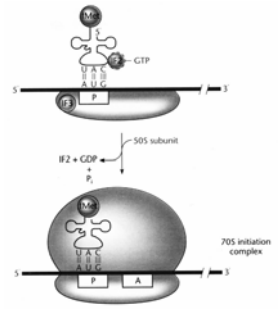
Protein Synthesis Inhibitors

- Macrolides - Lincosamides
- Aminoglycosides
- Tetracyclines
- Chloramphenicol
- Oxazolidinones
- Streptogramins

Lecture Outline

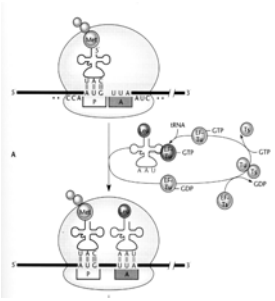
- Description of protein synthesis
- Antibiotics
  - Structure - function - classification
  - Mechanism(s) of action
  - Mechanism(s) of resistance
  - Spectrum of activity/Indications for use
  - Pharmacology
  - Toxicity
- Clinical examples

Overview of Translation (1)



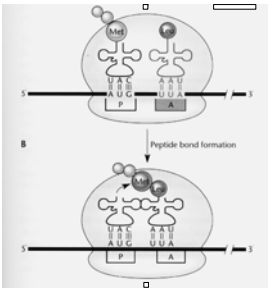
Initiation:
30S binds RBS of mRNA
AA binds tRNA using aminoacyl-tRNA synthetase
IF2 and fmet-tRNA binds 30S at P site
50S binds complex → 70S

Overview of Translation (2)



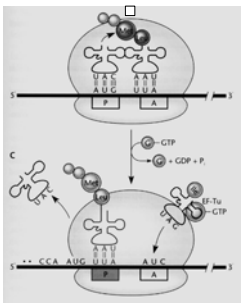
Initiation
- tRNA + AA binds translation elongation factor
- Enters ribosome and attaches at the A site

Overview of Translation (3)



Amino Acid Transfer
- Petidyltransferase on 50S ribosome attaches the next AA to the polypeptide
- Met added to Leu at A site

Overview of Translation (4)



Elongation

tRNA moved to P site by EF-G creating room at A site for next tRNA

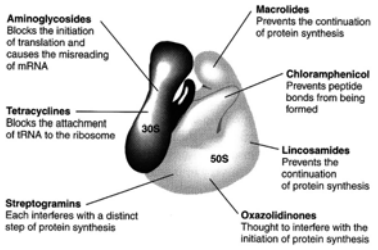
Translation termination

Occurs at nonsense codon sites *e.g.* UAA

Release factors

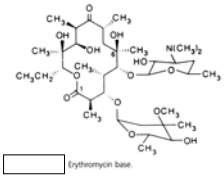
Ribosome dissociates

Mechanisms of Action - Protein Synthesis Inhibitors



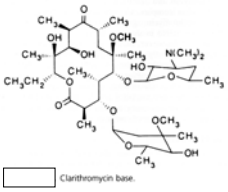
Macrolides

- Broad spectrum antibiotics
- Original agent: erythromycin
- Azalides: azithromycin and clarithromycin
  - selected antimicrobial and pharmacokinetic advantages



Large 14 member macrolactone ring with one or more deoxy sugars attached.

Inhibits formation of 50S ribosome blocking trans-peptidation or translocation.



Large 14 member lactone ring with modification of C6 to a methoxy group.

Azithromycin has a 15 membered lactone ring

Macrolides - Mechanisms of Resistance

- Mechanisms differ for different bacterial species
- Decreased permeability of envelope (*e.g.*, the enterobacteriaceae)
- Mutation of the 23S ribosomal RNA of the 50S ribosomal subunit (alters binding site)
  - Can be chromosomal, plasmid or on a transposon
  - Can confer resistance to macrolides, lincomycins and streptogramins (mls)
- Active efflux of antibiotic (plasmid-mediated) - mostly with Gram positive bacteria

Macrolides - Spectrum of Activity

- Erythromycin:
  - Gram positives: pneumococci, viridans streptococci, Group A streptococci, methicillin sensitive staphylococci
  - Gram negatives: bordetella, neisseria, campylobacter, ± hemophilus
  - Miscellaneous: mycoplasma, legionella, chlamydia, treponemes

