Peptide, Glycopeptide and Lipopeptide Antibiotics

Every organism produces some type of protective peptide antibiotic

- In multicellular organisms the peptides are made on ribosomes
  - Synthesized as large proteins and then post-translationally processed
  - Animals, including humans: defensins – 30-35 amino acid cationic peptides produced by neutrophils and macrophages as part of innate immune system
    - Inducible production upon exposure to microbes
  - Amphibians (magainins: short cationic peptides isolated from the skin of the African clawed frog *Xenopus leavis*. Have broad antibacterial and anticancer activity.)

- Most of these peptides form pores in bacterial membranes
  - e.g., magainin

\[
\text{H2N-GIGKFLHSAKKFVKMEIMNS-COOH}
\]

![Diagram of peptide structure and function](image)
Peptide Antibiotics

• Most microbial peptide antibiotics are not assembled on ribosomes. They are secondary metabolites synthesized by huge enzyme complexes called nonribosomal peptide synthetases (NRPSs)

• Create widely diverse structures

• Most contain non-proteinogenic amino acids
  – not limited to the 20 common L-amino acids found in proteins made on ribosomes

• Can be modified with fatty acyl groups or sugars

Peptide antibiotics

• Most are natural products isolated from bacteria and fungi

Mechanisms of action

• Most are bactericidal
  – Affect membrane permeability or function
    • Allow cellular contents to ‘leak’ out
  – Block cell wall biosynthesis
    • Bind to peptidoglycan and its precursors
  – Several inhibit protein biosynthesis

• Group suffers from a variety of problems inherent with peptide drugs
  – acid labile
  – short duration
  – some can be enzymatically hydrolyzed
  – can be antigenic

\[ \text{cyclosporin A (Tolypocladium niveum)} \]
Polymixins and Colistins

- Family of related, modified decapeptides
- All have a seven amino acid cyclic peptide core with a tripeptide linear extension
- All have a fatty acid ‘tail’ coupled to linear peptide portion
  - six amino acid residues are 2,4-diaminobutyric acid (Dab)
  - Very basic compounds
- Amphipathic ‘detergent-like’ molecules

![Polymyxin B1](image1)

![Polymyxin E1 (Colistin A)](image2)

Polymyxins: Mechanism of action

- Complex with lipid A, a constituent of the lipopolysaccharide
  - Lipopolysaccharide (LPS) – the major surface molecule of Gram-(-) bacteria – consists of three distinct structural components: O-antigen, polysaccharide core, and lipid A. Lipid A is also known as endotoxin
- Affect membrane permeability and disorganize membrane structure
  - Fatty acid ‘tail’ inserts into the lipid bilayer
  - Cationic ‘head’ interacts with anionic phospholipid head groups in outer membrane

![Lipid A](image3)

![polymyxin](image4)
Polymyxins

- Polymyxin activity is limited to Gram-(-) bacteria
- Topical use only
- No oral bioavailability
- Immobilized polymyxin on polymeric supports is being investigated to treat sepsis
  - Severe sepsis and septic (endotoxic) shock is common in critical care units
    - 750,000 cases/year in the US and a mortality rate of 30-50%
  - Sepsis occurs when the endotoxin is released from Gram-(-) bacteria that are lysed by macrophages. This endotoxin can produce a systemic inflammatory state that may lead to multiple organ failure and death.

Gramicidins

- Group of linear 15 residue peptides
- Have alternating D and L amino acids
- The peptides are not charged
  - $N$-terminus is formylated
  - $C$-terminus is derivatized as an ethanolamide
**Gramicidins: Mechanism of Action**

- Forms pores in Gram-(+) cytoplasmic membranes
  - Two gramicidin helices required to span the cytoplasmic membrane
  - Permits cytoplasmic cations to escape
  - Topical use only

**Bacitracins**

Complex mixture of cyclic peptides

- Major component is bacitracin A
- Activity is limited to Gram-(+)
- Used topically
- Often combined with polymixin

**Mechanism of Action**

- **Overall:** Inhibits peptidoglycan biosynthesis
- **Specifically:** Blocks the diphosphatase that converts undecalprenyl-diphosphate to undecaprenylphosphate
  - "inhibits translocation"
- Inhibits recycling of the undecalprenyl-diphosphate carrier
- Zn$^{2+}$ or Mg$^{2+}$ is required for activity
**Bacitracin: Mechanism of action**

