Oritavancin: Mechanism of Action

George G. Zhanel, Frank Schweizer, and James A. Karlowsky

Departments of Medical Microbiology and Chemistry, University of Manitoba, and Departments of Clinical Microbiology and Medicine, Health Sciences Centre, Winnipeg, Canada

Oritavancin is a semisynthetic lipoglycopeptide analogue of vancomycin that contains the heptapeptide core common to all glycopeptides. It differs from vancomycin by the presence of a hydrophobic N-4-(4-chlorophenyl)benzyl (also referred to as 4’-chlorobiphenylmethyl) substituent on the disaccharide sugar, the addition of a 4-epi-vancosamine monosaccharide to the amino acid residue in ring 6, and the replacement of the vancosamine moiety by 4-epi-vancosamine. One mechanism of action of oritavancin is inhibition of transglycosylation (important in peptidoglycan synthesis) by binding to D-alanyl-D-alanine stem termini in Gram-positive bacteria. The inhibition of peptidoglycan synthesis via inhibition of transglycosylation is common to all glycopeptides (vancomycin) and lipoglycopeptides. Secondary binding of oritavancin to the pentaglycyl (Asp/Asn) bridging segment in peptidoglycan also occurs, which distinguishes it from vancomycin and contributes to oritavancin’s activity versus vancomycin-resistant organisms. The presence of the hydrophobic 4’-chlorobiphenylmethyl group allows for interaction and disruption of the cell membrane, resulting in depolarization, permeabilization, and concentration-dependent, rapid cell death. This mechanism is shared with telavancin but not vancomycin and results in activity against daptomycin-nonsusceptible organisms. In conclusion, oritavancin’s mechanism of action involves at least 3 known mechanisms: inhibition of transglycosylation, inhibition of transpeptidation, and cell membrane interaction/disruption. Oritavancin’s multiple mechanisms of action confer activity against vancomycin-susceptible and -resistant organisms, as well as rapid, concentration-dependent killing versus actively growing, stationary phase, and biofilm-producing Gram-positive bacteria.

Reports of multidrug-resistant, Gram-positive pathogens such as methicillin-resistant Staphylococcus aureus (MRSA) appeared in the scientific literature as early as the 1960s [1, 2]. However, it was not until the 1980s that MRSA became widespread [1]. The steep rise in the prevalence of MRSA in hospitals during that decade resulted in a sharp increase in vancomycin use [1, 2]. Today, healthcare-associated MRSA is a worldwide concern, and the percentage of S. aureus isolates resistant to methicillin exceeds 60% in some intensive care units [1, 2]. Many countries, including the United States and Canada, are also experiencing a significant increase in the number of community-associated MRSA infections in otherwise healthy individuals [1, 2]. Compounding the problem is the emergence of vancomycin-intermediate S. aureus (VISA), vancomycin-resistant S. aureus (VRSA), and vancomycin-resistant Enterococcus (VRE), which has added a greater sense of urgency to the search for novel antimicrobials with activity against resistant Gram-positive pathogens [1, 3].

For decades vancomycin was viewed by many as the gold standard for the treatment of MRSA infections [4–6]. Although it is active against the vast majority of MRSA, vancomycin is plagued by poor penetration into certain tissues (eg, lung) and slow bactericidal activity [4, 6]. In addition, vancomycin’s minimum inhibitory concentration (MIC) for Gram-positive pathogens such as MRSA has been reported to be increasing, with therapeutic failures also being reported [7–11]. Furthermore, investigators are reporting the development of heteroresistant vancomycin-intermediate S. aureus (hVISA) and VISA [1, 3]. The rampant spread of MRSA and VRE and the inherent limitations of vancomycin therapy highlight the need for novel, rapidly bactericidal agents to combat infections with these pathogens [12].
Eli Lilly identified and developed vancomycin in the 1950s as a response to the emergence of penicillin-resistant *S. aureus* [6, 13, 14]. In the early 1980s, Eli Lilly renewed its efforts to identify other glycopeptides with enhanced properties relative to vancomycin (ie, active vs vancomycin-resistant strains, better pharmacodynamics, etc). They identified chloroeremomycin, a vancomycin analogue with a different vancosamine sugar than vancomycin and an additional vancosamine epimer not present in vancomycin’s structure (Figure 1) [13, 14]. Chloroeremomycin displayed modestly improved activity compared with vancomycin, against methicillin-susceptible *S. aureus* (MSSA), MRSA, and vancomycin-susceptible and -resistant strains of enterococci [13, 14]. Eli Lilly medicinal chemists synthesized >500 analogues of chloroeremomycin and identified a chlorobiphenylmethyl side chain analogue, LY333328 (later known as oritavancin) [13, 14]. From 1994 to 2001 Eli Lilly, in a screen to gain substantial anti-VRE activity while maintaining anti-MRSA activity, developed oritavancin, but terminated its antibacterial discovery/development program and sold oritavancin to InterMune, Inc, in 2002 [14]. InterMune conducted a variety of clinical trials with oritavancin but subsequently sold it to Targanta Therapeutics Corporation (now owned by The Medicines Company) in 2006 [14]. The Medicines Company is continuing to develop oritavancin, including performing additional clinical trials.

