ANTIMICROBIALS INHIBITING PROTEIN SYNTHESIS

- AMINOGLYCOSIDES
- MACROLIDES
- TETRACYCLINES AND CHLORAMPHENICOL
Protein synthesis
Diagram of protein synthesis involving ribosomes, tRNAs, and mRNA. The process includes the following steps:

1. Amino acid attachment to charged tRNA.
2. tRNA binding to the ribosome.
3. Transfer RNA selection by the ribosome.
4. Peptide bond formation.
5. tRNA movement within the ribosome.
6. Continued cyclic process.

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Aminoglycosides
1. Aminoglycosides are group of natural and semi-synthetic antibiotics. They have polybasic amino groups linked glycosidically to two or more aminosugar like: sterptidine, 2-deoxy streptamine, glucosamine

2. Aminoglycosides which are derived from: Streptomyces genus are named with the suffix –mycin. While those which are derived from Micromonospora are named with the suffix –micin.
Classification of Aminoglycosides

1. Systemic aminoglycosides
   - Streptomycin (*Streptomyces griseus*)
   - Gentamicin (*Micromonospora purpurea*)
   - Kanamycin (*S. kanamyceticus*)
   - Tobramycin (*S. tenebrarius*)
   - Amikacin (Semisynthetic derivative of Kanamycin)
   - Sisomicin (*Micromonospora inyoensis*)
   - Netilmicin (Semisynthetic derivative of Sisomicin)

2. Topical aminoglycosides
   - Neomycin (*S. fradiae*)
   - Framycetin (*S. lavendulae*)
Pharmacology of Streptomycin
Biological Source
It is a oldest aminoglycoside antibiotic obtained from Streptomyces griseus.

Antibacterial spectrum
1. It is mostly active against gram negative bacteria like *H. ducreyi*, *Brucella*, *Yersinia pestis*, *Francisella tularensis*, *Nocardia*, etc.
2. It is also used against *M. tuberculosis*
3. Few strains of *E.coli*, *V. cholerae*, *H. influenzae*, *Enterococci* etc. are sensitive at higher concentration.
Mechanism of action

Aminoglycosides bind to the 16S rRNA of the 30S subunit and inhibit protein synthesis.

1. **Transport of aminoglycoside through cell wall and cytoplasmic membrane.**
   a) Diffuse across cell wall of gram negative bacteria by porin channels.
   b) Transport across cell membrane by carrier mediated process liked with electron transport chain

2. **Binding to ribosome resulting in inhibition of protein synthesis**
Mature protein

Growing polypeptide

Direction of mRNA translation

aminoglycoside = ●

A

5' AUG 3'

Blocks initiation of protein synthesis

B

5' AUG 3'

Blocks further translation and elicits premature termination

C

5' AUG AUG 3'

Incorporation of incorrect amino acid
A. Aminoglycoside (represented by red circles) binds to the 30S ribosomal subunit and interferes with initiation of protein synthesis by fixing the 30S–50S ribosomal complex at the start codon (AUG) of mRNA. As 30S–50S complexes downstream complete translation of mRNA and detach, the abnormal initiation complexes, so-called streptomycin monosomes, accumulate, blocking further translation of the message. Aminoglycoside binding to the 30S subunit also causes misreading of mRNA, leading to

B. premature termination of translation with detachment of the ribosomal complex and incompletely synthesized protein or

C. incorporation of incorrect amino acids (indicated by the red X), resulting in the production of abnormal or nonfunctional proteins.
The Davis model for bactericidal activity of aminoglycosides.
Mechanism of resistance

1. Acquisition of cell membrane bound inactivating enzymes which phosphorylate/adenylate/acetylate the antibiotic
2. Decrease in affinity of antibiotic for ribosome due to mutation.
3. Decrease efficiency of aminoglycoside transporting mechanism.

Cross resistance: Only partial and unidirectional between streptomycin and other aminoglycosides
Pharmacokinetics

Absorption: Streptomycin is highly ionized. It is neither absorbed nor destroyed in GIT. However, absorption from injection site is rapid.

Distribution: Distributed on extracellularly. Vd is 0.3L/kg. Low concentration in synovial, pleural, peritoneal, serous fluids. Plasma $t_{1/2}$ is 2-4 hr.

Metabolism: It is not metabolized.

