Semisynthetic glycopeptide antibiotics

Groesen, E. van

Chapter 6 |

Summary

Summary

Vancomycin is a last-resort antibiotic for the treatment of many Gram-positive bacterial infections. Mechanistically, vancomycin functions by inhibiting cell-wall biosynthesis. More specifically, vancomycin binds to the D-Ala-D-Ala termini of the bacterial cell-wall precursor lipid II, preventing its crosslinking (**Fig. 1AB**). Worryingly, vancomycin resistance is becoming increasingly prevalent and can occur in multiple ways, including: 1) lipid II target modification from D-Ala-D-Ala to D-Ala-D-Lac, which is the most commonly encountered resistance mechanism (**Fig. 1C**); 2) lipid II target modification from D-Ala-D-Ala to D-Ala-D-Ser; and 3) cell-wall thickening coupled with decreased crosslinking activity, leading to increased D-Ala-D-Ala decoy targets. To tackle vancomycin-resistant bacteria, significant efforts have been spent in designing semisynthetic glycopeptide antibiotics with enhanced properties. Semisynthesis represents a convenient and cost-effective means to generate novel glycopeptide antibiotics. The work in this thesis describes the development and assessment of such novel semisynthetic glycopeptide antibiotics.

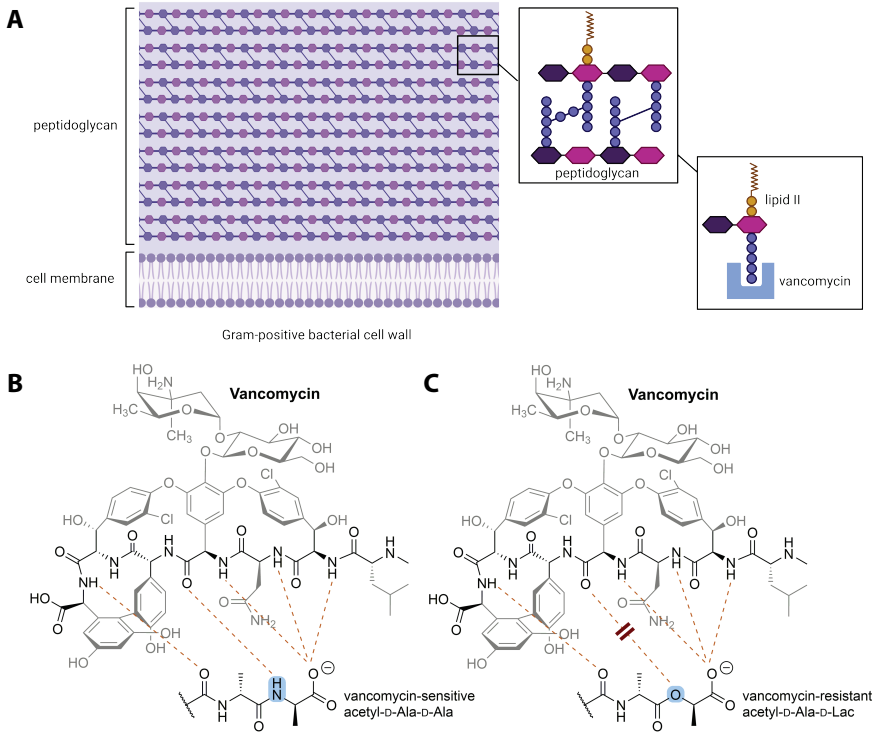

Fig. 1. Vancomycin activity and resistance against Gram-positive bacteria. (**A**) Gram-positive bacterial cell wall zoomed in on the peptidoglycan layer and vancomycin binding to lipid II. (**B**) Vancomycin binding to D-Ala-D-Ala with five hydrogen bonds. (**C**) Target modification to D-Ala-D-Lac results in loss of one hydrogen bond, leading to vancomycin resistance.

Chapter 1 describes all clinically approved and recently developed semisynthetic glycopeptide antibiotics with a focus on their structure, modes of action, resistance development, *in vitro* and *in vivo* antibacterial activity, pharmacokinetics, and toxicity. While the clinically used lipoglycopeptides telavancin, dalbavancin, and oritavancin partly overcome vancomycin resistance, they are generally characterized as having poor aqueous solubility, unusual pharmacokinetics (PK), and toxicity concerns. Therefore, the design of new glycopeptide antibiotics with enhanced antibacterial activity paired with improved PK and safety profiles continues to be of great importance. To address this, many different strategies have been employed, mostly focusing on enhancing glycopeptide binding to the bacterial surface often by including lipophilic and/or cationic charges. In addition, attempts at introducing multiple modes of action have also been reported, for example by conjugating glycopeptides to pyrophosphate-targeting groups or other antibiotics with alternative modes of action. Glycopeptide dimers that exhibit enhanced cell surface localization have also been developed. In addition, novel delivery systems have been described by which glycopeptides can be specifically targeted to relevant disease-related tissue. Beyond this, recent progress has even been made in expanding the spectrum of activity of glycopeptides to include Gram-negative strains.

