Renaissance of Vancomycin: Approaches for Breaking Antibiotic-Resistance in Multidrug-Resistant Bacteria

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Renaissance of Vancomycin:

Approaches for Breaking Antibiotic-Resistance in Multidrug-Resistant Bacteria

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Abstract:

The emergence of multidrug-resistant bacteria demands innovations in the development of new antibiotics. For decades, the glycopeptide antibiotic vancomycin has been considered as the “last resort” treatment of severe infections caused by Gram-positive bacteria. Since the discovery of the first vancomycin-resistant enterococci strains in the late 1980s, the number of resistances is steadily rising with often life-threatening consequences. As an alternative to the generation of completely new substances, novel approaches focus on structural modifications of established antibiotics such as vancomycin to overcome these resistances. Here, we provide an overview of several promising modifications of vancomycin in order to restore its efficacy against vancomycin-resistant enterococci.

Key words:

antibiotics
vancomycin
multidrug-resistant bacteria
enterococci
structural modification
**Antibiotic resistance – an emerging problem**

The highly efficient treatment of bacterial infections with antibiotics represents one of the most important achievements in modern medicine. Even though the post-antibiotic era was declared decades ago, antibiotic-resistant or even multidrug-resistant bacteria such as vancomycin-resistant enterococci (VRE) (Huycke et al. 1998) or methicillin-resistant *Staphylococcus aureus* (MRSA) tragically pose a greater threat to mankind than ever imagined (Goll et al. 2007). At the same time, the search for new antimicrobial substances decreased rapidly (Coates et al. 2011). In a recent study, the burden of infections due to resistant bacteria was estimated for the population of the European Union and the European Economic Area. Based on the calculated outcome of disability-adjusted life-years, the impact of antibiotic-resistant bacteria on human health turns out to be almost equal to that caused by influenza, tuberculosis and human immunodeficiency virus together (Cassini et al. 2019).

The considerable diversity of antimicrobial therapeutics is reflected by more than 20 different classes of antibiotics that are available today (Coates et al. 2011). In general, antibiotics are supposed to stop the growth of bacterial pathogens and to control the spreading of infections through bactericidal and bacteriostatic effects (Pankey and Sabath 2004). However, bacterial infections are still causing the death of approximately 700,000 people each year (Humphrey and Fleck 2016). Instead of developing new antibiotics which address novel targets, current approaches focus on structural modifications of well-established antibiotics such as vancomycin in order to restore their impact on resistant bacteria. Various further strategies to combat life-threatening bacterial infections, such as the use of bacteriophages (Matsuzaki et al. 2005), monoclonal antibodies (Lorenz et al. 2000; Motley et al. 2019), antimicrobial peptides (Fox 2013), metallic and polymeric nanoparticles (Beyth et al. 2015) and quorum-sensing inhibitors (Rasmussen and Givskov 2006), among others, are under extensive investigation (Simões et al. 2017). However, despite partially suggesting promising potential as effective alternative antimicrobials, up to date, none of these novel strategies have found significant broad application.

**The importance of vancomycin**

The glycopeptide antibiotic vancomycin was formerly designated as “last resort” for the control of several resistant Gram-positive bacteria. After its discovery in 1952 by E. C. Kornfield, it was successfully applied to treat especially severe infections of MRSA for more than 30 years (Levine 2006). In 1988, the first clinical isolates of vancomycin-resistant enterococci, namely *Enterococcus faecium* (*E. faecium*) and *Enterococcus faecalis* (*E. faecalis*), were discovered in Great Britain and shortly thereafter also in further European countries.
and the USA (Courvalin 2006). What started out as relatively harmless with an incident rate of 0.3% in 1989 swiftly increased to around 14% in 1998 (Bell et al. 1998). Already in 2009, studies of patients in intensive care units reported about 22% of nosocomial enterococci isolates as vancomycin-resistant (Pan et al. 2012). The strong increase in the rate of appearance of resistances against antibiotics such as vancomycin has become an extremely severe problem (Ashwin and Muralidharan 2015). This phenomenon can be traced back to the exorbitant use of antibiotics in the healthcare and agriculture sector for the last decades (Chang et al. 2015). The selection pressure on bacteria is dramatically increased by the exposure to antibiotics. Evading or neutralizing these antimicrobial substances is a way of Darwinian competition (Holmes et al. 2016). The careless mis- and overuse of antibiotics is therefore promoting the development of resistance mechanisms eventually leading to the increasing emergence of multi-resistant bacteria (Smith et al. 2005; Aslam et al. 2018). Once encoded in the bacterial DNA, the genetic elements mediating resistances can be transferred from one species to another (Hastings et al. 2004).