Excretion: It is excreted unchanged in urine by glomerular filtration.
Adverse effects
A) Toxicity

*Ototoxicity*

a) Cochlear damage
b) Vistibular damage

*Nephrotoxicity*

*Neuromuscular Blockade*

B) Skin reaction
Uses
Aerobic gram negative bacteria, *H influenzae, M catarrhalis, and Shigella* species; often used in combinations with beta-lactams.

Gonorrhea (spectinomycin, IM); tuberculosis (streptomycin, IM).

Brands
AMBISTRYN-S 0.75-1g powder per vial for inj.
TETRACYCLINES AND CHLORAMPHENICOL
TETRACYCLINES

History

i) The first member of the family was chlortetracycline derived from the soil organism *Streptomyces aureofaciens*. It was introduced by Benjamin M. Duggar in 1948.

ii) This was followed by oxytetracycline produced from *Streptomyces rimosus*.

iii) In 1952 by removing the chlorine atom from chlortetracycline, tetracycline was produced.

iv) Investigations on the mutant strains of *Streptomyces aureofaciens* in 1957 led to discovery of demethyltetracycline like, first member demeclocycline.
Chemistry

i) They are so named for their four ("tetra-") hydrocarbon rings ("-cycl-") derivation ("-ine").

ii) Groups R₁ - R₄ are the only functions which may be varied without affecting a substantial decrease in antimicrobial activity.

iii) Functional groups at positions 5, 6, and 7 may be removed without drastically altering the antimicrobial properties. The right configuration at C-5a and C-4 is essential for activity.

iv) Equilibration involving C-4 leads to the relatively inactive 4-epi-tetracyclines.

v) The hypothesis has been advanced that the principal active center is the C(11), C(12) diketone system of rings B and C.
Pharmacology of tetracycline

Drugs used for clinical use

i) Tetracycline
ii) Oxytetracycline
iii) Demeclocycline
iv) Doxycycline
v) Minocycline
MECHANISM OF ACTION
i) Tetracyclines enter Gram-negative bacteria by passive diffusion through porin proteins in the outer membrane, followed by active (energy-dependent) transport across the inner cytoplasmic membrane. Uptake into Gram-positive bacteria, such as *Bacillus anthracis* (the causative agent of anthrax), occurs similarly via an energy-dependent transport system. In contrast, mammalian cells lack the active transport system found in susceptible bacteria.

ii) Tetracyclines bind reversibly to the 16S rRNA of the 30S subunit and inhibit protein synthesis by blocking the binding of aminoacyl tRNA to the A site on the mRNA-ribosome complex. This action prevents the addition of further amino acids to the nascent peptide.
Antibacterial spectrum

i) Tetracyclines have broad spectrum of activity covers both gram positive and gram negative bacteria, Rickettsia, Traponema pallidum, mycoplasmas, and Chlamydiae, etc. They are primarily bacteriostatic.

ii) Gram positive bacilli e.g. Clostridia, Listeria, Corynebacteria, B. anthracis are inhibited.

iii) Gram negative bacterias like H.ducreyi, Calmmatobacterium granulomatis V.cholerae. are sensitive to tetracyclines
Antibacterial resistance

i) Resistance develops in graded manner.

ii) Bacteria acquire capacity to pump out the tetracycline out of the cell

iii) Acquire a plasmid mediated synthesis of a protection protein which protects the ribosomal binding site from tetracycline.

iv) Nearly complete cross resistance is seen among different members of tetracycline.
Pharmacokinetics

i) Absorption: They are usually given orally, old teracyclines have incomplete absorption due to low solubility and binding to Ca\(^{++}\), Al\(^{++}\), Fe\(^{++}\) and Mg\(^{++}\) in foods or drugs. Newer agents like Doxycycline and Monocycline are completely absorbed irrespective of food.

ii) Distribution: They widely distributed in the body (volume of distribution (>1L/Kg). Concentrated in liver, kidney, spleen, connective tissues in bone. Protein binding is variable. The CSF concentration is about \(\frac{1}{4}\)th of plasma concentration.

iii) Metabolism: They are partly metabolized with some degree of enterohepatic circulation.

iv) Excretion: They are primarily excreted by glomerular filtration so, has to be reduced in glomerular failure.
Adverse effects

i) Irritative effects: they can cause epigastric pain, nausea, vomiting and diarrhea by irritant property. Intramuscular injection is very painful.