Cation-dependent complexation with undecaprenylidiphosphate

**Glycopeptide antibiotics**

- Family of heavily modified peptides, usually heptapeptides, made rigid by multiple crosslinks between amino acid sidechains. All contain one or more aminosugar residues. Produced by various *Streptomyces* and related bacteria
  - Prototypes is *Vancomycin* (Vancocin®)
    - Complex tricyclic heptapeptide with a disaccharide attached
    - Rings are formed by oxidative couplings between tyrosine and phenylglycine residues
    - Activity is limited to Gram-(+) bacteria
      - Usually reserved for serious infections by β-lactamase resistant strains
      - Given IV — short term therapy
      - An oral form is available for diarrhea caused by *Clostridium difficile*
    - Usually bactericidal
      - Bacteriostatic against enterococci
    - Synergistic effect observed with some aminoglycosides
Vancomycin: Mechanism of Action

- Inhibits both transglycosylation and transpeptidation during peptidoglycan biosynthesis
- Forms a very strong noncovalent complex with the D-Ala-D-Ala terminus of the pentapeptide on lipid II and peptidoglycan
- Only affects reactions that occur on outer surface of cell
  - cannot cross cell membrane

Other glycopeptides

- **Teicoplanin** (Targocid®)
  - Closely related to vancomycin but several key differences
    - has a fatty acid ‘tail’
    - has 3 sugars and a different glycosylation pattern
    - Structure contains four rings
  - Causes less frequent and less severe side effects than vancomycin
    - the lipid portion increases protein binding and decreases frequency of injection when administered IM
  - Proposed dual mechanisms of action
    - One mechanism is identical with vancomycin
    - The second mechanism appears to be *direct* inhibition of transglycosylase
Glycopeptide resistance

- Vancomycin used for nearly 30 yrs before clinical resistance identified
  - Vancomycin Resistant Enterococcus VRE
- Most common resistant pathogens are *E. faecalis* (95% of VRE) and *E. faecium*.
- Some glycopeptide producing organisms use an efflux pump to keep intracellular concentrations of the peptide below the toxic concentration.
- Other glycopeptide producers have adapted a modified peptidoglycan structure that is not recognized by the antibiotics.
- Clinical resistance is largely due to this type of target modification.
- Resistant bacteria acquire a cassette of genes to produce a modified peptidoglycan with a terminal D-lactate residue instead of a D-alanine.

Glycopeptide resistance (cont’d)

- The modified lipid II is translocated and undergoes normal transglycosylation and crosslinking, BUT the lipid II and peptidoglycan are not bound strongly by vancomycin/teicoplanin.

Main clinical phenotypes of glycopeptide resistance:
- The most common are differentiated by sensitivity to teicoplanin and use the same molecular mechanism of resistance.
  - **VanA**: high-level resistance to both teicoplanin and vancomycin. Resistance genes are activated by presence of the antibiotics. Resistance gene cassette is transmitted between bacteria on a transposon (Tn1546). VanA is the enzyme (a ligase) that forms the D-Ala-D-Lac.
  - **VanB**: resistant to vancomycin but **susceptible** to teicoplanin. The resistance is only induced by vancomycin. VanB is the ligase that forms the D-Ala-D-Lac.
- A third, less common and less resistant form is also seen
  - **VanC**: Intrinsic resistance to vancomycin but not teicoplanin, found in *E. gallinarium* and *E. casseliflavus*. Constitutive expression of vanC gene - encodes a D-Ala-D-Ser ligase.

\[
\text{Dap} \quad \text{D-Glu} \quad \text{L-Ala} \\
\text{D-Ala} \quad \text{D-Lac}
\]
Glycopeptide resistance (cont’d)

**Induction of Vancomycin resistance**

- VanA and VanB resistance phenotypes are induced by antibiotics. Tn1546 carries seven genes – two, vanS and vanR encode a two-component regulatory system that activates transcription of the resistance genes that direct peptidoglycan remodeling.

VanS senses the presence of vancomycin and activates the response regulator VanR, which binds to a promoter region upstream of the resistance genes and activates transcription.

- VanH makes D-lactate from pyruvate, which is then coupled with D-Ala by Van A (or B) to give D-Ala-D-Lac. This is coupled with UDP-MurNAc-tripeptide by the normal enzyme, MurF, to give the altered UDP-MurNAc-pentapeptide.

- VanX and VanY prevent normal incorporation of D-Ala-D-Ala into the cell wall.

**VanH**

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**VanX and VanY**

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Glycopeptide resistance (cont’d)

- NOTE: Use of the glycopeptide avoparcin in livestock and birds as a growth promoter has been linked to the rise in antimicrobial resistance in Enterococci.
- Glycopeptide resistance is common in poultry given avoparcin. Avoparcin has the same mechanism of action as vancomycin and was banned in Europe in 1997 because of these suspicions.
  - Even after cleaning and disinfecting the poultry coops, resistant bacterial strains could be isolated 5 years later.
- Glycopeptide resistance has been found in poultry farmers and meat processors.

