Evaluation of Vancomycin in Combination with Piperacillin-Tazobactam or Oxacillin against Clinical Methicillin-Resistant Staphylococcus aureus Isolates and Vancomycin-Intermediate S. aureus Isolates In Vitro

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Vancomycin with piperacillin-tazobactam is used as empirical therapy for critically ill patients. Studies of this combination against methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-intermediate S. aureus (VISA) are limited, but β-lactams in combination with vancomycin have shown synergistic activity against MRSA and VISA. The goal of this study was to evaluate whether piperacillin-tazobactam and vancomycin were synergistic against MRSA and VISA in vitro. Bloodstream MRSA (n = 20) and VISA (n = 4) strains were selected. In vitro antimicrobial activities of piperacillin-tazobactam and oxacillin were evaluated by disk diffusion, and MICs were determined by Etest using Muller-Hinton agar with and without vancomycin at one-half the MIC. Time-kill studies evaluated 14 MRSA and all 4 VISA isolates using piperacillin-tazobactam at 300/35 mg/liter or oxacillin at 40 mg/liter alone and with vancomycin at one-half the MIC. Mean zones of inhibition for piperacillin-tazobactam and oxacillin increased with vancomycin against MRSA and VISA (P < 0.001 for all), and the MIC09 decreased with vancomycin against MRSA and VISA to values meeting susceptibility criteria for S. aureus (P < 0.001 for both antibiotics against MRSA). In MRSA time-kill studies, the mean 24-h reductions in inoculum for piperacillin-tazobactam, piperacillin-tazobactam with vancomycin, and oxacillin with vancomycin were 3.53, 3.69, and 2.62 log10 CFU/ml, respectively. The mean 24-h reductions in VISA inoculum for piperacillin-tazobactam, piperacillin-tazobactam with vancomycin, and oxacillin with vancomycin were 2.85, 2.93, and 3.45 log10 CFU/ml, respectively. Vancomycin with piperacillin-tazobactam or oxacillin demonstrated synergistic activity against MRSA and VISA. The clinical implications of these combinations against MRSA and VISA should be investigated.
VAN antimicrobial activity against MRSA and VISA. In order to meet this objective, the following outcomes were studied, with oxacillin (OXA) serving as a marker of NAF activity: (i) the antimicrobial activity of VAN in combination with TZP or OXA against clinical MRSA and VISA isolates using disk diffusion studies, (ii) the effect of VAN on the TZP and OXA MIC values for MRSA and VISA using the Etest methodology, and (iii) the effect of VAN in combination with TZP or OXA against clinical MRSA and VISA isolates in vitro using 24-h time-kill studies.

(A portion of this research was presented at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2012 [22].)

MATERIALS AND METHODS

Bacterial strains. We identified 20 clinical bloodstream MRSA isolates recovered from 20 different patients hospitalized at the University of New Mexico Health Sciences Center (UNMH) in Albuquerque, NM, from April 2007 to November 2011. Four VISA isolates were chosen for analysis: three clinical isolates recovered from blood and one ATCC strain (ATCC 700699; Mu50). Methicillin resistance and VAN MICs for all isolates were determined by using data from the BD Phoenix automated microbiological system (BD, Franklin Lakes, NJ) at the UNMH reference laboratory (Tricore Reference Laboratories Inc.).

Antibiotics and media. VAN and OXA analytical powder were obtained from Sigma-Aldrich Inc. (St. Louis, MO). TZP powder for injection (Wyeth Pharmaceuticals, Philadelphia, PA) was obtained from the UNMH inpatient pharmacy department. OXA and TZP disks were obtained from Becton, Dickinson ( Sparks, MD). VAN, TZP, and OXA Etests were obtained from AB Biodisk (Solna, Sweden). Mueller-Hinton broth obtained from Becton, Dickinson (Sparks, MD) and supplemented with calcium (25 mg/liter) and magnesium (12.5 mg/liter) (SMHB) was used for time-kill experiments. Mueller-Hinton agar (MHA) (Becton, Dickinson, Sparks, MD) was used for disk diffusion studies, Etest studies, and bacterial enumeration for time-kill studies.

