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B.Sc (HONOURS) MICROBIOLOGY (CBCS STRUCTURE)
C-13: MEDICAL MICROBIOLOGY (THEORY)
SEMESTER –VI

TOTAL HOURS: 60 **CREDITS: 4**

Unit 7 Antimicrobial agents: General characteristics and mode of action

No. of Hours: 8

Antibacterial agents: Five modes of action with one example each: Inhibitor of nucleic acid synthesis; Inhibitor of cell wall synthesis; Inhibitor of cell membrane function; Inhibitor of protein synthesis; Inhibitor of metabolism. Antifungal agents: Mechanism of action of Amphotericin B, Griseofulvin, Nystatin, Antiviral agents: Mechanism of action of Amantadine, Acyclovir, Azidothymidine, Antibiotic resistance.

Q. What is antimicrobial agent?

The term chemotherapy was coined by the German medical researcher Paul Ehrlich to describe the use of chemical substances to kill pathogenic organisms without injuring the host. Today, chemotherapy refers to the use of chemical substances to treat various aspects of disease. With this broad modern definition of chemotherapy, we describe a chemotherapeutic agent as any chemical substance used in medical practice. Such agents also are referred to as drugs. In microbiology we are concerned with antimicrobial agents, a special group of chemotherapeutic agents used to treat diseases caused by microbes.

Five different modes of action of antimicrobials are : (1) inhibition of cell wall synthesis, (2) disruption of cell membrane function, (3) inhibition of protein synthesis, (4) inhibition of nucleic acid synthesis, and (5) action as antimetabolites.

Q. Write the Antibacterial agents with one example each: Inhibitor of nucleic acid synthesis; Inhibitor of cell wall synthesis; Inhibitor of cell membrane function; Inhibitor of protein synthesis; Inhibitor of metabolism.

Ans: Inhibition of Cell Wall Synthesis

1. Many bacterial and fungal cells have rigid external cell walls, whereas animal cells lack cell walls. Consequently, inhibiting cell wall synthesis selectively damages bacterial and fungal cells. Bacterial cells, especially Grampositive ones, have a high internal osmotic pressure. Without a normal, sturdy cell wall, these cells burst when subjected to the low osmotic pressure of body fluids. Antibiotics such as penicillin and cephalosporin contain a chemical structure called a b-lactam ring, which attaches to the enzymes that cross-link peptidoglycans. By interfering with the cross-linking of tetrapeptides, these antibiotics prevent cell wall synthesis. Fungi and Archaea, whose cell walls lack peptidoglycan, are unaffected by these antibiotics.

Penicillin G is the drug of choice in treating infections caused by streptococci, meningococci, pneumococci, spirochetes, clostridia, and aerobic Grampositive rods. It is also suitable for treating infections caused by a few strains of staphylococci and gonococci that are not resistant to it. Because it retains activity in urine, it is suitable for treating some urinary tract infections. Infections caused by organisms resistant to penicillin G can be treated with semisynthetics such as nafcillin, oxacillin, ampicillin, or amoxicillin. Carbenicillin and ticarcillin are especially useful in treating Pseudomonas infections. Allergy to penicillin is rare among children but occurs in 1 to 5% of adults. Penicillins are generally nontoxic, but large doses can have toxic effects on the kidneys, liver, and central nervous system.

