Oritavancin Combinations with β-Lactams against Multidrug-Resistant Staphylococcus aureus and Vancomycin-Resistant Enterococci

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Oritavancin possesses activity against vancomycin-resistant enterococci (VRE) and methicillin-resistant Staphylococcus aureus (MRSA). In vitro data suggest synergy between beta-lactams (BLs) and vancomycin or daptomycin, agents similar to oritavancin. We evaluated the activities of BLs combined with oritavancin against MRSA and VRE. Oritavancin MICs were determined for 30 strains, 5 each of MRSA, daptomycin-nonsusceptible (DNS) MRSA, vancomycin-intermediate MRSA (VISA), heteroresistant VISA (hVISA), vancomycin-resistant Enterococcus faecalis, and vancomycin-resistant Enterococcus faecium. Oritavancin MICs were determined in the presence of subinhibitory concentrations of BLs. Oritavancin combined with ceftaroline, cefazolin, or nafcillin was evaluated for lethal synergy against MRSA, and oritavancin combined with ceftaroline, ampicillin, or ertapenem was evaluated for lethal synergy against VRE in 24-h time-kill assays. Oritavancin at 0.5× the MIC was combined with BLs at 0.5× the MIC or the biological free peak concentration, whichever one was lower. Synergy was defined as a ≥2-log10 CFU/ml difference between the killing achieved with the combination and that achieved with the most active single agent at 24 h. Oritavancin MICs were ≤0.125 μg/ml for all MRSA isolates except three VISA isolates with MICs of 0.25 μg/ml. Oritavancin MICs for VRE ranged from 0.03 to 0.125 μg/ml. Oritavancin in combination with cefazolin was synergistic against all MRSA phenotypes and statistically superior to all other combinations against DNS MRSA, hVISA, and VISA isolates (P < 0.02). Oritavancin in combination with cefazolin and oritavancin in combination with nafcillin were also synergistic against all MRSA strains. Synergy between oritavancin and all BLs was revealed against VRE strain 8019, while synergy between oritavancin and ampicillin or ertapenem but not ceftaroline was demonstrated against VRE strain R7164. The data support the potential use of oritavancin in combination with BLs, especially oritavancin in combination with ceftaroline, for the treatment of infections caused by MRSA.

Oritavancin (ORI) is a novel lipoglycopeptide antibiotic recently approved by the Food and Drug Administration for the treatment of acute bacterial skin and skin structure infections (ABSSSIs) (1–3). Unique to ORI among the agents approved for use for the treatment of ABSSSIs, the antibiotic is administered as a single dose of 1,200 mg as the entire treatment course. ORI is active against a wide range of Gram-positive organisms, including methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) (4). It possesses three mechanisms of action, acting via inhibition of both transglycosylation and transpeptidation at the cell wall, along with disruption of the cell membrane (5). ORI is a potent agent against Gram-positive organisms. Against over 9,000 S. aureus isolates isolated from bloodstream infections, ORI demonstrated an MIC<sub>90</sub> of 0.063 μg/ml, whereas the MIC<sub>90</sub> values of daptomycin (DAP) and vancomycin (VAN) were 0.5 and 1 μg/ml, respectively (6). Even against the subset of isolates with elevated VAN (2 μg/ml) and DAP (1 to 4 μg/ml) MICs, ORI maintained an MIC<sub>90</sub> of 0.125 μg/ml. ORI has demonstrated similar activity against VRE, with an MIC<sub>90</sub> of 0.063 μg/ml against the vancomycin-resistant Enterococcus faecium isolates studied thus far (7). The DAP MIC<sub>90</sub> for the same isolates was 32-fold higher. Notably, ORI demonstrated potent activity against isolates of VRE carrying the vanA gene, with an MIC<sub>90</sub> of 0.063 μg/ml. Telavancin and dalbavancin, the two other approved lipoglycopeptides, possess no activity against VRE isolates possessing this gene.

