La petite sous-unité et la fidélité du décodage

Le cas des aminoglycosides

Aminoglycosides

- Neomycin ($12.05/day), Gentamicin ($4.28/day), Tobramycin ($6.77/day), Amikacin ($7.81/day). (Additional $21.00/day with serum levels)
- Binds the 30S subunit.
- Only active against anaerobes because an oxygen dependent system is required to transport the molecules into the cell.
- Synergism with cell wall inhibitors is seen because they increase the permeability of the cell.
Aminoglycosides

► Antibacterial spectrum:
  ▪ Gram negatives: *Pseudomonas, Proteus, Serratia, E. coli, Klebsiella*
  ▪ Neomycin
    ► *S. aureus and Proteus*
    ► *Pseudomonas and Strep* are resistant
► Resistance – decreased uptake, decreased binding affinity, enzymes (plasmids).

Aminoglycosides

► Adverse effects:
  ▪ Ototoxic – associated with high peak levels and prolonged therapy. Pts on loop diuretics, vancomycin and cisplatin are at higher risk.
    ► Cochlear and vestibular.
    ► Concentrates in endolymph and perilymph.
  ▪ Nephrotoxic.
    ► Proximal tubule damage.
Classes of Aminoglycosides

Contain Deoxystreptamine derivatives

- 2-Deoxystreptamine
- Neomycin B, R=H

Lack Deoxystreptamine Derivatives

- Kanamycin A, R=OH
- Kanamycin B, R=NH₂
- Gentamicin A, R=H
- Gentamicin C₂, R=CH₃
- Streptomycin
- Hygromycin B
- Apramycin
Aminoglycosides (bactericidal)

**streptomycin**, kanamycin, gentamicin, tobramycin, amikacin, netilmicin, neomycin (topical)

- **Mode of action** - The aminoglycosides irreversibly bind to the 16S ribosomal RNA and freeze the 30S initiation complex (30S-mRNA-tRNA) so that no further initiation can occur. They also slow down protein synthesis that has already initiated and induce misreading of the mRNA. By binding to the 16 S t-RNA the aminoglycosides increase the affinity of the A site for t-RNA regardless of the anticodon specificity. May also destabilize bacterial membranes.

- **Spectrum of Activity** - Many gram-negative and some gram-positive bacteria; Not useful for anaerobic (oxygen required for uptake of antibiotic) or intracellular bacteria.

- **Resistance** - Common

- **Synergy** - The aminoglycosides synergize with ß-lactam antibiotics. The ß-lactams inhibit cell wall synthesis and thereby increase the permeability of the aminoglycosides.

Tetracyclines (bacteriostatic)

tetracycline, minocycline and doxycycline

- **Mode of action** - The tetracyclines reversibly bind to the 30S ribosome and inhibit binding of aminoacyl-t-RNA to the acceptor site on the 70S ribosome.

- **Spectrum of activity** - Broad spectrum; Useful against intracellular bacteria

- **Resistance** - Common

- **Adverse effects** - Destruction of normal intestinal flora resulting in increased secondary infections; staining and impairment of the structure of bone and teeth.
**Spectinomycin** (bacteriostatic)

- **Mode of action** - Spectinomycin reversibly interferes with mRNA interaction with the 30S ribosome. It is structurally similar to the aminoglycosides but does not cause misreading of mRNA.

- **Spectrum of activity** - Used in the treatment of penicillin-resistant *Neisseria gonorrhoeae*

- **Resistance** - Rare in *Neisseria gonorrhoeae*

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**Bacterial Specificity**

- Aminoglycosides are effective against a wide variety of aerobic, gram negative bacilli.

