Anti-Ribosomal Agents:
Aminoglycosides, Tetracyclines & Macrolides
- Common Origin and Mechanism

- The three major classes of agents discussed here have two features in common:
  a) they are all agents that are derived from microorganisms
  b) they all selectively interfere, in various manners, with bacterial translation of mRNA to protein by binding to bacterial ribosomes

'See figure 3.12 on page 30 of your text'

I. Aminoglycosides

A) Representative Structures
Aminoglycosides are a set of complex structures made by various microorganisms that have potent antimicrobial activity. Chemically, these compounds can be separated into two classes. The first class has the aminocyclitol streptidine as their core unit, with two or three sugars attached. The prototype of this class, and the first clinically useful aminoglycoside, is streptomycin (see below). However, most clinically used aminoglycosides (such as netilmicin) belong to the second class, which has the aminocyclitol 2-deoxystreptamine as their core unit. All aminoglycosides are highly charged, highly water soluble, chemically stable, poorly absorbed compounds. These common physical/chemical features have important implications for how these compounds are used clinically. For example, in general they are not active orally, and must be given by iv or im routes. These compounds are potently bacteriocidal to a wide range of susceptible gram-negative bacteria.
B) Mechanism of Action-Interfere with BACTERIAL Protein Synthesis

1) Outline of Normal Bacterial Protein Synthesis (Translation)

Since all of the antibiotics discussed in this section act by interfering with protein synthesis by binding to the bacterial ribosome, it is appropriate at this point to give a BRIEF (and necessarily incomplete) review of translation (refer to the figure below). The initiation complex is formed by the binding of the 50S and 30S subunits of the bacterial ribosome to the start codon of a molecule of mRNA. A molecule of tRNA charged with fMet then binds to the start codon at the P site of the ribosome. A second AA-charged tRNA, complementary to the next mRNA codon, then binds in the A site. In the transpeptidation step, a peptide bond is formed between the two amino acids, with a free molecule of tRNA being formed. The free tRNA is then released from the P site, and in an energy-dependent step translocation of the ribosome along the mRNA occurs. A new amino acid-charged tRNA, complementary to the third codon in the mRNA, then binds to the open A site. The entire cycle is repeated until the end of the coding sequence of the mRNA is reached. The specific steps in translation that are blocked by the various antibiotics are indicated in the sections below. The basic outlines of mammalian translation are very similar. However, antibiotics have selective effects on BACTERIAL protein synthesis because bacterial ribosomes are structurally distinct from ours, and thus antibiotics bind much more tightly to bacterial ribosomes.
2) Aminoglycoside Mechanism of Action

The mechanism of action of aminoglycosides (AG) is not as well established as for some of the other antibiotics discussed in this lecture, and several possible mechanisms of action have been proposed for AG. However, the one well-characterized action of AG on bacterial ribosomes is in the blockage of bacterial protein initiation. AG bind to the 30S subunit immediately after its assembly with the 50S subunit, and thus prevent the binding of the initial fMet-tRNA, blocking initiation of bacterial protein synthesis. The key to the potent bacteriocidal effects of AG appears to be due, at least in part, to the fact that binding of the AG to the ribosome is very tight and may be functionally irreversible. Thus, treatment of bacteria with AG leads to the accumulation of so-called “streptomycin monosomes” on mRNA strands.

![Diagram of aminoglycoside mechanism](image)

C) Metabolism and Elimination

As stated previously, all aminoglycosides are highly charged, highly water soluble, chemically stable, poorly absorbed compounds. As a result of these physical characteristics, most of a dose of AG is excreted unchanged in the urine. Renal disfunction can thus lead to a buildup of AG in the bloodstream. Due to their poor ability to cross membranes, AG also are strongly retained by tissues once they do penetrate, and their release from bound tissues is very slow.

D) Mechanisms of Toxicity

Toxicity is a very serious concern with all AG; the therapeutic window for these compounds is very narrow, and patients using them must be monitored carefully. The two main toxicities seen with AG are nephrotoxicity and ototoxicity. Nephrotoxicity is quite frequent (seen in 8-26% of patients) but is usually mild and almost always reversible upon withdrawl of the drug. On the other hand, AG-induced ototoxicity is rarer, but frequently leads to irreversible hearing loss, and serious and potentially permanent vertigo. The ototoxicity results from the fact that AG build up in the perilymph and endolymph of the ear. There, they bind specifically to phosphatidylinositols, particularly on hair cells of the inner ear, and this leads to the death of these cells.
II. Tetracyclines

A) Representative Structures

The natural product tetracycline (the parent member of this class, naturally) is not used clinically to any significant extent today. However, the semi-synthetic tetracycline derivatives minocycline and doxycycline are still used for a variety of different bacterial infections. These latter two compounds are more stable than the parent drug, which results in fewer adverse toxic effects (see below). They are also more hydrophobic and exhibit better oral bioavailability and other pharmacokinetic parameters. Tetracyclines exhibit very good oral bioavailability, and are very rarely used parenterally. Originally, the tetracyclines exhibited potent activity against a variety of gram-positive and gram-negative bacteria. However, due in large part to overuse, resistance emerged rapidly, and now these drugs are primarily used for the treatment of infections caused by mycoplasmas, chlamydiae, and rickettsiae.

