Athar Batch

Pharmacology

Lecture: 30

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(new class of antobiotics)

Protein Synthesis Inhibitors

Pharmacology and Toxicology

General Pharmacology

Second Year Medical Students

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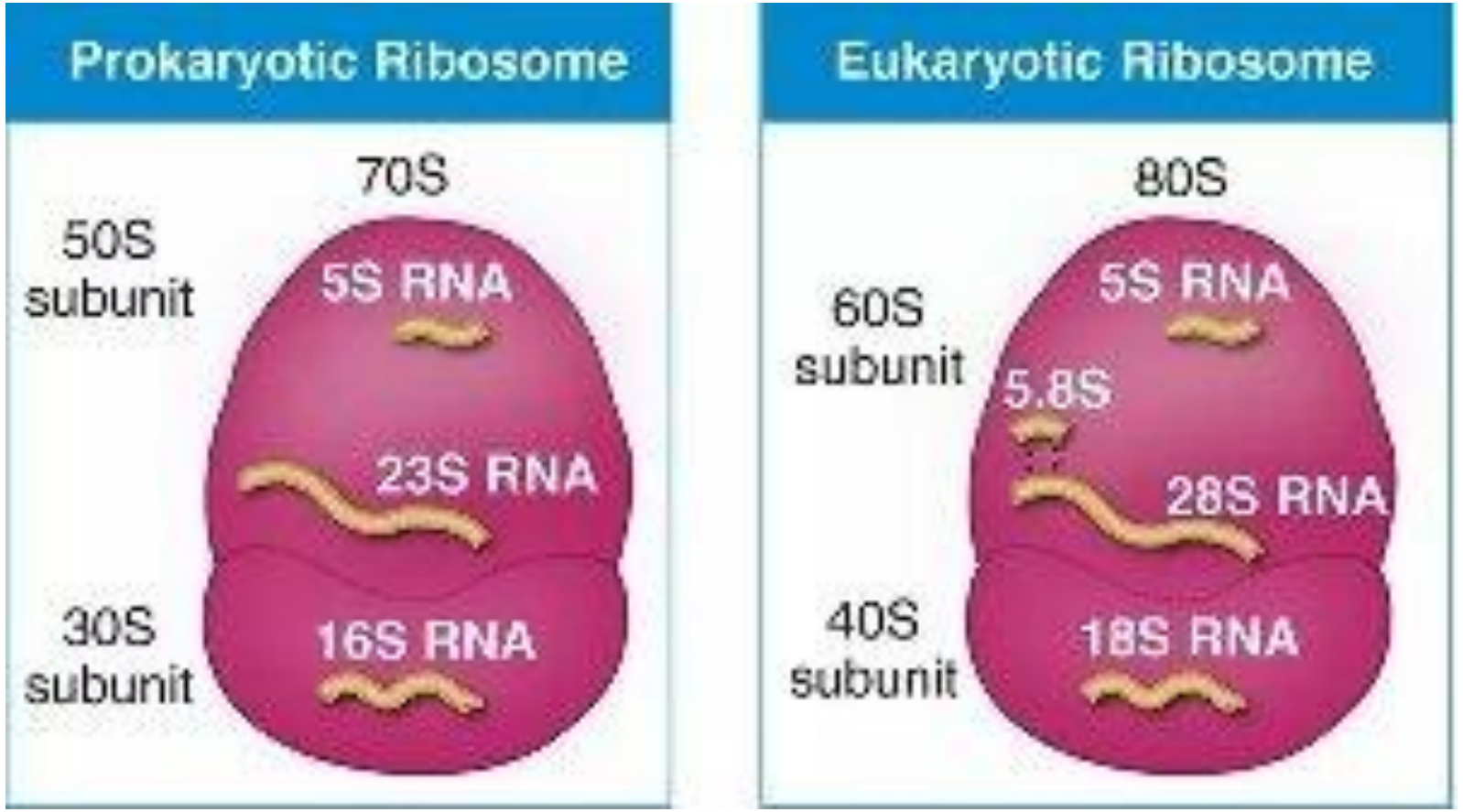
Faculty of Medicine