Oritavancin, a vancomycin analogue, is a semisynthetic lipoglycopeptide with activity against MRSA, VISA, VRSA, daptomycin-nonsusceptible *S. aureus*, and VRE (both VanA and VanB phenotypes) [3, 5, 13–21]. Oritavancin, which has novel pharmacokinetics [22, 23], is rapidly bactericidal in a concentration-dependent manner, and the area under the concentration–time curve to MIC ratio is the pharmacodynamic parameter that best describes its activity [5, 15, 24, 25]. This agent is currently in phase III development as a 1200-mg single dose for the treatment of acute bacterial skin and skin structure infections, including those due to MRSA [13, 14, 26, 27]. The purpose of this report is to review the data available on the mechanisms of action of oritavancin against Gram-positive pathogens.

**STRUCTURE OF ORITAVANCIN**

All glycopeptides, including vancomycin, contain a common heptapeptide core and are glycosylated (Figure 1) [5, 6, 13]. The heptapeptide core contains at least 5 amino acid residues bearing aromatic side chains (these 5 rings are highlighted in Figure 1). Oritavancin, a vancomycin analogue, is a synthetic derivative of the naturally occurring glycopeptide chloroeremomycin (Figure 1) [13, 14]. Chloroeremomycin belongs to the eremomycin class, which differs from vancomycin by the presence of a 4-epi-vancosamine monosaccharide at the amino acid residue in ring 6 and the substitution of the vancosamine by 4-epi-vancosamine (Figure 1) [13, 14]. These structural differences, compared with vancomycin, confer on chloroeremomycin an enhanced antimicrobial activity against both vancomycin-susceptible organisms (through increased affinity for the wild-type target), as well as improved activity against vancomycin-resistant organisms [14]. Oritavancin differs from chloroeremomycin by the addition of a 4'-chlorobiphenylmethyl substituent on the disaccharide sugar (Figure 1) [13, 14]. This structural alteration imparts improved activity against vancomycin-susceptible *Enterococcus* (VSE) and significantly improved activity against VRE, including VanA enterococci [13, 28, 29]. The lipophilic side chain in oritavancin anchors the compound to the cell membrane through hydrophobic interactions, and it stabilizes the oritavancin dimer (Figure 2) [15, 29–32]. The formation of dimers is achieved by interactions involving the disaccharides attached to ring 4 and the 4-epi-vancosamine on ring 6, as well as the chlorine on ring 2 [13, 32–35]. The dimers formed improve oritavancin’s ability to bind to its target, including D-alanyl-D-lactate (D-Ala-D-Lac) present in VanA enterococci [29].

**MECHANISM OF ACTION**

**Peptidoglycan Synthesis Inhibition**

The mechanism of action common to all members of the glycopeptide class, including vancomycin, is the inhibition of
bacterial cell wall synthesis [5, 6]. The cell wall and its major component, peptidoglycan, are responsible for maintaining cell shape and structural integrity of the bacterial cell. Peptidoglycan is a polymer composed of repeating disaccharide-pentapeptide units, which are synthesized in the cytoplasm. Transport of the disaccharide-pentapeptide units across the cell membrane occurs in the form of a complex with a lipid carrier (undecaprenyl pyrophosphate), known as lipid II. Translocation of lipid II across the cell membrane provides the substrate for transglycosylase enzymes to incorporate the disaccharide-pentapeptide monomer into the newly synthesized or nascent peptidoglycan. Vancomycin and other glycopeptides bind to the carboxyl-terminal acyl-D-alanyl-D-alanine (acyl-D-Ala-D-Ala) residues of the pentapeptide moiety of lipid II (mechanism 1 in Figure 2) [13]. This specific binding, which is referred to as the primary binding site, sterically hinders the transglycosylase enzyme from incorporating the disaccharide-pentapeptide monomer into nascent peptidoglycan. This inhibition of peptidoglycan synthesis by vancomycin results in a relatively slow (compared with β-lactams) and concentration-independent bactericidal activity [31].

