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Glycopeptides: Update on an old successful antibiotic class

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ABSTRACT

The natural product glycopeptides vancomycin and teicoplanin have come to play a significant role in the therapy for Gram-positive bacterial infections. In particular vancomycin is the choice for empiric therapy of these infections primarily due to its activity against and the significance of methicillin-resistant *Staphylococcus aureus*. While high-level problematic glycopeptide resistance among enterococci was observed initially and continues to increase, the slow creep of vancomycin intermediate susceptibility and the fear of frank resistance among the staphylococci have precipitated increasing work leading to creation of new semisynthetic analogs. These new agents, including dalbavancin and telavancin, are within 1–2 years availability in the clinic. Interestingly, chemical modifications resulting in these second-generation analogs and additional characterization have revealed new mechanisms of antibacterial action, and plasticity regarding additional properties including pharmacokinetics for the drug candidates. The unique beneficial properties of the near term vancomycin replacements, semisynthesis of additional important analogs, and advances in metabolic engineering resulting in novel scaffolds signal a new era for the glycopeptide antibiotics.

1. Introduction

The glycopeptide antibiotic vancomycin without question has a unique history [1–3]. Termed by some to be the agent of last recourse for refractory Gram-positive bacterial infections, this first and only commercially available glycopeptide in much of the world has become the drug of choice for empiric therapy, and is more widely utilized as a generic than during its entire branded life cycle [3–5]. The early pattern of use for vancomycin was set in play by a perception of inferiority relative to preferred beta-lactam antibacterials utilized in treating related infections, and believed toxicity associated with poor degree of purity [1–3]. However, upon the advent of the multidrug-resistant staphylococci, and in particular bacteria resistant to penicillinase-insensitive agents like methicillin, vancomycin use climbed to all time highs [1,3,4].

The acronym MRSA, for methicillin-resistant *Staphylococcus aureus*, is one of the most widely known terms to arise from the infectious disease field in modern time, ranking with the likes of TB, polio, and smallpox [6,7]. It is safe to say that nearly every lay person in developed countries let alone professionals working in the field have heard of this term, and likely know of someone who has suffered from one of these "hospital" or nosocomial-acquired infections. This pathogen is remarkable not only because of the significant associated morbidity and mortality, breadth of infectious syndromes, and very broad antibacterial resistance profile, but also because of the continuing and truly remarkable increase in the total numbers

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of infections. Today vancomycin use and MRSA are linked. In fact, MRSA alone might be the primary driver for the increased and continued use of parenteral vancomycin. And the next chapter is still unfolding in this remarkable story, as a related but distinct group of community-acquired MRSA has now cropped up in patients as disparate as children and professional sports athletes [7–9]. While the efficacious use of vancomycin, and the other commercially available glycopeptide teicoplanin, have been increasingly threatened by vancomycin-resistant *Enterococcus* spp. (VRE) in many countries, the more recent detection of hetero-resistance, intermediate susceptibility, and the long feared high-level resistance to glycopeptides among the staphylococci are of critical concern [10–19]. The end of effective antibiotic therapy for Gram-positive infections may not quite be in sight due to the newer agents including daptomycin and linezolid and near term development glycopeptide candidates, but reduced clinical efficacy due to tolerance and resistance are disturbing. These events, their significance to clinical therapy, how the findings have affected our understanding of glycopeptide antibiotics, and the future of drug discovery will be subsequently described in this brief review.

2. Vancomycin-resistant enterococci—the harbinger of things yet to come

In 1986 VRE were first detected in both France and Great Britain [20,21]. Within 1 year resistant strains were associated with infections in the U.S. [22,23]. This was immediately significant because glycopeptide resistance had not been observed since the discovery of vancomycin some 30 years earlier, other than among coagulase-negative staphylococci believed at the time to be contaminants and not clinically important [24–26]. Vancomycin was and is still often used for indications where glycopeptide-susceptible ampicillin-resistant enterococci may be encountered [10,27]. *Enterococcus* spp., primarily *Enterococcus faecalis* and *Enterococcus faecium*, are major pathogens encountered in the clinic accounting for 10–20% of nosocomial infections, and are the third most common cause of Gram-positive bacterial infections following the coagulase-negative staphylococci (CoNS) and *S. aureus* [28–34]. Concomitant with loss of glycopeptide susceptibility were frequent increases in beta-lactam resistance and high-level aminoglycoside resistance [13]. In some cases resistant enterococci have become refractory to nearly all commonly utilized antibiotics [35–37].