The Hashemite University

Textbook: Chapter 30 pp: 384-399

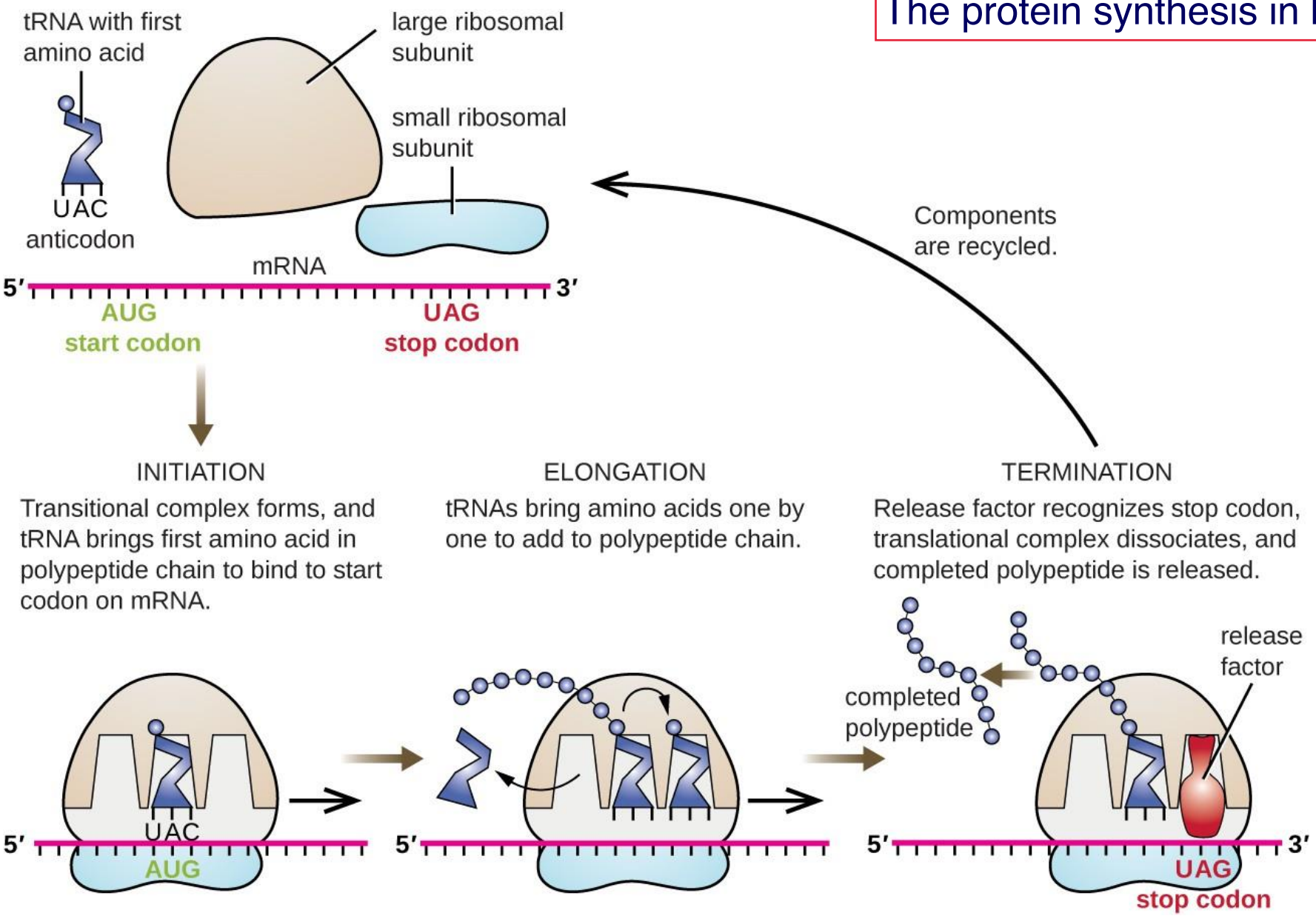
Bacterial Protein Synthesis



similar, but not identical

- bacteria protein synthesis is essential for biochemical process required for their survival. protein synthesis inhibitors are very effective antibiotics with very wide uses. the problem is that protein synthesis is not limited to bacteria (it occurs in both prokaryotic bacterial cells and eukaryotic human cells).
- The main organelle that is responsible for protein synthesis is the ribosome. despite the fact that bacteria protein synthesis and human protein synthesis are very similar process, they don't have identical ribosomes. Ribosomes are made up of proteins & r-RNA and composed of 2 subunits (large and small subunits).
- In bacteria, the sedimentation weight of the subunits are different from those on eukaryotic ribosomes. ribosomal bacteria are smaller in structural size. we can target the bacterial small subunit (30s) and sparing the eukaryotic small subunit (40s). this is the main basis of selective toxicity of protein synthesis inhibitors.
- Also, the steps of protein synthesis itself are different between bacteria and human cells. they are more complicated in eukaryotic human cells, with multi-steps and multi protein factors involved (more than that of the bacteria).
- that doesn't mean the protein synthesis inhibitors are completely safe or can't affect the human cells. drugs such as chloramphenicol and tetracyclines if used at high concentrations, they start affecting the protein synthesis process in human's cells and cause toxicity.

The protein synthesis in bacteria



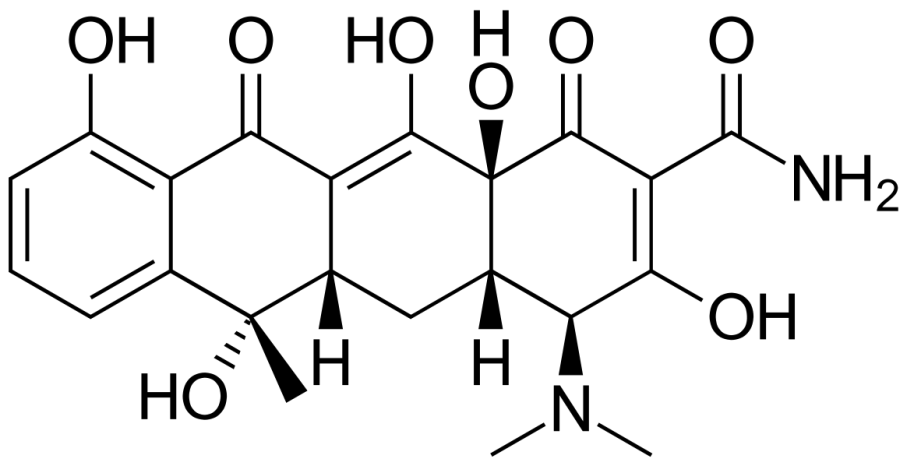
- to understand mechanism of action for protein synthesis inhibitors, we need to know protein synthesis process itself. it requires a machinery which is composed of the ribosome subunits. it also needs mRNA which carries the code for the protein to be synthesized and tRNA which has the anti-codon responsible to bind to the codon encoding for amino acid on mRNA and can attach to amino acid and carry it. in addition to these main structures, there's wide variety of factors (initiation, elongation, etc.) which help on the process. protein synthesis is an anabolic process which requires a lot of energy.

- It's divided into three main stages:
1. Initiation: (describes the process where synthesis starts. it begins by the assembly of protein synthesis complex. initiation complex process starts with binding of first amino-acid t-RNA on the start codon)
2. Elongation: (during elongation, there's farmeshifting of the mRNA with every new amino acid added)
* P-site: where the growing peptide enters.
* A-site: enters the new tRNA with the amino-acid to be transfered to the peptide.
3. Termination: (it's mediated by release factor that binds to a stop codon on mRNA and results in disassembly of ribosome mRNA complex and the completely synthesized polypeptide)

- there's multiple targets in protein synthesis machinery we can target with the drugs to interfere with the normal bacteria protein synthesis process(we can target 50s, 30s, binding of tRNA to mRNA, misreading the codons or etc.)

Tetracyclines

Tetracyclines



Tetracycline

TETRACYCLINES	
Demeclocycline	DECLOMYCIN
Doxycycline	VIBRAMYCIN
Minocycline	MINOCIN
Tetracycline	

- Tetracyclines: group of protein synthesis inhibitors that share chemical structure composed of four aromatic rings. named after the drug tetracycline which is the prototypical drug of this family.

- The most widely used drugs of this family are Tetracycline, Minocycline and specially **Doxycycline:** (has wide variety of uses in clinical medicine for treatment of bacterial infection, protozoan infection and even infection caused by spirochetes).

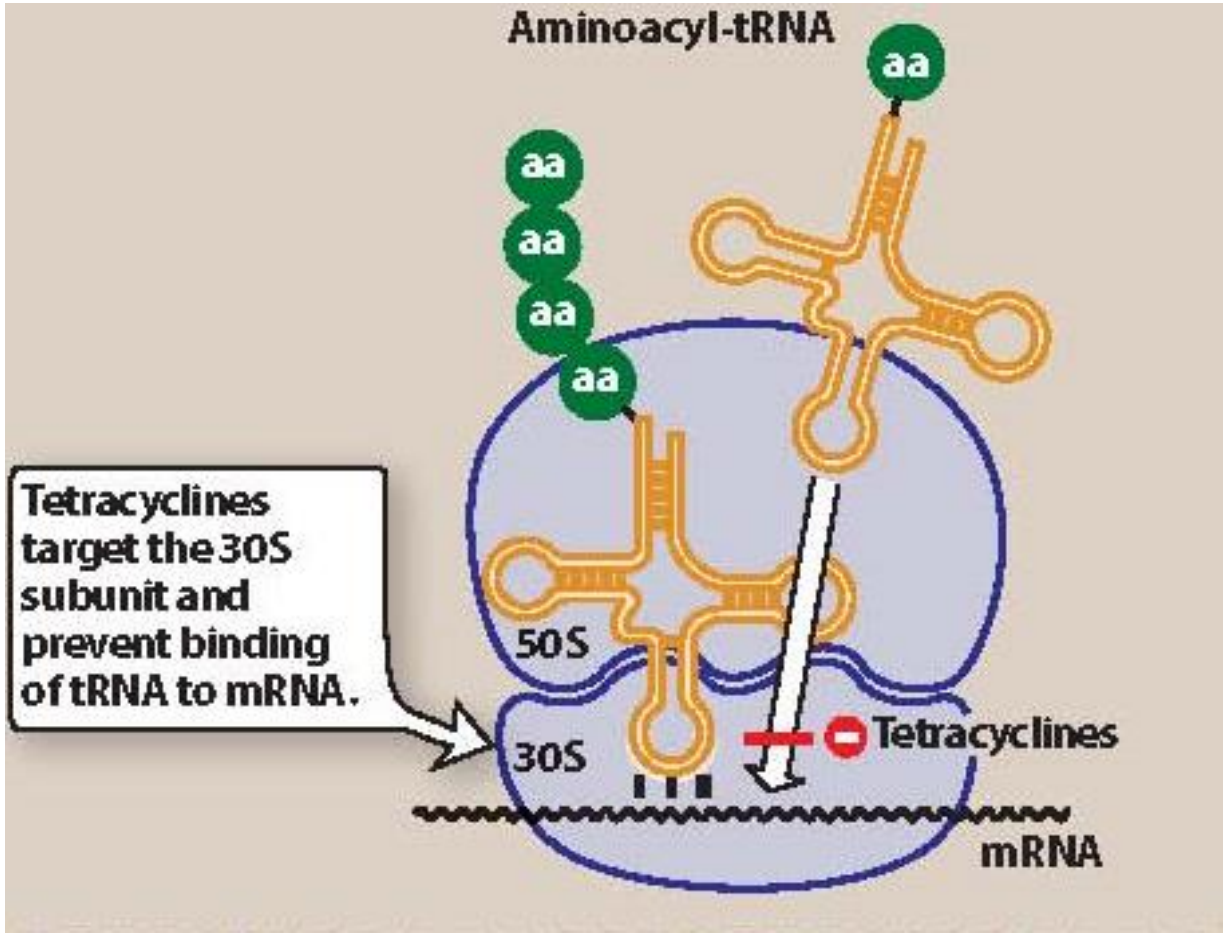
- the uses of tetracyclines are not just limited to bacteria as antibiotics. they can also have other effects targeting other pathogenic microorganisms.