Like vancomycin, oritavancin has also been reported to inhibit transglycosylation by binding to the primary site [28, 32, 33]. The addition of the hydrophobic side chain 4′-chlorobiphenylmethyl onto the disaccharide sugar confers on oritavancin an increased binding affinity to the target molecule lipid II (mechanism 1 in Figure 2). Oritavancin’s hydrophobic side chain allows bacterial cell membrane anchoring, which results in stabilizing the interaction with lipid II [32]. The self-association into dimers may promote cooperative interactions between dimers and adjacent pentapeptides of the peptidoglycan, which results in increased binding avidity [13, 15]. Unlike vancomycin, oritavancin is able to bind to depsipeptides, including D-Ala-D-Lac, which facilitates its inhibition of cell wall synthesis even in organisms that exhibit VanA-type resistance [28, 29]. Oritavancin forms homodimers prior to binding to D-Ala-D-Ala or D-Ala-D-Lac, which increases its binding affinity for the target site.

Unlike vancomycin, oritavancin has also been reported to inhibit transpeptidation by binding to a secondary site (mechanism 2 in Figure 2) [28, 33, 34]. Using solid-state nuclear magnetic resonance (NMR), the Schaefer group has described the molecular interactions of oritavancin with the cell wall and changes in cell wall structure as a result of these interactions [28, 33, 34]. These studies performed on either Staphylococcus aureus or Enterococcus faecium, using metabolic labeling of cell wall components with amino acids containing NMR-active nuclei, demonstrated that oritavancin is able to interact with newly made peptidoglycan components in a more extensive manner than does vancomycin. Specifically, oritavancin was reported to bind to the peptidic cross-linking portion of the cell wall of S. aureus (pentaglycine bridge [33, 34]) and of E. faecium (Asx

![Figure 2](https://academic.oup.com/cid/article-abstract/54/suppl_3/S214/290034/3217429034)

Figure 2. Proposed model depicting vancomycin and oritavancin mechanisms of action. Comparison of the single mechanism of action of vancomycin (inhibition of transglycosylation—mechanism 1) to the 3 different mechanisms of action of oritavancin (inhibition of transglycosylation—mechanism 1, inhibition of transpeptidation—mechanism 2, and disruption of bacterial membrane integrity—mechanism 3, depicting oritavancin dimers).
bridge [28]), in addition to binding the D-Ala-D-Ala termini of the stem peptide. Thus, oritavancin may retain microbiological activity against vancomycin-resistant organisms (such as VRE and VRSA, where the termini are modified to D-Ala-D-Lac) by instead binding the peptide bridge [12, 28, 34]. The addition of a hydrophobic side chain 4’-chlorobiphenylmethyl on the disaccharide sugar thus confers on oritavancin increased binding affinity to lipid II, resulting in inhibition of transglycosylation and a more pronounced impact on transpeptidation (mechanism 2 in Figure 2), owing to bridge peptide binding. Thus, the simultaneous accumulation of D-Ala-D-Ala stems and decrease in the level of cross-linking is suggestive of an agent acting on both transglycosylation and transpeptidation [28, 33].

**Cell Membrane Alterations**
The addition of a hydrophobic side chain 4’-chlorobiphenylmethyl on the disaccharide sugar confers on oritavancin not only increased binding affinity, which results in inhibition of transglycosylation and transpeptidation, but also cell membrane anchoring and self-association into dimers, which result in perturbation of cell membrane integrity in Gram-positive organisms [13, 29–32, 36]. Studies using the fluorescent probe 3,3’-dipropylthiadicarbocyanine iodide [DiSC₃(5)] as an indicator of the degree of depolarization of the plasma membrane following exposure to drug have shown that challenge with oritavancin but not vancomycin results in loss of membrane integrity [15, 31, 32]. The hydrophobic 4’-chlorobiphenylmethyl side chain of oritavancin was implicated in the mechanism, resulting in concentration-dependent membrane depolarization and increased permeability in various resistance phenotypes of S. aureus and enterococci, which resulted in cell death (VISA, VRE, VRE) [15]. Oritavancin interacts with the cell membrane, facilitating a prime antimicrobial position for peptidoglycan interactions, disrupting the bacterial membrane potential and increasing membrane permeability (mechanism 3 in Figure 2) [15]. Figure 2 compares the single mechanism of action of vancomycin (inhibition of transglycosylation) to the 3 different mechanisms of action of oritavancin (inhibition of transglycosylation, inhibition of transpeptidation, and disruption of bacterial membrane integrity).