The VRE have disseminated throughout much of the World [38–42]. In the U.S., VRE have exceeded 25% as the proportion of all enterococcal infections, whereas in Europe only Italy and Great Britain experience a high proportion of VRE infections in contrast to findings from other countries [31,43–45]. Epidemiological profiles are varied, the European experience impacted by previous use of the glycopeptide growth-promoter avoparcin which selects for cross-resistance, while in the U.S. a higher correlation is observed for use of third-generation cephalosporins, fluoroquinolones and anti-anaerobic antibiotics or combinations to which VRE are resistant and use of which may result in enhanced gastrointestinal colonization [46–49]. For an individual clinical institution initial colonization may be clonal. Following endemic colonization a more differentiated population is reported [50,51]. Interestingly, *E. faecalis* is more commonly isolated and associated with more severe infections, but a much greater proportion of *E. faecium* strains are vancomycin resistant [36,37,47].

VRE resistance to vancomycin and teicoplanin are mediated by synthesis of an altered cell wall precursor terminating in D-alanyl-D-lactate which exhibits a lower affinity for glycopeptide antibiotics due to reduced hydrogen bonding in contrast to the D-alanyl-D-alanine precursor from susceptible bacteria [52,53]. Glycopeptide binding blocks both transglycosylation and transpeptidation steps involved in cell wall synthesis [53,54]. This resistance requires multiple gene products to sense the antibiotics, synthesize the novel precursor, and to hydrolyze residual classical precursor to which the antibiotics might still bind [55–58]. In the case of the VanA enterococci, resistance is inducible by both vancomycin and teicoplanin and high-level resistance to both antibiotics is observed [10]. In contrast, for VanB enterococci vancomycin but not teicoplanin is sensed resulting in high-level vancomycin resistance with teicoplanin susceptibility [10]. Pre-growth in vitro in the presence of vancomycin results in resistance to teicoplanin as well for VanB strains. *vanA* and *vanB* resistance elements are carried on plasmids or transposons which may be transferred horizontally facilitating both intra- and inter-species spread of resistance [10,59,60,61]. A third principle mechanism, VanC, is also observed among other less frequently encountered species. In species including *Enterococcus casseliflavus*, *Enterococcus gallinarum*, and *Enterococcus flavescens* precursor terminating in D-alaynyl-D-serine is constitutively synthesized resulting in low-level resistance to vancomycin [62–65]. vanC genes are intrinsic and chromosomally located [64]. Other less frequent but related resistance mechanisms such as VanD and even vancomycin-dependency have been described [66].

3. The slippery slope—heteroresistance leading to intermediate susceptibility, and tolerance

In 1997 Hiramatsu et al. reported the first incidence of what they termed vancomycin-resistant *S. aureus* [67]. In much of the rest of the world the bacteria with this phenotype have become known as vancomycin-intermediate susceptible *S. aureus* (VISA) [12,67–72]. Initially it was feared that the vancomycin-resistance elements had been transferred from VRE to *S. aureus*. However, the VISA resistance mechanism was shown to be distinct. Underlying this reduced vancomycin susceptibility is substantially increased potential for cell wall synthesis, release of cell wall fragments, elevated cytoplasmic monomeric precursor with reduced amidation, reduced cell wall cross-linking, and reduced peptidoglycan recycling or autolysis for bacterial strains [73–76]. These biochemical changes result in reduced susceptibility in part due to binding and reduction of the effective glycopeptide antibiotic concentration by free D-alanyl-D-alanine termini from the thickened poorly cross-linked cell wall, and potentially due to a shift in the anabolic–catabolic balance in cell wall

synthesis [73–76]. Isolation of this type of bacterial strain while increasing has remained sparse, however, the frequency of infections due to vancomycin hetero-resistant, or more accurately hetero-VISA strains is increasing [68,77–83]. These latter bacteria may be the predecessor to VISA and thus might portend a coming surge in *S. aureus* infections where vancomycin efficacy is limited [84–86].

