Mechanisms of Action of Antimicrobial Agents

Selective Toxicity

An ideal antimicrobial agent exhibits selective toxicity, which means that the drug is harmful to a pathogen without being harmful to the host. Often, selective toxicity is relative rather than absolute; this implies that a drug in concentration tolerated by the host may damage an infecting microorganism.

Selective toxicity may be a function of a specific receptor required for drug attachment, or it may depend on the inhibition of biochemical drugs events essential to the pathogen but not to the host.

The mechanisms of action of antimicrobial agents can be discussed under four headings:

1- Inhibition of cell wall synthesis.
2- Inhibition of cell membrane function.
3- Inhibition of protein synthesis (i.e. inhibition of translation and transcription of genetic material).
4- Inhibition of nucleic acid synthesis.

Inhibition of Cell Wall Synthesis

Bacteria have a rigid outer layer, the cell wall. The cell wall maintains the shape and size of the microorganism, which has a high internal osmotic pressure. Injury to the cell wall (e.g. by lysozyme) or inhibition of its formation may lead to lysis of the cell.

The cell wall contains a chemically distinct complex polymer "**mucopeptide**" ("**peptidoglycan**") consisting of polysaccharides and a highly cross-linked polypeptide. The polysaccharides regularly contain the amino sugars *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM). The latter is found only in bacteria. To the amino sugars are

attached short peptide chains. The final rigidity of the cell wall is imparted by cross-linking of the peptide chains (eg. Through pentaglycine bonds) as a result of transpeptidation reactions carried out by several enzymes. The peptidoglycan layer is much thicker in the cell wall of G^+ than of G^- bacteria.

All β-lactam drugs are selective inhibitors of bacterial cell wall synthesis and therefore active against growing bacteria. The initial step in drug action consists of binding of the drug to cell receptors **(penicillin-binding proteins ; PBPs)**. There are three to six PBPs , some of which are transpeptidation enzymes. Different receptors have different affinities for a drug, and each may mediate a different effect. For example, attachment of penicillin to one PBP may result chiefly in abnormal elongation of the cell, whereas attachment to another PBP may lead to a defect in the periphery of the cell wall, with the resulting cell lysis.

After a β-lactam drug has attached to one or more receptors, the transpeptidation reaction is inhibited and peptidoglycan synthesis is blocked.

Examples of agents acting by inhibition of cell wall synthesis are pencillins, the cephalosporins, vancomycin, and cycloserine. Several other drugs, including bacitracin, teicoplanin, ristocetin, and novobiocin, inhibit early steps in the biosynthesis of the peptidoglycan.

Inhibition of Cell Membrane Function

The cytoplasmic membrane of bacteria and fungi has a structure different from that of animal cells and can be more readily disrupted by certain agents. Consequently, selective chemotherapy is possible.

Detergents, which contain lipophilic and hydrophilic groups, disrupt cytoplasmic membranes and kill the cell. One class of antibiotics, **the**

polymyxins, consist of detergent-like cyclic peptides that selectively damage membranes containing phosphatidylethanolamine, a major component of bacterial membranes. A number of antibiotics specifically interfere with biosynthetic functions of of the cytoplasmic membranes – eg, **nalidixic acid** and **novobiocin** inhibit DNA synthesis, and **novobiocin** also inhibits teichoic acid synthesis.

A third class of membrane-active agents is the **ionophores**, compounds that permit rapid diffusion of specific cations through the membrane. **Valinomycin**, for example, specifically mediates the passage of potassium ions. Some ionophores act by forming hydrophilic pores in the membrane ; other act by lipid-soluble ion carriers that behave as though they shuttle back and forth within the membrane potential, which is essential for oxidative phosphorylation, as well as for other membrane-mediated processes ; they are not selective for bacteria but act on the membranes of all cells.

Daptomycin is a new lipopeptide antibiotic that is rapidly bactericidal by binding to the cell membrane in a calcium-dependent manner causing depolarization of bacterial membrane potential. This leads to intracellular potassium release. Currently this agent is approved for use in the treatment of *Staphylococcus aureus* blood stream infections and skin and soft-tissue infections caused by gram-positive bacteria, particularly those organisms that are highly resistant to β-lactam agents and **vancomycin.**

Other examples of agents acting by inhibition of cell membrane function are **amphotericin B, colistin**, and **the imidazoles** and **triazoles**.

Inhibition of Protein Synthesis

Bacteria have 70S ribosomes, whereas mammalian cells have 80S ribosomes. The subunits of each type of ribosome, their chemical

composition, and their functional specificities are sufficiently different to explain why antimicrobial drugs can inhibit protein synthesis in bacterial ribosomes without having a major effect on mammalian ribosomes.