Kim et al have recently shown that the hydrophobic 4’-chlorobiphenylmethyl side chain of oritavancin results in interaction with isolated membrane protoplasts and increases membrane permeability in artificial liposomes composed of bacterial membrane phospholipids [32]. It is important to note that, although oritavancin’s effects and interactions with the bacterial cell membrane only occur above a threshold concentration, researchers have concluded that its rapid, concentration-dependent bactericidal effects against Gram-positive pathogens result from altering the integrity of the cell membrane [13, 15, 29, 31]. These findings also argue in favor of the currently studied dosing of oritavancin in clinical trials for acute bacterial skin and skin structure infections, that is, single-dose (1200 mg) therapy [27]. Belley et al have recently reported that, unlike vancomycin, oritavancin exhibited concentration-dependent bactericidal activity versus stationary phase inocula of MSSA, MRSA, and VRSA [29, 31]. As demonstrated for exponentially growing organisms, oritavancin induced membrane depolarization, increased permeability, and resulted in rapid cell death. These investigators reported a correlation between membrane depolarization and cell death [15]. Also, unlike vancomycin, oritavancin sterilized biofilms of MSSA, MRSA, and VRE at minimal biofilm eradication concentrations (MBECs) of between 0.5 and 8 µg/mL [31]. The bactericidal potency of oritavancin was evident as the MBECs were within 1 dilution of the respective MIC performed with planktonic cells [31]. The authors hypothesized that the hydrophobic 4’-chlorobiphenylmethyl side chain of oritavancin disrupts membrane potential and increases membrane permeability in Gram-positive pathogens. It is the concentration-dependent oritavancin-induced cell membrane perturbation and resulting permeability that results in cell death.

**Ultrastructural Changes**
The ultrastructural effects of oritavancin and vancomycin on MRSA and VRE were examined using transmission electron microscopy [29]. Both vancomycin-exposed (exposure at 16 µg/mL for 3 hours) and oritavancin-exposed (exposure at 1 µg/mL for 10 minutes) MRSA demonstrated cell wall thickening and membrane inclusions, whereas only oritavancin-exposed cells demonstrated deformed septa that were thickened and misshapen with loss of staining intensity of nascent septal cross walls. In VRE, oritavancin exposure (0.12 µg/mL or 1 µg/mL for 10 minutes) induced formation of large membrane inclusions and septal distortions. The authors hypothesized that oritavancin-induced septal defects may be due to membrane disruption because of oritavancin’s ability to disrupt and form cooperative interactions [29]. It is clear that in both MRSA and VRE, oritavancin demonstrates pronounced effects on cell division that are not observed with vancomycin. This is likely an indication that oritavancin is able to target critical yet vulnerable sites to achieve rapid concentration-dependent bactericidal activity.

**MECHANISM OF RESISTANCE**

Even though oritavancin demonstrates activity against clinical isolates of VanA, VanB, and VanC enterococci, it has been demonstrated in vitro using agar-based methods that moderate decreases in susceptibility level resistance to oritavancin can occur in enterococci isolates exhibiting the VanA or VanB phenotype [37]. However, resistance in isolates that harbor the vanB operon was only observed when the operon was altered
Such that it became inducible by teicoplanin or constitutively expressed [37]. Isolates that produce peptidoglycan precursors terminating in d-Lac may exhibit reduced susceptibility to oritavancin if all precursors terminating in d-Ala are eliminated [37]. This has been achieved in vitro by increasing resistance gene expression or reducing d-Ala-d-Ala production [37]. Such changes resulted in a maximum 16-fold increase in oritavancin MIC compared with the parent strain [37]. The expression of the vanZ gene of the vanA gene cluster also resulted in a 4-fold increase in MIC through an unknown mechanism [37]. Limited data are available regarding the selection of oritavancin-resistant mutants in the laboratory. This is likely because, in agar assays, oritavancin diffuses slowly and binds to agar, which makes interpretation of experiments to investigate single-step resistance selection difficult [38]. Bozdogan et al [38], studying a vancomycin-resistant S. aureus that was serially selected using vancomycin, showed a 32- to 64-fold increase in vancomycin MIC, a 16- to 128-fold increase in teicoplanin MIC, and a 16- to 64-fold increase in dalbavancin MIC, but only a 2- to 4-fold increase in oritavancin MIC. Thus far, it has not been possible to select for high-level oritavancin resistance in the laboratory, and there have been no instances of decreased oritavancin susceptibility emerging during or after oritavancin therapy in clinical studies to date [5, 13, 27]. Whether this is due to the fact that oritavancin demonstrates multiple different mechanisms of action is unknown.

CONCLUSIONS

Oritavancin’s mechanism of action involves at least 3 known mechanisms: inhibition of transglycosylation, inhibition of transpeptidation, and cell membrane interaction/disruption. Oritavancin’s multiple mechanisms of action confer activity against vancomycin-susceptible and -resistant organisms as well as rapid, concentration-dependent killing versus actively growing, stationary phase, and biofilm-producing Gram-positive bacteria.

Notes

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