Hetero-VISA as described by Liu and Chambers [71] are bacterial strains characterized by a subpopulation with elevated MICs to vancomycin and are descended from MRSA. The low-frequency reduced-susceptibility variants predominate in the presence of the selective pressure provided by vancomycin therapy resulting in reduced efficacy. They are hard to detect utilizing standard methods because the preponderance of the population is susceptible and masks their presence. Thus incidence of hetero-VISA may actually be greater than reported [26,68]. Hiramatsu advised use of a vancomycin agar screening plate for hetero-VISA due to the inadequacy of detection methods [87].

These bacteria are not only more difficult to treat therapeutically, but may also be more resistant to innate host defenses and result in more severe infections [88–92]. Many of the heteroresistant staphylococci are *agr*-group II strains, and have further exhibited both tolerance for vancomycin and reduced susceptibility to natural host defenses such as platelet microbicidal protein that play an important role particularly in the case of infections such as endocarditis [84,88–90]. Higher mortality has been reported from one study of infections due to hetero-VISA, but in another retrospective study no increase in associated mortality was detected [93–95]. In any case, poorer outcomes may often be expected from glycopeptide therapy for infections by these organisms which typically display MICs on the high end of the susceptible range [68,85–87,96–99].

4. Frank glycopeptide resistance among the MRSA

In 2002 the long feared event of high-level vancomycin resistance in MRSA (VRSA) was clinically manifested [14–16]. A handful of geographically distinct reports have been made subsequently [14–16]. The molecular basis for the resistance is the *vanA* determinant originating from a Tn1546-like element [53,100]. The *vanA* sequences from the original Michigan VRSA, and a vancomycin-resistant *E. faecalis* strain concomitantly infecting the patient, were identical confirming the potential for interspecies transfer of resistance from VRE [100]. For the Pennsylvania isolate differences in DNA sequences suggest instability of the vector plasmid and likely explain the lower MIC for vancomycin [15,100]. Some patients had not received vancomycin within five years of the detection of the VRSA, while in other patients vancomycin had been administered intermittently suggesting that this selective pressure may not be required for transfer of resistance [17]. For several of the VRSA automated susceptibility testing did not detect resistance, and the Center for Disease Control and Prevention recommended use of the vancomycin agar screening plate followed by broth microdilution testing was required [15,19]. The VRSA clinical isolates were susceptible to a number of older broad-spectrum or newer narrow spectrum antibiotics including chloramphenicol, minocycline, trimethoprim–sulfamethoxazole, linezolid, and quinupristin–dalfopristin but many of these agents are bacteriostatic and not first-choice for therapy of these infections [101,102].

The reason for concern generated by these findings is obvious. Increased frequency of infections caused by this pathogen resistant to the major empirical therapeutic is likely, and in fact incidence already may have been more widespread but were undetected [103]. These events resulted from clinical strains infecting patients with underlying illnesses. However, if transformation of *vanA* does occur in the community acquired MRSA or other more highly virulent strains of *S. aureus* numbers of VRSA infections, morbidity, and mortality may be greater as well. Vancomycin use may not be required as a selective pressure for the resistance transfer to occur between VRE and MRSA, therefore further limiting use of the antibiotic may not be an effective deterrent. Further, the rate of interstrain transfer of the genetic element between a VRSA and MRSA may be greater than interspecies transfer, and the staphylococci are more frequently encountered in the clinic. Additionally, resistance may arise at some point in the future due to use of the remaining active antibiotics against various Gram-positive bacterial infections, and one could speculate regarding potential for a VRSA resistant to most currently available antibiotics.

5. New clinical agents—the second-generation glycopeptide antibacterials

5.1. *Discovery of oritavancin—the first second-generation glycopeptide clinical candidate*

Oritavancin (LY 333328) (Fig. 1) is the first clinical candidate in what have been called the second-generation glycoepeptides [104–107]. These agents by virtue of their hydrophobic substituents have also been labeled lipoglycopeptides [54]. The goal of the project that culminated in the discovery of oritavancin was to improve over vancomycin's pharmacokinetic properties, and was based on an understanding of the relevant structure–activity relationship differences between vancomycin and teicoplanin. Improvements in alkylated and acylated analogs of vancomycin were deemed inadequate, and other natural product glycopeptides were subsequently evaluated as platforms [108–111]. Compounds like chloroeremomycin (LY264826) exhibiting better activity and spectrum were utilized as a starting point, and eventually leads were evolved to the resultant chlorobiphenyl-modified lipoglycopeptide that is oritavancin.