Tetracyclines

Mechanism of action

-bind reversibly to the 30S subunit of bacterial ribosome

-prevent the binding of tRNA to the mRNA-ribosome complex



- For tetracyclines and other protein synthesis inhibitors to work, they need to cross the plasma membrane and concentrate within the cytoplasm because the protein synthesis occurs inside the cell.

- tetracyclines can diffuse through the bacterial membranes passively (passive diffusion) or some tetracyclines can be shunted into the bacterial cell through special energy dependent transporters in susceptible organisms.

- once in the bacterial cell, they bind reversibly to the small subunit of the ribosome on the A-site. (the target of tetracyclines)

Tetracyclines

Antibacterial spectrum

- Bacteriostatic
- Effective against gram-positive, gram-negative, protozoa, spirochetes, atypical, etc

Commonly used for the treatment of:

1. Acne (doxycycline)
2. Chlamydia (doxycycline)
3. Peptic ulcer disease (tetracycline)
4. Lyme Disease (doxycycline)
5. Mycoplasma Pneumonia (doxycycline)

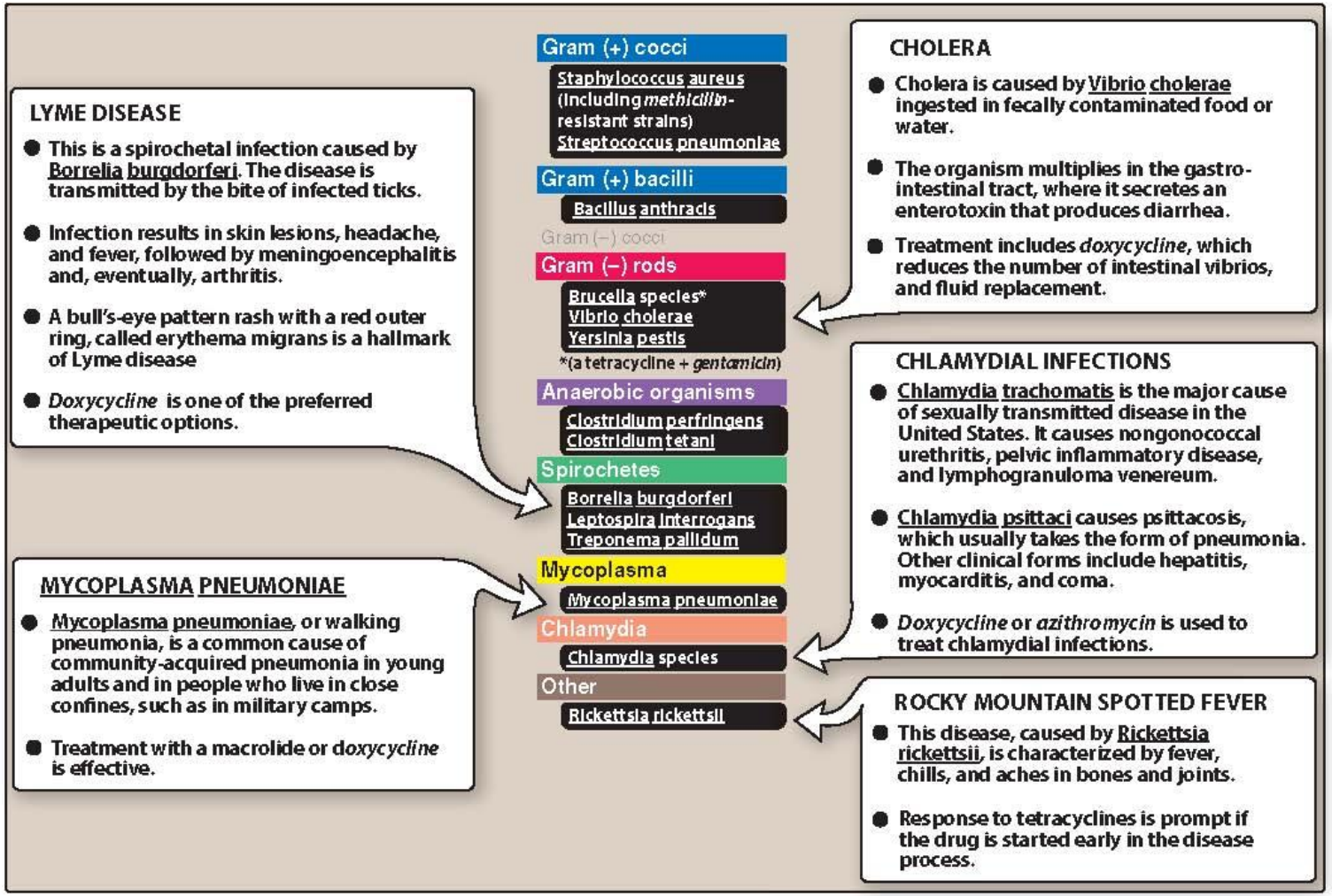
 Based on their mechanism of action, tetracyclines are bacteriostatic at clinically relevant concentration. they result in inhibition of bacterial cell growth rather bactericidal effect unlike cell wall inhibitors.

 they have wide antimicrobial coverage.

 used for:

- Chlamydia: there are different types. it cause sexually transmitted infection and may cause eye infection.
- Peptic ulcer disease: H. pylori is the bacteria responsible for most of peptic ulcers.
- Lyme Disease: caused by spirochete called Borrelia burgdorferi and involves multi-organs. common on areas of northern America.
- Mycoplasma Pneumonia: atypical bacteria which can cause atypical pneumonia which is a very common lung infection specially in young adults.

