Evaluation of Ceftaroline Activity against Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus* and Vancomycin-Intermediate Methicillin-Resistant *S. aureus* Strains in an *In Vitro* Pharmacokinetic/Pharmacodynamic Model: Exploring the “Seesaw Effect”

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A “seesaw effect” in methicillin-resistant *Staphylococcus aureus* (MRSA) has been demonstrated, whereby susceptibility to β-lactam antimicrobials increases as glyco- and lipopeptide susceptibility decreases. We investigated this effect by evaluating the activity of the anti-MRSA cephalosporin ceftaroline against isogenic pairs of MRSA strains with various susceptibilities to vancomycin in an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) model. The activities of ceftaroline at 600 mg every 12 h (q12h) (targeted free maximum concentration of drug in serum [\(C_{\text{max}}\], 15.2 µg/ml; half-life \([t_{1/2}]\), 2.3 h) and vancomycin at 1 g q12h (targeted \([C_{\text{max}}]\), 18 µg/ml; \([t_{1/2}]\), 6 h) were evaluated against 3 pairs of isogenic clinical strains of MRSA that developed increased MICs to vancomycin in patients while on therapy using a two-compartment hollow-fiber PK/PD model with a starting inoculum of \(~10^7\) CFU/ml over a 96-h period. Bacterial killing and development of resistance were evaluated. Expression of penicillin-binding proteins (PBPs) 2 and 4 was evaluated by reverse transcription (RT)-PCR. The achieved pharmacokinetic parameters were 98 to 119% of the targeted values. Ceftaroline and vancomycin were bactericidal against 5/6 and 1/6 strains, respectively, at 96 h. Ceftaroline was more active against the mutant strains than the parent strains, with this difference being statistically significant for 2/3 strain pairs at 96 h. The level of PBP2 expression was 4.4× higher in the vancomycin-intermediate *S. aureus* (VISA) strain in 1/3 pairs. The levels of PBP2 and PBP4 expression were otherwise similar between the parent and mutant strains. These data support the seesaw hypothesis that ceftaroline, like traditional β-lactams, is more active against strains that are less susceptible to vancomycin even when the ceftaroline MICs are identical. Further research to explore these unique findings is warranted.

Ceftaroline (CPT) is an anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) cephalosporin approved by the U.S. Food and Drug Administration as the prodrug ceftaroline-fosamil (CPT-F) for the treatment of community-acquired bacterial pneumonia and acute bacterial skin and skin structure infections. CPT inhibits cell wall synthesis by irreversibly binding penicillin-binding proteins (PBPs) 1 to 3, including the mutated PBP2a, which confers methicillin resistance, but like most β-lactams, CPT has minimal affinity for PBP2a (1–4). CPT maintains activity against MRSA isolates with reduced susceptibility to vancomycin (VAN) and daptomycin (DAP), including heteroresistant VAN-intermediate *S. aureus* (hVISA), VISA, VAN-resistant *S. aureus* (VRSA), and DAP-nonsusceptible *S. aureus* (DNSSA) (5, 6). Experiments evaluating CPT activity in a previously described *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) hollow fiber model against MRSA isolates with reduced susceptibility to VAN or DAP have suggested CPT may exhibit enhanced activity against hVISA and VISA compared to that in VAN-susceptible *S. aureus* (VSSA) (7, 8). Although this observation was unrelated to the objectives of these studies, it was noted with all of the hVISA and VISA strains, with the exception of Mu3, which is not susceptible to CPT.

The previously described “seesaw” effect in MRSA, in which isolates show increased oxacillin (OXA) susceptibility as VAN susceptibility decreases, may contribute to the enhanced bactericidal activity of CPT against hVISA and VISA isolates (9, 10). While the exact mechanism for increases in OXA susceptibility in the presence of the *mecA* gene is not fully elucidated, genetic and/or metabolic modifications in the expression or nature of PBPs due to VAN or DAP pressure may contribute (11). Comparisons of both unrelated clinical strains and *in vitro*-derived pairs have revealed a decrease or absence of PBP4 in VISA strains compared to VAN-susceptible MRSA (11–13). Studies examining both *in vitro* and clinical pairs have found increases in either the amount of PBP2, levels of expression of PBPs, or degree of PBP2 activity in VISA or VISA-like strains compared to those in VAN-susceptible *S. aureus* strains (13–15). A decrease in PBP4, to which CPT has minimal affinity, coupled with the increase in PBP2, to which CPT has high affinity, may explain the enhanced activity of β-lactams, including CPT, in hVISA and VISA strains.