Of the semisynthetic lipoglycopeptides, oritavancin exhibits the best in vitro activity against VanA enterococci and VRSA, good activity against the staphylococci, and truly exquisite activity against pneumococci [101,102,111–115] (Table 1). It is rapidly bactericidal against many species, and in particular for enterococci where vancomycin and teicoplanin are only bacteriostatic even against susceptible strains [113].

Oritavancin was active in rabbit models of *Streptococcus pneumoniae* meningitis with only 5% CSF penetration, and endocarditis due to *E. faecalis* [116,117]. Like all glycopeptides to date, oritavancin administration for therapy of systemic

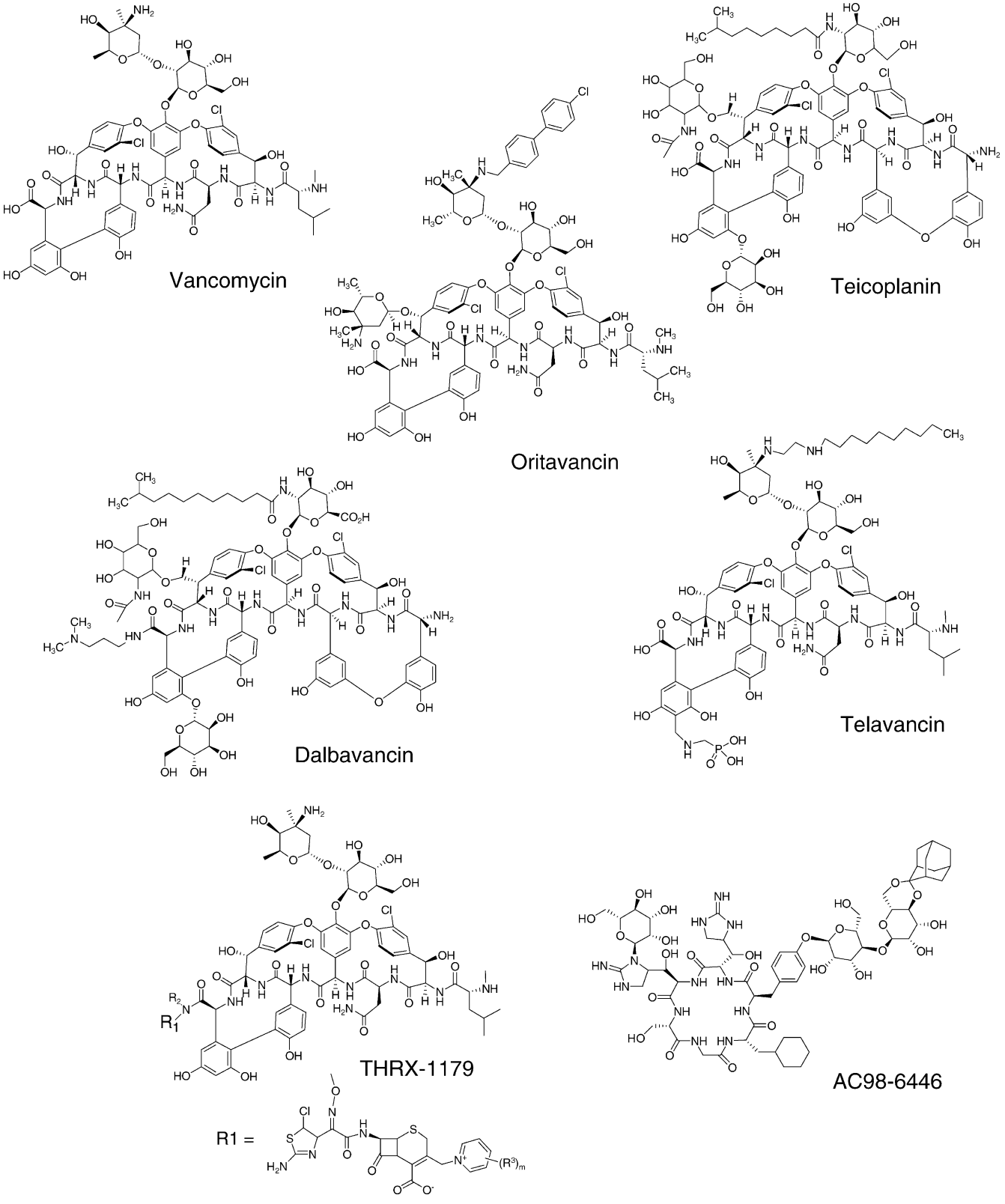

Fig. 1 – . Structures of glycopeptide antibiotics, near-term lipoglycopeptides, and interesting pre-clinical compounds. Structures: vancomycin; teicoplanin; oritavancin dalbavancin; telavancin; vancomycin–cephalosporin conjugate; mannopeptimycin analog AC98-6446.

Table 1 – Comparison of antibacterial activity of the semisynthetic lipoglycopeptide antibacterials with vancomycin[a,b]

		Oritavancin	Dalbavancin	Telavancin	Vancomycin
MRSA	MIC range	0.12–4	0.06–1	≤0.06–2	0.5–4
MSSA	MIC range	0.12–2	0.06–0.5	0.12–2	0.25–2
MR-CoNS	MIC range	0.25–4	0.06–1	0.12–2	1–4
MS-CoNS	MIC range	0.25–1	≤0.03–0.25	0.12–2	0.12–1
Streptococcus pneumoniae	MIC range	≤0.002–0.06	0.008–0.12	0.004–0.03	0.25–2
Beta-hemolytic streptococci	MIC range	0.016–0.12	≤0.03–0.12	0.03–0.12	0.5
Eneterococcus spp., vancomycin-susceptible	MIC range	0.06–0.25	≤0.03–1	0.06–1	0.25–4
Eneterococcus spp., VanB	MIC range	0.12–2	0.02–2	0.12–2	8–128
Eneterococcus spp., VanA	MIC range	1–4	0.5–>128	0.12–8	>128

[a] MIC, minimal inhibitory concentration (mg/L).
[b] References for susceptibility data [101,102,111–115,119–126,130–135].

