycin MICs increased 1- to 4-fold. Concentration-dependent activity was demonstrated by telavancin and daptomycin, in the presence and absence of serum. Telavancin and daptomycin were bactericidal against GISS and VRSA and performed similarly in the presence of serum. Quinupristin/dalfopristin demonstrated bactericidal activity at clinically achievable concentrations, whereas linezolid was bacteriostatic.

**Conclusions:** Telavancin demonstrated concentration-dependent bactericidal activity against GISS, hGISS and VRSA at concentrations equal to or above 4·MIC, which corresponds to therapeutic levels against GISS and clinically achieved concentrations against the VRSA. Similar to daptomycin, telavancin activity was diminished in the presence of serum but bactericidal activity was maintained. Further investigation with telavancin against GISS, hGISS and VRSA is warranted.

Keywords: methicillin-resistant *Staphylococcus aureus*, pharmacodynamics, daptomycin, VRSA

**Introduction**

*Staphylococcus aureus* has long been recognized as a serious pathogen responsible for a variety of human infections. Since the first report of methicillin resistance in the early 1960s, resistance associated with this pathogen has continued to increase worldwide.1,2 Until recently, all *S. aureus* remained susceptible to vancomycin, the first clinically available glycopeptide. The increase in
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Incidence of methicillin-resistant *S. aureus* (MRSA) worldwide has led to a dramatic rise in the use of vancomycin. An apparent consequence of sustained vancomycin prescribing pressure has been the development of a variety of staphylococci with reduced susceptibilities to vancomycin. These include glycopeptide-intermediate susceptible staphylococcal species (GISS) with MICs of 8–16 mg/L, heteroresistant staphylococcal species (hGISS) with MICs of 1–4 mg/L, and, the most worrisome, vancomycin-resistant *S. aureus* (VRSA), with MICs of 1024, 32, 64 and 256 mg/L, which have been reported recently.

Telavancin, a new lipoglycopeptide antimicrobial agent, is being investigated as a potential alternative treatment for resistant Gram-positive bacterial pathogens. Telavancin is rapidly bactericidal and demonstrates concentration-dependent effects. Recent pharmacokinetic data in healthy volunteers demonstrate achievable serum concentrations between 80 and 155 mg/L with a half-life of around 8 h, allowing for convenient once-daily administration. Similar to the approved glycopeptides vancomycin and teicoplanin, telavancin inhibits peptidoglycan synthesis. However, unlike these agents, telavancin also perturbs bacterial plasma membrane function, including dissipation of membrane potential and increases in permeability. This intramolecular synergy has been proposed as a possible mechanism to minimize the development of further resistance in these organisms.

Studies *in vitro* have demonstrated that telavancin possesses bactericidal activity with post-antibiotic effects against many strains of Gram-positive organisms including MRSA and GISS. Both telavancin and daptomycin have been noted to be highly protein bound, 93% and 92%, respectively. Pharmacokinetic studies in healthy volunteers along with documentation of concentration-dependent killing support once-daily administration.

The primary objective of the present study was to compare the *in vitro* activity of telavancin against GISS, hGISS and VRSA with those of vancomycin, linezolid, quinupristin/dalfopristin and daptomycin. Additionally, the effects of protein on the bactericidal properties at varying concentrations of telavancin were investigated.

**Materials and methods**

**Bacterial strains and antimicrobials**

Fifty clinical isolates of GISS and hGISS comprising 37 *S. aureus*, 9 *Staphylococcus epidermidis* and 4 *Staphylococcus haemolyticus* along with 3 VRSA isolates (VRSA_M1, VRSA_R1 and VRSA_NV) were obtained from Keiichi Hiramatsu (Japan), Centers for Disease Control and Prevention (CDC), Detroit Medical Center and the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA). Telavancin powder was obtained from Theravance, Inc., South San Francisco, CA, USA. Vancomycin analytical powder was commercially purchased from Sigma Chemical Company, St Louis, MO, USA. Linezolid (Pfizer) and quinupristin/dalfopristin (King Pharmaceuticals Inc.) were obtained commercially. Daptomycin powder was obtained from Cubist Pharmaceuticals, Lexington, MA, USA.

**Medium**

Mueller–Hinton broth (MHB; Difco Laboratories, Detroit, MI, USA) supplemented with magnesium (12.5 mg/L) and calcium (25 mg/L) (SMHB) was used for vancomycin, linezolid, quinupristin/dalfopristin and telavancin microdilution susceptibility testing and time–kill experiments. MHB, adjusted to contain the normal physiological calcium concentration (1.1–1.3 mmol/L), was used for all daptomycin experiments. Sigma S-7023 Human Serum Albumin (Sigma-Aldrich, St Louis, MO, USA) at 50% v/v was combined with SMHB for telavancin and daptomycin susceptibility and time–kill experiments. Processing of the serum included heating to 56°C for 1 h and then passage through 0.8, 0.5 and 0.22 micron filters. Brain–heart infusion agar (BHI; Becton–Dickinson, Sparks, MD, USA) for the representative GISS and VRSA isolates was used for bacterial quantification of samples from time–kill experiments.