Disk diffusion and Etest studies. MHA plates with or without VAN at one-half the MIC of the organism were used to examine the effects of TZP or OXA with VAN against all 20 MRSA strains and all 4 VISA strains using currently recommended Clinical and Laboratory Standards Institute disk diffusion methods (23). Strains were tested in duplicate to ensure reproducibility. A 0.5 McFarland suspension obtained from a culture grown overnight was used to inoculate MHA plates with and without VAN. Disks impregnated with OXA (1 mg/liter) or TZP (100/10 mg/liter) were placed on top of the MHA plates with and without VAN at one-half the MIC. Each plate was incubated at 37°C, and the zones of inhibition were measured after 24 h of incubation for MRSA isolates and after 24 to 48 h for VISA isolates. Additionally, for each isolate, TZP and OXA MICs were determined by using Etest methodology, as recommended by the manufacturer, in the presence or absence of VAN in the agar at one-half the MIC (24). VAN MICs were also determined by using Etest methodology in the presence or absence of either OXA or TZP in the agar at one-half the MIC (24). For Etest studies, plates were incubated at 37°C for 24 h; a 48-h incubation period was used for VAN susceptibility breakpoints for S. aureus are 13 mm and 18 mm, respectively.

In Vitro Combination Therapy for MRSA and VISA

<table>
<thead>
<tr>
<th>Organism</th>
<th>VAN at 1/2 MIC + OXA</th>
<th>P value</th>
<th>TZP at 1/2 MIC + TZP</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA (n = 20)</td>
<td>7.4 ± 2.4</td>
<td>&lt;0.01</td>
<td>17 ± 1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>VISA (n = 4)</td>
<td>14.2 ± 3.2</td>
<td>&lt;0.01</td>
<td>24.2 ± 2.5</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* All data are expressed as means ± standard deviations. The current OXA and TZP susceptibility breakpoints for S. aureus are 13 mm and 18 mm, respectively.

RESULTS

Bacterial strains. All 20 MSRA isolates used in the current study were susceptible to VAN, with MIC values ranging from 0.5 to 1.0 mg/liter, and all 4 VISA strains had a VAN MIC of 4 mg/liter by using automated methodology. Seventeen of the MRSA strains (85%) had a VAN MIC of 1.0 mg/liter, and three strains (15%) had a VAN MIC of 0.5 mg/liter. All MRSA and VISA isolates had an OXA MIC of >2 mg/liter. Of the MRSA isolates used in the time-kill studies, 11 (78.6%) had a VAN MIC of 1.0 mg/liter, and 3 (21.4%) had a VAN MIC of 0.5 mg/liter.

Disk diffusion and Etest studies. (i) MRSA isolates (n = 20). The mean zone of inhibition for OXA and TZP significantly increased in the presence of VAN (P < 0.001 for both). These results are shown in Table 1. The median TZP MIC determined by Etest decreased from 40 mg/liter (IQR, 24 to 64 mg/liter) to 0.625 mg/liter (IQR, 0.25 to 1.0 mg/liter) with the addition of VAN at one-half the MIC (P < 0.001). Additionally, the median OXA MIC determined by Etest decreased from 32 mg/liter (IQR, 13 to 108 mg/liter) to 0.75 mg/liter (IQR, 0.5 to 1.0 mg/liter) with the addition of VAN at one-half the MIC (P < 0.001). The associated MIC90 and MIC50 values for OXA and TZP with and without VAN are shown in Table 2. A representative example of the change in
TZP MIC with the addition of VAN is shown in Fig. 1. The median VAN MIC determined by Etest decreased from 1.5 mg/liter (IQR, 1.5 to 1.5 mg/liter) to 0.38 mg/liter (IQR, 0.25 to 0.50 mg/liter) and 0.38 mg/liter (IQR, 0.25 to 0.50 mg/liter) with the addition of OXA and TZP at one-half the MIC, respectively (P < 0.001 for both). The MIC<sub>50</sub> and MIC<sub>90</sub> for VAN with and without OXA and TZP at one-half the MIC are shown in Table 3.

(ii) VISA isolates (n = 4). For OXA and TZP, the mean zone of inhibition increased significantly with the addition of VAN at one-half the MIC (P < 0.001 for TZP and P = 0.007 for OXA). These mean zones of inhibition for each antibiotic or combination are shown in Table 1. The mean OXA MIC decreased from 139 ± 135.4 to 0.14 ± 0.14 mg/liter with the addition of VAN at one-half the MIC (P = 0.132). The mean TZP MIC decreased from 121 ± 121.9 to 0.04 ± 0.05 mg/liter with VAN at one-half the MIC (P = 0.141). The mean VAN MIC determined by Etest decreased from 4 ± 0 to 0.64 ± 0.42 mg/liter and 0.75 ± 0 mg/liter with the addition of OXA and TZP at one-half the MIC, respectively (P = 0.001 for OXA).