2. Natural cephalosporins (sefa-lo-spor0-inz), derived from several species of the fungus Cephalosporium, have limited antimicrobial action. Their discovery led to the development of a large number of bactericidal, semisynthetic derivatives of natural cephalosporin C. The nucleus of a cephalosporin is quite similar to that of penicillin; both contain b-lactam rings. Semisynthetic cephalosporins, like semisynthetic penicillins, differ in the nature of their side chains. Frequently used cephalospirins include cephalexin (Keflex), cephradine, and cefadroxil, all of which are fairly well absorbed from the gut and therefore can be administered orally. Other cephalosporins, such as cephalothin (Keflin), cephapirin, and cefazolin, must be administered parenterally, usually into muscles or veins. Although cephalosporins usually are not the first drug considered in the treatment of an infection, they are frequently used when allergy or toxicity prevents the use of other drugs. But because cephalosporins are structurally similar to penicillin, some patients who are allergic to penicillin may also be sensitive to the cephalosporins.. The development of new varieties of cephalosporins seems to be a race against the ability of bacteria to acquire resistance to older varieties. When organisms became resistant to early ‘‘first-generation’’ cephalosporins, new, ‘‘second-generation’’ cephalosporins, including cefuroxime and cefaclor, were produced . Now ‘‘third-generation’’ cephalosporins, such as ceftriaxone and cephtazidime, and ‘‘fourth-generation’’ cefepime, are used against organisms resistant to older drugs. These drugs are especially effective (for now) in dealing with hospital-acquired infections resistant to many antibiotics. They are being tried in patients with AIDS and other immunodeficiencies. (Do not confuse these with second- and third-line drugs, described earlier, which are not derivatives of one another.). Adverse effects from cephalosporins tend to be local reactions, such as irritation at the injection site or nausea, vomiting, and diarrhea when the drug is administered orally. Four to fifteen percent of patients allergic to penicillin also are allergic to cephalosporins. Moreover, newer cephalosporins have little effect on Gram-positive organisms, which can cause superinfections during the treatment of Gram-negative infections.

3. Carbapenems (kar0ba-pen-emz) represent a new group of bactericidal antibiotics with two-part structures. Primaxin, a typical carbapenem, consists of a b-lactam antibiotic (imipenem) that interferes with cell wall synthesis and cilastatin sodium, a compound that prevents degradation of the drug in the kidneys. As a group, the carbapenems have an extremely broad spectrum of activity. Bacitracin, a small bactericidal polypeptide derived from the bacterium Bacillus

licheniformis, is used only on lesions and wounds of the skin or mucous membranes because it is poorly absorbed and toxic to the kidneys. Vancomycin is a large, complex molecule produced by the soil actinomycete Streptomyces orientalis. It is too large a molecule to pass through pores in the outern membrane of Gram-negative cell walls, and is therefore not effective against most Gram-negative bacteria. It can be used to treat infections caused by methicillinresistant staphylococci and enterococci. It is also the drug of choice against antibiotic-induced pseudomembranous colitis (enteritis with the formation of false membranes in stool). Because it is poorly absorbed through the gastrointestinal tract, it must be administered intravenously. Vancomycin is fairly toxic, causing hearing loss and kidney damage, especially in older patients, if the drug is not monitored carefully.

Disruption of Cell Membrane Function

All cells are bounded by a membrane. Although the membranes of all cells are quite similar, those of bacteria and fungi differ sufficiently from those of animal cells to allow selective action of antimicrobial agents. Certain polypeptide antibiotics, such as polymyxins, act as detergents and distort bacterial cell membranes, probably by binding to phospholipids in the membrane. (With this distortion, the membrane is no longer regulated by membrane proteins, and the cytoplasm and cell substances are lost.) These antibiotics are especially effective against Gram-negative bacteria, which have an outer membrane rich in phospholipids. Polyene antibiotics, such as amphotericin B, bind to particular sterols, present in the membranes of fungal (and animal) cells. Thus, polymyxins do not act on fungi, and polyenes do not act on bacteria.

Five polymyxins, designated A, B, C, D, and E, have been obtained from the soil bacterium Bacillus polymyxa. Polymyxins B and E are the most common clinically. They are usually applied topically, often with bacitracin, to treat skin infections caused by Gramnegative bacteria such as Pseudomonas. Used internally, polymyxins can cause numbness in the extremities, serious kidney damage, and respiratory arrest. They are administered by injection when the patient is hospitalized and kidney function can be monitored.