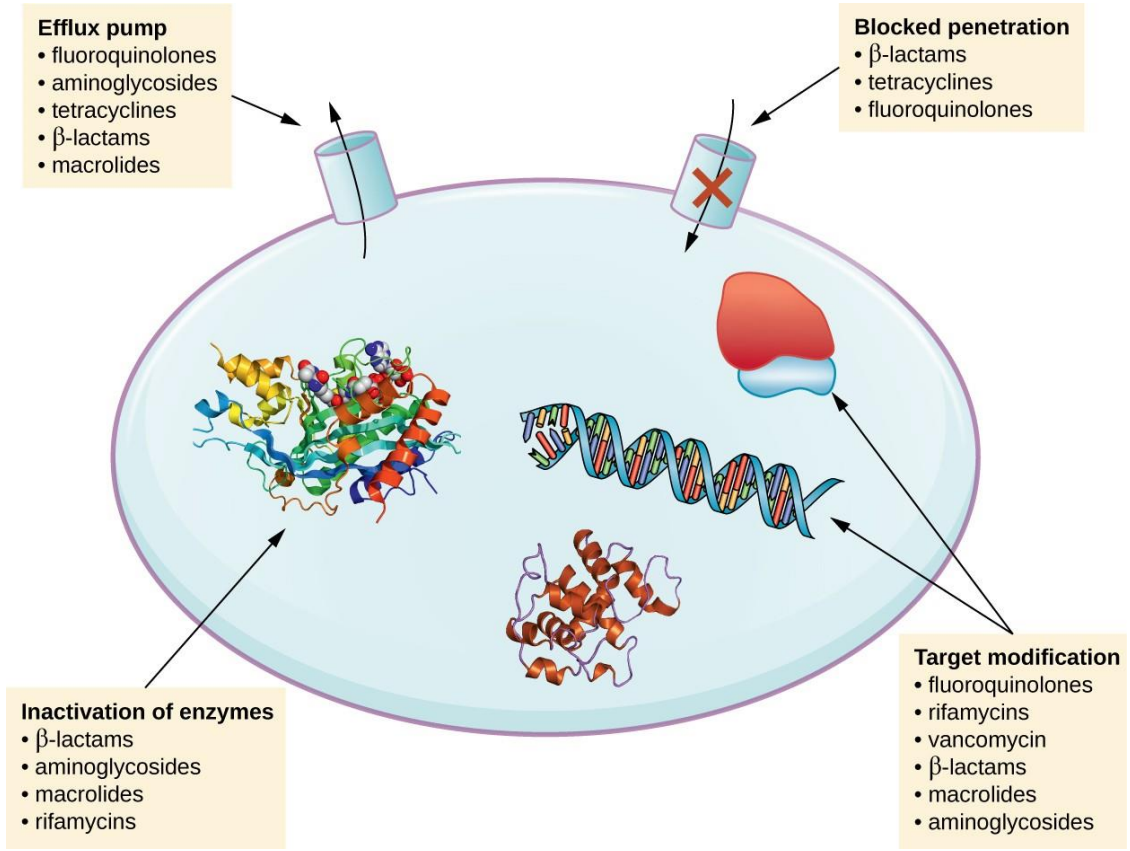
Therapeutic Spectrum of Doxycycline



Tetracyclines

Mechanisms of resistance

- Efflux pump (most common)
- Enzymatic inactivation of the drug
- Interfering with binding to ribosomes
- Cross-resistance is <u>not common</u>



- The drug resistance depends on either the special characteristics of the drug or their mechanism of action. Based on the mechanism of action of tetracyclines, we can expect the mechanism of resistance.

- Releasing B-lactamases against Tetracyclines or altering the PBP isn't useful because their structures don't have B-lactam ring and they don't target the PBPs.

1- the drug needs to enter the cell in order to inhibit protein synthesis. one of the resistance methods is that the bacteria develop efflux pumps located at plasma membrane to pump out drug molecules that enter the cell.
2- instead of releasing B-lactamase, bacteria release enzymes that are capable of inactivating tetracyclines.
3- certain bacteria alters the structure of the small subunit. so it will decrease the affinity of 30s to bind with tetracyclines.

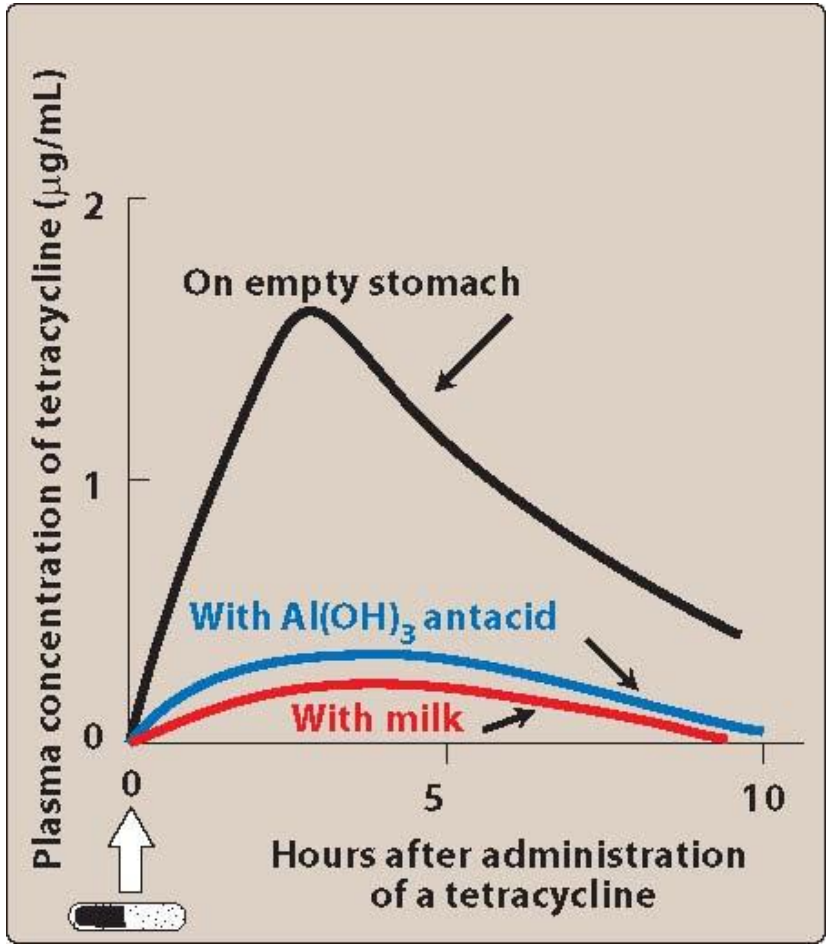
- if bacteria species are resistant to tetracycline, it's unlikely to be resistance to other types of tetracyclines. it also depends on the mechanism of resistance. if the mechanism is efflux pumps usually it won’t distinguish the type of tetracyclines and it's a very common mechanism of resistance on many types of bacteria.

Tetracyclines

Pharmacokinetics

Absorption

- Oral
- Adequately absorbed
- ↓ absorption when administered with dairy (high cations) → formation of nonabsorbable chelates



 Tetracyclines in general are given orally (most drugs of this family are available orally). it's an advantage because this allows for the use of the drug on outpatient level. they have very good absorption on gastrointestinal tract.

 Doxycycline and minocycline can also be given IV or IM if needed.

 problem of Tetracyclines is that they chemically like to bind with divalent and trivalent cations (positively charged ions) and forms insoluble complex. it is a problem if tetracycline were administered with food products that contain high content of cations (like dairy products), it will result in complexes which can't be absorbed.

 Antacids $Al(OH)_3$ are trivalent cations and can also decrease the absorption of tetracycline.

Tetracyclines

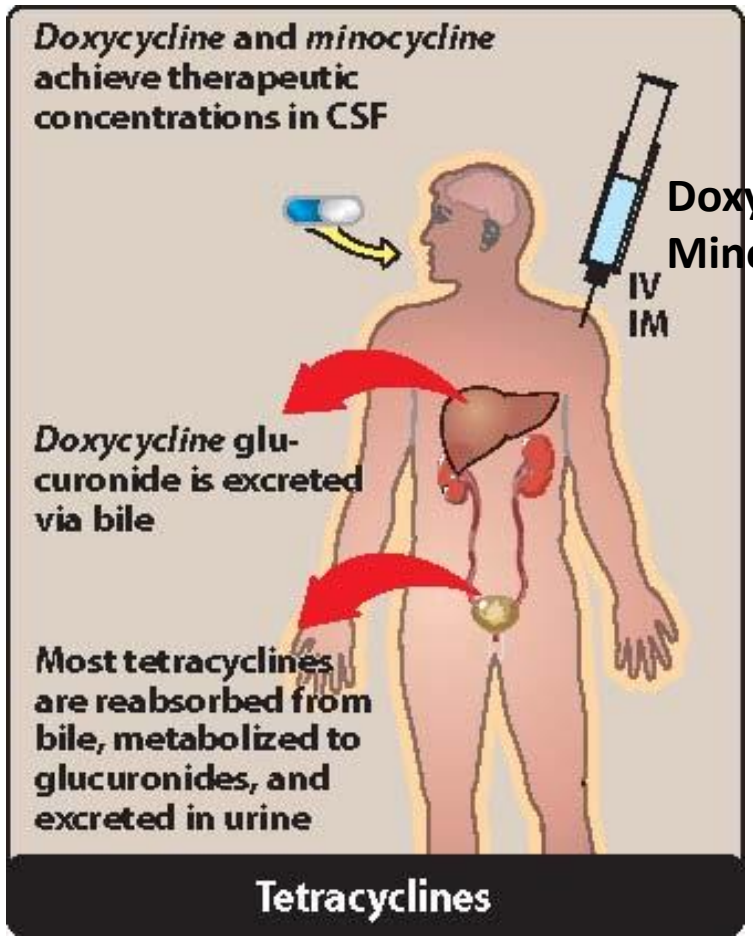
Pharmacokinetics

Distribution

- Distribute well in body fluids, including CSF
- Bind to tissues undergoing calcification e.g., bones, teeth.
- Cross placenta and deposit in fetal bones

Elimination

- Tetracycline eliminated unchanged in urine
- Doxycycline eliminated in bile/feces



Distribution:

Tetracyclines have a very good distribution profile, specially **doxycycline** and **minocycline.** they achieve the therapeutic concentration in CSF which means they can be used for treatment of central nervous system infection.