![Tetracycline](image1)

![Minocycline](image2)

![Doxycycline](image3)

B) Mechanism of Action

Tetracyclines act by binding to the A ("acceptor") site of bacterial ribosomes on the 30S subunit. It prevents the binding of a new tRNA to this site, blocking further protein synthesis (see figure below). Tetracyclines also bind (more weakly) to mammalian ribosomes and can inhibit mammalian protein synthesis in the same manner. However, these drugs have a very selective effect on bacterial cells because bacteria take up the tetracyclines much more avidly than do mammalian cells.

![Binding of tetracycline to A site of 30S](image4)

![Binding of new tRNA to open A site](image5)
C) Metabolism and Elimination
Primarily renal elimination is observed, but hepatic elimination also plays an important role, particularly with tetracycline and doxycycline. Thus, significant amounts can be recycled through intestinal uptake. Minocycline is extensively metabolized, in contrast to the other drugs.

D) Toxic Side Effects
1) Gastrointestinal irritation is frequently seen with these drugs, but is usually mild. In addition, however, these broad-spectrum antibiotics can lead, through disruption of normal colonic flora, to pseudomembranous colitis, a potentially life threatening side effect.
2) Photosensitivity - these drugs can enhance the sensitivity of patients to the sun, and this is particularly a problem in a small percentage of the population.
3) Hepatic toxicity - this is frequently seen with large parenteral doses, and in any dose given to a pregnant woman. This is thus one of two key reasons that tetracyclines are rarely if ever prescribed in pregnancy.
4) Renal toxicity - relatively rare, and is particularly rare with doxycycline.
5) Deposition in bones and teeth - treatment of infants and young children (particularly <5 yrs old) with tetracyclines can lead to the deposition of these compounds in the bones and teeth. This frequently leads to cosmetic discoloration of the teeth, and can occasionally interfere with the growth of developing bones. This can also occur in a fetus when the pregnant mother is treated with tetracyclines, and is the second reason that these drugs are not given to pregnant women.

E) Chemical Instability
Tetracycline itself is (relatively, compared to most drugs) unstable upon shelf storage, and this instability can lead to the formation of irritating or even toxic metabolites. Doxycycline and minocycline are significantly more stable, but patients should still be cautioned about the potential dangers of using older samples of tetracyclines. In addition, the unique chemical structure of all of the tetracyclines leads them to be excellent chelators of divalent metal ions such as calcium, iron, aluminum and magnesium (see below). For this reason, taking supplements of these minerals or drugs that contain them (particularly antacids), or milk can interfere with the absorption of tetracyclines, and thus this interaction should be avoided.
III. Macrolides

A) Structures

The macrolides are a class of structurally complex antibiotics derived from microorganisms. The first clinically important macrolide, erythromycin, is a natural product which is characterized by a 14-membered macrolide ring. Problems with gastric irritation and drug interactions (see below) led to the development of the 14-membered ring macrolide clarithromycin and the 15-membered “azalide” azithromycin. Both of these drugs are semi-synthetic derivatives of erythromycin. These drugs are usually given orally, but can also be given intravenously. Macrolides are particularly active against gram-positive cocci, and provide an important alternative to beta-lactam therapy. They are also active against mycoplasmal, chlamidial, and rickettsial. The primary advantages of the newer semisynthetic drugs are their reduced side effects and better pharmacokinetic properties, but note that azithromycin possesses improved activity against gram-negative bacteria.

![Structures of erythromycin, clarithromycin, and azithromycin]

B) Mechanism of Action

In contrast to the two antibiotic classes discussed previously in this section, the macrolides bind to a site on the 50S subunit of the bacterial ribosome. This binding occurs at a site between the A and P sites after the release of a free tRNA from the P site. It then blocks translocation of the nascent polypeptide chain/tRNA from the A site to the P site, blocking bacterial protein synthesis at this stage. The binding of the macrolide to this site is reversible. Note that macrolides bind very poorly to mammalian ribosomes due to the structural differences between the bacterial 50S subunit and our ribosomal subunit.

![Diagram of translocation and binding]

C) Metabolism and Elimination

Macrolides are excreted (primarily in unmodified form) in the bile and to a lesser extent in the urine. Erythromycin undergoes extensive oxidative metabolism by cytochrome p450 enzymes, but azithromycin and clarithromycin undergo significantly less metabolism.
D) Toxicity

Toxic reactions to macrolides are relatively rare. However, drug interactions can occur between macrolides and other drugs. This is particularly true for erythromycin, which competes with many other drugs for metabolism by p450. Erythromycin can decrease the rate of metabolism of corticosteroids, cyclosporin, digoxin, warfarin, and several other drugs. Azithromycin and clarithromycin are much less prone to exhibit drug interactions in this manner.