Although MRSA strains with reduced susceptibility to VAN and DAP, along with VRE strains resistant to DAP, are uncommon, their appearances in the medical literature have necessitated a look into novel ways to combat these problematic organisms (8–16). ORI possesses antibacterial activity against these organisms along with an ability to be administered by a novel, single-dose regimen. In vitro time-kill experiments have demonstrated the concentration-dependent bactericidal activity of ORI against daptomycin-nonsusceptible (DNS) MRSA, VAN-intermediate S. aureus (VISA), heteroresistant VISA (hVISA), and VRE in 24-h time-kill experiments (17–20). Recent data suggest that lipopeptides and glycopeptides demonstrate enhanced activity against these resistant, Gram-positive organisms when beta-lactams are added (9, 13, 15, 16, 21–35). However, the activity of ORI in combination with beta-lactams against multidrug-resistant MRSA or VRE strains has yet to be evaluated. Our objective was to evaluate the ability of beta-lactams to provide synergistic activity with ORI against resistant Gram-positive pathogens in combination MIC testing and 24-h time-kill experiments.
R7549) and two vancomycin-resistant lin (PIP), meropenem (MEM), and ertapenem (ERT) were purchased provided by The Medicines Company. DAP, VAN, cefazolin (CFZ), nafcillin (NAF), 5\(\text{g/ml}\) calcium according to CLSI guidelines (36, 37). The MIC values of all other studied antimicrobials were determined in duplicate by broth microdilution with an inoculum of \(10^6\) CFU/ml according to CLSI guidelines (37). The MIC for the respective organism or the biological free peak concentration of the beta-lactam, whichever one was lower, was used in combination with ORI. All agents were tested alone and in combination with ORI against each strain. Aliquots of 0.1 ml were obtained from each well at 0, 4, 8, and 24 h, serially diluted to the appropriate concentrations, and plated using automatic spiral plating (Whitley automated spiral plater; Don Whitley Scientific, West Yorkshire, England) for the best enumeration of the number of CFU per milliliter and avoidance of antibiotic carryover. After 24 h of growth on tryptic soy agar (TSA; Difco, Detroit, MI) for MRSA or brain heart infusion agar (BHI; Difco, Detroit, MI) for VRE, bacterial colonies were counted using a laser colony counter (ProtoCOL). Time-kill curves were generated by plotting the mean colony counts (log\(_{10}\) number of CFU per milliliter) versus time to compare the 24-h killing effects of single agents and combination antimicrobial exposures. synergy was defined as a greater than or equal to 100-fold increase in bacterial killing compared to that achieved with the most active single constituent that had no important effect on organism growth (38). Bactericidal activity was defined as a greater than or equal to 3-log\(_{10}\) CFU/ml reduction of the bacterial count at 24 h from that at the baseline.

**Statistical analysis.** Changes in the number of CFU per milliliter at 24 h were compared by one-way analysis of variance (ANOVA) for time-kill assays. A \(P\) value of \(<0.05\) was considered significant. All statistical analyses were performed using SPSS Statistical Software (release 23; SPSS, Inc., Chicago, IL).

**RESULTS**

**MIC measurement.** The range of MICs of ORI alone and in combination with beta-lactams against MRSA and VRE are listed in Tables 1 and 2, respectively. Against MRSA, ORI MICs ranged from 0.03 to 0.25 \(\mu\text{g/ml}\), with the MIC values for 17/20 (85\%) isolates being in the susceptible range \(\leq 0.125\ \mu\text{g/ml}\) (1). The three strains with an ORI MIC of 0.25 \(\mu\text{g/ml}\) were \(S.\ aureus\) strains of the vancomycin-intermediate phenotype. CFZ, NAF, and CPT in combination with ORI demonstrated the greatest effects on the ORI MIC for MRSA, with the MICs of ORI in the combination for some strains being up to 32-fold lower than the MICs of ORI alone (Table 1). The ORI MICs in combinations of ORI with either FEP, PIP, or the carbapenems were up to 4-fold lower than the ORI MICs without the presence of any beta-lactam.

