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The accessory gene regulator is a quorum-sensing operon which coordinates the expression of secreted and cell-associated virulence factors and controls several metabolic pathways in Staphylococcus aureus in a growth phase-related fashion (4, 5, 7, 8). S. aureus strains which exhibit a dysfunction accessory gene regulator (agr) locus may possess an intrinsic survival advantage under vancomycin-selective pressure (11–14). However, the correlation between vancomycin exposure using the area under the concentration-time curve (AUC/MIC) necessary to suppress the development of vancomycin-intermediate resistance and agr function or group has not been investigated. We examined the relationship between vancomycin exposure and the development of intermediate-level resistance in agr groups I, II, III, and IV using an in vitro pharmacodynamic model. All agr groups developed intermediate resistance to vancomycin after subtherapeutic exposure. The free unbound fraction of the area under the concentration-time curve (AUC/MIC) required to suppress resistance was fourfold higher (P < 0.001) in agr dysfunctional strains (112 to 169) than that in parent wild-type strains (28).

Simulated therapeutic vancomycin exposures were evaluated against agr wild-type and knockout Staphylococcus aureus groups I, II, III, and IV using an in vitro pharmacodynamic model. All agr groups developed intermediate resistance to vancomycin after subtherapeutic exposure. The free unbound fraction of the area under the concentration-time curve (AUC/MIC) required to suppress resistance was fourfold higher (P < 0.001) in agr dysfunctional strains (112 to 169) than that in parent wild-type strains (28).

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RN6390, RN6607, RN3984, and RN4850 represent agr-positive S. aureus prototype strains carrying agr groups I, II, III, and IV, respectively, and were obtained from the Network on Antimicrobial Resistance in Staphylococcus aureus (NARSA), supported under NIAID/NIH contract N01-AI-95359, and Richard P. Novick. RN6911, RN9120, and RN9121 are the agr-null derivatives of groups I, II, and IV (NARSA), respectively. RN3984-M is the null derivate of RN3984, which demonstrated a loss of agr function by a lack of production of delta-hemolysin.

Vancomycin analytical-grade powder was commercially purchased (Sigma, St. Louis, MO). Stock solutions were freshly prepared at the beginning of each week and kept frozen daily at −4°C. Mueller-Hinton broth (Difco, Detroit, MI) supplemented with 25 μg/ml calcium and 12.5 μg/ml magnesium (SMHB) was used for in vitro pharmacodynamic models and susceptibility testing involving vancomycin. Colony counts were determined using tryptic soy agar (TSA; Difco, Detroit, MI) plates. MICs were determined by broth microdilution in SMHB according to CLSI guidelines (2). The function of the agr operon was qualitatively assessed in all isolates using S. aureus RN4420 (NARSA) for delta-hemolysin expression, as previously described (13).

An in vitro pharmacodynamic model was utilized as previously described for the collection of bacterial and antimicrobial dosing and sampling (1). All model simulations were conducted over 72 h and were performed in triplicate to ensure reproducibility. Vancomycin regimens of 62.5, 125, 250, 500, 750, and 1,000 mg every 12 h (free unbound fraction of the AUC [AUC/MIC/24] exposures of 14 to 225 μg/ml/h; half-life 6 h) were simulated according to the manufacturer’s recommendations for a patient with normal renal function. A protein binding level of 55% was utilized for all model simulations (9). Antibiotic concentrations were determined from each model at 0 to 72 h for pharmacokinetic analysis. The AUC from 0 to 24 h was calculated using the linear trapezoid method. Vancomycin concentrations were determined by fluorescence polarization immunoassay (TDx; Abbott Diagnostics) as previously described (16). Differences between regimens in log10 CFU/ml at 72 h were determined using analysis of variance with Tukey’s test for multiple comparisons. All statistical analyses were performed using SPSS statistical software (Release 12.0; SPSS, Inc., Chicago, Illinois).

The development of resistance was evaluated at multiple time points throughout the simulation at 0, 24, 48, and 72 h for all model simulations. Resistance was determined by the detection of growth on TSA plates containing 3 and 6 μg/ml and by changes in MIC via Etest of all model samples.

All of the characterized S. aureus strains evaluated were susceptible to vancomycin MICs of 1.0 μg/ml. Observed pharmacokinetic parameters for all tested therapeutic regimens are shown in Table 1. Quantitative changes in log10 CFU/ml over 72 h are graphically displayed in Fig. 1. Changes in MIC sec-
against all agr-null strains (agr group I, II, III, and IV), the exposure of an fAUC/MIC of 14 resulted in a subsequent increase in MIC at 72 h. In agr-null group II, S. aureus increases to a MIC of 8 μg/ml were noted secondary to exposure as high as an fAUC/MIC of 112. Parent wild-type strains (agr groups I, II, III, and IV [RN6390, RN6607, RN3984, and RN8450, respectively]) also resulted in the development of intermediate resistance after exposure to an fAUC/MIC of 14, although the magnitude of MIC increases were lower compared with that in agr-null isolates: MIC increases of 3 to 4 μg/ml were noted in agr-positive isolates relative to increases of 6 to 8 μg/ml in agr-null isolates. The vancomycin exposure necessary to suppress the development of resistance in S. aureus agr knockout isolates was fourfold greater with fAUC/MICs of 112 to 169 versus an fAUC/MIC of 28 for wild-type, agr-positive isolates (P < 0.001).