In normal microbial protein synthesis, the mRNA message is simultaneously "read" by several ribosomes that are strung out along the mRNA strand. These are called **polysomes.**

Examples of drugs acting by inhibition of protein synthesis are the **erythromycins, lincomycins, tetracyclines, glycyclines, aminoglycoside, and chloramphenicol.**

1-Aminoglycosides

Such as **streptomycin**, the **first step** of the mode of action is the attachment of the aminoglycoside to a specific receptor protein in (P 12 in the case of streptomycin) on the 30S subunit of the microbial ribosome. **Second**, the aminoglycoside blocks the normal activity of the "initiation complex" of peptide formation (mRNA+ formyl methionine + tRNA). **Third**, the mRNA message is misread on the "recognition region" of the ribosome ; consequently, the wrong amino acid is inserted into the peptide, resulting in a nonfunctional protein. **Fourth,** aminoglycoside attachment results in the breakup of polysomes and their separation into **monosomes** incapable of protein synthesis. The overall effect is usually an irreversible event – killing of the bacterium.

2- Macrolides, Azalides, and Ketolides

These drugs **(erythromycins, azithromycin, clarithromycin, roxithromycin, the ketolide, and telithromycin)** bind to the 50S subunit of the ribosome, and the binding site is a 23S rRNA. They may interfere with formation of inhibition complexes for peptide chain synthesis or may interfere with aminoacyl translocation reactions.

3- Lincomycins

Clindamycin binds to the 50S subunit of the microbial ribosome and resembles macrolides in binding site, antibacterial activity, and mode of action.

4- Tetracyclines

Tetracyclines bind in the 30S subunit of microbial ribosomes. They inhibit protein synthesis by blocking the attachment of charged aminoacyl-tRNA. Thus, they prevent introduction of new amino acids to the nascent peptide chain. The action is usually inhibitory and reversible upon withdrawal of the drug.

5- Glycylcyclines

The glycylcyclines are synthetic analogues of the tetracyclines. The glycylcyclines inhibit protein synthesis in a manner similar to the tetracyclines ; however, they are bactericidal, likely due to their more avid binding to the ribosome.

6- Chloramphenicol

Chloramphenicol binds to the 50S subunit of the ribosome. It interferes with the binding of new amino acids to the nascent peptide chain, largely because chloramphenicol inhibits peptidyl transferase. Chloramphenicol is mainly bacteriostatic, and growth of microorganisms resumes when the drug is withdrawn.

7- Streptogramins

Quinupristin/dalfopristin is a combination of two pristinamycin derivatives. These two agents act synergistically to achieve bactericidal activity against gram-positive bacteria not seen with rither agent alone. The mechanism of action appears to be irrevrsible binding to different sites on 50S ribosome.

8- Oxazolidinones

The oxazolidinones are a relatively new class of antimicrobial agents that possess a unique mechanism of inhibition of protein synthesis primarily in gram-positive bacteria. These compounds interfere with translation by inhibiting the formation of *N*-formylmethionyl-tRNA, the initiation complex at the 30S ribosome. **Linezolid** is the agent that is currently commercially available.

Inhibition of Nucleic Acid Synthesis

Examples of drugs acting by inhibition of nucleic acid synthesis are the **quinolones, pyrimethamine, rifampin, sulfonamides, trimethoprim,** and **trimetrexate.** Rifampin inhibits bacterial growth by binding strongly to the DNA-dependent RNA polymerase of bacteria. Thus, it inhibits bacterial RNA synthesis. Rifampin resistance results from a change in RNA polymerase due to a chromosomal mutation that occurs with high frequency. The mechanism of rifampin action on viruses is different. It blocks a late stage in the assembly of poxiviruses.

All quinolones and fluoroquinolones inhibit microbial DNA synthesis by blocking DNA gyrase.

For many microorganisms, *ρ*-aminobenzoic acid(PABA) is an essential metabolite. The specific mode of action of PABA involves an adenosine triphosphate (ATP)-dependent condensation of a pteridine with PABA to yield dihydropteroic acid, which is subsequently converted to folic acid. PABA is involved in the synthesis of folic acid, an important precursor to the synthesis of nucleic acids. Sulfonamides are structural analogs of PABA and inhibit dihydropteroate synthetase.

Sulfonamides can enter into the reaction in place of PABA and compete for the active center of the enzyme. As a result, nonfunctional analogs of folic acid are formed, preventing further growth of the

bacterial cell. The inhibiting action of sulfonamides on bacterial growth can be counteracted by an excess of PABA in the environment (competitive inhibition). Animal cells cannot synthesize folic acid and must depend upon exogenous sources.

Trimethoprim (3,4,5-trimethoxybenzylpyrimidine) inhibits dihydrofolic acid reductase 50,000 times more efficiently in bacteria than in mammalian cells. This enzyme reduces dihydrofolic to tetrahydrofolic acid, a stage in the sequence leading to the synthesis of purines and ultimately of DNA. Sulfonamides and trimethoprim each can be used alone to inhibit bacterial growth. If used together, they produce sequential blocking, resulting in a marked enhancement (synergism) of activity. Such mixtures of sulfonamide (five parts) plus trimethoprim (one part) have been used in the treatment of pneumocystis pneumonia, malaria, shigella enteritis, systemic salmonella infections, urinary tract infections, and may others.

Pyrimethamine also inhibits dihydrofolate reductase, but it is more active against the enzyme in mammalian cells and therefore is more toxic than trimethoprim. Pyrimethamine plus sulfonamide or clindamycin is the current treatment of choice in toxoplasmosis and some other protozoal infections.