ANTIBIOTICS - PROTEIN SYNTHESIS, NUCLEIC ACID SYNTHESIS AND METABOLISM

I. Major Principles and Definitions

A. Selectivity - Clinically effective antimicrobial agents all exhibit selective toxicity toward the bacterium rather than the host. It is this characteristic that distinguishes antibiotics from disinfectants. The basis for selectivity will vary depending on the particular antibiotic. When selectivity is high the antibiotics are normally not toxic, however, even highly selective antibiotics can have side effects.

B. Therapeutic Index - the therapeutic index is defined as the ratio of the toxic dose to the effective therapeutic dose. The higher the therapeutic index, the better the antibiotic.

C. Categories of Antibiotics - Antibiotics are categorized as **bactericidal** if they kill the susceptible bacteria or **bacteriostatic** if they reversibly inhibit the growth of bacteria. In general the use of bactericidal antibiotics is preferred but many factors may dictate the use of a bacteriostatic antibiotic. When a bacteriostatic antibiotic is used, the duration of therapy must be sufficient to allow cellular and humoral defense mechanisms to eradicate the bacteria.

If, possible bactericidal antibiotics should be used to treat infections of the endocardium or the meninges. Host defenses are relatively ineffective in these sites and the dangers imposed by such infections require prompt eradication of the organisms.

D. Antibiotic Susceptibility Testing - The basic quantitative measures of the in vitro activity of antibiotics are the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The MIC is the lowest concentration of the antibiotic that results in inhibition of visible growth (i.e. colonies on a plate or turbidity in broth culture) under standard conditions. The MBC is the lowest concentration of the antibiotic that kills 99.9% of the original inoculum in a given time.

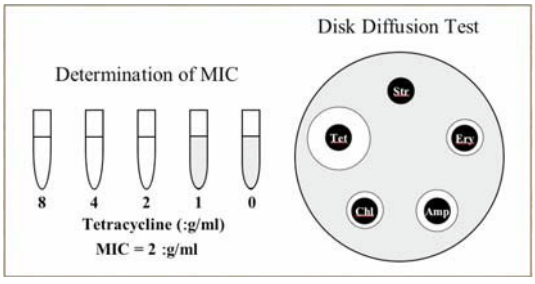

Figure 1 illustrates how to determine the MIC of an antibiotic. For an antibiotic to be effective the MIC or MBC must be able to be achieved at the site of the infection. The pharmacological absorption and distribution of the antibiotic will influence the dose, route and frequency of administration of the antibiotic in order to achieve an effective dose at the site of infection.

In clinical laboratories a more common test for antibiotic susceptibility is a disk diffusion test (Figure 1). In this test the bacterial isolate is inoculated uniformly onto the surface of an agar plate. A filter disk impregnated with a standard amount of an antibiotic is applied

to the surface of the plate and the antibiotic is allowed to diffuse into the adjacent medium. The result is a gradient of antibiotic surrounding the disk. Following incubation, a bacterial lawn appears on the plate. Zones of inhibition of bacterial growth may be present around the antibiotic disk. The size of the zone of inhibition is dependent on the diffusion rate of the antibiotic, the degree of sensitivity of the microorganism, and the growth rate of the bacterium. The zone of inhibition in the disk diffusion test is inversely related to the MIC.

The test is performed under standardized conditions and standard zones of inhibition have been established for each antibiotic. If the zone of inhibition is equal to or greater than the standard, the organism is considered to be sensitive to the antibiotic. If the zone of inhibition is less than the standard, the organism is considered to be resistant.

E. Combination Therapy - Combination therapy with two or more antibiotics is used in special cases:

(1) To prevent the emergence of resistant strains
(2) To treat emergency cases during the period when an etiological diagnosis is still in progress
(3) To take advantage of antibiotic synergism

Antibiotic synergism occurs when the effects of a combination of antibiotics is greater than the sum of the effects of the individual antibiotics. **Antibiotic antagonism** occurs when one antibiotic, usually the one with the least effect, interferes with the effects of another antibiotic.

