Impact of Glycopeptide Resistance in *Staphylococcus aureus* on the Dalbavancin In Vivo Pharmacodynamic Target

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ABSTRACT

Dalbavancin is a novel lipoglycopeptide with activity against *S. aureus*, including glycopeptide resistant isolates. The *in vivo* investigation reported here tested the impact of infection higher MICs for this antibiotic against 7 *S. aureus* isolates, including several vancomycin-intermediate strains. A 1-log and 2-log kill was achieved against 6 and 7 of the isolates. The mean free drug AUC/MICs for net stasis, 1-log kill and 2-log kill were 27.1, 53.3, and 111.1, respectively.

MANUSCRIPT TEXT

The increasing rates of resistance among both hospital- and community-acquired bacterial pathogens such as *Staphylococcus aureus*, coagulase-negative staphylococci, and enterococci have prompted attempts to discover new antimicrobials with activities against multidrug-resistant gram-positive pathogens (1-6). Dalbavancin is a new lipoglycopeptide antibiotic with activity against multidrug-resistant gram-positive organisms (4, 7-9). In addition to enhanced antimicrobial potency, the compound possesses a unique pharmacokinetic profile that includes an extremely prolonged elimination half-life of more than 1 week (10-12). Clinical development of the compound has thus far demonstrated success for the treatment of skin and soft tissue and of catheter-related bloodstream infections (13-18). Once weekly administration of the dosing regimens used in these trials has been shown to produce free-drug trough concentrations exceeding the MIC₉₀ₛ of gram-positive pathogens from large surveillance databases (17, 19-23).
The current studies were designed to define the pharmacodynamics target for dalbavancin against *S. aureus* strains with dalbavancin MICs at or above the current FDA breakpoint (≥0.12 μg/mL) and some of which were vancomycin intermediate (VISA) (24-29). The results from these studies provide a pharmacodynamic rationale in support of the current clinical dosing regimens. Furthermore, the data provide a starting point for the development of revised susceptibility breakpoints for this new compound.

Seven strains of *Staphylococcus aureus* (including 4 vancomycin intermediate *S. aureus* [VISA]) were studied (Table 1). The MIC values of dalbavancin and vancomycin were determined in triplicate using CLSI reference broth microdilution methodology in the presence P80 (30). The dalbavancin MIC range for the *S. aureus* isolates was 0.12 to 0.50 μg/mL. Animals were maintained in accordance with the criteria of the American Association for Accreditation of Laboratory Animal Care. All animal studies were approved by the Animal Research Committee of the William S. Middleton Memorial VA Hospital. The neutropenic murine thigh infection model was used for all studies. Mice were inoculated with 10⁷ CFU/ml of each strain. Single-dose plasma pharmacokinetic studies were performed with thigh-infected mice given intraperitoneal doses (0.2 ml/dose) of dalbavancin (2.5, 10, 40, 80 and 160 mg/kg). Dalbavancin plasma concentrations were measured by an LC/MS/MS assay (Figure 1). The lower limit of quantification for the assay was 0.05 μg/ml. Sample analysis QC precision (%CV) ranged from 5% to 6.4% and accuracy (%bias) ranged from -3.5% to -10.0%. Peak levels were observed by 2-6 h. Dalbavancin exhibited relatively linear pharmacokinetics based upon the dose-AUC relationship. The half-life was prolonged and varied from 4.1-9.31 h. A protein binding value of 98.4% was utilized based upon prior studies in this model (31).

The in vivo virulence of the *S. aureus* isolates was similar in the untreated control mice based upon the increase in thigh burden over the treatment period, 2.30 ± 0.14 log₁₀ CFU/thigh. Two
hours after infection, dalbavancin was administered via the intraperitoneal route with one of seven two-fold-escalating intraperitoneal doses of dalbavancin every 12 h (2.5, 5, 10, 20, 40, 80, and 160 mg/kg) for a six day treatment period. Untreated control groups were sampled at the start of therapy and end of study. The thighs were removed from each animal and immediately processed for CFU determination. The results of these studies were analyzed by using the sigmoid dose-effect model (32). The magnitude of the PK/PD index associated with each endpoint dose was calculated from the following equation: 

\[
\log_{10} D = \log_{10} \left( \frac{E}{(E_{\text{max}} - E)} \right) / (N + \log_{10} ED_{50}),
\]

where \( E \) is the control growth for the static dose \( D \), \( E \) is the control growth - 1 log unit for a \( D \) of 1-log killing, and \( E \) is the control - 2 log units for a \( D \) of 2-log killing.

A 1-log and 2-log kill was achieved against 7 and 6 of the isolates, respectively (Figure 1A and Table 2). The dalbavancin in vivo exposure response data was also considered relative to the PK/PD linked driver, AUC/MIC, using free drug concentrations. Drug accumulation was calculated and included in AUC estimates. Using a sigmoid Emax model the data fit was strong for the seven strain dataset (\( R^2 \) 0.86) as shown in Figure 2B. The numeric AUC/MIC values associated with each of the three treatment endpoints are also shown in Table 2. Net stasis was observed with a dalbavancin fAUC/MIC value near 25. fAUC/MIC values near 50 and 100 were associated with 1- and 2-log reductions in organism burdens in the neutropenic mice, respectively.

