Assessment by Time-Kill Methodology of the Synergistic Effects of Oritavancin in Combination with Other Antimicrobial Agents against Staphylococcus aureus

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Oritavancin is a semisynthetic lipoglycopeptide in clinical development for serious gram-positive infections. This study describes the synergistic activity of oritavancin in combination with gentamicin, linezolid, moxifloxacin, or rifampin in time-kill studies against methicillin-susceptible, vancomycin-intermediate, and vancomycin-resistant Staphylococcus aureus.

Oritavancin is a semisynthetic lipoglycopeptide in clinical development that has activity against methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci. It differs from other glycopeptides such as vancomycin and teicoplanin in that its bactericidal activity in vitro is rapid and concentration dependent (1). Recent work demonstrated that oritavancin binds avidly to glass and plastic labware surfaces, causing its potency to be significantly underestimated during susceptibility testing and other microbiological assays (3). The Clinical Laboratory Standards Institute (CLSI) recent update to include polysorbate-80 at 0.002% throughout oritavancin broth microdilution testing (9), which limits binding of oritavancin to vessel surfaces, has prompted reevaluation of oritavancin activity in a range of in vitro microbiological assays.

The potential benefits of combination antimicrobial chemotherapy over monotherapy include decreased resistance development, synergistic antibacterial activity, and a broadened antibacterial spectrum (10, 12). Previous studies examining the activity of oritavancin in combinations were performed in the absence of polysorbate-80, which may have affected assay results (5, 15, 17, 20, 22). We have thus revisited oritavancin combination testing using time-kill methodology in the presence of 0.002% polysorbate-80 to determine whether combinations of oritavancin with either gentamicin, moxifloxacin, or rifampin exhibit synergistic antibacterial activity against methicillin-susceptible S. aureus (MSSA), vancomycin-intermediate S. aureus (VISA), and vancomycin-resistant S. aureus (VRSA).

(Part of this work was presented at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 17 to 20 September 2007 [6].)

Oritavancin diphosphate powder (Targanta Therapeutics, Cambridge, MA) was dissolved in water containing 0.002% polysorbate-80 (9), and polysorbate-80 was maintained at this concentration to minimize oritavancin loss to the surface of vessels during in vitro testing (3). VISA isolate NRS402 and VRSA isolate VRSS (both obtained from Network on Antimicrobial Resistance in Staphylococcus aureus) were grown overnight in brain heart infusion broth containing 4 μg/ml vancomycin (to ensure the VISA and VRSA phenotypes). For time-kill assays, bacteria were subcultured in cation-adjusted Mueller-Hinton broth (CAMHB) until exponential phase (optical density at 600 of approximately 0.25), diluted to approximately 5 × 10⁶ CFU/ml in CAMHB containing antimicrobial agents alone or in combination, and exposed for 24 h at 37°C (21). Inclusion of polysorbate-80 did not substantially affect killing kinetics for comparator agents compared to assays performed in its absence (data not shown). To prevent drug carryover during serial dilution plating, aliquots of the drug-challenged culture were added to an equal volume of a 25-mg/ml activated charcoal suspension. Synergy was defined as a ≥2-log₁₀ decrease in CFU/ml between the combination and its most active constituent after 24 h (at least one of the drugs must be present at a concentration that does not affect the growth curve of the test organism), and the number of surviving organisms in the presence of the combination must be ≥ 2 log₁₀ CFU/ml beyond the starting inoculum (2). Bacteriostatic and bactericidal activities were defined as <3-log₁₀ and ≥3-log₁₀ reductions in CFU/ml at 24 h, respectively, relative to the starting inoculum (21). All experiments were repeated at least three times, and results of a representative experiment are presented; data points are averages from duplicate CFU/ml determinations within an experiment.

Oritavancin concentrations in the combination time-kill studies were selected to allow for assessment of synergy: oritavancin at concentrations below its MIC exerted transient antibacterial activity against the S. aureus isolates such that either an initial lag in growth or decrease in CFU was observed following addition of oritavancin (Fig. 1). In all cases, regrowth occurred to various levels by 24 h (Fig. 1).

Against the MSSA reference strain S. aureus ATCC 29213, combinations of oritavancin with either gentamicin, moxifloxacin, or rifampin were synergistic and bactericidal at 24 h (Fig. 1A; Table 1). Knowledge of whether antimicrobial combinations exert bacteriostatic or bactericidal effects could be important for treatment outcomes in certain infections (14). Syn-
ergy was not observed with the combination of oritavancin and linezolid, a protein synthesis inhibitor (Table 1).