**Susceptibility testing**

The MIC and MBC for each isolate was determined using microdilution technique with an inoculum of 5 × 10^6 cfu/mL according to the Clinical and Laboratory Standards Institute guidelines and incubated for 24 h at 35°C. Samples (5 μL) from visually clear wells were plated onto BHI plates for the determination of MBCs, and all samples were incubated for 24 h at 35°C. MICs and MBCs were determined in duplicate. Telavancin and daptomycin MICs and MBCs in addition were determined in the presence of SMHB or SMHB-Ca^2+ (daptomycin) combined with 50% human serum.

**Time–kill curves**

Time–kill experiments were performed in triplicate for all antibiotics against two GISS and two VRSA strains. Time–kill curve experiments for telavancin and daptomycin were performed in the presence and absence of serum. Using a starting bacterial density of 1 × 10^8 cfu/mL during mid-exponential phase, the organism was diluted to 5 × 10^7 cfu/mL into each of the different growth media and the growth control. Mid-exponential phase was determined by obtaining spectrometer readings of OD ~0.3 at 625 nm, which corresponds to ~1 × 10^5 cfu/mL. Telavancin, along with each of the other drugs, was tested at concentrations of 1×, 2×, 4×, 8×, 16× and 32× the respective MIC against the GISS and VRSA. Various concentrations of telavancin were used to evaluate organism killing along with the extent of protein binding and concentration dependent activity of the compound. Aliquots (0.1 mL) were removed from cultures at 0, 1, 4, 8 and 24 h and diluted in 0.9% sodium chloride for colony counting. Colony counts were performed on BHI using an automatic spiral plater (DW Scientific; Frederick, MD, USA) followed by incubation at 35°C for 24 h. We determined these methods to have a lower limit of reliable detection of 2 log_{10} cfu/mL. Time–kill curves were constructed by plotting mean colony counts (log_{10} cfu/mL) versus time. In order to account for antibiotic carryover, all samples were diluted and/or filtered sufficiently prior to plating, therefore reducing the antibiotic concentration below the MIC of the drug. Bactericidal activity was defined as a >3 log_{10} cfu/mL reduction in bacterial density (99.9% kill) from the starting inoculum. Time to 99.9% kill (T_{99.9}) was determined by linear regression of the sample points if r^2 ≥ 0.95 or by visual inspection.

**Statistical analysis**

All statistical analyses were performed using SPSS statistical software (release 11.5.2.1; SPSS, Inc. Chicago, IL, USA). Colony counts at 24 h were compared between groups using one-way ANOVA followed by Tukey’s post hoc test for multiple comparisons. A P value of ≤0.05 indicated statistical significance.
Results

Susceptibility results

Telavancin, daptomycin and quinupristin/dalfopristin demonstrated potent activity against all GISS and hGISS organisms tested. All organisms were susceptible to linezolid as well. Results for MIC50 and MIC90, the MIC range and MBC ranges are shown in Table 1. The MIC and MBC for the two GISS and VRSA utilized in the time–kill analysis are summarized in Table 2. In the presence of serum the MIC of telavancin was noted to increase 2- to 8-fold (Table 1) regardless of the organism. Like telavancin, daptomycin also demonstrated an increase in MICs (1- to 4-fold) in the presence of human serum.

Table 2. MICs/MBCs for all 50 GISS and hGISS strains

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>MICb (mg/L)</th>
<th>MBC range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Linezolid</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Quinupristin/dalfopristin</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Daptomycin + serum</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Telavancin</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Telavancin + serum</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

*aIncludes all staphylococci species: 37 strains of Staphylococcus aureus, 9 of S. epidermidis and 4 of S. haemolyticus.