F. Antibiotics and Chemotherapeutic agents - The term **antibiotic** strictly refers to substances that are of biological origin whereas the term **chemotherapeutic agent** refers to a synthetic chemical. The distinction between these terms has been blurred because many of our newer "antibiotics" are actually chemically modified biological products or even chemically synthesized biological products. The generic terms to refer to either antibiotics or chemotherapeutic agents are **antimicrobic** or antimicrobial agent. However, the term antibiotic is often used to refer to all types of antimicrobial agents.

II. **Review of Protein Synthesis and Site of Action of Antimicrobials that Inhibit Protein Synthesis**

A. Initiation of Protein Synthesis - Figure 2 illustrates the initiation of protein synthesis and the site of action of antimicrobials that inhibit this process.

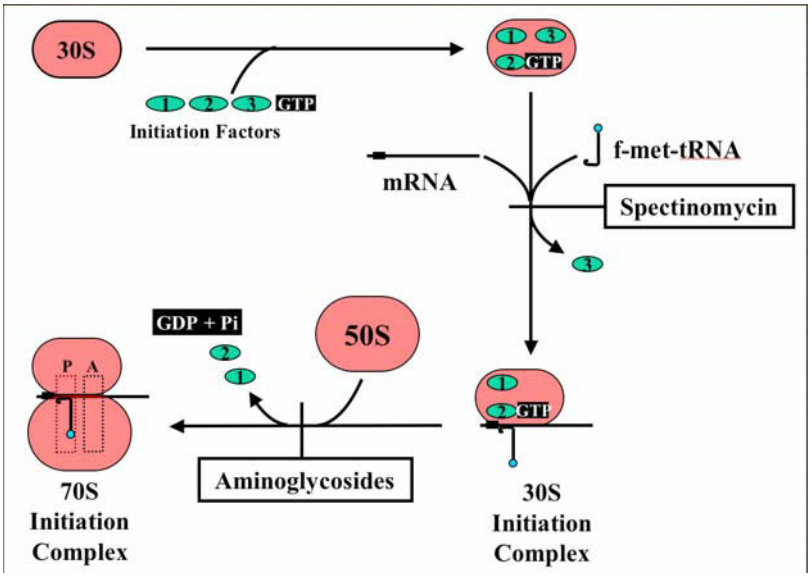

Figure 2. Initiation of Protein Synthesis

B. Elongation - Figure 3 illustrates the process of elongation and the site of action of antimicrobials that inhibit this process.

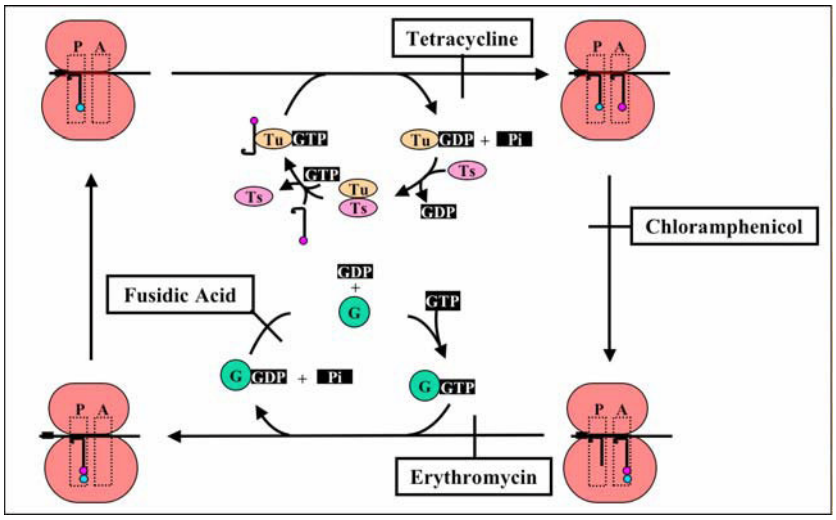

Figure 3. Elongation during protein synthesis.

III. **Inhibitors of Protein Synthesis (most bacteriostatic)**

The selectivity of these agents is a result of differences in the prokaryotic 70S ribosome and the 80S eukaryotic ribosome. Since eukaryotic mitochondrial ribosomes are similar to prokaryotic ribosomes these antimetabolites can have some toxicity.