- High contents of Ca^{+2} and Mg^{+2} are found in tissues undergoing calcification (bone & teeth), so tetracyclines concentrate there. it's **contraindicated** for children under 8 years of age who still have bone calcification going on and growth for their bone because they can cause permanent tooth discoloration and can also affect a child's growth (bone malformation).
- Tetracyclines can cross the placenta and may result in teratogenicity effect. they're **contraindicated** in pregnancy.

Elimination:

Tetracycline are eliminated unchanged or minimally hepatic metabolized. **Doxycycline** is heavily metabolized in the liver by glucuronidation and heavily excreted in the bile and feces (can be used for patients with renal failure). other tetracyclines undergo normal hepatic metabolism into inactive metabolite and eliminated by renal excretion in the urine.

Tetracyclines

Adverse effects

- **Gastric discomfort:**

-irritation of gastric mucosa

-esophagitis

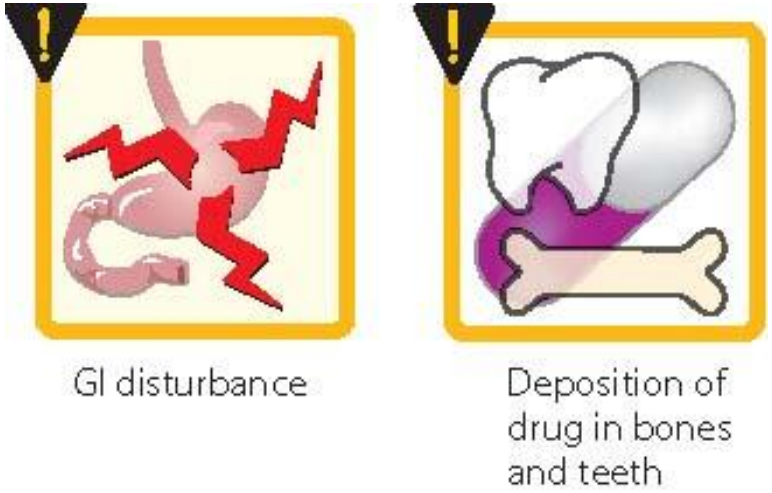
- **Effects on calcified tissues**

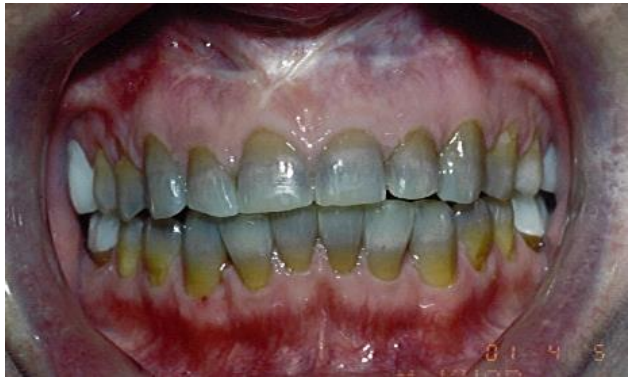
-deposited in tissues undergoing calcification, e.g., bones in children.

-dental hypoplasia

-growth problems

-pediatric use is limited





 One of the most common adverse effects of tetracyclines is gastric discomfort which is probably associated with nausea and vomiting. if severe, it may cause inflammation of the esophagus.

- we can reduce the irritation by combining them with food (not dairy product) except tetracycline should always be given on empty stomach.

 One of the adverse effects caused by tetracyclines specially in children is a condition called dental hypoplasia charactrized by discoloration of teeth and sometimes malformations of teeth growth. (see the picture in the previous slide)

Tetracyclines

Adverse effects

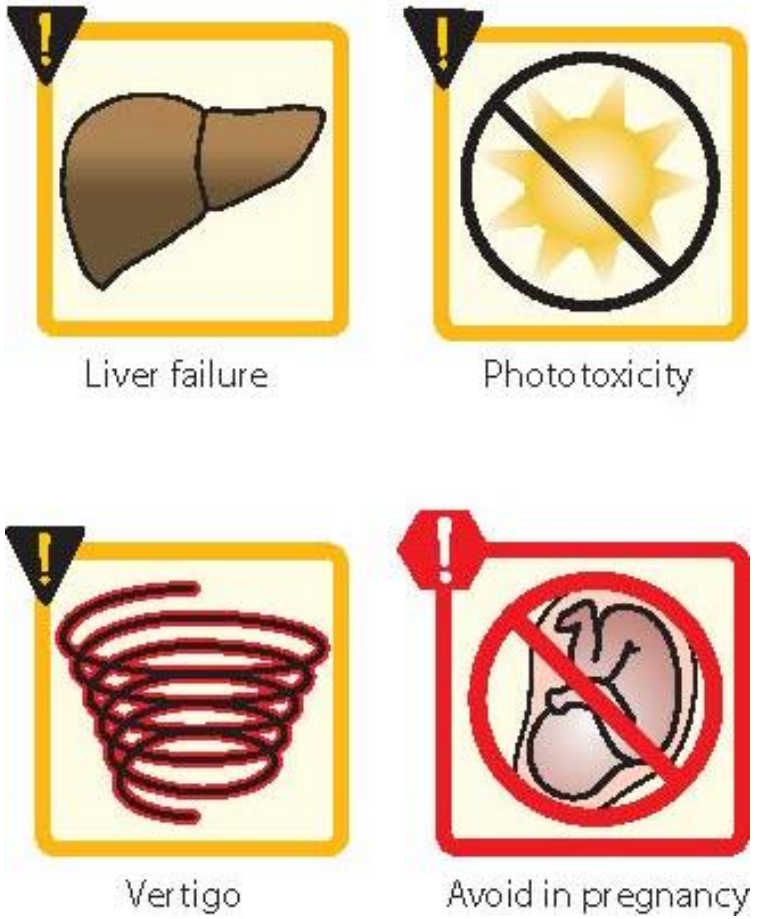
- **Hepatotoxicity**
- **Phototoxicity:**

-severe sunburns (recommended to wear sun protection)

- **Vestibular dysfunction:**

-dizziness, vertigo, tinnitus

- **Pseudotumor cerebri**



 since some tetracyclines are metabolized in the liver (such as Doxycycline or minocycline), they can also cause hepatotoxicity. it's more susceptible to develop hepatic toxicity if they were given at high doses or in pregnant women.

 Tetracyclines can cause phototoxicity. mean they increase the sensitivity of skin to sun light. patients who use tetracyclines are more susceptible of developing sever sunburns. it's recommended to use sunscreens and avoid unnecessary exposer to sunlight.

 the structure in the inner ear that is responsible for hearing and balance are the cochlea (hearing) and the **vestibule** (maintaining balance). Tetracyclines can result in vestibular dysfunction resulting in vertigo and dizziness. **vertigo** (sensation of self-movement or the movement of your surroundings) is different than **dizziness** (feeling lightheaded, unsteadiness, fatigue).

 CSF is on a closed circulation. if there's an obstruction or the volume of CSF increased, the CSF pressure will increase (intra-cranial hypertension). one of the causes is pseudotumor cerebri (false brain tumor / no tumor). Tetracyclines may cause elevation intra-cranial pressure.