Chapter 2 describes the synthesis of a panel of semisynthetic lipoglycopeptide antibiotics named the guanidino lipoglycopeptides (**Fig. 2**). The guanidino lipoglycopeptides consist of vancomycin, which is semisynthetically modified to include an aryl linker, a guanidine moiety (which is positively charged at physiological pH), and a lipophilic tail. The guanidino lipoglycopeptides are readily synthesized from vancomycin in a two-step process. Although no anti-Gram-negative activity was detected, the guanidino lipoglycopeptides display potent activity *in vitro* against a range of clinically relevant Gram-positive bacteria, including methicillin-resistant *S. aureus* (MRSA), *S. pneumoniae*, and *Clostridia* as well as vancomycin-intermediate and vancomycin-resistant strains, such as VISA, VRSA, and VRE. The *in vitro* activity of the guanidino lipoglycopeptides is vastly superior to that of vancomycin and generally equipotent or superior to the clinically used semisynthetic lipoglycopeptides telavancin, dalbavancin, and oritavancin.

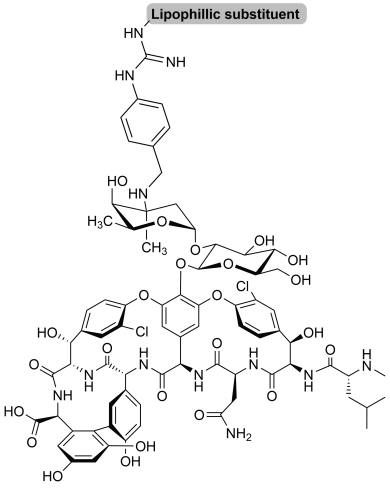

Fig. 2. General structure of the guanidino lipoglycopeptides

Building upon these findings, **Chapter 3** describes a more elaborate *in vitro* and *in vivo* assessment of the guanidino lipoglycopeptides, which revealed the guanidino lipoglycopeptides have anti-*S. aureus*-biofilm activity. Furthermore, they indicate low-plasma protein binding and display similar killing kinetics as observed for clinically used glycopeptides. Moreover, these compounds show lower mammalian cell toxicity compared to clinically used lipoglycopeptides and exhibit minimal propensity for resistance selection. Mechanistically, the guanidino lipoglycopeptides retain the cell-wall biosynthesis inhibitory mode of action of vancomycin, by binding to the D-Ala-D-Ala moiety of lipid II with significantly enhanced binding affinity. Notably, the guanidino lipoglycopeptides were also found to maintain binding to the resistant version of lipid II, containing D-Ala-D-Lac. In addition, no significant membrane depolarization and permeabilization was detected in bacterial cells treated with the guanidino lipoglycopeptides, also supporting a specific, targeted mechanism of action. The most promising guanidino lipoglycopeptide, containing a linear fully saturated C_7 lipid, was selected for *in vivo* assessment, which revealed it to be well tolerated while maintaining blood concentrations above the minimum inhibitory concentration for >8 hours in mice when dosed at a low 3 mg/kg. *In vivo* efficacy studies showed, both in a *S. aureus* murine thigh infection and a 7-day sepsis survival study, that this C_7 guanidino lipoglycopeptide was significantly superior to vancomycin in reducing the bacterial burden and increasing survival (**Fig. 3**). Ongoing work is aimed at further characterizing the toxicity and PK profile of the guanidino lipoglycopeptides.

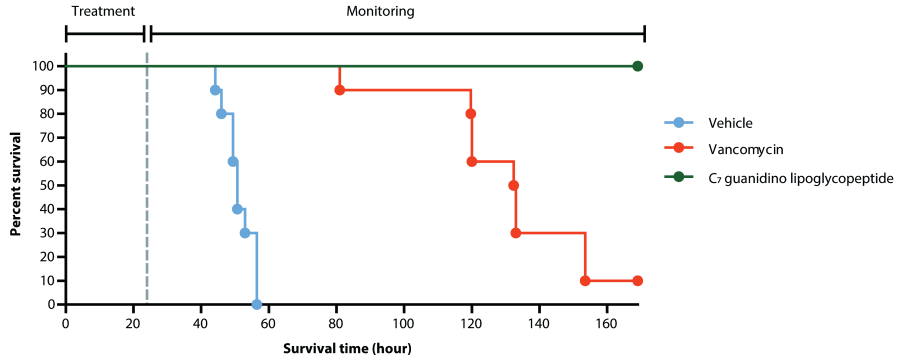

Fig. 3. Murine *S. aureus* sepsis survival upon treatment with vehicle (blue), vancomycin (orange), and C_7 guanidino lipoglycopeptide (green).