ii) Liver damage: Fatty infiltration of liver and jaundice occurs occasionally.

iii) Kidney damage: Prominent only in presence of existing kidney disease Fancony syndrome produced by outdated tetracycline due to degraded products like epiteracycline, anhydroteracycline and epianhydroteracycline

iv) Phototoxicity: Higher incidence are seen with demeclocycline and doxycycline.

v) Teeth and bones: Calcium tetracycline chelate gets deposited in developing teeth and bone. If given in mid-pregnancy to 5 months of extra-uterine life leads to brown discoloration, ill-formed teeth more susceptible to caries. Given in late pregnancy lead or childhood can cause temporary suppression of bone growth.

vi) Antianabolic effect: Reduced protein synthesis so have catabolic effects They induce negative nitrogen balance and can increase blood urea.

vii) Diabetes insipidus: Demeclocycline antagonizes ADH action and reduce urine concentration.

viii) Vestibular toxicity: Minocycline has produced ataxia, vertigo and nystagmus.
Precautions
i) Not to be used in pregnancy, lactation, and in children.
ii) Avoided in patients on diuretics.
iii) Use cautiously in renal and hepatic insufficiency.
iv) Never use expired preparations
v) Do not mix with penicillin inactivation occurs.
iv) Do not inject intrathecally.

Uses
i) Empirical therapy
ii) First choice drugs in: Venereal diseases, Atypical pneumonia, Cholera, Brucellosis, Plague, Relapsing fever etc.
iii) Second choice drugs in: Tetanus, anthrax, lasteria, patients allergic to penicillin etc.
iv) Urinary tract infections, Amoebiasis, acne vulgaris, prophylactic use in COPD
Marketed Preparations
i) Doxycycline: TERADOX, BIODOXI, DOXT, 100mg cap
ii) Tetracycline: ACHROMYCYIN, HOSTACYCLINE 250, 500mg cap
iii) Minocycline: CYANOMYCYIN 50, 100 mg caps
CHLORAMPHENICOL
History
Cholramphenicol was isolated from *Streptomyces venezuelae* in 1947. It is now synthesized chemically and is commercially available as the synthetic product.

Chemistry
i) Replacement of the -NO$_2$ group with a number of other substituents including CN, CONH$_2$, NH$_2$, OH results in loss of anti-bacterial activity.

ii) The phenyl group has been replaced with other aromatic or alicyclic groups such as naphthyl, pyridyl, thienyl, cyclohexyl etc. Only the nitro thienyl compound has antibacterial activity and it was less potent.

iii) Only the d-threo compounds of the 4 stereo isomers is anti-bacterial activity. The primary alcohol group seems to be imperative. Alteration of any kind leads to loss of activity.

iv) The propanediol side chain too is non alterable.

v) Replacement of the di-chloro acetyl group with other halogens such as bromine resulted in loss of potency.
Mechanism of action

i) It inhibits protein synthesis by interfering with transfer of the elongating peptide chain to the newly attached aminoacyl-tRNA at the ribosome-mRNA complex.

ii) It attaches specifically to 50s ribosome and thus may hinder the access of aminoacyl-tRNA to acceptor site for amino acid incorporation.
Antibacterial spectrum

i) It is primarily bacteriostatic, and bacteriocidal at high concentration.

ii) It is a broad spectrum antibiotic

iii) Highly active against Salmonella including S. typhi.

iv) More active than tetracyclines against H. influenzae, B. pertussis, Klebsiella

v) Less active against gram positive bacteria

Resistance

i) Resistance in graded manner

ii) Resistance mainly due to acquisition of R plasmid encoded for acetyl transferase enzyme which inactivate chloramphenicol.

iii) Decreased permeability and into the resistant bacteria cells.

iv) Lower affinity of bacterial ribosome for chloramphenicol
Pharmacokinetics

Absorption: Rapidly and completely absorbed from GIT.

Distribution: It is 50-60% bound to plasma protein and very widely distributed. Volume of distribution is 1L/kg. Pass freely in serous cavities and BBB. Crosses placenta and is secreted in bile and milk.

Metabolism: Primarily conjugated with glucuronic acid in liver. Plasma $t_{1/2}$ is 3-5 hrs.