**TABLE 1 MICs of ORI alone and in combination with beta-lactams against MRSA**

<table>
<thead>
<tr>
<th>Strain</th>
<th>ORI alone</th>
<th>CFZ</th>
<th>NAF</th>
<th>CPT</th>
<th>FEP</th>
<th>PIP</th>
<th>MEM</th>
<th>ERT</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNS MRSA</td>
<td>0.03–0.12</td>
<td>&lt;0.01–0.06</td>
<td>&lt;0.01–0.12</td>
<td>0.03–0.12</td>
<td>0.03–0.12</td>
<td>0.03–0.12</td>
<td>0.02–0.12</td>
<td>0.02–0.12</td>
</tr>
<tr>
<td>VISA</td>
<td>0.13–0.25</td>
<td>&lt;0.01–0.25</td>
<td>&lt;0.01–0.25</td>
<td>&lt;0.01–0.12</td>
<td>0.03–0.25</td>
<td>0.06–0.25</td>
<td>0.06–0.12</td>
<td>0.06–0.25</td>
</tr>
<tr>
<td>hVISA</td>
<td>0.03–0.12</td>
<td>0.02–0.06</td>
<td>0.01–0.06</td>
<td>0.03–0.12</td>
<td>0.03–0.06</td>
<td>0.03–0.06</td>
<td>0.03–0.06</td>
<td>0.03–0.06</td>
</tr>
<tr>
<td>MRSA</td>
<td>0.03–0.06</td>
<td>&lt;0.01–0.03</td>
<td>0.03–0.06</td>
<td>0.02–0.06</td>
<td>0.02–0.06</td>
<td>0.02–0.03</td>
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</table>

\(^a\) ORI, oritavancin; CFZ, cefazolin; NAF, nafcillin; CPT, ceftaroline; FEP, cefepime; PIP, piperacillin; MEM, meropenem; ERT, ertapenem.

\(^b\) Five isolates of each strain type were tested.
Against vancomycin-resistant *E. faecium*, ORI MICs ranged from 0.03 to 0.125 μg/ml, while all vancomycin-resistant *E. faecalis* strains possessed ORI MICs of 0.125 μg/ml. When beta-lactams were placed in the medium, ORI MICs for vancomycin-resistant *E. faecium* strains were not affected to the same extent that they were for the MRSA strains, although broth containing CPT did reduce the ORI MIC for one vancomycin-resistant *E. faecium* strain, 8019, up to 16-fold (Table 2). The MICs of ORI in combination with the carbapenems, AMP, and CPT in broth were up to 16-fold lower than the MICs of ORI alone against several vancomycin-resistant *E. faecalis* strains. ORI MICs for VRE were not affected by the presence of PIP and FEP in broth to the same extent that they were by the presence of the other beta-lactams tested.

**Time-kill assays.** In time-kill studies against MRSA (Fig. 1), CPT, CFZ, and NAF all showed a similar extent of killing at 24 h, and all showed synergistic activity with ORI against DNS MRSA, VISA, and MRSA. Each of these combinations was also superior to any single agent against isolates of all three of these phenotypes, and each was bactericidal as well ($P < 0.001$ for all comparisons). Against hVISA, however, only CPT in combination with ORI was bactericidal, and this combination was superior to CFZ and NAF in combination with ORI ($P < 0.001$ for ORI in combination with CPT compared to ORI in combination with CFZ or ORI in combination with NAF), although the activity of ORI combined with any of the beta-lactams was superior to that of any agent alone ($P < 0.001$ for all dual-agent and single-agent comparisons).

Time-kill studies did not demonstrate the same level of synergy against VRE overall as that against MRSA (Fig. 2). Against *E. faecium* 8019, CPT, AMP, and ERT all demonstrated synergy with ORI, and each combination was superior to any single-agent regimen ($P < 0.001$). Against *E. faecalis* R7164, no regimen was bactericidal, but ERT and AMP were synergistic with ORI and superior to all single-agent regimens and ORI in combination with ERT was superior to ORI in combination with AMP ($P = 0.01$). ORI in combination with CPT demonstrated the greatest reduction in the count of the VRE at 24 h and was superior to ORI in combination with AMP ($P = 0.001$). Against ORI combined with CPT resulted in a less than 100-fold reduction in the number of CFU per milliliter at 24 h compared to that achieved with treatment with CPT alone. Both *E. faecium* R6815 and *E. faecalis* R7549 demonstrated slight regrowth at 24 h with all single-agent and combination regimens.