While the guanidino lipoglycopeptides are promising semisynthetic glycopeptides with potent activity against Gram-positive bacteria, **Chapter 4** focuses on glycopeptide derivatives with activity against Gram-negative organisms. Vancomycin is generally inactive against Gram-negative bacteria as it cannot cross the outer membrane

(OM) present in these strains, preventing the antibiotic from reaching its target (**Fig. 4**). To address this, we covalently linked the known OM disruptor polymyxin E nonapeptide (PMEN) to vancomycin, creating the vancomyxins (**Fig. 5**). Exogenous supplementation of PMEN to vancomycin results in improved *in vitro* activity against Gram-negative strains, however, covalent conjugation to PMEN, as present in the vancomyxins, further enhances potency, especially against *E. coli* and *K. pneumoniae*. Notably, the vancomyxins maintain activity against vancomycin-sensitive Gram-positive microorganisms and even overcome vancomycin resistance in some Gram-positive strains. Antagonization studies indicate that the vancomyxins are able to bind to the lipopolysaccharide present in the OM. Furthermore, the vancomyxins were found to be non-hemolytic and displayed low cytotoxicity against proximal tubular epithelial cells, as their 50% cytotoxic concentration was multiple orders of magnitude higher than the concentrations required to inhibit bacterial cell growth.

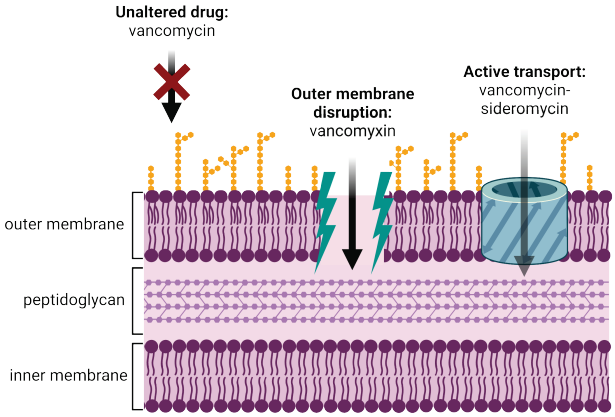

Fig. 4. Strategies for potentiation of vancomycin against Gram-negative bacteria.

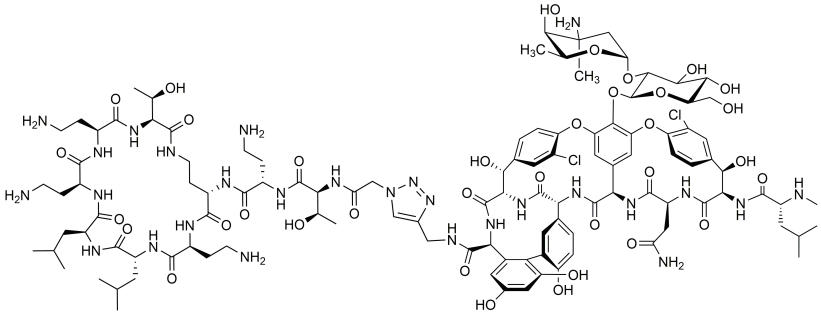

Fig. 5. Representative vancomyxin

As an alternative to OM disruption, **Chapter 5** focuses on hijacking bacterial active transport systems to facilitate the passage of vancomycin across the OM in an attempt to confer activity against Gram-negative bacteria (**Fig. 4**). To this end, the iron-chelating siderophore trihydroxymate was covalently linked to vancomycin (**Fig. 6**). The working hypothesis in this case relates to the knowledge that siderophores sequester iron in the environment and are actively transported to the periplasm, where the vancomycin target lipid II resides. The vancomycin-trihydroxymates prepared were found to retain some of the bacterial cell wall biosynthesis inhibition of the parent glycopeptide, however they showed diminished activity in preventing the growth of Gram-positive organisms and were inactive against siderophore producing Gram-negative bacteria. This lack of activity against the Gram-negative strains initially tested was attributed to the lower chelating efficiency for ferric iron of trihydroxymates compared to the naturally produced siderophores. However, against Gram-negative *E. coli* deficient of its own siderophore biosynthesis or export, the vancomycin-trihydroxymates did display enhanced *in vitro* potency relative to vancomycin. Whether this effect is due to active uptake or iron deprivation of the bacterial cells remains to be fully elucidated.

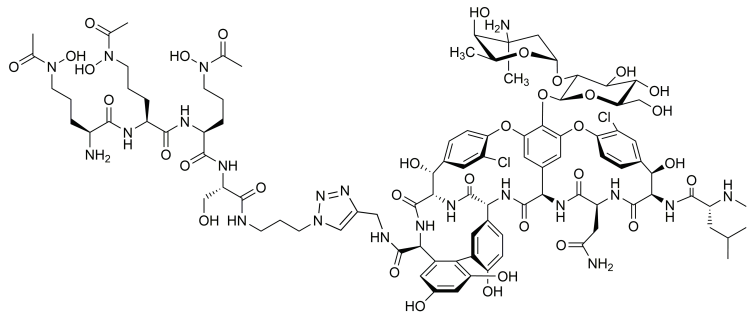

Fig. 6. Representative vancomycin-sideromycin