Azalides - Spectrum of Activity

- Spectrum similar to erythromycin
- Increased activity against hemophilus, *Mycobacterium avium intracellulare*, toxoplasma
- Azithromycin > Gram negative activity
- Clarithromycin > Gram positive activity

Macrolides - Pharmacology

- Can be administered orally or parenterally
  - Well absorbed - especially azalides
- t1/2 erythromycin - 1.4h
- Azalides have long t1/2
  - Clarithromycin 3-7h
  - Azithromycin 2-4 days
- Well distributed, CNS penetration limited except with inflammation

Macrolides - Pharmacology

- High concentrations in alveolar cells and polymorphonuclear leukocytes, especially azalides
- Most of drug is concentrated in the liver and excreted in the bile. Some is inactivated in the liver by demethylation.

Macrolides - Indications for Use

- Community acquired pneumonia: mycoplasma, legionella, chlamydia
- Pertussis
- *Campylobacter jejuni* gastroenteritis
- MAC (azalides)
- Alternative agents for: group A,C,G streptococcal infections, rheumatic fever prophylaxis, *C. trachomatis* urethritis, anthrax

Macrolides - Indications for Use

- Novel indication for use
  - Potential antibacterial (vs. anti-inflammatory) effects in the treatment of *P. aeruginosa* infections in Cystic Fibrosis

Macrolides - Toxicity

- Generally well tolerated
- Gastrointestinal symptoms - cramps, diarrhea secondary to motility stimulating effects of antibiotic. Motilin receptor agonist
- Cholestatic hepatitis (rare)
- Drug interactions - erythromycin > clarithromycin interferes with the cytochrome P450 enzymes leading to increased levels of other drugs *e.g.* dilantin, warfarin, cyclosporine

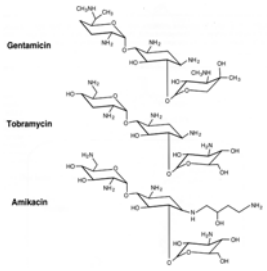
Clindamycin (Lincosamide)

- MOA similar to macrolides
- Bacteristatic activity against Gram positive bacteria and anaerobes - also toxoplasma
- Pharmacology - high bone concentrations
- Toxicity - diarrhea, allergy
- Indications - penicillin-resistant anaerobic infections

Aminoglycosides

- Complex sugars with glycosidic linkages
- Bactericidal antibiotics with activity primarily directed against aerobic Gram negative bacteria
- Narrow therapeutic window with significant toxicity
- Primarily used as a second therapeutic agent in the treatment of serious Gram negative or enterococcal infections

Aminoglycosides



Polycationic molecule with at least 2 aminosugars linked by glycosidic bonds to an amino-cyclitol ring

Removal of amino or hydroxyl groups correlates with loss of antibacterial activity and toxicity

Water soluble - limited ability to cross lipid membranes

Aminoglycosides - Mechanism of Action

- Diffuses through porin channels in outer membrane of Gram negative bacteria
- Binds to and alters bacterial cell membrane causing leakage of the outer Gram negative membrane and disruption of the cell wall
- *Interferes with mRNA translational accuracy primarily at the 30S ribosome causing misreading and premature chain termination
- Bactericidal activity appears to be multifactorial

Aminoglycosides - Mechanism of Resistance

- *Enzymatic modification of the aminoglycoside by adenylation, phosphorylation or acetylation
  - Usually found on plasmids or transposons
- Anaerobes are resistant because they lack an O_2 dependent transport system
- Chromosomal mutations can also cause alterations in binding and uptake e.g. *S. aureus*

Antibacterial Spectrum

- Aerobic gram negative bacilli
- *Pseudomonas aeruginosa*
- Gram positive bacteria (used for synergy) *Staphylococcus spp., Enterococcal spp.*
- Selected aminoglycosides have activity against: *Mycobacteria spp., Yersinia pestis*
- No activity against: hemophilus, anaerobes, pneumococcus, neisseria

Aminoglycosides - Indications for Use

- Empiric therapy: life-threatening infections that require broad spectrum coverage
- Specific therapy: synergistic antimicrobial activity
  - Enterococcal endocarditis
  - Pseudomonas infections
- Monotherapy: rarely used, inhalational therapy for CF patients with pseudomonal pneumonia