**DISCUSSION**

The data from this study represent a sampling of data for Gram-positive isolates with reduced susceptibilities to commonly used agents, such as DAP and VAN, tested for their ORI MICs and the time-kill efficacy of ORI both alone and in combination with beta-lactams. Here, we have demonstrated, using the methodology for ORI testing in 2015 CLSI guidelines (37), that several beta-lactam agents positively affect ORI MIC values against both MRSA and VRE strains.
FIG 2 Results of 24-h time-kill studies of 0.5× the MIC of ORI in combination with beta-lactams and VRE. The beta-lactam concentrations used in the combinations were 0.5 × the MICs listed below or the biological free peak concentrations presented in the text, whichever one was lower. (A) Strain 8019; ORI MIC, 0.125 μg/ml; AMP MIC, >128 μg/ml; ERT MIC, >32 μg/ml; CPT MIC, 32 μg/ml; (B) strain R6815; ORI MIC, 0.125 μg/ml; AMP MIC, >64 μg/ml; ERT MIC, >32 μg/ml; CPT MIC, >64 μg/ml; (C) strain R7164; ORI MIC, 0.125 μg/ml; AMP MIC, 8 μg/ml; ERT MIC, >32 μg/ml; CPT MIC, 8 μg/ml; (D) strain R7549; ORI MIC, 0.125 μg/ml; AMP MIC, 8 μg/ml; ERT MIC, >32 μg/ml; CPT MIC, 8 μg/ml. Circles with dashed lines, growth control; squares with dashed lines, ORI at 0.5× the MIC; triangles with dashed lines, AMP at 0.5× the MIC; inverted triangles with dashed lines, ERT at 0.5× the MIC; diamonds with dashed lines, CPT at 0.5× the MIC; triangles with solid lines, ORI in combination with AMP; inverted triangles with solid lines, ORI in combination with ERT; circles with solid lines, ORI in combination with CPT.

Oritavancin Synergy with β-Lactams against MRSA and VRE

In time-kill assays, however, beta-lactams were reliably synergistic with ORI only against the MRSA isolates tested.

The ORI MICs in this study were comparable to those that have been previously published. In the case of standard MRSA, all isolates in the present study were inhibited by ORI at a concentration not exceeding 0.063 μg/ml, a value that corresponds to the published MIC < sub>90 from surveillance of over 9,000 S. aureus isolates (6). With regard to strains of the more resistant phenotypes, such as hVISA, VISA, and DNS MRSA, the ORI MIC values obtained in our study were lower than those reported previously, where the ORI MICs against hVISA, VISA, and DNS MRSA isolates ranged up to 2 μg/ml (6, 18). In our study, 12 of 15 (80%) hVISA, VISA, or DNS MRSA isolates were susceptible to ORI, with MIC values being 0.25 μg/ml for only three VISA isolates. These apparent discrepancies in the in vitro potency of oritavancin may be due to the small numbers of multidrug-resistant isolates tested; therefore, more isolates need to be studied. However, in all cases the ORI MICs were severalfold lower than the VAN and DAP MICs in the present studies. Also, population pharmacokinetic data demonstrate that free, non-protein-bound ORI concentrations remain above 0.25 μg/ml out to at least 1 week after administration of a 1,200-mg dose, suggesting that ORI concentrations are above the MIC values for even our VISA isolates for a long duration (39). Clinical data for S. aureus isolates with the vancomycin-intermediate resistance profile and MIC values of 0.25 μg/ml are currently lacking, however.

When the S. aureus isolates were exposed to subinhibitory concentrations of beta-lactams in combination with ORI, the ORI MICs were generally lower than those achieved with ORI alone. In particular, the presence of CPT in broth in combination with ORI resulted in lower ORI MIC values for isolates of all phenotypes compared with those achieved with ORI alone, and none of the isolates remained nonsusceptible to ORI. This is the first study, to the best of our knowledge, that has evaluated the effect of beta-lactams on the activity of ORI against MRSA. Although combination MIC values may not translate directly into clinical benefit, the results of our time-kill study serve to provide further evidence of the beneficial effects of beta-lactams in combination with ORI against MRSA.