A. Antimicrobials that bind to the 30S Ribosomal Subunit

1. Aminoglycosides (bactericidal) - **Streptomycin**, kanamycin, gentamicin, tobramycin, amikacin, netilmicin and neomycin (topical)

a. 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 r-RNA the aminoglycosides increase the affinity of the A site for t-RNA regardless of the anticodon specificity. May also destabilize bacterial membranes.

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

c. Resistance - Common

d. Synergy - The aminoglycosides synergize with β-lactam antibiotics. The β-lactams inhibit cell wall synthesis and thereby increase the permeability of the bacteria to aminoglycosides.

2. Tetracyclines (bacteriostatic) - **tetracycline**, minocycline and doxycycline

a. 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.

b. Spectrum of Activity - Broad spectrum; Useful against intracellular bacteria

c. Resistance - Common

d. Adverse Effects - Destruction of normal intestinal flora resulting in increased secondary infections; staining and impairment of the structure of bone and teeth

3. **Spectinomycin** (bacteriostatic)

a. Mode of Action - Spectinomycin reversibly interferes with m-RNA interaction with the 30S ribosome. Spectinomycin is structurally similar to aminoglycosides but does not cause misreading of mRNA

b. Spectrum of Activity - Used in the treatment of penicillin-resistant Neisseria gonorrhoeae

c. Resistance - Rare in Neisseria gonorrhoeae

B. Antimicrobials that Bind to the 50S Ribosomal Subunit

1. **Chloramphenicol**, lincomycin, clindamycin (bacteriostatic)

a. Mode of Action - These antimicrobials bind to the 50S ribosome and inhibit peptidyl transferase activity.

b. Spectrum of Activity -
(1) Chloramphenicol - Broad range
(2) Lincomycin -and clindamycin - Restricted range

c. Resistance - Common

d. Adverse Effects - Chloramphenicol is toxic (bone marrow suppression) but it is used in the treatment of bacterial meningitis.

2. Macrolides (bacteriostatic) - **Erythromycin**

a. Mode of Action - The macrolides inhibit translocation.
b. Spectrum of Activity - Gram-positive bacteria, Mycoplasma, Legionella
c. Resistance - Common

C. Antimicrobials that Interfere with Elongation Factors

1. **Fusidic Acid** (bacteriostatic)

a. Mode of Action - Fusidic acid binds to elongation factor G (EF-G) and inhibits release of EF-GDP from the EF-G/GDP complex.

b. Spectrum of Activity - Gram-positive cocci

IV. **Inhibitors of Nucleic Acid Synthesis and Function**

The selectivity of these agents is a result of differences in prokaryotic and eukaryotic enzymes affected by the antimicrobial agent.

A. Inhibitors of RNA Synthesis and Function

1. **Rifampin**, rifamycin, rifampicin (bactericidal)

a. Mode of Action - These antimicrobials bind to DNA-dependent RNA polymerase and inhibit initiation of RNA synthesis.

b. Spectrum of Activity - Wide spectrum but is used most commonly in the treatment of tuberculosis

c. Resistance - Common

d. Combination Therapy - Since resistance is common, rifampin is usually used in combination therapy

B. Inhibitors of DNA Synthesis and Function (bactericidal)

1. Quinolones - **nalidixic acid, ciprofloxacin**, oxolinic acid

a. Mode of Action - These antimicrobials bind to the A subunit of DNA gyrase (topoisomerase) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis.

b. Spectrum of Activity - Gram-positive cocci and urinary tract infections

c. Resistance - Common for nalidixic acid; developing for ciprofloxacin

V. **Antimetabolite Antimicrobials**

A. Inhibitors of Folic Acid Synthesis - The selectivity of these antimicrobials is a consequence of the fact that bacteria cannot use pre-formed folic acid and must synthesize their folic acid. In contrast, mammalian cells use folic acid obtained from food.

1. Review of Folic Acid Metabolism - Figure 4 summarizes the pathway of folic acid metabolism and indicates the sites at which antimetabolites act.