Tetracyclines

Contraindications

1. Pregnant women
2. Breast-feeding women
3. Pediatric age group <8 years

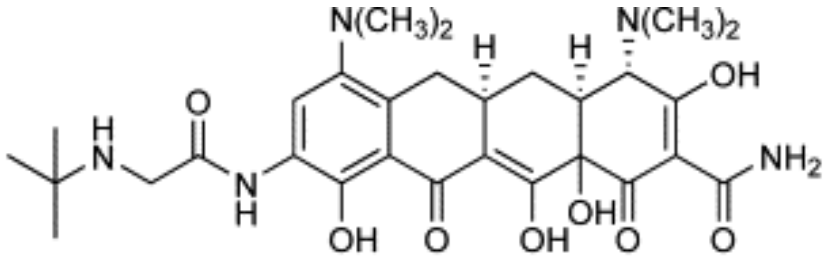
1. can cross placental barrier
2. can be excreted in breast milk and ingested by the fetus
3. deposition on bone tissues

Glycylcyclines

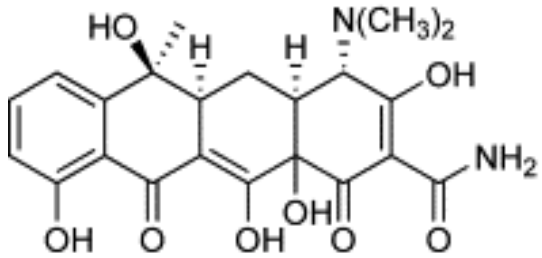
ex: Tigecycline

Tigecycline

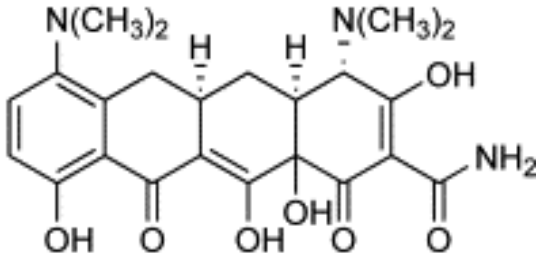
- Derivative of minocycline
- Same mechanism of action as tetracyclines
- Similar mechanisms of resistance



Tigecycline (**58**)



Tetracycline (**59**)



Minocycline (**60**)

Tigecycline

Antibacterial spectrum

- Effective against MRSA
- Effective against multi-drug resistant streptococci
- Effective against vancomycin-resistant enterococci (VRE)
- Effective against ESBL gram-negative bacteria
- Effective against Acinetobacter spp
- NOT effective against Pseudomonas

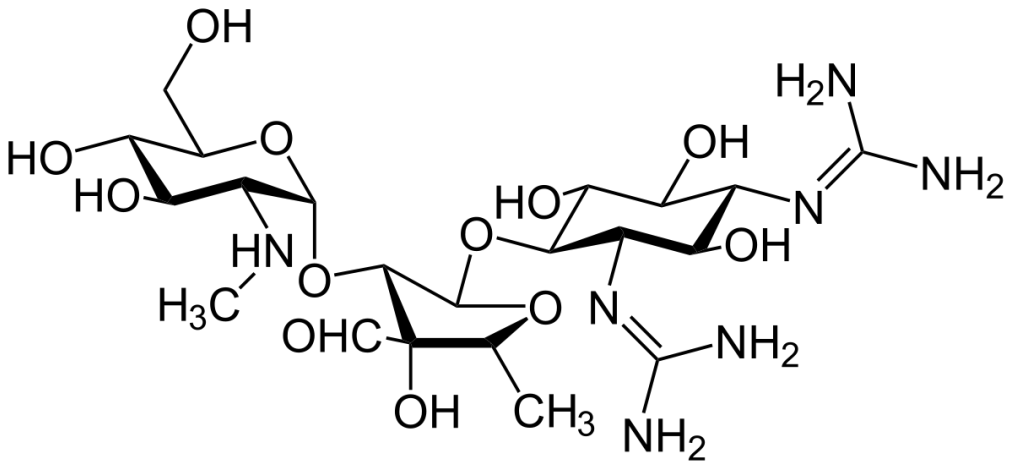
 the difference between tigecycline and tetracyclines is that tigecycline has a wider and more effective antibacterial spectrum.

 it doesn't have a beta lactam ring, so it's effective against extended spectrum beta-lactamase (ESBL) producing bacteria.

 Tigecycline is a powerful drug with very good antibacterial coverage. However, it can be associated with adverse effects. it can severe inflammation of the pancreas (pancreatitis, a life threatening condition). Tigecycline should always be kept as a last option to treat a resistant infection (not first line treatment).

Aminoglycosides

Aminoglycosides



AMINOGLYCOSIDES

Amikacin

Gentamicin GARAMYCIN

Neomycin NEO-FRADIN

Streptomycin

Tobramycin TOBREX

 Amikacin & **Gentamicin** are the mainly or commonly used drugs of this family.

 Neomycin is only limited to topical use for skin infections because it's very toxic if given systemically.

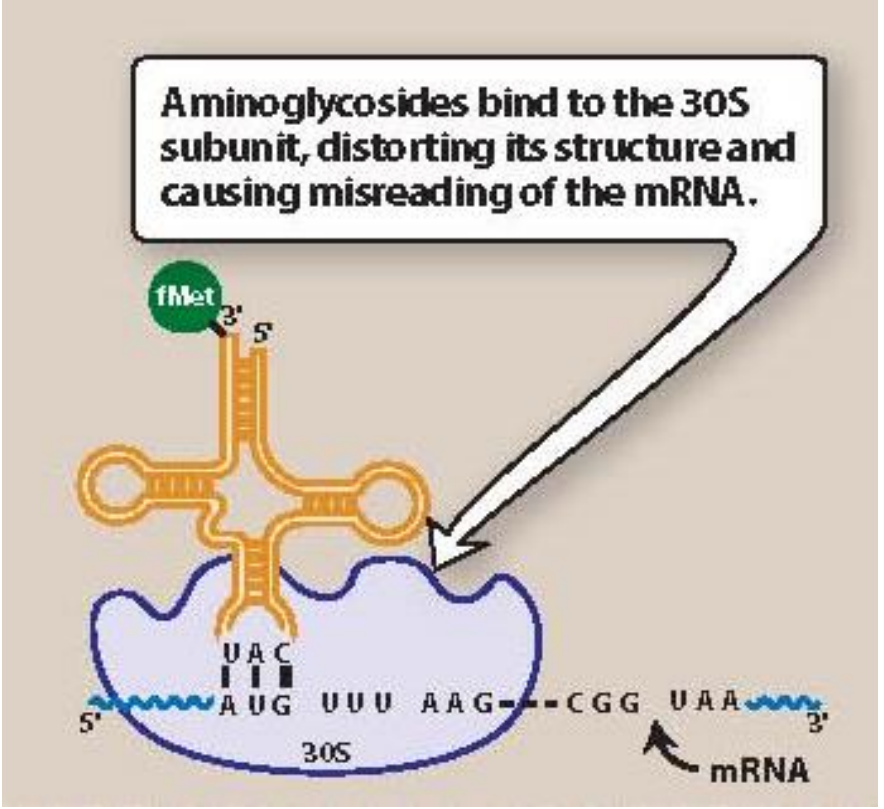
 Tobramycin is very important and frequently used for treatment of respiratory tract infections specially those caused by *pseudomonas aeruginosa*.

 Streptomycin is an antitubercular drug (used to treat tuberculosis), but not any more because it's avery toxic drug. its uses now are very limited.