- These antibiotics are also effective on some gram-positive bacteria, including mycobacteria

- Streptomycin is not effective against Gram negative

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1- [http://rgd.mcw.edu/tools/ontology/ont_annot.cgi?term_key=120392&ontology=wo](http://rgd.mcw.edu/tools/ontology/ont_annot.cgi?term_key=120392&ontology=wo)

Discovery of Aminoglycosides\textsuperscript{1,2}

- In 1943, *streptomycin* was the first aminoglycoside found in various species of *Micromonospora* and *Streptomyces*.
- It was an exciting discovery because it was able to kill many bacteria that penicillin could not, including *Mycobacterium tuberculosis*.
- All aminoglycosides are derived from actinomycete or they are semi synthetic derivatives.
- Aminoglycosides are all poly-cationic and water soluble and contain aminosugars.


Aminoglycosides

- Streptomycin
- Gentamicin
- Tobramycin
- Netilmicin
- Amikacin
- Neomycin

- Little used except for TB
- Broad gram negative coverage
- Like gentamicin, but better activity against *Pseudomonas*
- Often effective for bacteria resistant to gentamicin
- Low resistance or toxicity
- Bowel sterilization, topical
General Mechanism of Action

- Aminoglycosides act as antibiotics by inhibiting bacterial protein synthesis:

- The drugs target bacterial ribosomes and interact with the A-site rRNA on the 16S domain of the 30S ribosomal subunit.1, 2

- Streptomycin promotes miscoding by attracting random tRNAs. Other aminoglycosides restrict ribosome movement. 3


Actions of aminoglycosides

- Blocks initiation of protein synthesis
- Blocks further translation and elicits premature termination
- Incorporation of incorrect amino acid
Clinical Use of Aminoglycosides

• Parenteral
  - Slow IV (1-2 hr) to avoid neuromuscular blockade
  - Synergistic with carbenicillin, but do not give in one IV (drugs react, breaking down carbenicillin)
  - Exception is use of oral neomycin to sterilize bowel for surgery
• Limited due to toxicity
  - Avoid use with other ototoxic drugs (e.g., ethacrynic acid)
• Generally used only for serious gram negative infections
  - Third generation cephalosporin usually preferred
• For UTI, give bicarbonate to alkalinize urine
  - Only taken up well by bacteria at neutral to alkaline pH
  - Not effective in abscesses

Aminoglycosides: Narrow Therapeutic Index

• Renal toxicity in 10-20% of patients
  – Check creatinine clearance before and during therapy
• Ototoxicity
  – Hearing and vestibular defects
  – Can be irreversible
• Neurotoxicity on administration
  – Neuromuscular blockade
  – Can be risk for myasthenia gravis
Streptomycin Biosynthesis

In vivo, a series of enzymatic steps converts dihydrostreptose and streptidine 6-phosphate into streptomycin.

Structure-Activity Relationship

- As we just saw, aminosugars are conserved in all of these antibiotics – hence the name aminoglycosides.
- The positively charged amino groups are critical for binding to the negatively charged rRNA.
- The hydroxyl groups are also important for H-bonding to rRNA, but to a lesser extent than the electrostatic interactions.
- The number of amino groups on the aminoglycoside directly affects the inhibitory ability of the antibiotic. ¹

Binding modes

the antibiotics interact in many ways with RNA: (i) only with the phosphate groups (streptomycin); (ii) mainly with bases (hygromycin, spectinomycin); (iii) with a mixture of both (paromomycin, geneticin); (iv) via magnesium ions (tetracycline) or a protein side chain (streptomycin). The antibiotics can mimick base stacking (pactamycin) or form pseudo base pairing interactions with ribosomal bases (paromomycin and related aminoglycosides).

Parallel focusing: ribosome-antibiotic interactions

- Determine binding energies between 30S ribosomal subunit and aminoglycoside antibiotics
Aminoglycoside Improvements

Since naturally occurring aminoglycosides may cause hearing loss and can also damage the kidney due to their toxicity, attempts have been made to minimize the drug so that it is stripped down to its essential parts.¹

Many groups have utilized the neamine core as a starting point for synthesis of novel aminoglycosides.

Others have tried to further minimize the recognition motif to a 1,3-hydroxyamine core.