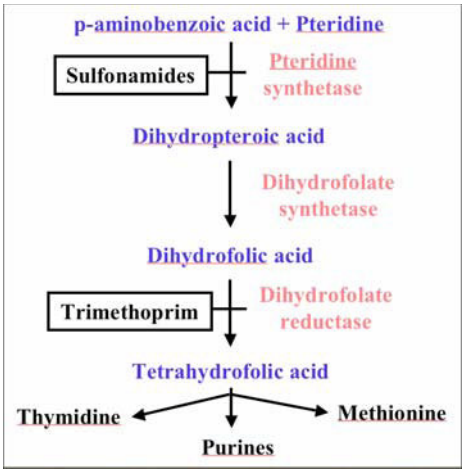

Figure 4. Synthesis of tetrahydrofolic acid and antibiotics that inhibit its synthesis.

1.**Sulfonamides, sulfones** (bacteriostatic)

a. Mode of Action - These antimicrobials are analogues of para-aminobenzoic acid and competitively inhibit pteridine synthetase and therefore block the formation of dihydropteroic acid.

b. Spectrum of Activity – Broad range activity against gram-positive and gram-negative bacteria; used primarily in urinary tract infections and in *Nocardia* infections.

c. Resistance - Common

d. Combination Therapy - The sulfonamides are used in combination with trimethoprim; this combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

2. **Trimethoprim**, methotrexate, pyrimethamine (bacteriostatic)

a. Mode of Action - These antimicrobials bind to dihydrofolate reductase and inhibit formation of tetrahydrofolic acid.

b. Spectrum of Activity - Broad range activity against gram-positive and gram-negative bacteria; used primarily in urinary tract infections and in *Nocardia* infections.

c. Resistance - Common

d. Combination Therapy - These antimicrobials are used in combination with the sulfonamides; this combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

B. Anti-Mycobacterial Agents - Antimycobacterial agents are generally used in combination with other antimicrobials since treatment is prolonged and resistance develops readily to individual agents.

1. **Para-aminosalicylic acid** (PSA) (bacteriostatic)

a. Mode of Action - Similar to sulfonamides

b. Spectrum of Activity - Specific for *Mycobacterium tuberculosis*

2. **Dapsone** (bacteriostatic)

a. Mode of Action - Similar to sulfonamides

b. Spectrum of Activity - Used in treatment of leprosy

3. **Isoniazid** (INH) (bacteriostatic)

a. Mode of action - Isoniazid inhibit synthesis of mycolic acids.

b. Spectrum of activity - Used in treatment of tuberculosis

c. Resistance - Has developed

VI. Antimicrobial Drug Resistance

A. Principles and Definitions

1. Clinical resistance to an antimicrobial agent occurs when the MIC of the drug for a particular strain of bacteria exceeds that which is capable of being achieved with safety in vivo. Resistance to an antimicrobial can arise (1) by mutation in the gene that determines sensitivity/resistance to the agent or (2) by acquisition of extrachromosomal DNA (plasmid) carrying a resistance gene. Resistance that appears after introduction of an antimicrobial agent into the environment usually results from a selective process, i.e. the agent selects for survival of those strains possessing a resistance gene. Resistance can develop in a single step or it can result from the accumulation of multiple mutations.

2. Cross-resistance implies that a single mechanisms confers resistance to multiple antimicrobial agents while multiple resistance implies that multiple mechanisms are involved. Cross-resistance is commonly seen with closely related antimicrobial agents while multiple resistance is seen with unrelated antimicrobial agents.

B. Mechanisms of Resistance

1. Altered permeability of the bacteria to an antimicrobial agent - Altered permeability may be due to the inability of the antimicrobial agent to enter the bacterial cell or alternatively to the active export of the agent from the cell.

2. Inactivation of the antimicrobial agent - Resistance is often the result of the production of an enzyme that is capable of inactivating the antimicrobial agent.

3. Altered target site - Resistance can arise due to alteration (mutation) of the target site for the antimicrobial agent.

4. Replacement of a sensitive pathway - Resistance can result from the acquisition of a new enzyme to replace the sensitive one.