Evaluation of Telavancin Activity versus Daptomycin and Vancomycin against Daptomycin-Nonsusceptible \textit{Staphylococcus aureus} in an \textit{In Vitro} Pharmacokinetic/Pharmacodynamic Model

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Daptomycin-nonsusceptible (DNS) \textit{Staphylococcus aureus} strains have been reported over the last several years. Telavancin is a lipoglycopeptide with a dual mechanism of action, as it inhibits peptidoglycan polymerization/cross-linking and disrupts the membrane potential. Three clinical DNS \textit{S. aureus} strains, CB1814, R6212, and SA-684, were evaluated in an \textit{in vitro} pharmacokinetic/pharmacodynamic (PK/PD) model with simulated endocardial vegetations (starting inoculum, $10^{6.5}$ CFU/g) for 120 h. Simulated regimens included telavancin at 10 mg/kg every 24 h ($t_{1/2}$, 8 h), vancomycin at 1 g q12h (peak, 30 mg/liter; $t_{1/2}$, 6 h), and daptomycin at 6 mg/kg (peak, 95.7 mg/liter; $t_{1/2}$, 8 h). Differences in CFU/g between regimens at 24 through 120 h were evaluated by analysis of variance with a Tukey’s post hoc test. Bactericidal activity was defined as a $\geq3$-log$_{10}$ CFU/g decrease in colony count from the initial inoculum. MIC values were 1, 0.25, and 0.5 mg/liter (telavancin), 4, 2, and 2 mg/liter (daptomycin), and 2, 2, and 2 mg/liter (vancomycin) for CB1814, R6212, and SA-684, respectively. Telavancin displayed bactericidal activities against R6212 (32 to 120 h; $-4.31$ log$_{10}$ CFU/g), SA-684 (56 to 120 h; $-3.06$ log$_{10}$ CFU/g), and CB1814 (48 to 120 h; $-4.9$ log$_{10}$ CFU/g). Daptomycin displayed initial bactericidal activity followed by regrowth with all three strains. Vancomycin did not exhibit sustained bactericidal activity against any strain. At 120 h, telavancin was significantly better at reducing colony counts than vancomycin against all three tested strains and better than daptomycin against CB1814 ($P < 0.05$). Telavancin displayed bactericidal activity \textit{in vitro} against DNS \textit{S. aureus} isolates.

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MATERIALS AND METHODS

\textbf{Bacterial strains.} A total of three clinical DNS \textit{S. aureus} isolates were evaluated: SA-684 (a MRSA strain recovered from a patient during therapy for tricuspid endocarditis; provided by G. W. Kaatz, J. Dingell VA Hospital, Detroit, MI); CB1814 (a methicillin-susceptible \textit{S. aureus} isolate from the daptomycin bacteremia and endocarditis clinical trial); R6212 (a heteroresistant vancomycin-intermediate \textit{S. aureus} [hVISA] isolate from Detroit Medical Center) (17). In all isolates, the DNS was stable to 5 serial passages on tryptic soy agar and was confirmed by daptomycin population analysis (data not shown).

\textbf{Antimicrobials.} Telavancin (Theravance, Inc., South San Francisco, CA) was provided by the manufacturer. Daptomycin was commercially purchased (Cubist Pharmaceuticals). Vancomycin was obtained from Sigma Chemical Company (St. Louis, MO).

\textbf{Media.} Mueller-Hinton broth II (Difco, Detroit, MI) with 25 mg/liter calcium and 12.5 mg/liter magnesium (MHB II) was used for all in vitro PK/PD models used to evaluate telavancin and vancomycin. Supplemented Mueller-Hinton broth (SMHB) supplemented to 75 mg/liter calcium (equivalent to 50 mg/liter of calcium in the presence of albumin) was used for all in vitro PK/PD models due to the dependency of daptomycin on calcium.
on calcium for antimicrobial activity (1, 19). MHB II and agar (Bacto; Difco, Detroit, MI) supplemented with 50 mg/liter of calcium were used for population analysis and daptomycin drug plates. Brain heart infusion agar (Difco, Detroit, MI) was used for vancomycin drug plates. Colony counts were determined using tryptic soy agar (TSA; Difco, Detroit, MI) plates.