The objective of the present study was to investigate the poten-

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performed (NP) on known VISA strains. Vancomycin population analysis was not considered positive for the hVISA phenotype. Vancomycin population analysis profiles (PAPs) were calculated by dividing the AUC of the test organism by the AUC of hVISA strain Mu3. A VAN PAP AUC ratio of 0.9 is considered positive for the hVISA phenotype (16).

Materials and Methods

Bacterial strains. Three isogenic clinical MRSA strain pairs, which developed reduced susceptibility to VANC and/or DAP, were evaluated. The hVISA strain Mu3 was used as a reference strain for population analysis experiments as previously described (16). The VISA strain Mu50 was also included in the CPT population analysis experiments as a comparator. The strain information is summarized in Table 1.

Antimicrobials and media. CPT was provided by its manufacturer (Forest Laboratories, Inc., New York, NY), and VAN and DAP were commercially purchased (Sigma-Aldrich Co., St. Louis, MO, and Cubist Pharmaceuticals, Lexington, MA, respectively). Mueller-Hinton broth (MHB; Difco, Detroit, MI) with 25 mg/liter calcium and 12.5 mg/liter magnesium was used for all in vitro experiments. MHB supplemented to 50 mg/liter of calcium was used for DAP MIC testing. Colony counts were determined using tryptic soy agar (TSA; Difco) plates. Brain heart infusion agar (BHA; Difco) plates, supplemented with VAN or CPT, were used for resistance screening and population analysis experiments. Antibiotic medium agar 11 (BHA; Difco, Detroit, MI) was used for bioassays performed for pharmacokinetic analysis.

SUSCEPTIBILITY TESTING

The MICs of study antimicrobial agents were determined by broth microdilution (BMD) according to Clinical and Laboratory Standards Institute (CLSI) guidelines and by Etest (17). All samples were incubated at 35°C for 24 h before the MICs were read.

Modified PAP. A bacterial suspension of $1 \times 10^8$ CFU/ml was plated with an automatic spiral-plating device (WASP; DW Scientific, West Yorkshire, United Kingdom) onto freshly prepared BHIA plates containing 0.6 to 2.5 µg/ml of CPT or 0.25 to 8 µg/ml of VAN (7, 16). After 48 h of incubation at 35°C, colony counts were determined using an automated colony counter (ProtoCOL; Synoptics, Ltd., Frederick, MD). The lower limit of detection for colony counts is 2 log10 CFU/ml. Colony counts were plotted against increasing concentrations of VAN or CPT, and the areas under the curve (AUC) of the resultant curves were calculated using SigmaPlot (version 10.0; Systat Software, Inc., San Jose, CA). For VAN population analysis profiles (PAPs), AUC ratios were calculated by dividing the AUC of the test organism by the AUC of hVISA strain Mu3, which was determined on the same day. Any strain with an AUC ratio of $\geq 0.9$ was considered positive for the hVISA phenotype (16).

Table 1. Vancomycin phenotypes, susceptibilities, and population analyses of the strains tested in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>MIC (µg/ml) by BMD (MIC by Etest)</th>
<th>VAN PAP AUC ratioa</th>
<th>CPT PAP AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>JH1</td>
<td>VSSA</td>
<td>1 (2) 0.5 (0.5) 0.25 (0.38)</td>
<td>0.8247</td>
<td>7.95</td>
</tr>
<tr>
<td>JH9</td>
<td>VISA</td>
<td>8 (6) 0.5 (0.5) 1 (1.5)</td>
<td>NP</td>
<td>7.45</td>
</tr>
<tr>
<td>R6911</td>
<td>hVISA</td>
<td>2 (4) 0.5 (0.5) 2 (1.5)</td>
<td>1.2528</td>
<td>10.13</td>
</tr>
<tr>
<td>R6913</td>
<td>VISA</td>
<td>4 (8) 0.5 (0.5) 4 (8)</td>
<td>NP</td>
<td>8.99</td>
</tr>
<tr>
<td>T51643</td>
<td>VSSA</td>
<td>1 (2) 1 (1) 0.5 (0.5)</td>
<td>0.774</td>
<td>12.21</td>
</tr>
<tr>
<td>H9749-2</td>
<td>hVISA</td>
<td>2 (3) 1 (0.5) 0.5 (1.5)</td>
<td>1.167</td>
<td>10.32</td>
</tr>
<tr>
<td>Mu3</td>
<td>hVISA</td>
<td>2 (3) 2 (1.5)</td>
<td>13.42</td>
<td>11.34</td>
</tr>
<tr>
<td>Mu50</td>
<td>hVISA</td>
<td>4 (4) 1.75 (0.75)</td>
<td>8.16</td>
<td></td>
</tr>
</tbody>
</table>