infections is via parenteral route. The molecule exhibits a three-compartment clearance profile with a beta half-life of 18 h [105,107]. In a Phase III complicated skin and skin structure infection (CSSSI) clinical trial 3 mg/kg QD dosing of oritavancin achieved non-inferiority compared to vancomycin and cephelexin, but with a shorter mean duration of therapy [107]. A subsequent Phase III CSSSI trial utilized a standard 200 mg QD dose of oritavancin and non-inferiority criteria were met as compared to a 15 mg/kg bid dose of vancomycin followed by oral cephelexin. Again the mean length of therapy for oritavancin (5.3 days) was substantially shorter than for vancomycin (10.9 days) [107]. One curious in vitro finding was that abnormalities in lipid storage were observed in macrophages and fibroblasts exposed to the antibiotic [118]. How this observation may relate to safety aspects of the molecule is unknown. Further development of oritavancin ceased some 3 years ago while Intermune, the current licensee, sought a partner to assume responsibility for completion of the work. Targanta Therapeutics recently announced acquisition of oritavancin from Intermune.

5.2. *Dalbavancin—a once weekly super-teicoplanin*

Dalbavancin (BI-397) is the most clinically advanced of the second-generation lipoglycopeptide antibacterials and the NDA for this molecule has been filed with the U.S. Food and Drug Administration [119–122]. This molecule was derived from a rational design approach intended to improve both activity against coagulase-negative staphylococci and pharmacokinetic properties, while retaining activity against VanB enterococci [122]. Dalbavancin is a semi-synthetic derivative of the teicoplanin-related glycopeptide A40926 modified with an amide appendage at the c-terminus and an alteration of the hydrophobic acylglucosamine substituent, and like teicoplanin is active against VanB enterococci as well as the staphylococci and other important species [120–127]. Similar to teicoplanin, dalbavancin is also composed of a complex of related analogs with a preponderant component [121,122] (Fig. 1).