Echoing the results of our combination MIC testing, NAF, CFZ, and CPT provided synergistic activity against both DNS MRSA and VISA when they were used in combination with ORI in time-kill studies. However, only CPT in combination with ORI was able to demonstrate synergy against standard MRSA and hVISA strains as well. Previously, a study evaluated the activity of ORI in combination with gentamicin, rifampin, and linezolid against MRSA, and the authors demonstrated synergistic effects between ORI and each of these agents (18). However, ours is the first study to evaluate ORI in combination with beta-lactam antibiotics. Previously, beneficial effects against MRSA, particularly MRSA strains with reduced susceptibility to VAN or DAP, have...
been demonstrated when beta-lactams were combined with VAN or DAP, and the findings of our study suggest that beta-lactams confer similar benefits upon ORI in vitro (22, 23, 25–27, 32–35). With regard to enterococci, this is the first study to evaluate the combination of ORI and beta-lactams in time-kill analyses. Previous data have suggested that beta-lactams are synergistic with DAP against enterococci, even against strains resistant to DAP (40). Notably, this previous study tested the synergy between beta-lactams and DAP against strain 5938 under experimental conditions similar to those used in our current study (40). Synergistic activities between beta-lactams and DAP in that study and beta-lactams and ORI in our study appear similar. On the basis of these limited comparative data, it would appear that ORI and DAP may possess similar synergistic activity when strains of similar phenotype are used, although further work is necessary.

As in these previous studies, it appears that as the MICs of VAN and DAP increase, the synergistic activity between ORI and beta-lactams is greater, possibly owing to the seesaw effect, the phenomenon in which glycopeptide and lipopeptide MICs are inversely proportional to beta-lactam MICs (41). Because the mechanism of action of ORI bears similarities to the mechanisms of action of both VAN and DAP, the seesaw effect hypothesis likely does help explain the synergistic activity. It is also of interest that CPT appears to possess the most beneficial effect when it is combined with ORI, at least compared to the effect of ORI in combination with CFZ or NAF.

Previously, an affinity for certain penicillin-binding proteins (PBPs) has been demonstrated to predict an enhancement of DAP activity, with activity against PBP 1 in staphylococci and PBP 5 in enterococci being important (23, 40, 42–44). The most recent data available suggest that the synergy achieved with the carbapenems ertapenem and imipenem, which are selective for PBP 1, in combination with DAP is superior to that achieved when DAP is used in combination with other beta-lactams, including NAF, which possesses nonselective activity against PBPs (44). Interestingly, our data demonstrated that ORI MIC values were lower in the presence of NAF than in the presence of MEM or ERT, although these tests were limited to comparisons in combination MIC measurement.

Other work has demonstrated that CPT has a pronounced effect on both VAN and DAP activity, perhaps due to its intrinsic anti-MRSA activity through high-affinity binding to mutant PBP 2a (32, 34, 35). Our ORI MIC data and data from time-kill analyses demonstrate that ORI has synergistic activity against MRSA when it is used in combination with CPT, perhaps suggesting that PBP 2a may play an important role in the synergistic activity between CPT and this new lipoglycopeptide against MRSA.

With regard to enterococci, previous data suggest that CPT, ERT, or AMP in combination with DAP has synergistic effects (30, 40). These synergistic effects are possibly mediated by the powerful binding of CPT to several enterococcal PBPs, including PBP 5, as well as the similarly broad PBP-binding profile of ERT (42, 45, 46). Here, however, synergy was limited to two of the tested VRE isolates, and not all three of the agents showed synergistic activity with ORI against both isolates. It is possible that although DAP and ORI share at least one similar mechanism of action, synergistic effects among beta-lactams are driven by different interactions. Further work to characterize the mechanism behind the increased ORI activity in the presence of beta-lactams will help us better understand this relationship, and in vitro and in vivo pharmacokinetic/pharmacodynamic models using humanized concentrations of ORI and beta-lactams will help us to better assess and understand these synergistic interactions.

There are limitations to the current study. The data represent the findings of only 24-h, static experiments, which may not provide results completely representative of those associated with the in vivo activity of ORI alone or in combination. There were also a limited number of strains, limiting the generalizability of our results to all staphylococci and enterococci. Investigation of more examples of both MRSA and VRE strains is warranted to confirm that these results can be replicated.

Conclusion. The rising rates of nonsusceptibility to commonly used agents, such as DAP and VAN, among Gram-positive bacteria necessitate the use of novel approaches to therapy. Therapy with ORI, with its three mechanisms of action and in vitro activity against isolates with reduced susceptibility to DAP and VAN, is a promising option. With effective treatment of infections caused by MRSA and VRE frequently requiring combination therapy, the time-kill data from this study suggest that ORI is capable of acting synergistically with beta-lactams, at least against MRSA.

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