Time-kill studies. (i) MRSA isolates (n = 14). The mean log<sub>10</sub> CFU/ml reductions in inoculum size at 24 h and standard deviations for each antibiotic or antibiotic combination are shown in Table 4, and a representative time-kill curve is shown in Fig. 2. There was a statistically significant difference in the reduction of the inoculum size at 24 h depending on antibiotic exposure [F(5) = 88.832; P < 0.001]. The mean 24-h reduction in inoculum for VAN with TZP was significantly greater than that for VAN or OXA alone (P < 0.001 for both) but not for TZP alone (P = 0.999). The mean 24-h reduction in inoculum for OXA with VAN was also significantly greater than that for VAN or OXA alone (P < 0.001 for both) but not for TZP alone (P = 0.335). VAN with TZP had a greater reduction in inoculum at 24 h than did OXA with VAN, but the difference was not statistically significant (P = 0.170). VAN did not decrease the inoculum against any isolate tested. VAN with TZP was bactericidal against all isolates tested, with a mean time to bactericidal activity of 8.9 ± 6.7 h. VAN with OXA was bactericidal against half of the clinical isolates tested and demonstrated synergy compared to OXA alone in 9/14 isolates tested, additivity in 4/14 isolates tested, and indifference in 1 isolate tested. The mean time to bactericidal activity for VAN with OXA was 19.4 ± 7.8 h. No antagonism was observed between either antibiotic combination.

(ii) VISA isolates (n = 4). The mean 24-h reductions in inoculum size for each antibiotic and combination are shown in Table 4, and a representative time-kill curve is shown in Fig. 2. The mean 24-h reductions in inoculum for TZP and VAN with TZP were greater than that for OXA alone but less than that for VAN with OXA. VAN with TZP demonstrated bactericidal activity against two isolates, and against one other isolate, it achieved bactericidal activity at 4 h but then experienced regrowth from 2.0 log<sub>10</sub> CFU/ml to 2.8 log<sub>10</sub> CFU/ml at 8 and 24 h. OXA with VAN demonstrated synergy compared to OXA alone and was bactericidal against all four isolates tested. No antagonism was observed between either antibiotic combination against the VISA isolates.

DISCUSSION

The results of this study show that, regardless of the in vitro methodology used, VAN with TZP or OXA has great potential for

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**TABLE 2 MRSA in vitro antibiotic susceptibilities determined by using TZP and OXA Etests**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/liter)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (mg/liter)</th>
<th>MIC range (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA</td>
<td>32</td>
<td>256</td>
<td>6–256</td>
</tr>
<tr>
<td>VAN at 1/2 MIC + OXA</td>
<td>0.75</td>
<td>2</td>
<td>0.38–256</td>
</tr>
<tr>
<td>TZP</td>
<td>32</td>
<td>96</td>
<td>8–192</td>
</tr>
<tr>
<td>VAN at 1/2 MIC + TZP</td>
<td>0.5</td>
<td>2</td>
<td>0.19–4</td>
</tr>
</tbody>
</table>

**TABLE 3 MRSA in vitro antibiotic susceptibilities determined by using VAN Etests**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/liter)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (mg/liter)</th>
<th>MIC range (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAN</td>
<td>1.5</td>
<td>2</td>
<td>1–2</td>
</tr>
<tr>
<td>VAN at 1/2 MIC + OXA</td>
<td>0.38</td>
<td>0.50</td>
<td>0.125–0.75</td>
</tr>
<tr>
<td>VAN at 1/2 MIC + TZP</td>
<td>0.38</td>
<td>0.50</td>
<td>0.19–0.50</td>
</tr>
</tbody>
</table>

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FIG 1 Representative 24-h time-kill curve (MRSA strain M3615).
TABLE 4 Mean 24-h change in inoculum based on antibiotic exposure

<table>
<thead>
<tr>
<th>Organism</th>
<th>GC VAN at 1/2 MIC</th>
<th>OXA OXA + VAN at 1/2 MIC</th>
<th>TZP</th>
<th>TZP + VAN at 1/2 MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA (n = 14)</td>
<td>+2.8 ± 0.5</td>
<td>+2.5 ± 0.9</td>
<td>+0.6 ± 1.9</td>
<td>−2.6 ± 1.8</td>
</tr>
<tr>
<td>VISA (n = 4)</td>
<td>+3.4 ± 0.3</td>
<td>+2.7 ± 0.8</td>
<td>+1.35 ± 2</td>
<td>−3.5 ± 0.1</td>
</tr>
</tbody>
</table>

a The lower limit of reliable detection was 2.0 log10 CFU/ml. GC, growth control.