Inhibition of Protein Synthesis

In all cells, protein synthesis requires not only the information stored in DNA, plus several kinds of RNA, but also ribosomes. Differences between bacterial (70S) and animal (80S) ribosomes allow antimicrobial agents to attack bacterial cells without significantly damaging animal cells—

that is, with selective toxicity. Aminoglycoside antibiotics, such as streptomycin, derive their name from the amino acids and glycosidic bonds they contain. They act on the 30S portion of bacterial ribosomes by interfering with the accurate reading (translation) of the mRNA message—that is, the incorporation of the correct amino acids. Chloramphenicol and erythromycin act on the 50S portion of bacterial ribosomes, inhibiting the formation of the growing polypeptide. Because animal cell ribosomes consist of 60S and 40S subunits, these antibiotics have little effect on host cells. (Mitochondria, however, which have 70S ribosomes, can be affected by such drugs.)

Inhibition of Nucleic Acid Synthesis

Differences between the enzymes used by bacterial and animal cells to synthesize nucleic acids provide a means for selective action of antimicrobial agents. Antibiotics of the rifamycin family bind to a bacterial RNA polymerase and inhibit RNAsynthesis.

From among the rifamycins produced by Streptomyces mediterranei, only the semisynthetic rifampin is currently used. Easily absorbed from the digestive tract except when taken directly after a meal, it reaches all tissues and body fluids. Rifampin blocks RNA transcription. Although it is bactericidal and has a wide spectrum of activity, it is approved in the United States only for treating tuberculosis and eliminating meningococci from the nasopharynx of carriers. Rifampin can cause liver damage but usually does so only when excessive doses are given to patients with preexisting liver disease. It is unusual among antibiotics in its ability to interact with other drugs, and possibilities of such interactions should be considered before the drug is given. Taking rifampin concurrently with oral contraceptives has been implicated in an increased risk of pregnancy and menstrual disorders. Dosages of anticoagulants must be increased while a patient is taking rifampin to achieve the same degree of reduction in blood clotting. Finally, drug addicts who are receiving methadone sometimes suffer withdrawal symptoms if they are given rifampin without an increase in methadone dosage. One explanation for these diverse effects is that rifampin stimulates the liver to produce greater quantities of enzymes that are involved in the metabolism of a variety of drugs.

Action as Antimetabolites

The normal metabolic processes of microbial cells involve a series of intermediate compounds called metabolites that are essential for cellular growth and survival. Antimetabolites are substances that affect the utilization of metabolites and therefore prevent a cell from carrying out necessary metabolic reactions. Antimetabolites function in two ways: (1) by competitively inhibiting enzymes and (2) by being erroneously incorporated into important molecules such as nucleic acids. Antimetabolites are structurally similar to normal metabolites. The actions of antimetabolites are sometimes called molecular mimicry because they mimic, or imitate, the normal molecule, preventing a reaction from occurring or causing it to go awry. In competitive inhibition an enzymatic reaction is inhibited by a substrate that binds to the enzyme's active site but cannot react. While this competing substrate occupies the active site, the enzyme is unable to function, and metabolism will slow or even cease if enough enzyme molecules are inhibited. Consider sulfanilamide and para-aminosalicylic acid (PAS), which are chemically very similar to para-aminobenzoic acid (PABA). They competitively inhibit an enzyme that acts on PABA. Many bacteria require PABA in order to make folic acid, which they use in synthesizing nucleic acids and other metabolic products. When sulfanilamide or PAS instead of PABA is bound to the enzyme, the bacterium cannot make folic acid. Animal cells lack the enzymes to make the folic acid and must obtain it from their diets; thus their metabolism is not disturbed by these competitive inhibitors. Antimetabolites such as the purine analog vidarabine and the pyrimidine analog idoxuridine are erroneously incorporated into nucleic acids. These molecules are very similar to the normal purines and pyrimidines of nucleic acids. When incorporated into a nucleic acid, they garble the information that it encodes because they cannot form the correct base pairs during replication and transcription. Purine and pyrimidine analogs are generally as toxic to animal cells as to microbes because all cells use the same purines and pyrimidines to make nucleotides. These agents are most useful in treating viral infections, because viruses incorporate analogs more rapidly than do cells and are more severely damaged.