MICs for dalbavancin are markedly lower against many susceptible strains than those of vancomycin and several of the other glycopeptides [119–126] (Table 1). However, dalbavancin's MBC/MIC ratios are somewhat higher than for vancomycin and teicoplanin, and dalbavancin's high-level serum protein binding (98%) adversely impacts in vitro antibacterial action reducing activity to near-vancomycin levels in some cases [122]. A prolonged half-life also due to the exceptional serum protein binding is observed, but in this case the effect benefits both pharmacodynamic and potential pharmaco-economic properties [122]. Studies in animal models have suggested that serum concentrations greater than 5 mg/L are required for extended activity, and serum bactericidal titers of 2 could be determined from human volunteers when serum concentrations were in the range of 20 mg/L [122,127].

Numerically better activity for dalbavancin (2 dose regimen) versus comparator for clinical and microbiological success at follow up have been reported from a Phase II CSSSI trial [128]. In three subsequent Phase III CSSSI studies, similar findings of numerically better activity for dalbavancin versus vancomycin were reported against all *S. aureus*. In the case of comparison with linezolid no difference was noted, and in both trials activity against MRSA was similar to that observed against combined susceptible and MRSA [122,129]. In the trial comparing dalbavancin and cefazolin no differences were noted between efficacies of the two compounds for susceptible bacteria [122,129]. In general, findings largely suggest that dalbavancin may perform similarly to teicoplanin but with the advantage of better activity against the coagulase-negative staphylococci, and sustained serum levels providing better pharmacodynamic potential and a 2-week course of once-weekly dosing. These properties may allow for community or outpatient use against some infections. Vicuron (Versicor-Biosearch Italia) has recently been purchased along with dalbavancin by Pfizer.

5.3. *Telavancin—a new and improved vancomycin?*

Telavancin (TD-6424) (Fig. 1) is another in the line of second-generation semisynthetic lipoglycopeptide antibacterial agents [130–133]. Based on a vancomycin scaffold, this molecule exhibits potent in vitro antibacterial action against a broad array of important Gram-positive pathogens [130–135]. Goals of the project leading to the discovery of telavancin were to substantially increase the bactericidal activity over that of vancomycin, to regain activity against vancomycin-resistant species, and to improve the PK/ADME properties such that once daily administration could become standard [130,132,133]. Like for oritavancin this was achieved by alkylation of the vancosamine substituent with a hydrophobic

moiety, but in the telavancin case a decyl-aminopropyl derivative was used. Vancomycin was selected as the backbone because it was expected that introduction of the hydrophobic substituent would increase the half-life for the analog, and that use of a glycopeptide core with an already extended half-life as in the case of oritavancin or teicoplanin would result in a derivative with less desirable properties. It was felt that an exceptionally long half-life might be a liability if the semisynthetic lipoglycopeptide should be responsible for an unexpected adverse event. To impart a more drug-like nature to earlier leads in the series, and arrive at a molecule with the optimum distribution, clearance, and safety properties, a balancing hydrophilic methylamino-phosphonate substituent was introduced at the resorcinol position [132]. Solubility and ease of formulation have been further facilitated by use of hydroxyl-propyl-beta-D-cyclodextrin excipient in parenteral preparations [136].

Telavancin is active in vitro against nearly all Gram-positive pathogens including VanA enterococci [130–135] (Table 1). Highly potent, it is active against other glycopeptide-resistant bacteria including both the Michigan and Pennsylvania VRSA [130,133]. Like for oritavancin extreme potency is observed for the streptococci and in particular *S. pneumoniae* [130,133,134]. Minimal effect of serum on activity against staphylococci has been noted. Telavancin is rapidly bactericidal against staphylococci including VISA, and glycopeptide tolerant strains, and is bactericidal for enterococci in contrast to vancomycin [130,131,133,137]. This agent also exhibits an extended post-antibiotic effect relative to that of vancomycin [131]. An interesting and potentially important finding was that telavancin was active in an in vitro biofilm model where vancomycin and a number of other antibiotics were much less effective [138].

Telavancin is highly efficacious in animal models of relevant infections [139,140]. Notable among these observations are the narrow differences in the effective dose for infections in immuno-compromised as compared with -competent animals, and in the rabbit model of endocarditis. In this latter stringent model, telavancin was efficacious and sterilized MRSA and VISA valve vegetations [140]. This remarkable activity likely results from its potent bactericidal activity for staphylococci, and perhaps its tissue distribution although this latter property has not been evaluated relative to the infected site, i.e. vegetations [131,140].