New Glycopeptides

- Dalbavancin
  - Chlorobiphenyl derivative of chloroeremomycin
  - Active vs both VanA and VanB phenotype VRE
  - Strong bactericidal activity in conditions where vancomycin is bacteriostatic
  - Once-a-day dosing is proposed

- Oritavancin
  - Derivative of teicoplanin
  - Longer acyl chain, GlcNAc is removed and N,N-dimethyl propionamide is formed
  - Active vs VanB but not VanA phenotype VRE
  - Greater potency than vancomycin or teicoplanin
  - Once-a-week dosing is planned
Streptogramin peptide antibiotics

Quinupristin/dalfopristin (Synercid)
- Combination of two structurally unrelated, semisynthetic streptogramin-type peptides
  - quinupristin is derived from pristinamycin I (a type B streptogramin)
  - dalfopristin is derived from pristinamycin IIA (a type A streptogramin)

- Each streptogramin alone has modest bacteriostatic activity
- A 7:3 combination of Type A:Type B results in a synergistic effect that is bactericidal
- The pristinamycin combination is used in France as an oral antibiotic
  - Poor water solubility limits clinical use
- Quinupristin and dalfopristin were developed as an injectable form

Metabolism
- Quinupristin and dalfopristin are converted to several active major metabolites:
  - two conjugated metabolites of quinupristin (one with glutathione and one with cysteine)
  - hydrolyzed dalfopristin
- Synercid has been shown to be a strong inhibitor of CYP3A4.

Mechanism of Action
- Both type A and B streptogramins target the 50S bacterial ribosomal subunit
  - the A class (e.g., dalfopristin) binds to the peptidyl transferase site
    - Only bind in absence of loaded tRNA and block early steps in elongation
    - Binding causes a conformational change that increases affinity of quinupristin
  - the B class (e.g., quinupristin) binds to the same region as the macrolide antibiotics
    - Can bind ribosome at any time during protein synthesis
    - Prevent extension of protein chain and can cause release of incomplete proteins
- Action of the individual components is bacteriostatic
- When combined the action is synergistic and can be bactericidal
- Approved for serious or life-threatening VRE and certain MRSA infections
Streptogramins (cont’d)

Resistance – two mechanisms

• Drug modifying enzymes
  – type A streptogramins – several O-acetyltransferases are known

  ![Diagram of ValD and Ac-CoA reaction]

  ![Diagram of Vgb lyase catalyzing hydrolysis of lactone and elimination of water]

  ![Diagram of Vgb enzyme complex]

  ![Diagram of Vgb substrate interaction]

– type B streptogramins
  • Vgb lyase: catalyzes hydrolysis of lactone and elimination of water

  ![Diagram of Vgb catalytic mechanism]

• Target modification
  – Presence of a ribosomal methyl transferase confers resistance to quinupristin (type B)

  ![Diagram of ribosomal methyl transferase]

Daptomycin

• **Daptomycin** (Cubicin®) is a branched cyclic lipopeptide produced by *S. roseosporus*.

  ![Diagram of daptomycin structure]

• Approved for treatment of complicated skin and skin structure infections caused by Gram-(+)
  – including both MRSA and MSSA

• In Phase III for the treatment of infective endocarditis and bacteremia and in the treatment of VRE infections.

Resistance

• Resistance to daptomycin is rare, but can develop
  – There are no known transferable genetic elements that confer daptomycin resistance

• Cross-resistance has not been observed with any other class of antibiotic
  – Indication of the novel mechanism of action
Daptomycin: Mechanism of Action

- Requires Ca\(^{2+}\) for binding and insertion into bacterial membranes
  - oligomerizes to form a channel
  - causes rapid depolarization of membrane potential
- The loss of membrane potential leads to inhibition of protein, DNA and RNA synthesis

\[
\text{Ca}^{2+} \rightarrow \text{daptomycin} \rightarrow \text{membrane} \rightarrow \text{loss of membrane potential} \rightarrow \text{inhibition of protein, DNA and RNA synthesis}
\]

Ramoplanin

- Cyclic Lipoglycopeptide produced by an *Actinoplanes* sp.
- Active against many Gram-(+) including MRSA and VRE
- Still in clinical trials

**Mechanism of action**
- Blocks peptidoglycan biosynthesis by complexing as a dimer with lipid II
  - Recognizes the muramyl portion along with the adjacent diphosphate, *not* the peptide portion like vancomycin.