The combination of oritavancin and gentamicin was previously shown to be synergistic against two VISA isolates by time-kill methodology without polysorbate-80 (15). These findings were confirmed and extended in the current study: against the VISA isolate S. aureus NRS402, oritavancin with gentamicin or linezolid was also synergistic (Fig. 1B and C; Table 1); these combinations were bactericidal at the 24-h time point. Oritavancin in combination with gentamicin or linezolid was also synergistic and bactericidal against the VISA isolate VRSS (Fig. 1D; Table 1). Conceivably, the ability of oritavancin to

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**TABLE 1. Summary of in vitro time-kill assays of oritavancin combinations against S. aureus isolates**

<table>
<thead>
<tr>
<th>Agent</th>
<th>MSSA ATCC 29213</th>
<th>VISA NRS402</th>
<th>VISA VRSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc (µg/ml)²</td>
<td>Fold MIC in combination</td>
<td>Δlog CFU at 24 h vs:</td>
</tr>
<tr>
<td>ORI</td>
<td>0.031</td>
<td>0.5</td>
<td>−0.34 NA</td>
</tr>
<tr>
<td>GEN</td>
<td>0.25</td>
<td>0.5</td>
<td>−0.81 −7.3</td>
</tr>
<tr>
<td>LZD</td>
<td>1</td>
<td>0.5</td>
<td>−0.64 −1.2</td>
</tr>
<tr>
<td>MOX</td>
<td>0.063</td>
<td>1</td>
<td>−1.5 −6.3</td>
</tr>
<tr>
<td>RIF</td>
<td>0.008</td>
<td>1</td>
<td>−5.9 −2.3</td>
</tr>
</tbody>
</table>

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¹ Abbreviations: ORI, oritavancin; GEN, gentamicin; LZD, linezolid; MOX, moxifloxacin; RIF, rifampin.
² Concentration of the antimicrobial agent tested as a single agent and used in combination in the time-kill assay.
³ Multiple of the broth microdilution MIC (determined as per the CLSI guidelines) of oritavancin or other agent used in combination.
⁴ Values presented are the log₁₀ change in CFU (a negative value indicates a decrease) at 24 h for the single agent relative to the growth control (C) or for the combination of the indicated agent with oritavancin relative to the most active agent of the combination (A) or the inoculum (I). Values shown are the means of duplicate determinations from a representative experiment repeated at least three times.
⁵ NA, not applicable.
⁶ ND, not determined since inherent resistance or nonsusceptibility to the combination agent precluded testing in combination with oritavancin.
⁷ The value after the slash is for oritavancin used in combination with linezolid.
increase membrane permeability (19) may facilitate entry of gentamicin into the cell, as has been shown with sesquiterpenoids, agents that increase S. aureus membrane permeability and increase susceptibility to gentamicin (7). The combination of oritavancin and rifampin was synergistic and bacteriostatic against VRSA at the 24-h time point (Fig. 1D; Table 1). That this combination was also synergistic against MSSA suggests a common killing mechanism of these two strains. We have observed that oritavancin inhibits RNA synthesis in S. aureus RN4220, a methicillin-susceptible laboratory strain (4). Loss of the permeability barrier function has been linked to inhibition of macromolecular synthesis, including RNA synthesis (25). Putative leakage of RNA precursors from the cell due to perturbation of cell membrane barrier function by oritavancin, coupled with inhibition of RNA polymerase by rifampin, may explain the synergy between these two agents.

Despite the synergy exhibited by certain combinations of antimicrobial agents in vitro, the overall benefit of combinations in clinical practice remains controversial (12, 16). For example, a recent meta-analysis examining inclusion of an aminoglycoside with a β-lactam for the treatment of endocarditis demonstrated no benefit in clinical outcome over β-lactam monotherapy and increased the frequency of nephrotoxicity (13). However, combination therapy may be beneficial for treatment of certain infections that harbor bacteria either in a tolerant state or in a biofilm, such as those associated with indwelling devices (8, 11, 18, 26), or for tuberculosis (24). Recent in vitro findings that the combination of a β-lactam with vancomycin evokes synergistic activity against methicillin-resistant VRSA (23) highlight the potential of antimicrobial combination therapy and thus the importance of in vitro synergy testing.

In conclusion, using newly approved methodology that for broth microdilution assays maintain oritavancin at its intended concentration, we have demonstrated in vitro synergy between oritavancin and representative, clinically used antimicrobial agents against drug-susceptible and -resistant S. aureus strains. Future studies in vivo infection models should provide a better understanding of the therapeutic potential of oritavancin combinations.

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REFERENCES