**Mechanisms of vancomycin resistances**

Highly pathogenic microorganisms have managed to evade most of the mechanisms developed by mankind aiming to destroy them. Antibiotics are designed to specifically interfere with at least one of the physiological pathways that are essential to bacteria. Five main targets are preferred, because of their efficacy without harming the host organism, since they significantly differ from eukaryotic to prokaryotic systems. These include bacterial protein synthesis, membrane permeability, function of the bacterial DNA, folic acid synthesis as well as cell wall synthesis (Steinhilber et al. 2010). The latter is targeted by the glycopeptide antibiotic vancomycin in Gram-positive bacteria. It binds to the basic building block of the bacterial cell wall by forming hydrogen bonds with D-alanyl-D-alanine (D-Ala-D-Ala), the C-terminal peptide motif of the peptidoglycan precursor (Fig. 1). Upon interaction with vancomycin, the precursors cannot be processed by the transglycosylase, thereby preventing functional cell wall assembly (Murray 1997). Subsequently, replicating bacteria cannot survive due to an incomplete and corrupted cell wall, which makes them vulnerable to external forces such as osmotic pressure (Walsh 1999). Due to their different cell wall morphology Gram-negative bacteria are not susceptible to vancomycin (Johnson et al. 1990). In order to circumvent the deleterious impact of vancomycin, most resistant Gram-positive bacteria rely on similar mechanisms. By substituting the terminal D-alanine of the peptidoglycan precursor by D-lactate, the regular binding site of vancomycin is disturbed (Fig. 1) (Boneca and Chiosis 2003). Thus, a lower number of hydrogen bonds can be formed between vancomycin and the precursor. This results in a
dramatic decrease of the binding affinity for the antibiotic compound. Other resistant strains express a D-alanyl-
D-serine (D-Ala-D-Ser) motif which is believed to exert steric hindrance. In general, bacterial resistances to
vancomycin can be classified into six groups, namely vanA, -B, -C, -D, -E and -G. While vanA, -B and -D use
the D-Ala-D-Lac precursor to evade the effect of vancomycin (Fig. 1), vanC, -E and -G make use of the
alternative D-Ala-D-Ser C-terminus. These types of resistances also differ in their sensitivity to vancomycin and
teicoplanin (Courvalin 2006). The clinical most relevant vanA and -B resistances mainly differ in their
susceptibility to teicoplanin. While the vanA-type is resistant to teicoplanin, vanB only withstands lower
concentrations (Moellering 1998). These strategies of circumventing the antibiotic impact are a prime example
for the four common mechanisms that are used by organisms to evade antimicrobial treatment as depicted in
Fig. 2. As such resistances can be triggered even by small alterations of the genome (Park and Walsh 1997) and
in most cases the mutation rate is high (Tippin et al. 2004; Perfeito et al. 2007), consequently, the pathogens
seem to be always one step ahead.