a The AUC ratio is relative to the PAP AUC of Mu3. A VAN PAP AUC ratio of $\geq 0.9$ is considered positive for the hVISA phenotype.
FIG 1 Activity of simulated drug regimens tested against each isogenic strain pair in the in vitro hollow fiber PK/PD model.
RESULTS

Susceptibility and population analysis data for each pair are summarized in Table 1. The CPT MICs were 0.5 or 1 μg/ml, with no differences between the parent and mutant strains for any of the three isogenic pairs by standard BMD methods. VAN MICs were 1 or 2 μg/ml in parents and increased to 2 to 8 μg/ml in VISA and/or hVISA derivatives by standard BMD methods. DAP MICs were 0.25/1 μg/ml, 2/4 μg/ml, and 0.5/0.5 μg/ml for the parent/derivative isogenic strain pairs, respectively, by standard BMD methods. Etest MICs were higher than the BMD values for VAN but were similar for CPT and DAP.

The average observed $f_{c_{\text{max}}}$ values for VAN and CPT were 21.5 ± 0.2 μg/ml (target, 18 μg/ml) and 14.63 ± 0.3 μg/ml (target, 15.2 μg/ml), respectively. The average observed half-lives for VAN and CPT were 5.4 ± 0.16 h (target, 6 h) and 2.27 ± 0.26 h (target, 2.3 h), respectively. The VAN $f_{\text{AUC}_{0-24}}$ was 218 ± 3.25 mg · h/ml. For CPT, the free-drug times above the MIC were 91.35% of the dosing interval for the strains with a MIC of 0.5 μg/ml and 72.3% for the strains with a MIC of 1 μg/ml.

Bacterial survival against each antimicrobial regimen over time for each strain pair is summarized in Fig. 1A to C. CPT was significantly more active against the mutant strain than the parent strain by 96 h in 2/3 pairs and maintained bactericidal activity against 5/6 strains by 96 h. VAN was bacteriostatic against 5/6 strains by 96 h. VAN, at a simulated dose of 1 g intravenous (i.v.) every 12 h, was less active than CPT in this model against all strains except for one VAN-susceptible MRSA strain (T-51643). VAN was significantly more active against JH1 (MIC, 1 μg/ml) than its VISA mutant, JH9 (MIC, 8 μg/ml), and was somewhat more active against T-51643 than its hVISA derivative, H9749-2, but was similarly active against the hVISA-VISA pair R6911 and R6913. The emergence of resistance was not detected from any of the models.

VAN PAP was conducted on non-VISA strains in order to identify hVISA. R6911 and H9749-2 were both positive for the hVISA phenotype, as indicated by a PAP AUC ratio to hVISA control strain Mu3 of ≥0.9 (Table 1) (16). CPT population analysis was also performed in order to describe heterogeneous susceptibility to CPT (Fig. 2). Values representing heterogeneity of susceptibility to CPT and VAN, reported as PAP AUC or AUC ratios to Mu3, are listed in Table 1. The CPT AUC values were higher in the more VAN-susceptible than in the less-susceptible mutant strain in each pair. A larger difference in CPT AUC between parent and mutant strains seemed to correlate with a larger difference in CPT killing in the model; however, there was poor correlation between CPT AUC and killing when all strains were compared in all models.