Telavancin has been extensively characterized for potential adverse events including an additional Phase I human clinical study to evaluate the effect on cardiac repolarization (QTc interval duration) [141,142]. Telavancin had a minimal potential for affecting QTc in comparison to the positive control moxifloxacin [141]. In a published Phase II CSSSI evaluation, telavancin (7.5 mg/kg QD) achieved slight numerically greater activity than for the standard of care [143]. Telavancin is currently undergoing human evaluation at higher doses in multiple Phase III and Phase II clinical studies [144]. These advanced studies for CSSSI and hospital acquired pneumonia include arms intended to evaluate superiority of telavancin over vancomycin for treatment of infections due to MRSA [144]. Telavancin was recently licensed from the originator Theravance by Astellas (Yamanouchi–Fujisawa Pharmaceuticals).

5.4. *Mechanism of action—the unique diversity of an antibacterial platform*

One of the important outcomes from the quest for activity against glycopeptide-resistant bacteria has been the improved understanding of how glycopeptides work. Another remarkable finding has been the diversity of action observed among the semi-synthetic analogs as a result of the introduction of modifications [145–150]. These compounds do bind the lipid II substrate and inhibit peptidoglycan synthesis [145–151]. Additionally, the second-generation lipoglycopeptides may act by direct-binding and inhibition of the transglycosylase in some bacterial strains, inhibition of bacterial lipid synthesis, or direct action on the bacterial membrane [146–150].

Work by Allen and colleagues suggested that the hydrophobic moiety may benefit dimerization of the semisynthetic analogs with resultant improvements in substrate affinity and peptidoglycan synthesis inhibition [145,151,152]. These compounds may also exhibit increased binding potential facilitated through increased membrane anchoring as described by the Williams group for teicoplanin [145,151–153]. The extensive and remarkable work by the Kahne-Walker group demonstrated absence of a requirement for substrate binding of these lipoglycopeptide analogs for the cryptic activity against vancomycin-resistant bacteria, and along with their collaborators at Merck instead ascribed activity to direct interaction of the antibiotics with the transglycosylase in inhibition of cell wall synthesis [146–148]. Studies from the Theravance group have demonstrated effects of the semisynthetic antibiotics on bacterial lipid synthesis inhibition as well that could explain the diverse effects in different bacterial species, and direct action against bacterial membranes resulting in loss of function and cell viability [149,150]. It is likely that these effects may be variable between different bacterial species such that one target predominates over another in staphylococci versus enterococci for example. It is also likely that the lipoglycopeptides exert many of these actions concomitantly and that this combined action is the basis for their remarkable bactericidal activity and spectrum. A further area of interest and current uncertainty is how much each of these putative mechanisms contributes to the overall antibacterial action, but this challenge certainly portends great opportunity. Additional studies may establish clearer structure–activity relationships to the point where desirable attributes might be dialed in to third-generation analogs using a rational design methodology.

6. Future directions

6.1. *Self-association and covalent dimers*

A substantial body of work has accumulated supporting dimerization or self-association in solution as a beneficial property of some glycopeptide antibiotics resulting in higher substrate affinity due to a multivalent effect [154–156]. Covalently linked dimers have been systematically prepared and these compounds do exhibit greater target affinity and antibacterial activity, and in some cases recovered activity against vancomycin-resistant bacteria [154–156]. Hydrophobically-substituted vancomycin covalent dimers also were

synthesized and exhibited extremely potent bactericidal activity [155]. Unfortunately while these compounds possess expected superior antibacterial properties as compared with traditional glycopeptides and are efficacious in animal models of infection, their pharmacokinetic attributes are unfavorable and toxicity in animal models is apparent [155]. Specifically, deposition in liver and kidney are observed along with nephrotoxicity in mice. While these agents provide unique probes of the relationship of physico-chemical properties of glycopeptide antibiotics and biological activity, converting them into a drug would provide substantial challenge.