**50% and 90%, MICs at which 50 and 90 per cent of strains are inhibited, respectively.

Table 2. MICs/MBCs for the strains used in time–kill analysis

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Mu50 MIC (MBC) mg/L</th>
<th>NJ992 MIC (MBC) mg/L</th>
<th>VRSA*MIC (MBC) mg/L</th>
<th>VRSA*PA MIC (MBC) mg/L</th>
<th>VRSA*NY MIC (MBC) mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>4 (4)</td>
<td>8 (8)</td>
<td>1024 (&gt;2048)</td>
<td>32 (64)</td>
<td>64 (128)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1 (32)</td>
<td>1 (2)</td>
<td>1 (16)</td>
<td>2 (4)</td>
<td>0.25 (0.5)</td>
</tr>
<tr>
<td>Quinupristin/dalfopristin</td>
<td>0.5 (0.5)</td>
<td>0.5 (1)</td>
<td>0.5 (4)</td>
<td>0.25 (0.5)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0.25 (0.25)</td>
<td>0.25 (0.25)</td>
<td>0.125 (0.125)</td>
</tr>
<tr>
<td>Daptomycin + serum</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>0.5 (1)</td>
<td>0.25 (0.5)</td>
<td>0.25 (0.25)</td>
</tr>
<tr>
<td>Telavancin</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>4 (8)</td>
<td>2 (2)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Telavancin + serum</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>32 (32)</td>
<td>8 (8)</td>
<td>4 (4)</td>
</tr>
</tbody>
</table>

*Not used in time–kill analysis.

Discussion

Antibiotic protein binding and its impact on clinical outcomes has been a controversial subject for a number of years. Biologically, only free drug is available to interact with the targeted bacterial cells, and therefore the per cent free fraction has been utilized.
in most pharmacodynamic simulations. It has been observed in vitro that the susceptibility profiles of drugs that are highly protein bound (i.e. ≥90%) are affected by the presence of serum or albumin. Although highly variable, a 1- to 8-fold increase in MICs in the presence of serum has been described for antibiotics such as teicoplanin, daptomycin and ceftriaxone against S. aureus.\textsuperscript{21–23} In addition, the degree and association of binding affinity may also play a role. For example, a drug that has a high degree of binding affinity may be less available at the site of infection.

Previous investigations have demonstrated that telavancin’s protein binding in human serum is ~93%.\textsuperscript{10} In the present study, telavancin MIC in the presence of serum increased on average 2-fold. Despite the increase in MIC, this effect had no

\begin{table}
\centering
\caption{GISS change from baseline (log\textsubscript{10} cfu/mL) at 24 h\textsuperscript{d}}
\begin{tabular}{cccccc}
\hline
\times MIC & V & QD & L & D & T \\
\hline
1 & $-1.47$ & 0.25$^d$ & 1.25 & 2.23 & 2.05$^e,f$ \\
2 & $-2.71$ & -0.13 & -0.05 & 2.23 & -1.99$^c$ \\
4 & -3.30 & -2.16 & -3.19 & -4.12 & -4.04$^d$ \\
8 & -4.26 & -4.21 & -4.27 & -4.16 & -4.10 \\
16 & -4.23 & -4.07 & -4.33 & -4.12 & -4.12 \\
32 & -4.23 & -4.07 & -4.33 & -4.12 & -4.12 \\
\hline
\end{tabular}
\begin{tabular}{cccccc}
\hline
\times MIC & V & QD & L & D & T \\
\hline
1 & -2.34 & -0.97 & 2.08 & 1.01 & 1.22$^{a,e}$ \\
2 & -2.26 & -2.84 & 0.56 & 0.20 & 0.16$^{a,e}$ \\
4 & -2.88 & -3.80 & -4.05 & -3.92 & -4.07 \\
8 & -3.98 & -3.92 & -4.09 & -3.90 & -4.25 \\
16 & -4.01 & -4.13 & -4.12 & -3.91 & -4.26 \\
\hline
\end{tabular}
\end{table}

\textbf{V}, vancomycin; QD, quinupristin/dalfopristin; L, linezolid; D, daptomycin; T, telavancin.

\textit{a}$^d$Note that positive values indicate regrowth.

\begin{table}
\centering
\caption{VRSA change from baseline (log\textsubscript{10} cfu/mL) at 24 h\textsuperscript{i}}
\begin{tabular}{cccccc}
\hline
\times MIC & V & QD & L & D & T \\
\hline
1 & 1.84$^a$ & 1.98 & 2.04 & 1.93 & 0.80$^d$ \\
2 & 1.76 & 1.88 & 1.95 & 1.94 & -1.51$^d$ \\
4 & 1.71 & -3.05 & -1.31 & 2.59 & -4.13$^d$ \\
8 & 1.40 & -4.00 & -1.55 & -3.92 & -4.13$^b$ \\
16 & -0.70 & -4.17 & -4.19 & -3.96 & -4.18 \\
32 & -2.79 & -4.17 & -4.17 & -4.03 & -4.06 \\
\hline
\end{tabular}
\begin{tabular}{cccccc}
\hline
\times MIC & V & QD & L & D & T \\
\hline
1 & -1.05 & 1.75 & 0.41 & 1.97 & -1.80$^d$ \\
2 & -1.80 & 0.77 & -2.56 & 1.87 & -2.00$^{h}$ \\
4 & -2.06 & -3.28 & -3.24 & -3.98 & -2.89$^b$ \\
8 & -2.30 & -3.61 & -4.20 & -4.01 & -3.99 \\
16 & -2.40 & -4.91 & -4.16 & -4.14 & -4.16 \\
32 & -2.90 & -3.82 & -4.11 & -4.10 & -4.19 \\
\hline
\end{tabular}
\end{table}

\textbf{V}, vancomycin; QD, quinupristin/dalfopristin; L, linezolid; D, daptomycin; T, telavancin.