Aminoglycosides - Pharmacology

- Minimal absorption after oral administration
- Limited tissue distribution due to polarity
- Not metabolized, excreted by the kidney
- Rapid absorption after IM administration

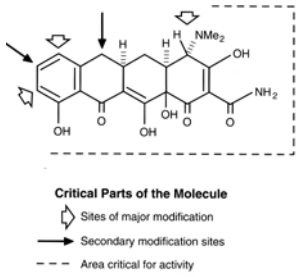
Aminoglycosides - Toxicity

- Nephrotoxicity: incidence 5-25%
  - Damages proximal tubular cells,
- Ototoxicity: Cochlear 3-14%, Vestibular 4-6%
  - Long otic fluid t1/2
  - Cochlear damage to the outer hair cells of the organ of Corti
  - Vestibular damage to type 1 hair cell of the summit of the ampullar cristae

Tetracyclines

- Broad spectrum bacteristatic agents
- Grouped based on differences in t1/2 - short, intermediate and long-acting
- Not used for treatment of staphylococcal or Gram negative bacterial infections because of the rapid emergence of resistance

Tetracyclines



Basic structure consists of 4 fused 6 carbon rings - hydronaphthacene nucleus with modifications at selected positions

Binds to the 30S portion of ribosome, prevents access of aminoacyl tRNA molecules to the mRNA ribosome-peptide complex

Tetracyclines - Mechanism of Resistance

- Common in both Gram positive and negative bacteria
- Generally, but not exclusively, plasmid-mediated
- *Decreased uptake and increased excretion of the drug (pump)
- Resistance is conferred to all tetracyclines
- Has been associated with the extensive use of tetracyclines in animal food

Tetracyclines - Antimicrobial Spectrum

- Gram positives: *S. pneumoniae, S. pyogenes, S. agalactiae,* enterococci
- Gram negatives: *E. coli, Neisseria spp., Hemophilus spp., Shigella spp.*
- Miscellaneous: Spirochetes - Borrelia, Rickettsiae, Chlamydiae, Mycoplasma, Legionella, Pasteurella, Ehrlichia (Anaplasma)
- Tigecycline: new broad spectrum glycylcycline with activity against resistant gram positives and negatives

Tetracyclines - Indications for Use

- Treatment of chlamydia, mycoplasma, brucella, vibrio, helicobacter, rickettsia, borrelia, ehrlichia (anaplasma) infections
- *Mycobacterium marinum* infections
- Acne
- Rarely the first drug of choice

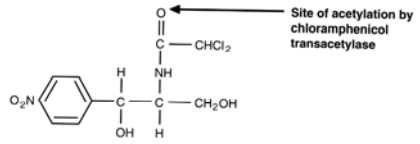
Tetracyclines - Pharmacology

- Primarily oral agents
- Cations Ca^{2+}, Mg^{2+} interfere with absorption by chelating tetracyclines, *e.g.*, dairy products
- t1/2 varies with agent as does extent of excretion by the kidney.
  - Doxycycline excreted in the feces
  - Minocycline metabolized in the liver
- In general excellent tissue distribution
- Concentrated in the bile, achieves levels of 10-26% of serum in CSF

Tetracyclines - Toxicity

- Gastrointestinal symptoms: common
- Photosensitivity
- Discoloration of teeth due to binding to calcium - not reversible
- Hypersensitivity reactions: rash, urticaria, anaphylaxis (rare)
- Hepatotoxicity - especially during pregnancy

Chloramphenicol



Binds to peptidyl transferase, component of 50S ribosomal subunit. Resistance usually due to presence of above enzyme

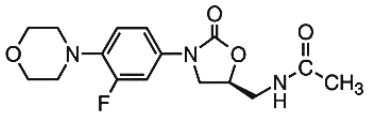
Chloramphenicol

- Broad spectrum, mostly bacteristatic, covers aerobic Gram positives and most Gram negatives, most anaerobes and rickettsia
  - May be bactericidal against pneumococcus, neisseria
- Higher levels achieved following oral rather than intravenous administration
- Well distributed throughout the body
- Metabolized to inactive metabolite in the liver

Chloramphenicol

- CSF levels 30-50% of serum without inflammation
- Rarely used because of concern about toxicity - bone marrow aplasia, Gray baby syndrome
- Indications include: not the drug of choice for any infection. Used to treat typhoid fever (*S. typhi*), meningitis in penicillin allergic, and rickettsial infections
- Has been used as a marker of bad medical practice!