**Counteracting strategies**

In order to stop the rise of multidrug-resistant enterococci, the seemingly obvious idea would be the development
of completely new substances that possess different modes of action. However, the number of antimicrobial
targets is limited, and many recent publications suggest structural modification of vancomycin as a promising
attempt. Several strategies were applied to enhance the antibiotic potential of vancomycin to eradicate otherwise
resistant strains. One promising approach of modification combines the properties of lipophilic membrane
anchors with cationic cell-penetrating peptides as an electrostatic effector which is supposed to interact with the
negatively charged bacterial cell wall (Fig. 3a) (Blaskovich et al. 2018). Yarlagadda et al. were able to show that
a modification with a single cationic quaternary ammonium in combination with a lipophilic element at the same
position can tremendously increase the antimicrobial potential of vancomycin against resistant strains (Fig. 3d)
(Yarlagadda et al. 2014). As ammonium-based cations tend to be more prone to excretion by the liver and the
kidneys, another study reveals the modification of vancomycin with sulfonium moieties (Fig. 3b, e, g, h) (Guan
et al. 2019). Interestingly, sulfonium modifications yield an even higher antimicrobial potential when
additionally combined with lipophilic elements such as alkyl chains. These findings demonstrate that
combinations of certain modification approaches can enhance the efficiency even more (Guan et al. 2019).
Another strategy, which was postulated by an earlier work of Yarlagadda et al. is a dipicolyl-vancomycin
conjugate to further impede the cell wall synthesis (Fig. 3c). The additional dipicolyl extension acts as a zinc-
binding element allowing vancomycin to bind essential pyrophosphates in the bacterial peptidoglycan building machinery (Yarlagadda et al. 2016). Modifying the disaccharide of vancomycin with chlorobiphenyl also reactivates its ability to tackle resistant enterococci (Fig. 3i). Chen et al. showed that even after damaging the D-Ala-D-Ala binding pocket the chlorobiphenyl conjugate was still able to affect resistant bacteria. This indicates a different mode of action (Chen et al. 2003). An alteration of the binding pocket itself was also used to restore the efficacy of vancomycin. The most effective binding pocket modification investigated was the exchange of oxygen to nitrogen in the peptide backbone of vancomycin (Fig. 3f). A combination with a chlorobiphenyl moiety enhanced the antimicrobial activity even further (Okano et al. 2017).

Overall, vanA-resistant strains of E. faecium are tolerant to vancomycin concentrations of up to 1024 µg/mL (Landman et al. 1993). Bacterial strains which can resist concentrations of vancomycin above 4 µg/mL are classified as vancomycin resistant (The European Committee on Antimicrobial Susceptibility Testing 2019). If the minimal inhibitory concentration (MIC) of a vancomycin derivative is below 4 µg/mL, it is considered to be resistance breaking. Comparing the MIC values of the different vancomycin conjugates reveals that not all modifications actually lead to resistance-breaking compounds in the tested setup (Table 1). While the lipopeptide and the dipicolyl conjugate as well as the binding pocket modification are able to overcome resistance, the quaternary ammonium and the chlorobiphenyl strategy could not reach the breakpoint. In contrast, combining a chlorobiphenyl moiety (Fig. 3i) with a modification of the binding site (Fig. 3f) can decrease the MIC by 100-fold when compared to the binding site modification alone. The sulfonium conjugates allow a comparison between position of modification and length of the alkyl chain. The C₁₂H₂₅ alkyl chain modifications seem to break resistance in vanA-resistant E. faecium whereas the conjugates with a C₁₀H₂₁ alkyl chain do not reach the breakpoint. Nevertheless, all shown conjugates improve the antibacterial potential of vancomycin noticeably.

Given the fact that the resistance genes of enterococci can be easily transferred to other bacterial species such as staphylococci, the emergence of vancomycin-intermediate and vancomycin-resistant Staphylococcus aureus (VISA, VRSA) will certainly become a serious issue for future medicine (de Niederhäusern et al. 2011). Therefore, activity testing of the vancomycin derivatives on bacterial strains with either reduced susceptibility or already acquired resistance will be highly needed. Some of the presented compounds have already been tested on VISA strains with promising results (Blaskovich et al. 2018; Yarlagadda et al. 2014; Yarlagadda et al. 2016; Guan et al. 2019). However, vancomycin is still considered to be the gold standard for the therapy of susceptible representatives of this species (Uhl et al. 2017). Furthermore, it was already approved for therapeutic treatment...
of infections with *Clostridium difficile* (*C. difficile*) (Bartlett 2008). So far, there are only few clinical reports on vancomycin-resistant clostridia (Peng et al. 2017), whereas mutants of *C. difficile* exhibiting reduced sensitivity to vancomycin were generated in an *in vitro* simulation model (Leeds et al. 2014). With respect to these findings, the most promising modifications should also be tested on these bacteria.

