Vancomycin and Daptomycin Pharmacodynamics Differ against a Site-Directed Staphylococcus epidermidis Mutant Displaying the Small-Colony-Variant Phenotype

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Catheter-related bloodstream infections due to slow-growing Staphylococcus epidermidis small-colony variants (SCVs) are extremely difficult to treat. Daptomycin and vancomycin pharmacodynamics were evaluated against a site-directed hemB mutant of S. epidermidis displaying the SCV phenotype and compared to that of the parental strain. The maximal killing effect decreased by 7.7-fold for vancomycin and 1.5-fold for daptomycin against the SCV mutant and were well characterized by a Hill-type mathematical model ($R^2 > 0.97$).

Staphylococcus epidermidis is the most common pathogen involved in catheter-related bloodstream infections (CRBSIs) (8). Clinical experience demonstrates that agents with proven in vitro activity are often unable to cure these infections when infected-device salvage is attempted. The persistent and often recurrent course of device-associated infections has been linked, in part, to the ability of S. epidermidis to establish adherent, multilayered biofilms on surfaces of inserted or implanted foreign bodies (15–17). In addition, infections caused by these pathogens are extremely difficult to treat due to the emergence of multidrug resistance and reduced susceptibility to vancomycin (5, 8).

A number of recent foreign-body-associated infections due to coagulase-negative staphylococcal small-colony variants (SCVs), including several pacemaker-related infections, have been described (2, 9, 13, 16). Reduced susceptibility and tolerance to a variety of antimicrobials, including aminoglycosides, trimethoprim-sulfamethoxazole, and vancomycin, have been described and may complicate management of infections due to phenotypic variants. While antibiotic killing activity and pharmacodynamic parameters have been studied in detail for Staphylococcus aureus SCVs (3, 9, 12), data on such phenotypic variants of Staphylococcus epidermidis are missing. Since SCVs recovered from clinical specimens have been genetically undefined and exhibit a high rate of reversion to the large-colony form, a clinically derived, site-directed S. epidermidis mutant was constructed by interrupting one of the hemin-biosynthetic genes, hemB, in S. epidermidis by inserting an ermB cassette into hemB (1). Therefore, this S. epidermidis mutant along with the corresponding parent strain with normal phenotype was utilized as a tool to compare vancomycin and daptomycin pharmacodynamic parameters.

The bacterial strains utilized in this study were S. epidermidis O-47 and its respective hemB mutant O-47::ermB, which exhibits a stable SCV phenotype (1, 6). The construction of the mutant and its characteristics were previously described (1). Analytical-grade daptomycin powder was obtained from Cubist Pharmaceuticals, Lexington, MA. Analytical-grade vancomycin powder was obtained from Sigma Chemical Co., St. Louis, MO. Fresh working solutions of daptomycin and vancomycin were made prior to each experimental run. Mueller-Hinton broth (Difco, Detroit, MI) supplemented with 25 mg/liter calcium and 12.5 mg/liter magnesium was utilized for all experiments involving vancomycin; Mueller-Hinton broth supplemented with 50 mg/liter calcium and 12.5 mg/liter magnesium was utilized for all experiments involving daptomycin. MICs were determined by quadruplicate broth microdilution techniques in accordance with standards of the Clinical and Laboratory Standards Institute (4). Time-kill experiments were performed as previously described (12). The following concentrations for daptomycin and vancomycin were evaluated: 0, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 mg/liter against a starting inoculum of S. epidermidis of approximately $10^7$ CFU/ml. Samples were withdrawn for determination of bacterial counts at 0, 2, 4, 8, and 24 h. All time experiments were completed in duplicate.