¹ Current Topics in Medicinal Chemistry, 2003, 3(9): 101-1019.

ImprovementAttempts

A combinatorial library approach was used with the 1,3-hydroxyamine core to create novel aminoglycosides.

These structures were screened on an artificial 27 nucleotide model mimicking the A-site binding region.

Alterning neomycin to so that it lacks the 5 member ring improves antibiotic activity.
Aminoglycoside Resistance ¹, ²

Antibiotic resistance can develop from one of three mechanisms:

- **Mutation of on the ribosome**
  - If a mutation is in the binding site, it can reduce affinity for the drug

- **Reduced permeability of the antibiotic**
  - If it can’t get into the cell, it can’t interact with the ribosome

- **Acquire enzymes that degrade aminoglycosides**
  - Aminoglycosides can be inactivated by phosphorylation, acetylation, or adenylation

1- Drugs. **2001**. 61(6): 713-721
2- Current Drug Targets – Infectious Disorders. **2002**, 2: 143-1604

Improvements for Resistance ¹

There are two techniques to overcome bacterial resistance to aminoglycosides:

1 – Synthesis of novel aminoglycosides

2 – Development of enzyme inhibitors

Modified Aminoglycosides

The first rationally designed aminoglycoside was Dibekacin, a variation of Kanamycin B, lacking the 3' and 4' hydroxyls.

This alteration makes it unrecognizable to the deactivating enzyme, so the antibiotic can freely inhibit the ribosome.

Modifying existing drugs is a very common way to try to bypass resistances.

More Modifications

Modifications done to Kanamycin B to try to prevent inactivation by enzymes produced in resistant bacteria.

Enzyme Inhibitors

By inhibiting the enzymes that deactivate aminoglycosides, the once resistant drugs can work again as antibiotics.

This is an example of an enzyme inhibitor that includes the structure of an aminoglycoside, neamine. It is linked to an adenosine and blocks the binding site to the aminoglycoside deactivating enzyme.

Strategies to overcome phosphorylating enzymes that act on antibiotics.
3D structure of streptomycin bound to the 30S ribosome (1FJG.pdb). Streptomycin is shown as red spheres, proteins are coloured green, RNA is coloured grey; b) Surface of the binding pocket showing the region within 8Å of streptomycin. c) Residues and helices within 8Å of streptomycin. d)-k) Interactions of streptomycin with the 30S particle. Hydrogen bonds with donor-acceptor distance of less than 3.5Å are shown.

a) 3D structure of hygromycin bound to the 30S ribosome (1HNZ.pdb). Hygromycin is shown as red spheres. b) Close up of the hygromycin binding site (helix h44 residues 1401-1410 and 1490-1501). Hygromycin is shown as ball and sticks. c) Secondary structure diagram of parts of h44 that interact with hygromycin (red boxed area) and paromomycine (green boxed area). Tertiary interactions are indicated using the Leontis-Westhof nomenclature. d)-i) Interactions of hygromycin with the 30S particle. Hydrogen bonds with donor-acceptor distance of less than 3.5Å are shown.
3D structure of paromomycin bound to the 30S ribosome (1FJG.pdb). Paromomycin is shown as red spheres, proteins are coloured green, RNA is coloured grey. b) Paromomycin binding site taken from a structure presenting the minimal A-site (1J7T.pdb). c) Superposition of aminoglycoside antibiotics containing the neamine moiety onto paromomycin (yellow). Colour coding: geneticine (cyan), gentamicin A (magenta), kanamycine A (green), lividomycine (deep-salmon), neamine (grey), neomycine B (slate–blue), ribostamycine (orange), tobramycine (marine). For pdb codes and references see table 1. d) Superposition of aprromycin (1YRJ.pdb blue-green) onto paromomycine (yellow) e)-i) Common interactions of ring I and II of neamine containing aminoglycosides; paromomycine is shown as example. Common hydrogen bonds with donor-acceptor distance of less than 3.5Å are shown.