PBP4 was expressed to a lesser extent in the strain with reduced susceptibility to VAN in each pair; however, this difference was too small (<4-fold) to be considered a substantial change in expression. JH1 expressed 1.3 times more PBP4 than JH9, R6911 expressed 1.1 times more PBP4 than R6913, and T51643 expressed 2 times more PBP4 than H9749-2. PB2 expression was substantially induced in JH9 relative to that in JH1 but was moderately downregulated in the mutant strains of the other pairs. PB2 was expressed 4.4 times higher in JH9 than in JH1, but expression was 63% lower in R6913 than that in R6911 and 28% lower in H9749-2 than that in T51643.

DISCUSSION

The inverse correlation between β-lactam susceptibility and lipopeptide susceptibility in MRSA, known as the seesaw effect, has been shown to affect traditional antistaphylococcal β-lactams. However, it is unknown whether this relationship holds true for the anti-MRSA cephalosporin CPT. In this study, we evaluated differences in CPT activity in closely related isolates that differed only in the susceptibilities to VAN and DAP. Interestingly, in all three pairs the mutant and the parent strain had the same CPT MIC. We demonstrated that CPT was significantly more active against MRSA strains with reduced susceptibility to VAN relative to their more susceptible parent strains for 2/3 pairs tested, despite the fact that both parent and mutant strains had the same CPT MIC. This observation supports the hypothesis that CPT is also affected by the seesaw effect and may be more active against strains with a higher VAN and/or DAP MIC. Even though there was not a measurable difference in the CPT MIC for the mutant versus the parent strain in any of the pairs, using a population analysis profile to characterize less-susceptible subpopulations we revealed that mutant strains were more uniformly susceptible than their corresponding parent strains, as evidenced by their lower CPT PAP AUC. The relative decrease in heterogeneity between the parent and mutant strains seemed to be proportional to the increased level of killing observed in the model. However, there was a poor correlation between the log$_{10}$-CFU/ml reduction in bacterial load and the CPT PAP AUC across all strains (data not shown). The simulated CPT regimen resulted in greater bacterial killing than the simulated VAN regimen against all strains, except for T51643 (MRSA). It is interesting to note that this strain was the most susceptible to VAN (VAN MIC, 1 μg/ml; VAN PAP AUC ratio, 0.77) and the least susceptible to CPT (CPT MIC, 1 μg/ml; CPT PAP AUC, 12.21). The improved activity of CPT simulations

FIG 2 Ceftriaxone population analysis profile for various strains tested in this study. Shown are the ceftriaxone population analysis profiles for Mu3 (hVISA; VAN MIC, 2 μg/ml; CPT MIC, 2 μg/ml), JH1 (VSSA; VAN MIC, 1 μg/ml; CPT MIC, 0.5 μg/ml), JH9 (VISA; VAN MIC, 8 μg/ml; CPT MIC, 0.5 μg/ml), R6911 (hVISA; VAN MIC, 2 μg/ml; CPT MIC, 0.5 μg/ml), R6913 (VISA; VAN MIC, 4 μg/ml; CPT MIC, 0.5 μg/ml), T51643 (VSSA; VAN MIC, 1 μg/ml; CPT MIC, 1 μg/ml), and H9749-2 (hVISA; VAN MIC, 2 μg/ml; CPT MIC, 1 μg/ml).
relative to VAN was not surprising, as the VAN exposures in the model were only optimized for the two VSSA strains with MICs of 1 μg/mL. Suboptimal VAN exposure in this model does not allow for clinical extrapolation of the comparison between CPT and VAN; however, this was not the goal of this experiment.

The improvement in CPT killing against less-susceptible strains was not associated with a substantial reduction in transcription of PBP4, as was hypothesized. The expected increase in PBP2 expression in VAN nonsusceptible strains was only observed between JH1 and JH9. Other investigators have shown that PBP2 and PBP2a are both induced in the presence of cell wall active agents, including VAN and OXA (15, 20). While some have suggested that reduction of PBP4 transcription plays an important role in the expression of the VISA phenotype, others have found that PBP4, as well as other PBP subtypes, may be increased or unchanged in glycopeptide-nonsusceptible strains (11, 12, 21). It is possible that differential PBP expression is a common but not universal mechanism for reduced VAN susceptibility and the seesaw effect. Further research is warranted to clarify the role of variable PBP expression in glycopeptide-nonsusceptible strains.

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