Aminoglycosides

Mechanism of action

- Bind to 30S ribosomal subunit
- Interfere with assembly of the functional ribosomal apparatus
- Cause the 30S subunit of the completed ribosome to misread the genetic code



 aminoglycosides have the same target as tetracyclines, small ribosomal subunit. However, their binding site is different than that of tetracyclines which means the results is different.

 aminoglycosides prevent the assembly of the entire ribosomal apparatus. they prevent the binding of the small and large subunits.

 if the small subunit binds with mRNA, aminoglycosides can interfere with reading of the code and result in misreading of the codons on mRNA. (misread the genetic code)

Aminoglycosides

Antibacterial spectrum

- Bactericidal
- Concentration-dependent
- Exhibit PAE
- Effective against gram-negative bacilli (INCLUDING multi-DRUG resistant *P. aeruginosa*)
- Used in combination with β-lactams

- aminoglycosides are bactericidal drugs unlike tetracyclines.

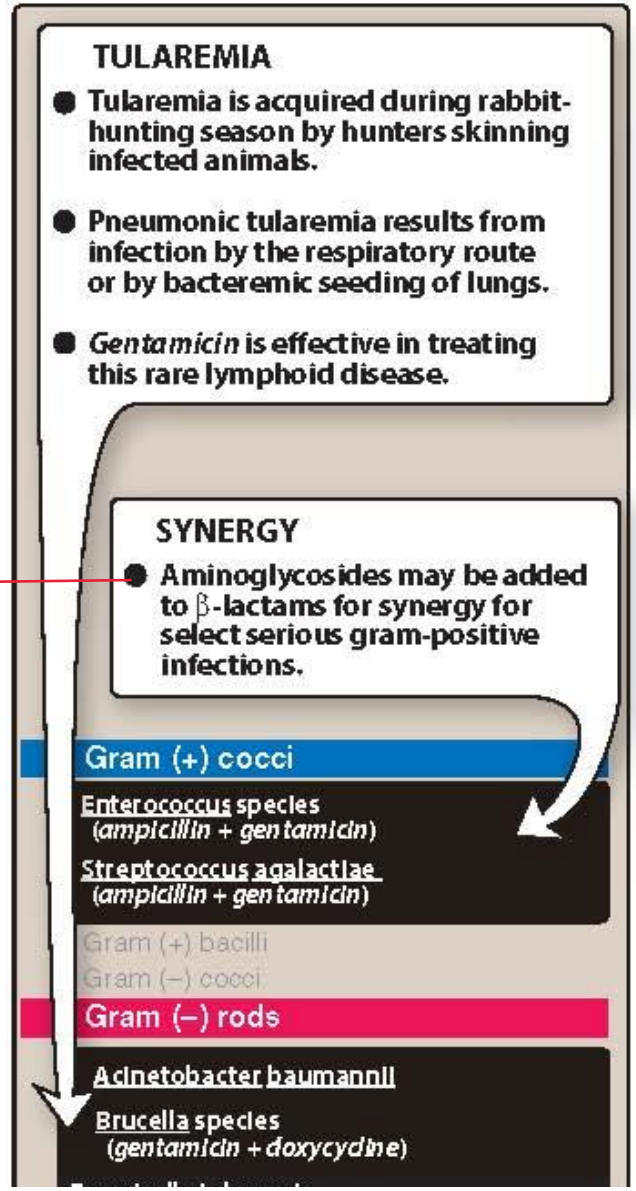
- they're concentration dependent. as further we increase the dose, we will get more bacteria cell killing.

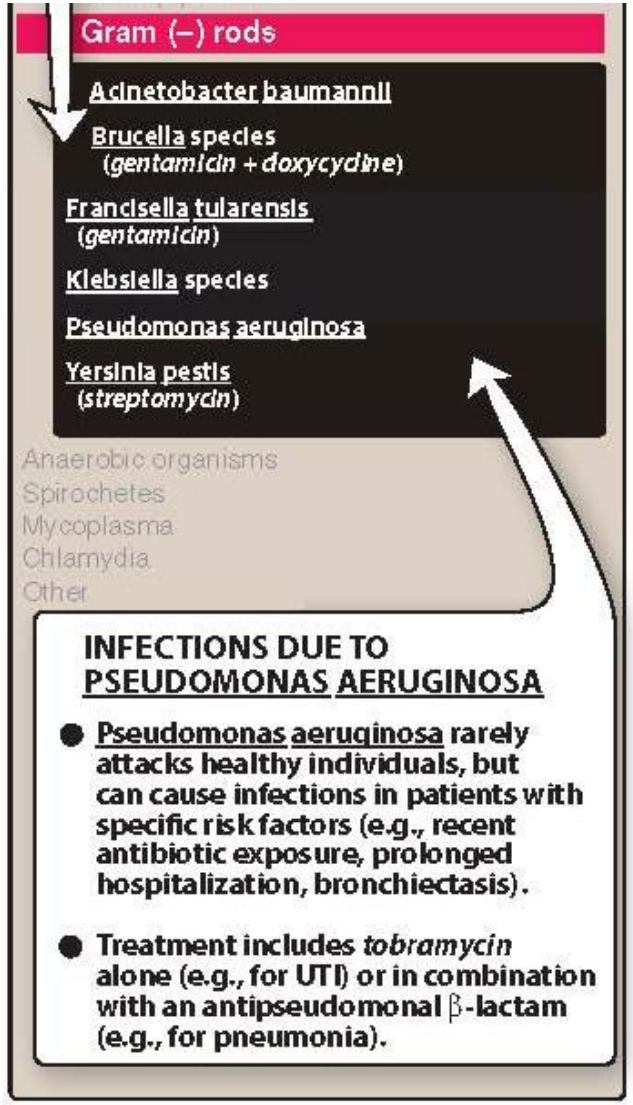
- they're a good example of PAE (postantibiotic effect). it's the ability of the antibiotic to exert an antibacterial effects even after its plasma concentration fall bellow the MIC.

- the main coverage of aminoglycosides is gram(-) bacilli. unlike tetracyclines and tigecycline, aminoglycosides (specially tobramycin) are effective against *P. aeruginosa*.

- one of the very famous combination in clinical medicine is the combination of **B-lactams** penicillins + **aminoglycosides** usually for treatment of gram (+) bacteria.

B-lactams + aminoglycosides have a synergistic effect.





Some clinical uses of aminoglycosides

Aminoglycosides

Mechanisms of resistance

1) efflux pumps
2) decreased uptake
3) modification and inactivation by plasmid-associated synthesis of enzymes that hydrolyze aminoglycosides

   -Amikacin is less vulnerable to these enzymes

- mechanism of resistance for aminoglycosides are:

1. **efflux pumps**: actively pump aminoglycosides outside the bacteria cell. so it will stop them from attaching to ribosomes.

2. **decrease uptake**: prevent the uptake of aminoglycosides through modifying the porins structure (decreased cell permeability).

3. releasing certain specialized **enzymes** through plasmid mediated resistance mechanism. these enzymes can inactivate (hydrolyze) the molecules of aminoglycosides.

Aminoglycosides

Pharmacokinetics

Absorption

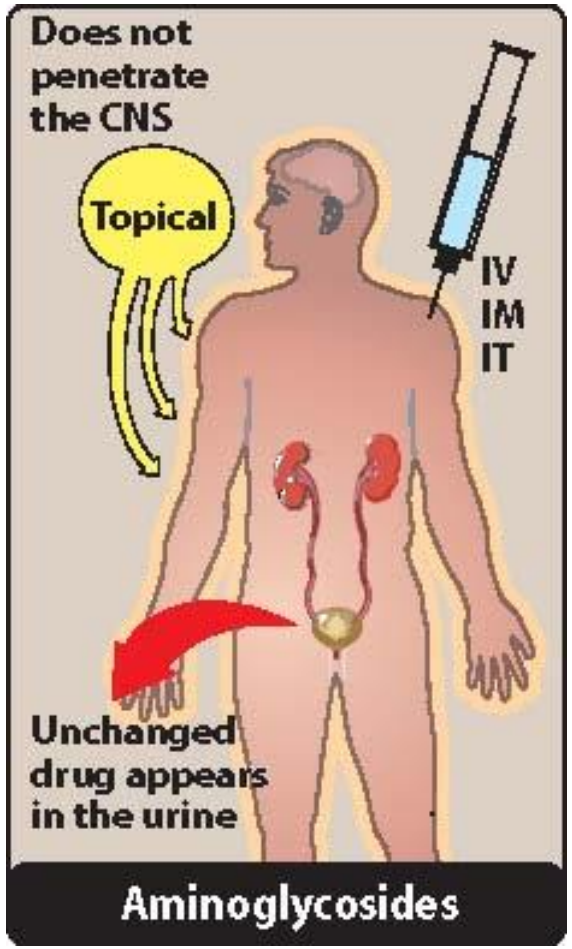
-all are given IV (except neomycin)

Distribution

-variable distribution in body fluids

-inadequate distribution in CSF

-cross the placenta



- The majority of aminoglycosides are given IV, IM or even IT (intrathecally, an injection into the spinal canal, or into the subarachnoid space).