**Susceptibility testing.** MICs were determined by broth microdilution to 10^-6 according to the Clinical and Laboratory Standards Institute guidelines (7). All samples were incubated at 35°C for 24 h.

**SEVs.** Simulated endocardial vegetations (SEVs) were prepared as previously described (1, 5, 6, 15, 20, 29, 32–34). Organism stocks were prepared by inoculating three TSA plates with lawns for overnight growth at 35°C. Organisms were swabbed from the growth plates into 5-ml test tubes of SMHB, resulting in a concentration of approximately 10^10 CFU/ml. SEVs were prepared in 1.5-ml siliconized Eppendorf tubes by mixing 0.05 ml of diluted organism suspension (final inoculum, 8.5 log_{10} CFU/0.5 ml), 0.5 ml of cryoprecipitated human antihemophilic factor from volunteer donors (American Red Cross, Detroit, MI), and 0.025 ml of platelet suspension (platelets mixed with normal saline; 250,000 to 500,000 platelets per clot). A volume of 0.05 ml of bovine thrombin (5,000 units/ml; GenTrac, Inc., Middleton, WI) was added to each tube after insertion of a sterile monofilament line into the mixture. The resultant simulated vegetations were then transferred from the Eppendorf tubes by using a sterile disposable plastic needle (Becton Dickinson, Sparks, MD) and introduced into the model. This methodology resulted in each SEV consisting of approximately 3 to 3.5 g/dl of albumin and 6.8 to 7.4 g/dl of total protein.

**In vitro PK/PD model.** A two-compartment in vitro model consisting of a 250-ml two-compartment glass apparatus with ports, in which the SEVs were suspended, was utilized for all simulations (1, 5, 6, 15, 20, 29, 32–34). The apparatus was prefilled with medium, and antibiotics were administered as boluses over a 120-h time period into the central compartment via an injection port. The model apparatus was placed in a 37°C water bath throughout the procedure, and a magnetic stir bar was placed in the medium for thorough mixing of the drug in the model. Fresh medium was continuously supplied and removed from the compartment along with the drug via a peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, IL) set to simulate the half-lives of the antibiotics. Simulated antibiotic regimens included telavancin at 10 mg/kg every 24 h (peak, 95.7 mg/liter; average half-life, 8 h), and vancomycin or daptomycin to assess the development of resistance. Plates were examined for growth after 24 to 48 h of incubation at 35°C. Since we observed additional drug carryover in the SEVs for telavancin (due to lower MIC values), the SEV samples for telavancin could not be plated directly onto MHA to assess the development of drug resistance. Therefore, the telavancin population analysis was performed on the initial isolate and the isolate at 120 h to assess any shifts in the population susceptibility from baseline.

**Statistical analysis.** Changes in CFU/g at 24, 48, 72, 96, and 120 h were compared by two-way analysis of variance with Tukey’s post hoc test. A P value of ≤0.05 was considered significant. All statistical analyses were performed using SPSS Inc. (Chicago, IL) statistical software (release 10.07).

**RESULTS**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>TLV</td>
</tr>
<tr>
<td>CB1814</td>
<td>1</td>
</tr>
<tr>
<td>R6212</td>
<td>0.25</td>
</tr>
<tr>
<td>SA-684</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*TLV, telavancin; DAP, daptomycin; VAN, vancomycin.*
get, 8 h) values of 101.8 to 111.1 μg/ml and 7.1 to 9.1 h, respectively.

The quantitative changes in the log10 CFU/g for the tested regimens against the three strains are displayed in Fig. 1A to C. As shown, telavancin displayed sustained bactericidal activities against all three strains. Against CB1814, telavancin displayed sustained bactericidal activity (~4.9 log10 CFU/g at 120 h) and a statistically significantly greater reduction in CFU/g at 120 h than daptomycin or vancomycin (P < 0.05). Telavancin also displayed early (32 h) and sustained bactericidal activity (~4.31 log10 CFU/g at 120 h) against R6212. Against SA-684, telavancin maintained bactericidal activity at 120 h (~3.06 log10 CFU/g). Vancomycin did not display sustained bactericidal activity against any strain. At 120 h, telavancin reduced the CFU/g significantly more than vancomycin against R6212 and SA-684 (P < 0.05). Daptomycin displayed initial bactericidal activity followed by regrowth for all three strains. No isolates with additional nonsusceptibility to daptomycin or resistance to vancomycin were recovered. Evaluation of isolates by telavancin population analysis revealed similar profiles before and after exposure to telavancin in the SEV in vitro PK/PD model, with a slight shift for SA-684 (Fig. 2).