\textit{a}$^h$Note that positive values indicate regrowth.

\begin{table}
\centering
\caption{Change from baseline at 24 h with 50% v/v serum (log\textsubscript{10} cfu/mL)$^a$}
\begin{tabular}{cccccc}
\hline
\times MIC & D & T & D & T & D & T \\
\hline
1 & 0.25$^a$ & -1.10$^a$ & -2.33 & -3.88$^*$ & 1.07 & -2.16$^*$ \\
2 & -2.41 & -3.10 & -3.09 & -3.90 & 0.55 & -4.20$^*$ \\
4 & -4.18 & -4.11 & -3.98 & -3.94 & 0.96 & -4.07$^*$ \\
8 & -4.18 & -4.14 & -4.00 & -3.91 & -1.38 & -4.18$^*$ \\
16 & -4.19 & -4.18 & -4.03 & -4.10 & -3.98 & ND \\
32 & -4.22 & -4.03 & -4.04 & -4.06 & -4.09 & ND \\
\hline
\end{tabular}
\begin{tabular}{cccccc}
\hline
\times MIC & D & T & D & T & D & T \\
\hline
1 & 0.81 & -4.10$^*$ & 0.81 & -4.10$^*$ & 0.69 & -4.11$^*$ \\
2 & 0.72 & -4.23$^*$ & 0.72 & -4.23$^*$ & 0.72 & -4.23$^*$ \\
4 & -1.31 & -4.16$^*$ & -1.31 & -4.16$^*$ & -1.31 & -4.16$^*$ \\
16 & -4.08 & ND & -4.08 & ND & -4.08 & ND \\
32 & -4.18 & ND & -4.18 & ND & -4.18 & ND \\
\hline
\end{tabular}
\end{table}

\textbf{D}, daptomycin plus serum; T, telavancin plus serum; ND, not done.

\textit{a}$^i$Note that positive values indicate regrowth.

\textit{b}MIC values determined in presence of 50% v/v serum were used.
impact on bactericidal activity as demonstrated by kill curves at concentrations of 8× MIC or greater. This may be due to a weaker protein binding association constant than predicted by protein binding experiments.\textsuperscript{10,24} Alternatively, the second mechanism (an effect on bacterial membrane integrity) may be less affected by protein binding, as suggested by Hegde \textit{et al.}\textsuperscript{25} Notably, the concentration-dependent activity of telavancin was apparent both in the presence and absence of serum.

\textbf{Figure 1.} Sample time–killing curves at 8× MIC. (a) Mu50; (b) NJ992; (c) VRSA\textsubscript{ATCC}; and (d) VRSA\textsubscript{AP}. Filled circles, growth control; open circles, growth control in serum; filled upside-down triangles, vancomycin; open triangles, linezolid; filled squares, quinupristin/dalfopristin; open squares, daptomycin; filled diamonds, daptomycin in serum; open diamonds, telavancin; and filled triangles, telavancin in serum.

\textbf{Figure 2.} Concentration-dependent bactericidal activity of telavancin against Mu50. (a) Telavancin alone; (b) telavancin with 50\% human serum. Filled circles, growth control; open circles, 1× MIC; filled upside-down triangles, 2× MIC; open triangles, 4× MIC; filled squares, 8× MIC; open squares, 16× MIC; and filled diamonds, 32× MIC.
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The present study evaluated telavancin and comparator agents against a large number of GISS, hGISS and VRSA clinical isolates. The susceptibility data against GISA strains are consistent with previous studies evaluating telavancin, daptomycin and linezolid. In the present study, telavancin demonstrated concentration-dependent activity against GISA, hGISS and VRSA clinical isolates. Telavancin’s activity appeared similar to other antimicrobials against the GISA and hGISS as exhibited by killing curve experiments. Some differences between activities were noted against the VRSA compared with daptomycin at serum concentrations achievable with a 12.5 mg/kg dose of telavancin. Similar to daptomycin, the rate of telavancin killing was decreased in the presence of human serum; however, bactericidal activity was maintained. Further investigation of telavancin as an alternative treatment for GISA, hGISS and VRSA infections is warranted.

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Transparency declarations

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References