Recently, prolonged administration of vancomycin has been linked with vancomycin tolerance and down-regulation of the agr locus. In addition, S. aureus exposure to trough vancomycin concentrations of <10 μg/ml has been associated with an increase in the MIC and development of glycopeptide-intermediate S. aureus-like characteristics (15a, 17). In the present investigation, we sought to determine whether a relationship exists between the development of heterogeneous resistance among all wild-type and knockout agr isolates using an in vitro pharmacodynamic model simulating vancomycin therapeutic dosing. S. aureus isolates that were dysfunctional in agr required vancomycin doses and fAUC/MICs more than fourfold higher than that necessary for wild-type isolates to prevent the development of resistance. Interestingly, although we observed MIC increases of up to 3 to 4 μg/ml (which may be currently classified as reduced susceptibility) in wild-type, agr-positive isolates, the alterations in MIC in agr dysfunctional isolates

TABLE 1. Pharmacokinetic and pharmacodynamic parameters obtained with in vitro models

<table>
<thead>
<tr>
<th>Targeted fAUC/MIC</th>
<th>Simulated regimen</th>
<th>Achieved fAUC/MIC</th>
<th>Cmax/MIC</th>
<th>Cmin/MIC</th>
<th>Half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>225</td>
<td>1,000</td>
<td>240.3 ± 8.8</td>
<td>18.8 ± 2.0</td>
<td>5.1 ± 0.8</td>
<td>6.1 ± 0.3</td>
</tr>
<tr>
<td>169</td>
<td>750</td>
<td>175.9 ± 10.8</td>
<td>13.7 ± 0.6</td>
<td>3.4 ± 0.5</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>112</td>
<td>500</td>
<td>118.8 ± 6.6</td>
<td>9.6 ± 0.6</td>
<td>2.3 ± 0.3</td>
<td>6.5 ± 0.8</td>
</tr>
<tr>
<td>56</td>
<td>250</td>
<td>51.4 ± 1.6</td>
<td>4.3 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>6.5 ± 0.2</td>
</tr>
<tr>
<td>28</td>
<td>125</td>
<td>32.2 ± 6.5</td>
<td>2.3 ± 0.3</td>
<td>0.6 ± 0.1</td>
<td>5.9 ± 0.2</td>
</tr>
<tr>
<td>14</td>
<td>62.5</td>
<td>16.8 ± 2.3</td>
<td>1.2 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>6.8 ± 0.5</td>
</tr>
</tbody>
</table>

a Cmax, maximum concentration of drug in serum; Cmin, minimum concentration of drug in serum. Values (μg/ml) are shown as means ± standard deviations unless indicated otherwise.

b Values are expressed as micrograms per millilter.

c Values are expressed as milligrams per every 12 h.

FIG. 1. Representative activity of vancomycin at simulated regimens over 72 h against agr group II (RN6607 agr II wild type and the null derivate RN9120). Error bars indicate standard deviations.
were typically at least onefold higher at 6 to 8 μg/ml at the same vancomycin exposure. Therefore, in all agr dysfunctional isolates, the development of intermediate resistance was evident after subtherapeutic vancomycin exposure using both the previous (≤4 μg/ml) and newer breakpoints of susceptibility (≤2 μg/ml) (2). Additionally, although we found the development of resistance in all agr dysfunctional groups, the magnitude of increase in vancomycin MIC was greatest in the agr-nul l group II isolate. This is in agreement with previous reports that this genotype may possess an intrinsic survival advantage (6, 15), as this isolate demonstrated the largest increase in MIC (8 μg/ml), and the greatest exposure of vancomycin was necessary to suppress the development of resistance.

We conclude that the development of vancomycin-intermediate resistance may be driven by suboptimal vancomycin exposure in the setting of dysfunction in the agr locus in S. aureus. These findings suggest the use of more aggressive vancomycin-dosing strategies to maintain optimal exposures. These findings highlight the potential problems associated with suboptimal vancomycin exposures, which ultimately impact the susceptibility of this antibiotic with possible consequences to other classes of antimicrobials (3, 10, 11, 17). More attention to optimal dosing of vancomycin may be important in managing methicillin-resistant Staphylococcus aureus infections.

REFERENCES