**DISCUSSION**

The optimal treatment for DNS *S. aureus* infections remains to be defined, and current treatment options are based on limited evidence (4, 21). Increasing the treatment challenges associated with these infections, DNS *S. aureus* is most commonly found in deep-seated high-inoculum infections, such as osteomyelitis, septic arthritis, and endocarditis, which require long-term treatment (4). In this study we evaluated the activity of the new lipoglycopeptide telavancin against DNS *S. aureus* in an *in vitro* PK/PD model of simulated endocardial vegetations. This *in vitro* model incorporates a high inoculum of bacteria embedded into human fibrin and platelets, which are subsequently exposed to antibiotics dosed to achieve a simulation of human pharmacokinetics over the course of the 5-day evaluation period. Under these experimental conditions, telavancin displayed bactericidal activities against all three strains tested and was statistically significantly more active than vancomycin. This additional activity compared to vancomycin is likely attributable to the dual mechanism of action of telavancin. Daptomycin displayed activity initially against all strains tested; however, regrowth occurred due to DNS.

The mechanism by which *S. aureus* develops nonsusceptibility to daptomycin is not fully understood; however, it appears to be due to a series of incremental changes commonly but not universally found in all DNS *S. aureus* strains (8, 12, 14, 16, 17, 26, 35–37). In general, DNS *S. aureus* strains are associated with increased cell surface charge, increased cell wall thickness, changes in membrane fluidity, and decreased cytoplasmic membrane depolarization by daptomycin (11, 25, 26). The increase in positive cell surface charge is hypothesized to decrease daptomycin activity via repulsion of the active positively charged daptomycin-Ca2+ complex and inhibition of daptomycin-induced membrane perturbation (18). Mutations in the mprF gene leading to overexpression of the MprF protein contribute to increased positive surface charge via translocation of positively charged phospholipids to the outer side of the cytoplasmic membrane and by lysinylation of membrane phosphatidylglycerol (11, 26). Increased D-alanylation of cell wall teichoic acids via increased expression of the dltABCD operon also contributes to the increased positive surface charge (35). An increase in cell wall thickness, which is most commonly observed in isolates with concurrent decreased susceptibility to vancomycin, may contribute to DNS via an affinity trapping mechanism similar to vancomycin in VISA strains (8–10, 36).

Our results demonstrate telavancin’s activity against daptomycin-nonsusceptible strains of *S. aureus*. Indeed, the bactericidal activi-

**FIG 1** Activity of telavancin, daptomycin, and vancomycin against CB1814 (A), R6212 (B), and SA-684 (C). Telavancin, open triangles; daptomycin, open squares; vancomycin, open circles; growth control, filled circles; dashed line, telavancin limit of accuracy.
ties and decreases in colony counts in this study are similar to results of a previously published study examining the activity of telavancin against MRSA, hVISA, and VISA isolates (20). It is unknown, based on current data, however, if telavancin would maintain activity against DNS S. aureus isolates that were also VISA, as these strains were not included in either study.

Possible limitations of this study include its short duration and lack of strains displaying reduced susceptibility to both vancomycin and daptomycin. It is possible that the study period of 120 h (5 days) was not sufficient to elicit the full impact of a telavancin-DNS S. aureus interaction that might occur with longer exposures. As DNS in S. aureus has been associated with reduced susceptibility to vancomycin, it is likely that the activity telavancin displayed against DNS S. aureus in this study does not extrapolate to all strains of DNS S. aureus (24). In conclusion, telavancin displays bactericidal activity against DNS S. aureus and is more active than vancomycin. Further research is warranted to explore telavancin’s activities against these strains.

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**REFERENCES**


