Vancomycin: Resistance in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a gram-positive cocci bacteria that can be the cause of skin and soft tissue infections (SSTIs). Typically, infections caused by *S. aureus* are treated using a beta-lactam antibiotic, but when the bacteria express a foreign penicillin-binding protein, PBP2a, they can become resistant to beta-lactams[1]. Resistant *S. aureus*, such as methicillin-resistant *Staphylococcus aureus* (MRSA), is usually treated using vancomycin. Vancomycin is a glycopeptide antibiotic that targets the cell wall of gram-positive bacteria; this leads to inhibited bacterial cell growth. When there is a mutation in the bacterial cell wall, vancomycin can no longer bind properly and therefore no longer inhibits cell growth.

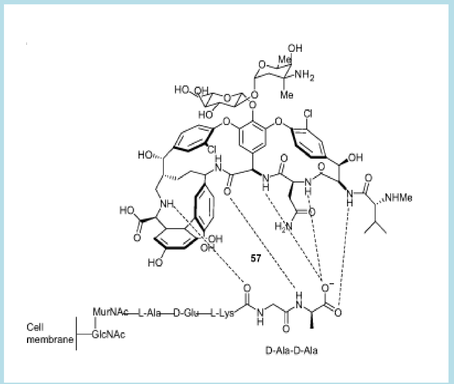

Fig. 1: Structure of Vancomycin[2].

Introduction

XX, a 39 year-old female patient, presented to Aurora Hospital complaining of reddened, painful, and swollen area in her left axilla region. XX was admitted with a normal white blood cell count (WBC), had a low-grade fever, and was suspected to have thrombophlebitis. Due to the presentation of symptoms, the physician believed XX had an SSTI possibly caused by MRSA. While the blood culture results were pending, the patient was started on empiric vancomycin therapy.

The infusion was given over two hours to reduce the chance of Red Man Syndrome. This adverse reaction, not an allergic reaction, is caused when vancomycin is infused too quickly, resulting in the release of histamine. This presents as a erythematous rash of the face, neck, and upper body. This reaction does not affect how well vancomycin works. The efficacy of vancomycin in treating an infection is based on trough levels. The trough level represents the minimum concentration of drug needed to treat the infection.

Vancomycin is a time-dependent antibiotic that is administered intravenously due to its lipophilic nature causing poor oral absorption[3]. The drug binds the D-Ala-D-Ala of the growing peptidoglycan backbone of the cell wall, inhibiting the growth of the bacteria. Some bacteria can become resistant to vancomycin by mutating their D-Ala-D-Ala to D-Ala-D-Lac, causing the drug to be unable to bind properly.

Molecular Story

Vancomycin, a large glycopeptide antibiotic, interacts with the D-Ala-D-Ala portion of a growing bacterial cell wall via hydrogen bonding with the backbone. There are 5 total hydrogen bonds that occur:

- Beginning with the terminal alanine on the bacterial cell wall, the first 3 hydrogen bonds are between amino hydrogens on 2 tyrosine residues and the carboxylate group of the terminal alanine.
- The fourth hydrogen bond occurs between the carbonyl group of an ethanoic acid moiety and an amide group of the terminal alanine.
    - This hydrogen bond is the focus of vancomycin-resistance. When resistant bacteria substitute a D-Lactic Acid for the terminal D-Alanine, an oxygen takes the place of the amide nitrogen (highlighted in purple in figure 3), resulting in an ester group that is unable to hydrogen bond with the ethanoic acid residue of vancomycin.
    - The absence of just one hydrogen bond is enough to weaken the affinity of vancomycin for the bacterial cell wall to a point where growth is possible and the bacteria survive.
- Finally, the fifth hydrogen bond is between another tyrosine residue on vancomycin and the carbonyl group on the second D-Alanine.

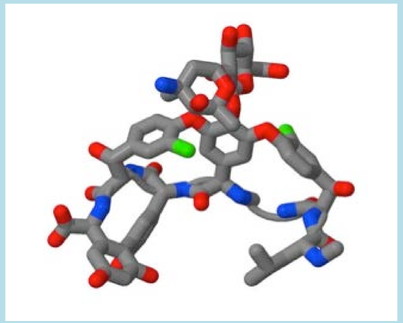

Fig. 2: With a molecular mass of 1449.3 Daltons. Vancomycin is a large antibiotic that targets gram-positive bacteria[4]. Nitrogens are colored **BLUE** oxygens are colored **RED** and chlorines are colored **GREEN**.