Linezolid - Oxazolidinone



Binds the 50S ribosomal subunit and inhibits formation of the initiation complex. Does not (at present) exhibit cross resistance with other protein synthesis inhibitors

Linezolid - Oxazolidinone

- Bacteristatic synthetic antibiotic with activity against Gram positive cocci - even those resistant to other agents
- Administered parenterally or orally
- Excellent distribution, metabolized by liver, excreted in urine
- Resistance by mutation of 23s RNA (50S subunit)
- Indication: primarily for treatment of drug-resistant enterococcal or staphylococcal infections

Streptogramins

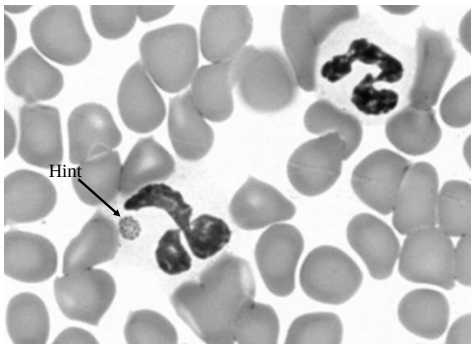
- Bactericidal (often) combination derived from pristinamycin: quinupristin (30%) and dalfopristin (70%)
- Both interfere with 50S ribosomal subunit: Quinupristin inhibits peptide chain elongation and dalfopristin interferes with peptidyl transferase
- Resistance primarily occurs by methylation of MLS binding site (plasmid-mediated) also drug modification or efflux (less common)

Streptogramins

- Limited spectrum of activity: Gram positive cocci - staphylococci, streptococci, enterococci (only *E. faecium* not *E. faecalis*)
- Only parenteral, well distributed, metabolized in the liver to inactive metabolites
- Used to treat vancomycin or methicillin-resistant infections Gram positive infections
- Toxicity: phlebitis, myalgias, arthralgias

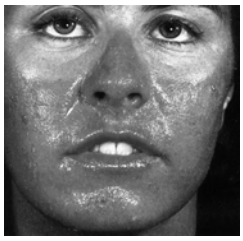
Clinical Scenario 1

A 25 year old female returns from a camping trip in Maine in late July. She develops fever, malaise and myalgias. Other than her obvious discomfort her physical examination is unrevealing, however her laboratory examination is of note. She is both thrombocytopenic and leukopenic. Her peripheral smear is shown on the next slide.



Clinical Scenario 1 (cont'd)

She is placed on the therapy you select and much to your chagrin she develops the following reaction while on therapy



Clinical Scenario 2

You have decided to take an elective in tropical medicine and arrive in sub-Saharan Africa only to learn that this is the meningitis belt. There is an enormously high incidence of meningococcal meningitis in this area. Given all the limitations of therapy in this economically impoverished area which antimicrobial agent might be a reasonable choice to treat the children with meningitis in this region?

Clinical Scenario 3

A 78 year old man develops a urinary tract infection caused by a vancomycin-resistant enterococcus. He refuses hospitalization noting that his granddaughter is getting married this coming Weekend. What to do?

Clinical Scenario 4

A 19-year old college student with a prosthetic mitral valve secondary to rheumatic fever as a child needs prophylaxis against Group A streptococcus. He has a documented history of an anaphylactic allergic reaction to penicillin. What should be used as an alternate?

Hint - he is warned to reduce his dose of coumadin.

Clinical Scenario 5

A 70-year-old man with a history of refractory leukemia is re-hospitalized with a pseudomonas pneumonia. He develops renal failure in his second week of combination antibiotic therapy. Which antibiotic is the likely cause of this complication

What I Think You Should Know

- The mechanisms of action of the different families of antibiotics
- The major mechanisms of resistance
- The spectrum of activity
- Pharmacology of the antibiotic - *i.e.* distribution, toxicity, mode of excretion
- General indications for use
- *NB -You will need to be able to integrate this information and apply to clinical scenarios*