**Conclusion**

While there are numerous drugs available for the therapy of bacterial infections, there are only a few compounds capable to serve as “last resort” for severe infections. At the same time, the number of such infections is steadily increasing. Vancomycin seemed to be the pivotal antibiotic for the treatment of multidrug resistant infections caused by Gram-positive bacteria. Unfortunately, vancomycin-resistant bacteria have become an increasingly difficult-to-treat cause of nosocomial infections. Due to the limited availability of novel bacterial targets, various attempts were made to obtain vancomycin derivatives which are able to overcome vancomycin resistance. Most strategies focus on the general idea to introduce cationic charges as well as lipophilic elements into the structure of vancomycin to target the bacterial cell membrane. Some of the modifications presented show promising results in restoring the capability of vancomycin to control even vancomycin-resistant bacteria. Nevertheless, further investigations with respect to the pharmacological properties of the conjugates like their cytotoxicity and *in vivo* characteristics are needed to further expand the antibiotic armory not solely, but in particular, against emerging multidrug-resistant bacteria.
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deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and


Tab. 1. Minimal inhibitory concentrations (MICs) of vancomycin conjugates.

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<tr>
<th>Type of modification</th>
<th>Site of modification</th>
<th>MIC [µg/mL]</th>
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<th>Reference</th>
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<tr>
<td>None</td>
<td>R₁ OH R₂ CH R₃ O R₄ NH R₅ H</td>
<td>1024</td>
<td>ATCC 51559</td>
<td>Landman et al. 1993</td>
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<tr>
<td>Lipopeptide</td>
<td>a R₁ CH R₂ O R₃ NH R₄ H</td>
<td>0.02</td>
<td>ATCC 51559</td>
<td>Blaskovich et al. 2018</td>
</tr>
<tr>
<td>Quaternary ammonium</td>
<td>d R₁ CH R₂ O R₃ NH R₄ H</td>
<td>13</td>
<td>ATCC 51559</td>
<td>Yarlagadda et al. 2014</td>
</tr>
<tr>
<td>Dipicolyl</td>
<td>c R₁ CH R₂ O R₃ NH R₄ H</td>
<td>3.5</td>
<td>n.s.*</td>
<td>Yarlagadda et al. 2016</td>
</tr>
<tr>
<td>Sulfonium</td>
<td>b R₁ OH R₂ CH R₃ O R₄ NH R₅ H</td>
<td>8</td>
<td>Efm-HS-0649</td>
<td>Guan et al. 2019</td>
</tr>
<tr>
<td>Chlorobiphenyl</td>
<td>e R₁ OH R₂ CH R₃ O R₄ NH R₅ H</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Binding pocket</td>
<td>f R₁ OH R₂ CH R₃ O R₄ NH R₅ H</td>
<td>0.005</td>
<td>ATCC BA-2317</td>
<td>Okano et al. 2017</td>
</tr>
</tbody>
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Note: The MICs of the compounds with respective modifications (a – i) as presented in Fig. 3 and their correspondent strain of vanA-resistant E. faecium are shown. The unmodified vancomycin serves as reference. Resistance-breaking MICs are highlighted.

*n.s., not specified
Fig. 1. Interaction of vancomycin with the bacterial cell wall precursor D-Ala-D-Ala. Vancomycin forms hydrogen bonds (dotted lines) enabling inhibition of cell wall synthesis. In case of vanA-mediated resistance, the terminal D-alanine (X = NH) of the peptidoglycan precursor is replaced by D-lactate (X = O) causing a significant reduction of its binding affinity to vancomycin.

Fig. 2. General resistance mechanisms of bacteria. (A) Modification of the target structure to decrease the affinity for antibiotics. (B) Efflux pumps reduce the concentration of the antimicrobial substance in the bacterial cell. (C) Enzymatic degradation of antibiotics. (D) Modifications within the cell membrane and the cell wall decrease their permeability thereby reducing the presence of the harmful drug in the bacterium.

Fig. 3. Modification strategies of vancomycin. The five modification sites of vancomycin are marked as R₁, R₂, R₃, R₄ and R₅. As shown on the left side, R₁ can be modified by (a) a lipopeptide, (b) a sulfonium, (c) a dipicolyl and (d) a quaternary ammonium moiety. Further modifications comprise the introduction of a sulfonium moiety at the positions (e) R₂, (g) R₄ and (h) R₅. (f) An amide-amidine-exchange at site R₃ alters the characteristic of the binding pocket. (i) A chlorobiphenyl moiety has been conjugated at position R₅.
Modified target structure

Enzymatic degradation

Modified cell wall and cell membrane

Efflux pump

Antibiotic

A

B

C

D

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