6.2. *A two-pronged attack*

Antibacterial synergy is an important phenomenon and its clinical significance is evident from the frequent combination therapy with antibiotics for serious infections [157–159]. A number of groups have tried to take application of this principle to the next level by creating a variety of bifunctional antibacterial agents that concomitantly inhibit two distinct targets [160–162]. More recently, glycopeptide-beta-lactam hybrids have been created by conjugating vancomycin and cephalosporin analogs active against Gram-positive bacteria [163–165]. The expectation is that by targeting two topologically adjacent targets substantially improved affinity will be achieved through a multivalent effect. By additionally inhibiting two sequential/related enzymatic steps or the same process by both substrate and enzyme inhibition even greater activity will result than can be achieved with a combination of the unlinked or parent molecules [157–159,163–165]. This appears to be a clever approach and time will tell whether these molecules will bear up to their potential.

6.3. *Distant cousins*

Ramoplanin is another in the series of remarkable discoveries by the former Lepetit/Biosearch Italia group (now Vicuron/Pfizer) [166]. This cyclic lipoglycopeptide agent is undergoing clinical evaluation as an oral disinfectant for gastrointestinal colonization by vancomycin-resistant enterococci [166]. Ramoplanin is active against the VRE, and while unfortunately too toxic for systemic administration, may be appropriate for the role under evaluation since it like most glycopeptides exhibits poor oral bioavailability [166].

AC98-6446 is a novel semisynthetic derivative of the cyclic glycopeptide mannopepti- mycins and also is an inhibitor of peptidoglycan synthesis [167] (Fig. 1). It is active in vitro against many staphylococci, streptococci, and enterococci, and parenteral administration is effective in animal models of infection where AC98-6446 potency ranges from 10 to 40-fold greater than that of vancomycin [168,169]. This looks to be an interesting pre-clinical molecule although scant data regarding safety have been reported.

6.4. *Metabolic engineering and enzyme-based semi-synthesis*

While the new semi-synthetic antibacterials have focused on vancomycin and teicoplanin like platforms, nearly 200 natural product glycopeptides have been discovered in the past 50 years [10]. Many of these may not have had desirable properties, but the glycopeptide scaffold appears to be a relatively unexploited source on which to base discovery of new antibacterial drugs. New technologies including use of precursor-directed biosynthesis and mutasynthesis, manipulation of halogenases, tailoring of glycopeptide aglycon scaffolds via acyltransferases, and alteration of carbohydrate decoration with glycotransferases or semisynthesis may increase the diversity of the platform for the glycopeptide class [168–176]. For example, Kruger et al. [174] have used chemoenzymatic synthesis to create a series of vancomycin and teicoplanin lipoglycopeptide analogs. Sun et al. [177] explored the importance of the disaccharide moiety of vancomycin, and actually created a hybrid molecule comprised of the vancomycin aglycon and a disaccharide fragment of the transglycosylase inhibitor moenomycin [178]. Composition of the carbohydrate as well as substitution of the hydrophobic moieties appears important based on the role of the sugar residue in molecular recognition by the glycopeptides [179,180]. Additional further work has included other semisynthetic bifunctional molecules created by Shionogi scientists similar in nature to telavancin, and synthesis of analogs with putatively metabolizable hydrophobic substituents to aid in clearance [181,182]. Great opportunity to create new important antibacterial agents would be afforded by utilization of a combination of approaches and considering available MOA and SAR findings to date.

7. Summary

Significant growth has occurred in the area of glycopeptide antibiotics particularly in the past 20 years. This is very important considering the significant healthcare challenge of vancomycin resistance faced by the clinical community. Work in the area of semisynthetic analogs based on traditional glycopeptide scaffolds has exploded and resulted in three clinical candidates, two of which appear near term. Other distinct related natural products have been described, and semisynthetic analogs of these have been prepared and characterized as well. Diversity of MOA for the second-generation lipoglycopeptides is complex and potentially important from the standpoint of utilizing diverse SAR to deliver additional promising agents. The significance of these new agents and their unique properties, should they achieve regulatory approval and commercialization, will be increasingly felt as the diversity of glycopeptide resistance mechanisms and numbers of resistant strains increase particularly among the staphylococci. While glycopeptide resistance is of substantial concern, and rightly so, there is promise of new clinical agents to fill the increasing void, perhaps with even better properties than the glycopeptide antibiotics in use today.

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