Oritavancin Disrupts Membrane Integrity of *Staphylococcus aureus* and Vancomycin-Resistant Enterococci To Effect Rapid Bacterial Killing

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Oritavancin is an investigational lipoglycopeptide in clinical development for the treatment of acute bacterial skin and skin structure infections. In this study, we demonstrate that oritavancin causes bacterial membrane depolarization and permeabilization leading to cell death of Gram-positive pathogens and that these effects are attributable to the 4′-chlorobiphenylmethyl group of the molecule.

Vancomycin-resistant *Staphylococcus aureus* (VRSA) and enterococci (VRE) exhibit high-level resistance to vancomycin that is caused by alteration of the bacterial cell wall target C-terminal acyl-d-alanyl-d-alanine dipeptide to the depsipeptide d-alanyl-d-lactate (6). Intermediate-level resistance to vancomycin in *S. aureus*, referred to as vancomycin-intermediate *S. aureus* (VISA) and heterogeneous VISA (hVISA), has also been described and poses a clinical challenge (1, 17). The investigational lipoglycopeptide oritavancin demonstrates *in vitro* activity against vancomycin-nonsusceptible isolates (hVISA, VISA, VRSA, and VRE), exhibiting MIC₉₀ values between 0.25 μg/ml and 2 μg/ml for the various phenotypes in recent studies (2, 3). Its activity is unaffected by methicillin resistance in staphylococci (2). Oritavancin is derived from the addition of a 4′-chlorobiphenylmethyl side chain...
demonstrated that oritavancin probably kills bacterial membrane phospholipids (7, 10, 15). Our earlier studies increases the permeability of artificial liposomes composed of that oritavancin interacts with isolated membrane protoplasts and is distinct from that of vancomycin. Recent studies showed Gram-positive organisms (13), signifying a mechanism of action activity against VRSA and VRE isolates (15). Oritavancin typi-
glycan, the pentaglycyl bridge, and to account in part for its micro-Aldrich, St. Louis, MO) on bacterial membrane depolar-
microbial Resistance in 700698, VISA ATCC 700699, VRSA VRSS (Network on Anti-

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The bacterial isolates used in this study were hVISA ATCC 700699, VISA ATCC 700699, VRSA VRSS (Network on Anti-

The rapid bactericidal pharmacodynamics of oritavancin against S. aureus and enterococci in vitro differ from those of vancomycin and teicoplanin (13). To understand these differences, we assessed the effect of oritavancin challenge on membrane depolarization. The addition of oritavancin to exponential-phase hVISA caused an immediate and concentra-
tion-dependent increase in the fluorescence of DiSC3(5) (3,3'-
dipropylthiadicarbocyanine iodide) that was preloaded into cell membranes (Fig. 1A), an indication of membrane depo-
larization. Depolarization has also been described with the lipoglycopeptide telavancin (8, 11). In contrast, fluorescent signals from cells exposed to 16 \( \mu \)g/ml chloroeremomycin or vancomycin remained constant over the exposure period, indicating that membrane potential was unaffected. These results demonstrate that the 4′-chlorobiphenylmethyl group of orita-
vancin is responsible for membrane depolarization in S. aureus. Further study of lipoglycopeptide side chains (16) could eluci-
date the structure-activity relationship required for depolarization.

Exposure of hVISA to oritavancin also caused immediate and concentration-dependent increases in membrane perme-

Effects of oritavancin on cell viability were monitored under conditions similar to those used in the membrane integ-

FIG. 2. Oritavancin-induced membrane depolarization and cell killing of VISA, VRSA, and VREF isolates are tightly correlated. (A to C) Correlations were determined for VISA ATCC 700699 (A), VRSA VRSS (B), and VanB VREF ATCC 51299 (C). Oritavancin was used at 0.5 \( \mu \)g/ml (△), 1 \( \mu \)g/ml (▲), 2 \( \mu \)g/ml (■), 4 \( \mu \)g/ml (□), 8 \( \mu \)g/ml (○), and 16 \( \mu \)g/ml (●).
of either chloroeremomycin or vancomycin had no effect on cell viability within the time frame of the assay. These results imply that loss of membrane potential and increased permeability, attributed to the 4-′chlorobiphenylmethyl group of oritavancin, are responsible for its rapid bactericidal activity. The correlation between membrane depolarization (timing and extent) and cell viability of hVISA yielded $r = 0.78$ (Fig. 1D). The correlations were also strong for the VISA ($r = 0.85$), VRSA ($r = 0.90$), and VRE ($r = 0.82$) isolates (Fig. 2). Unlike prior studies with telavancin (8) and the lipopeptide daptomycin (18), this is the first study to demonstrate and quantify a correlation between disruption of membrane potential and bacterial killing for both staphylococci and enterococci.

Although we cannot exclude the possibility that oritavancin inhibition of peptidoglycan cross-linking via binding of the pentaglycyl bridge (9) causes the rapid bactericidal effect, it seems unlikely in that oritavancin retains bactericidal activity against stationary-phase S. aureus inoculated into nutrient-depleted medium, conditions under which cell wall synthesis is expected to be minimal (4). This idea is further supported by the results of a study demonstrating negligible killing of stationary-phase S. aureus by vancomycin and the β-lactam nafcillin (12).

We conclude that the addition of the 4-′chlorobiphenylmethyl group to the natural product chloroeremomycin provides for profoundly increased antibacterial activity of the resultant molecule, oritavancin. Most notably, oritavancin effects rapid and concentration-dependent bactericidal killing against Gram-positive pathogens, including vancomycin-nonsusceptible phenotypes, via perturbation of membrane integrity. Although it remains to be determined whether the multiple mechanisms of action of oritavancin act simultaneously to effect cell death, it is possible that they reduce the propensity to select for resistance.

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REFERENCES