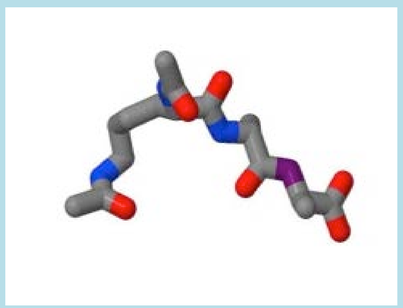

Fig. 3: D-Ala-D-Ala portion of the bacterial cell wall. The nitrogen highlighted in **PURPLE** is changed to an oxygen in vancomycin-resistant bacteria, resulting in an ester that cannot hydrogen bond with the drug.

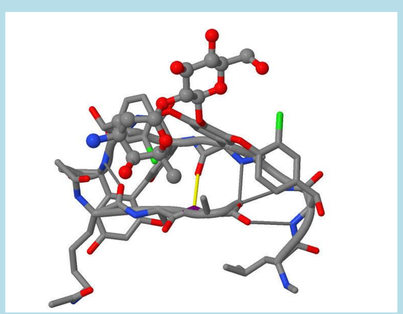

Fig. 4: Vancomycin bound to the D-Ala-D-Ala portion of the cell wall. Hydrogen bonds are shown in gray with the crucial ethanoic acid hydrogen bond highlighted in **YELLOW.** It is this bond that is lost when bacteria substitute a D-Lactic Acid for the terminal D-Alanine. This results in reduced affinity to bacterial cell walls, leading to potential treatment failure due to resistance.

Images rendered from 1FVM.pdb, and coloring is consistent with CPK coloring

Next Generation Drug Design

Vancomycin-resistance is becoming more prevalent because of the dipeptide mutation in gram-positive bacteria. Due to the intricate nature of the peptide portion of vancomycin, it is nearly impossible to restructure the peptide backbone. According to a study by Ge et al., adding hydrophobic substituents on the vancosamine nitrogen increases activity against vancomycin-resistant strains of bacteria[5]. These hydrophobic substituents facilitate dimerization, and there is attachment of the glycopeptide antibiotic to the cell surface of the bacteria. This attachment suggests that the substituted carbohydrates directly interact with the proteins involved in transglycosylation therefore inhibiting transglycosylation[5]. This would be able to occur even though the drug would not be able to effectively bind to D-Ala-D-Lac.

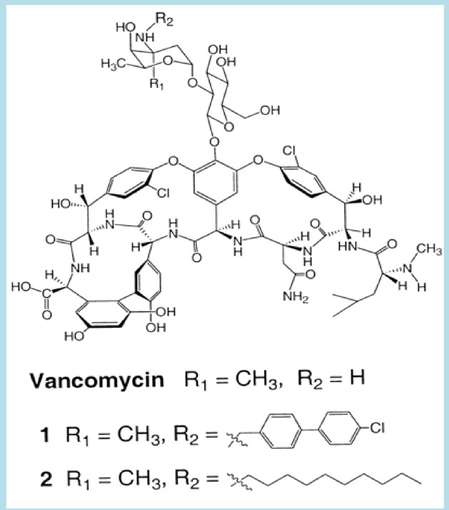

Fig. 5: Vancomycin structure with R1 and R2 substituents. Substituents 1 and 2 are groups that can be added to decrease vancomycin-resistance[5].

Summary

Vancomycin is a bactericidal antibiotic that binds to D-Ala-D-Ala on the growing peptidoglycan backbone of gram-positive bacteria[6]. Gram-positive bacteria can become resistant to vancomycin by a mutation in the peptidoglycan backbone resulting in D-Ala-D-Lac. A bacteria's resistance to vancomycin is one potential cause of an infection's failure to respond to treatment. After an increase in dose due to low trough levels, XX's symptoms diminished indicating she did not have a vancomycin-resistant form of MRSA.

References

1. Stapleton, et al. (2002) *Science Progress*, 85, 57.
2. Abreu, et al. (2003) *Journal of Brazilian Chemical Society*, 14, 675
3. Rybak. (2006) *Clinical Infectious Diseases*, 42, S35.
4. Hirao, et al. (2012) *BioMed Central Microbiology*, 12,69.
5. Ge, et al. (1999) *Science*, 284, 507.
6. Sakoulas, et al. (2004) *Journal of Clinical Microbiology*, 42, 2398.

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