These PK/PD targets are lower than those observed previously with wild-type \( S. \) aureus strains in this same model(31). This is in part due to lower pharmacokinetic values measured in the present study, perhaps due to differences in the drug assay method. Of note, the present kinetic study included a robust sampling scheme and a more sensitive and accurate drug assay method compared to the prior animal model investigation. Specifically, we used specific LC-MS/MS assay compared to the prior bioassay. The treatment studies were otherwise similar.
with regard to animal species, neutropenia, antibiotic (dalbavancin), drug preparation, route of 
administration, treatment duration, study endpoints, and data analysis.

The present studies were designed to discern the PK/PD impact of infection with less common, 
*S. aureus* that had dalbavancin MICs at or above the current dalbavancin FDA breakpoint 
(<0.12 µg/mL). Dalbavancin demonstrated potent in vivo activity against *S. aureus* strains with 
higher MICs, including those exhibiting a VISA phenotype. While it will be important to 
corroborate these preclinical findings with data from patients, consideration of the AUC/MIC 
target from these studies in the context of human pharmacokinetics would suggest a safe 
treatment margin against these higher MIC isolates. If one considers the steady state kinetics 
of dalbavancin in patients relative to the stasis, 1 and 2 log AUC/MIC targets in this study, the 
MIC breakpoints would be revised to 4, 2, and 1 µg/ml, respectively.
Table 1. Study Strains and Dalbavancin In vitro Susceptibility

<table>
<thead>
<tr>
<th>S. aureus Isolate</th>
<th>Dalbavancin MIC (mg/L)</th>
<th>Vancomycin MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSI653</td>
<td>0.25</td>
<td>2</td>
</tr>
<tr>
<td>LSI1848</td>
<td>0.12</td>
<td>2</td>
</tr>
<tr>
<td>LSI1854</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>LSI1856</td>
<td>0.25</td>
<td>4 VISA</td>
</tr>
<tr>
<td>LSI1857</td>
<td>0.25</td>
<td>4 VISA</td>
</tr>
<tr>
<td>LSI1861</td>
<td>0.25</td>
<td>4 VISA</td>
</tr>
<tr>
<td>LSI1862</td>
<td>0.5</td>
<td>4 VISA</td>
</tr>
</tbody>
</table>

Table 2. In vivo efficacy of dalbavancin against select S. aureus isolates using AUC/MIC as the predictive pharmacodynamic index

<table>
<thead>
<tr>
<th>Strain</th>
<th>Static Dose</th>
<th>1 Log Kill</th>
<th>2 Log Kill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24h Dose (mg/kg)</td>
<td>24h fAUC/MIC</td>
<td>24h Dose (mg/kg)</td>
</tr>
<tr>
<td>LSI1848</td>
<td>15.17</td>
<td>56.49</td>
<td>31.45</td>
</tr>
<tr>
<td>LSI1861</td>
<td>13.55</td>
<td>25.00</td>
<td>24.63</td>
</tr>
<tr>
<td>LSI1857</td>
<td>14.34</td>
<td>26.59</td>
<td>26.72</td>
</tr>
<tr>
<td>LSI1854</td>
<td>15.00</td>
<td>13.95</td>
<td>35.80</td>
</tr>
<tr>
<td>LSI1862</td>
<td>12.64</td>
<td>11.60</td>
<td>32.92</td>
</tr>
<tr>
<td>LSI653</td>
<td>14.93</td>
<td>27.77</td>
<td>27.20</td>
</tr>
<tr>
<td>LSI1856</td>
<td>15.21</td>
<td>28.32</td>
<td>30.88</td>
</tr>
<tr>
<td>Mean</td>
<td><strong>14.41</strong></td>
<td><strong>27.10</strong></td>
<td><strong>29.94</strong></td>
</tr>
<tr>
<td>Median</td>
<td><strong>14.93</strong></td>
<td><strong>26.59</strong></td>
<td><strong>30.88</strong></td>
</tr>
<tr>
<td>SD</td>
<td><strong>0.98</strong></td>
<td><strong>14.62</strong></td>
<td><strong>3.93</strong></td>
</tr>
</tbody>
</table>

SD represents standard deviation
Figure 1. Plasma pharmacokinetics of dalbavancin in mice following intraperitoneal administration. Each symbol represents the mean and standard deviation from three mice. The drug concentration values presented represent total (protein bound and unbound) drug. The AUC values represent 0 to infinity.

Figure 2: A. In vivo dose effect of dalbavancin against seven select S. aureus isolates using a neutropenic mouse thigh model. Each symbol represents the mean and standard deviation from four thighs. B. In vivo dose effect of dalbavancin against seven S. aureus isolates using a neutropenic mouse thigh model. Each symbol represents the mean and standard deviation from four thighs. The dalbavancin exposure is expressed as the free drug 24-h AUC/MIC. The burden of organisms was measured at the start and end of therapy. The horizontal line at 0 represents the burden of organisms in the thighs of mice at the start of therapy. Data points below the line represent killing and points above the line represent growth. The $R^2$ represents the coefficient of determination. The ED$_{50}$ represents the AUC/MIC associated with 50% of the maximal effect ($E_{max}$), and $N$ is the slope of the relationship or the Hill coefficient. The line drawn through the data points is the best fit line based upon the sigmoid $E_{max}$ formula.
REFERENCES