added activity against MRSA and VISA. Previous studies have found synergy with ASBLs in combination with VAN against MRSA and VISA, and our study substantiates those findings (15–21). However, previous studies using TZP in combination with VAN were limited in scope and by the number of isolates tested (15–17). Our study is comprehensive in terms of the number of isolates tested and the number of in vitro tests used, but it is also the first study to test VAN in combination with TZP against VISA. Rochon-Edouard and colleagues tested VAN in combination with TZP using disk diffusion methodology and found a positive antibacterial effect against 16/32 MRSA isolates tested (15). We utilized their methodology against 20 MRSA isolates and found that the TZP zone of inhibition for all isolates tested significantly increased with subinhibitory VAN concentrations in the agar. VAN with TZP or NAF has also shown synergistic activity against 3 MRSA isolates in 48-h time-kill studies with infected fibrin clots (17). We tested 14 MRSA isolates in 24-h time-kill studies using the same concentrations of VAN, TZP, and OXA and found similar results. In time-kill experiments, VAN with TZP decreased the inoculum against all isolates tested. VAN with OXA decreased the inoculum against many isolates tested and showed synergistic activity against the majority of MRSA isolates and all VISA isolates tested. This confirms the synergy between VAN and OXA against MRSA and VISA demonstrated by Leonard and by Werth et al. (18, 21). Our study is the first to demonstrate synergistic activity of VAN with TZP against VISA.

While our results show that there is a synergistic effect between VAN and TZP or OXA, proposed mechanisms to understand the interaction between ASBLs and VAN against MRSA and VISA are not clearly elucidated. TZP and OXA may provoke some alteration in the MRSA cell wall that allows for improved VAN activity even if the MRSA strain is susceptible to VAN. Werth and colleagues recently described synergy between VAN and ceftaroline or OXA against VISA and heterogeneous VISA (hVISA) isolates, despite β-lactam exposure reducing overall VAN-Bodipy binding by approximately 50% (21). Those authors hypothesized that beta-lactam exposure may have improved vancomycin interactions with the cell wall. Given these results and the marked decrease in VAN MICs against MRSA and VISA with the addition of subinhibitory OXA and TZP concentrations observed in our study, this is a reasonable explanation of the synergy between ASBLs and VAN. Some VISA isolates have also demonstrated an increased susceptibility to β-lactams in the face of rising VAN and daptomycin MICs, the “seesaw effect” (26). While none of the VISA isolates tested in our study were determined to be susceptible to OXA or TZP, both ASBLs in combination with VAN demonstrated enhanced antibacterial activity against VISA compared to either antibiotic alone as well as VAN alone in all studies. These findings suggest that the seesaw effect may have contributed to the enhanced antibacterial activity observed with both antibiotic combinations against the VISA isolates. An alternative explanation of the synergistic activity between VAN and TZP or OXA may be that VAN alters the composition of the MRSA cell wall, allowing increased TZP and OXA binding. This mechanism may be a more plausible explanation for the results of our study, given that a subinhibitory VAN concentration was used for all experiments. In Etest studies, VAN at subinhibitory concentrations was shown to decrease all TZP and almost all OXA MICs to values less than or equal to their current susceptibility breakpoints for S. aureus. Inhibition of MRSA peptidoglycan synthesis by VAN has been shown to decrease methicillin resistance and cause MRSA to change from a homogeneous methicillin-resistant phenotype to a

FIG 2 Representative 24-h time-kill curve (VISA strain C44).
heterogeneous methicillin-resistant phenotype (27). It also appears that there is a strain-dependent phenomenon which explains the synergistic activity between these antibiotics against S. aureus. For example, VAN and TZP demonstrated enhanced activity against MRSA, whereas VAN and OXA demonstrated enhanced activity against VISA. However, despite a known mechanism, VAN with TZP or OXA demonstrated synergistic activity against MRSA and VISA in vitro.

The current study is not the first to mention the antimicrobial effects of an ASBL with VAN against MRSA or VISA, but it is the first to mention such effects of VAN with TZP against MRSA using numerous MRSA isolates. It is also the first study to mention such an effect of VAN and TZP against VISA. When considering the results of the current study in broader contexts, it is important to note that peak TZP concentrations were used for all time-kill studies. A previous time-kill study by Palmer and Rybak using TZP at 300/35 mg/liter against MRSA led to the use of this TZP concentration in the current study (17). Future studies using in vitro PK/PD models that simulate in vivo antibiotic exposure of both antibiotics against MRSA and VISA would further enhance our understanding of VAN-ASBL combination therapy. More in-depth analyses of the manner in which TZP and VAN interact with MRSA and VISA cell walls are needed as well.

In conclusion, with the incidence of MRSA and VISA infections on the rise coupled with their high rate of treatment failures, investigators and clinicians are searching for novel treatment options for these life-threatening infections, including combination antibiotic therapy. In this study, increased antimicrobial activity against MRSA and VISA was observed when TZP or OXA was combined with subinhibitory concentrations of VAN in vitro. VAN with TZP is used often clinically, and we must be cognizant of the potential beneficial effect of VAN with TZP against S. aureus. Given the rising number of MRSA and VISA treatment failures, it is prudent to investigate the clinical effect of this antibiotic combination for the treatment of invasive MRSA and VISA infections.

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REFERENCES