Excretion: Metabolites excreted mainly in urine.

Adverse effects
i) Bone marrow depression
ii) Hypersensitivity reactions
iii) Irritative effects
iv) Superinfections
v) Gray baby syndrome
Interactions

i) Chloramphenicol inhibits metabolism of tolbutamide, chlorpropamide, warfarin, cyclophosphamide and phenytoin

ii) Phenobarbitone, phenytoin, rifampin enhances chloramphenicol metabolism

iii) It can antagonize the cidal action of β-lacta/aminoglycosides.

Precautions

i) Never use for minor infections and unidentified etiology

ii) Do not use for infections treatable by safer antibiotics

iii) Avoid repeated courses.

iv) Daily dose not exceed 2-3g
Uses
i) Enteric fever
ii) Pyogenic meningitis
iii) Anaerobic infections
iv) Introcular infections
v) Topically for conjunctivitis

Marketed Preparation
i) CHLOROMYCETIN, PARAXIN, 200mg, 500mg
ii) VANMYCETIN 0.4% eyes drops
• MACROLIDES, AMINOGLYCOSIDES,
• POLYENE AND POLYPEPTIDE ANTIBIOTICS
MACROLIDES

History
i) It was isolated from *Streptomyces erythreus* in 1952.
ii) Erythromycin is the first member discovered in the 1950s.

Chemistry
i) Because erythromycin is basic in nature, they can be inactivated in acids due to formation of a spiral ketal. Specifically, the 6-hydroxyl, 9-ketone, and possible the 12-hydroxyl are involved in the acid instability.

ii) The only chemical modification that improves stability, potency, and oral activity is removal of the 9-ketone group. Other modifications and their effect on activity have not been studied considerably.
Antibacterial Spectrum

i) Antibacterial spectrum between penicillin G and tetracycline.

ii) Narrow spectrum of activity mostly gram positive and few gram negative.

iii) Highly active against *Str. Pyrogens* and *Str. Pneumonia*, *N. gonorrhea*, *clostridia*, *C.diphtheriea*, *Lasteria*, *H. influenza*, *H.ducreyi*, mycoplasma not affected by penicillin.

iv) Enterobacteriaceae, other gram negative bacilli and B. fragilis are not inhibited
• Mechanism of action
i) **Act by inhibiting bacterial protein synthesis.** It combines with 50S ribosomal subunit and interferes with translocation process.

ii) After peptide bond formation between newly attached amino acid and nascent peptide chain at the acceptor (A) site, the elongated peptide is translocated back to the peptidyl (P) site, making the site A available for next aminoacyl tRNA attachment. This is prevented by erythromycin and ribosome fail to move along the mRNA to expose the next codone.

iii) As a result peptide chain may be prematurely terminated.
Resistance

i) All cocci readily develop resistance to by mechanism which render them less permeable to erythromycin

ii) Resistant Enterobacteriaceae found to produce to esterase.

iii) Alteration in the ribosomal binding site for erythromycin by plasmid encoded methylase enzyme.

iv) Cross resistance occurs between the group and also between Clindamycin and Chloramphenicol.
Pharmacokinetics

Absorption: It is acid liable. Given as enteric coated tablets which absorption. It is incomplete and food delays absorption. Its acid esters are better absorbed.

Distribution: Widely distributed in body, enters cells and abscesses, cross serous membrane, and placenta but not BBB. Attains therapeutic concentration in Prostate. 70-80% plasma protein bound, plasma $t_{1/2}$ is 1.5hr.

Metabolism: Metabolized and excreted in bile in active form.

Excretion: Mainly through renal excretion.
Adverse effects

i) Gastrointestinal: Epigastric pain and diarrhoea

ii) Hypersensitivity: Rashes, fever, etc

Drug Interactions

1. Rise of plasma levels of theophylline, carbamazepine, valproate, ergotamine and warfarin.

2. Several cases of QT prolongation serious ventricular arrhythmias and death reported due inhibition of CYP3A4. Increase blood levels of terfenadine/astemizole/cisapride.

Uses

1. As an alternative to penicillin

2. As first choice of drug for M. pneumoniae, Whooping cough, Chancroid.

Brand name: ROXID, ROXIBID, RULIDE 150, 300mg tab.