An integrated pharmacokinetic-pharmacodynamic area measure (log ratio area) was applied to all CFU data as previously described (13), using equation 1. The traditional approach (log ratio change) was also used (equation 2). See reference 13 for more details.

$$\log \text{ratio area} = \log_{10}\left(\frac{\text{AUCFU}_{\text{drug}}}{\text{AUCFU}_{\text{growth control}}}\right) \quad (1)$$

$$\log \text{ratio change} = \log_{10}\left(\frac{\text{CFU}_{24\,\text{h}}}{\text{CFU}_{0\,\text{h}}}\right) \quad (2)$$

A four-parameter concentration-effect Hill-type model was fit to the effect parameter using Systat (version 12; Systat Software Inc., San Jose, CA) as previously described (12) and equation 3.
AU CFU<sub>drug</sub> is the number of CFU of the culture with drug (in arbitrary units), AU CFU<sub>growth control</sub> is the number of CFU of the culture with controlled growth, the dependent variable (E) is either the log ratio area or the log ratio change, E<sub>0</sub> is the measured effect at a zero drug concentration, E<sub>max</sub> is the maximal effect, C is the concentration of drug, EC<sub>50</sub> is the concentration for which there is a 50% maximal effect, and H is the Hill or sigmoidicity constant.

Against <i>S. epidermidis</i> strain O-47 and its <i>hemB</i> mutant, which displays the SCV phenotype, the MICs of vancomycin were 2.0 and 4.0 mg/liter and of daptomycin were 0.25 and 0.25 mg/liter, respectively. The antibacterial activities and pharmacodynamics of vancomycin and daptomycin against the O-47 strain and the <i>hemB</i> mutant are displayed in Fig. 1. Vancomycin achieved bactericidal activity against the parent strain with
a normal phenotype at concentrations of >4 mg/liter, which occurred at 24 h. At higher concentrations, vancomycin demonstrated concentration-independent killing, with apparent thresholds of 4 and 8 mg/liter, after which increases in drug concentration did not result in subsequent increases in killing activity. Daptomycin displayed bactericidal activity against the parent strain at concentrations of >2 mg/liter. However, with daptomycin, a greater concentration-dependent trend was observed, with increasing concentrations resulting in greater reduction in bacterial colonies. In contrast, vancomycin achieved little activity against the hemB mutant, which displayed the SCV phenotype, even at a concentration of 128 mg/liter, with maximal reductions of less than 1 log in bacterial counts after 24 h. However, for daptomycin, although early killing of the mutant was attenuated, with concentrations of >16 mg/liter achieving bactericidal activity by 8 h, all concentrations of >4 mg/liter were able to achieve bactericidal activity at 24 h.

Model-fitted parameter estimates for vancomycin and daptomycin against both strains are shown in Table 1. Model fits for the Hill-type model to the data were excellent, with all coefficients of determination \( R^2 \) being >0.97. There was a difference in the pharmacodynamic activities of both drugs against the strains with the two different phenotypes, with the \( E_{\text{max}} \) and \( E_{\text{C50}} \) of vancomycin and daptomycin decreasing for the mutant may potentially be explained, in part, by the strain’s enhanced adhesive properties, as hemB mutants have been shown to express increased amounts of polysaccharide intercellular adherin and stronger adhesion properties (1, 3, 11). This increased adhesion may potentially contribute to enhanced biofilm properties and cell wall sequestration mechanisms hampering antimicrobial activity. Interestingly, vancomycin was previously shown to display little activity against mexitillin (meticillin)-resistant Staphylococcus aureus embedded in biofilm, whereas daptomycin achieved bactericidal activity against biofilm-embedded methicillin-resistant Staphylococcus aureus (10). Additionally, as the SCV phenotype is characterized by its slow growth, it has previously been demonstrated that vancomycin ceases to achieve bactericidal activity in stationary-phase S. aureus but that daptomycin’s bactericidal activity is not hampered (7, 14). The current findings are also consistent with previous studies of hemB mutants of S. aureus where vancomycin failed to achieve bactericidal activity (12). Taken together with the results of other studies, these findings may provide additional insight into the mechanisms of vancomycin tolerance and allow us to explore potential alternatives against difficult-to-treat, persistent infections. It is important to note that the definitive treatment of S. epidermidis SCV infections may involve surgical-device removal, which is frequently performed in clinical practice, as these results may apply only to situations of device salvage, such as with infected prosthetic valves or pacemakers. Although only one stable hemB SCV mutant of S. epidermidis has been constructed to date, further investigations with additional stable SCV mutant clinical isolates are necessary to confirm these findings before these results can be applied to clinical practice.

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REFERENCES