- Cholestatic hepatitis can be seen, particularly with ester derivatives of erythromycin.
- Macrolides can cause gastric irritation.
- Rarely, allergic reactions to macrolides can be seen.

IV. Miscellaneous Antiribosomal Agents

A) Clindamycin

1. Structure

Clindamycin is a semisynthetic, chlorinated derivative of the naturally occurring antibiotic lincomycin. Clindamycin is much more active than the macrolides against anaerobes, both gram-positive and gram negative. It penetrates into bone and thus is valuable for the treatment of osteomyelitis. Clindamycin can be given orally or parenterally. It also exhibits good cellular penetration.

\[
\text{CH}_3 \quad \text{Cl}\quad \text{H}\quad \text{N}\quad \text{O}\quad \text{H}\quad \text{OH}\quad \text{SCH}_3
\]

2. Mechanism of Action

The mechanism of action of clindamycin is very similar to that of the macrolides. It binds to the same site on the 50S ribosome subunit, and thus clindamycin and a macrolide should not be used together.

3. Toxicity

Diarrhea is frequently seen in patients treated with clindamycin. This may lead to pseudomembranous colitis due to colonization of the colon by C. difficile. This potentially serious side effect was first seen with clindamycin, but can also occur with other potent broad-spectrum antibiotics.
B) Chloramphenicol

1. Structure

Chloramphenicol is an unusual natural product that was originally isolated from a Streptomycin strain. It is now made by total synthesis. It is well absorbed orally, but can also be given by IV. It is an inexpensive, broad spectrum antibiotic, but its utility is limited by a rare but serious side effect. It is a valuable treatment for bacterial meningitis, due to its ability to achieve high concentrations in the CSF.

\[
\begin{align*}
\text{Cl} & \hspace{1cm} \text{C} & \hspace{1cm} \text{H}_2\text{O}R \\
\text{N} & \hspace{1cm} \text{H} & \\
\text{O} & \hspace{1cm} \text{H} & \\
\text{NO}_2 & \hspace{1cm} & \\
\end{align*}
\]

Chloramphenicol (R = H)

2. Mechanism of Action

Chloramphenicol binds to a site on the 50S subunit of bacterial ribosomes. Its binding there blocks peptide bond formation. Note that it also binds to the large subunit of mammalian mitochondrial ribosomes, and this may contribute to its toxic effects on immune system cells.

3. Toxicity

The two main toxic effects of chloramphenicol are:

a) a dose related, reversible erythroid suppression of bone marrow production. This can be reversed with withdrawal of the drug.

b) a rare, idiosyncratic reaction causing aplastic anemia, which is not dose dependent and is irreversible. This reaction only occurs with a frequency of 1/30,000 patients, but is frequently fatal.
IV. New Antiribosomal Agents

A) Synercid (Quinupristin/Dalfopristin)

1. Structure
Synercid is a combination of the antibacterial agents quinupristin and dalfopristin, in a ratio of 30:70. Pristinamycin, a compound closely related to dalfopristin, is an oral antibiotic that has been used for several years in Europe. However, while pristinamycin, dalfopristin, or quinupristin alone are bacteriostatic, Synercid is a bacteriocidal agent against gram-positive and certain gram-negative bacteria. Synercid can only be given parenterally.

![Synercid Structure](image)

**Quinupristin**

![Quinupristin Structure](image)

**Dalfopristin**

![Dalfopristin Structure](image)
2. Mechanism of Action

Synercid specifically targets the bacterial ribosome, like the other drugs discussed herein. However, it has a unique mechanism of action, which involves synergy at the molecular level. Both quinupristin and dalfopristin bind to the 50S ribosome subunit. Dalfopristin binds to 50S and interferes with elongation by preventing the binding of a new aa-tRNA to the acceptor site. Quinupristin binds to a different site on 50S and stimulates the premature dissociation of the peptidyl-tRNA, and may also interfere with the release of the completed polypeptide after synthesis, by blocking a channel on the 50S subunit. The synergy between the two results from dalfopristin binding first, causing a conformational change in the ribosome, and enhancing its affinity for quinupristin.

B) Linezolide (Zyvox™)

1. Structure

Linezolid is the first member of a new, mechanistically and structurally novel class of antibiotics, the oxazolidinones (named after the key central structural feature of these compounds). It is an entirely synthetic compound, and was approved for use in April 2000. Linezolid can be given orally or parenterally. It also exhibits good cellular penetration. It exhibits good activity versus a wide variety of resistant gram positive bacteria, but little useful activity against gram negative bacteria.

2. Mechanism of Action

Linezolid and related oxazolidinones selectively and uniquely bind to the bacterial 50S ribosomal subunit and inhibit bacterial translation at the initiation phase of protein synthesis.