- Exception: **Neomycin** is given topically because it cause nephrotoxicity if given Intravenously.

- they distribute very well in body fluids, but not as well as tetracyclines. they don't cross the CNS.

- they still can cross the placenta.

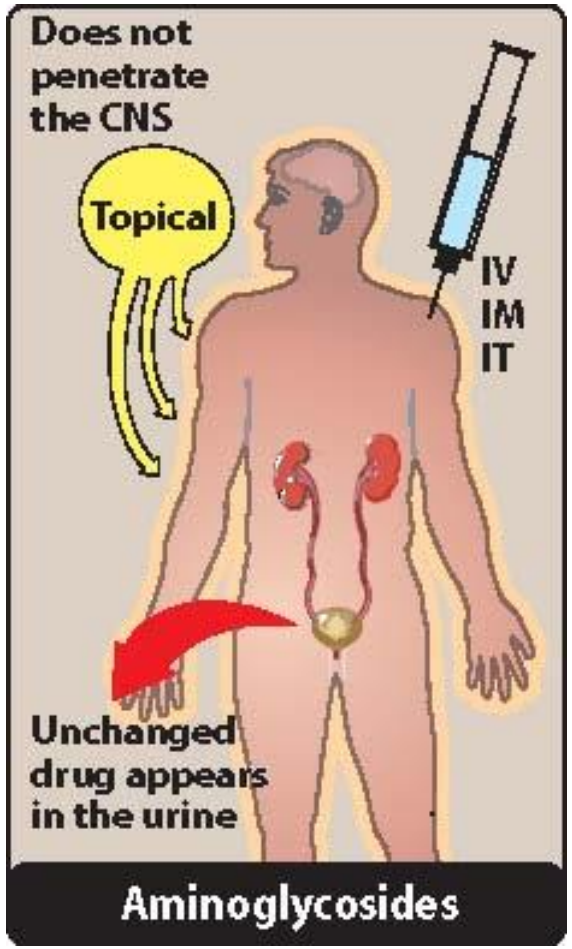
Aminoglycosides

Pharmacokinetics

Elimination

-90% are excreted unchanged in the urine

-accumulation occurs in cases of renal dysfunction



- the main method of elimination for aminoglycosides is through the kidney excretion.

- they are NOT heavily hepatic metabolized like some tetracyclines. they're relatively safe to be used in patients with hepatic dysfunction.

- we need to be careful with giving these drugs to patients with acute renal injury, renal dysfunction, etc. either give them an alternative drug or decrease the dose.

Aminoglycosides

Adverse effects

- **Ototoxicity (vestibular and auditory)**

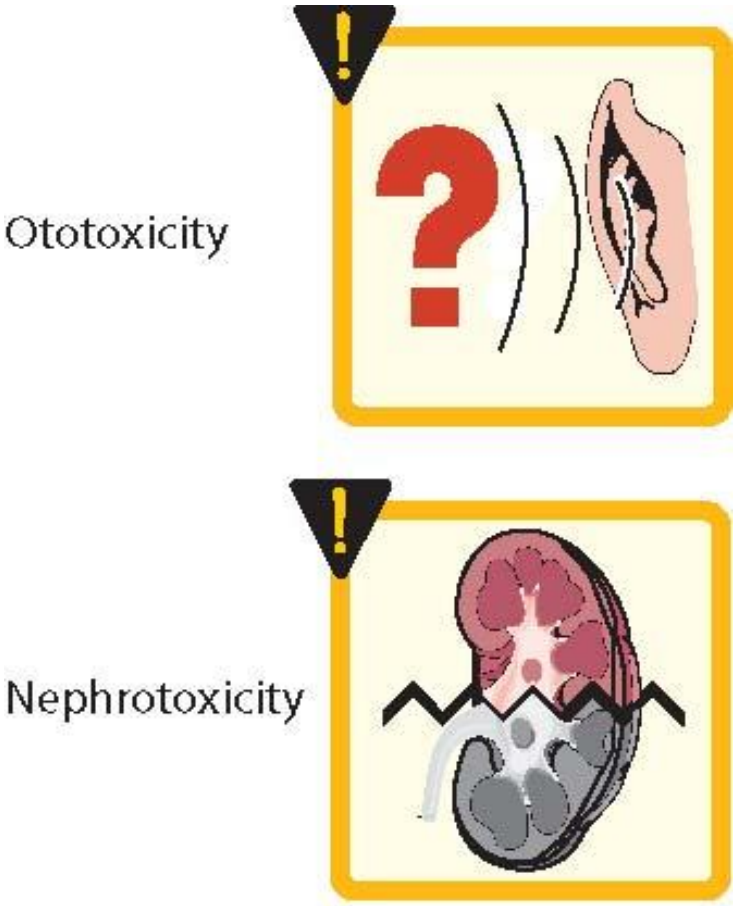
-might cause irreversible deafness

-Vertigo (especially with streptomycin)

- **Nephrotoxicity**

-disrupt Ca^{++}-mediated transport processes

-from mild reversible renal impairment to irreversible acute tubular necrosis



 Aminoglycosides are toxic compounds and not easy or safe to use. they're associated with different adverse effects.

1. **ototoxicity:** injury/ toxicity to the inner ear responsible for hearing and balance. if used at a very high concentration for a long period of time, it may cause permanent deafness. their effect on vestibule may cause vertigo (streptomycin is not used commonly because of its toxicity).

2. **nephrotoxicity:** kidney injury. it can be mild or very severe. these drug needs to be used with caution in patients with kidney disease or even avoid it.

Aminoglycosides

Adverse effects

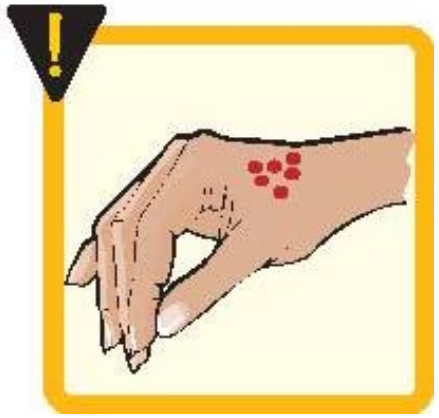
- <u>Neuromuscular paralysis</u>

-patient with myasthenia gravis are at risk

- <u>Allergic reaction</u>

- Mostly contact dermatitis with topical neomycin

Paralysis



Skin rash

3. **Neuromuscular paralysis:** specially if the patient has myasthenia gravis it may worsen the case (the disease is characterized by the formation auto-antibodies directed against acetylcholine receptors resulting in destruction of the communication between nerves and muscles).

4. **Alergic reaction:** specially if given topically (neomycin). may cause contact dermatitis or alergic reaction of the skin in response to the drug.

Why these protein synthesis inhibitors have many side effects?

high concentrations of drugs such as chloramphenicol or the tetracyclines may cause toxic effects as a result of interaction with mitochondrial mammalian ribosomes, because the structure of mitochondrial ribosomes more closely resembles bacterial ribosomes.