Q. Write the Antifungal agents: Mechanism of action of Amphotericin B, Griseofulvin, Nystatin.

1. Imidazoles and Triazoles

The imidazoles and triazoles comprise a large group of related synthetic fungicides. Several agents, including clotrimazole, ketoconazole, miconazole, and fluconazole, are currently in use; many are available without prescription. The imidazoles and triazoles appear to affect fungal plasma membranes by disrupting the synthesis of membrane sterols. All these agents are used topically in creams and solutions to control fungal skin infections (dermatomycoses) and Candida yeast infections of the skin, nails, mouth, and vagina. Ketoconazole has also been given orally to treat systemic fungal infections, especially when other antifungal agents have not been effective. Some patients, however, have experienced mild to severe skin irritations with the topical agents. Furthermore, potentially severe drug interactions may occur, especially with certain antihistamines and immunosuppressants.

2. **Polyenes**

The polyene family of antibiotics consists of antifungal agents that contain at least two double bonds. Amphotericin B and nystatin are two of the most common polyene antibiotics.

3. Amphotericin B.

The fungicidal antibiotic amphotericin B (Fungizone) is derived from Streptomyces nodosus. This drug binds to plasma membrane ergosterol (a crystallizing sterol) found in fungi and some algae and protozoa but not in human cells. Amphotericin B increases membrane permeability such that glucose, potassium, and other essential substances leak from the cell. The drug is poorly absorbed from the digestive tract and so is given intravenously. Even then, only 10% of the dose given is found in the blood. Excretion persists for up to 3 weeks after treatment is discontinued, but it is not known where the drug is sequestered in themeantime. Amphotericin B is the drug of choice in treating most systemic fungal infections, especially cryptococcosis, coccidioidomycosis, and aspergillosis. Although fungi are not known to develop resistance to this agent, side effects are numerous and sometimes severe. They include abnormal skin sensations, fever and chills, nausea and vomiting, headache, depression, kidney damage, anemia, abnormal heart rhythms, and even blindness. Because some of the fungal infections are fatal without treatment, patients, especially those who are immunocompromised or have AIDS, have little choice but to risk these unfortunate side effects.

4. Nystatin.

The polyene antibiotic nystatin (Mycostatin) is produced by Streptomyces noursei. This drug has the same mode of action as amphotericin B but is also effective topically in the treatment of Candida yeast infections. Because it is not absorbed through the intestinal wall, it can be given orally to treat fungal superinfections in the intestine, which often occur after long-term treatment with antibiotics. Nystatin was named for the New York State Health Department, where it was discovered.

5.Griseofulvin.

Griseofulvin, originally derived from Penicillium griseofulvum, is used primarily for superficial fungal infections. This fungistatic drug is incorporated into new cells that replace infected cells; it interferes with fungal growth, probably by impairing the mitotic spindle apparatus used in cell division. Although griseofulvin (Fulvicin) is poorly absorbed from the intestinal tract, it is given orally and appears to reach the target tissues through perspiration. It is ineffective against bacteria and most systemic fungal agents but is very useful topically in treating fungal infections of the skin, hair, and nails. Most infections are cured within 4 weeks, but recalcitrant infections associated with fingernails and toenails may persist even after a year of treatment. Reactions to griseofulvin are usually limited to mild headaches but can include gastrointestinal disturbances, especially when prolonged treatment is required. It is also one of the antibiotics suspected, but not proven, to reduce effectiveness of birth control pills.

Q. Write the Antiviral agents: Mechanism of action of Amantadine, Acyclovir, Azidothymidine.

Currently available antiviral agents inhibit some phase of viral replication, but they do not kill the viruses.

1. **Purine and Pyrimidine Analogs**

Several purine and pyrimidine analogs are effective antiviral agents. All cause the virus to incorporate erroneous information (the analog) into a nucleic acid and thereby interfere with the replication of viruses. The drugs include **idoxuridine, vidarabine, ribavirin, acyclovir, ganciclovir, and azidothymidine (AZT).**

Idoxuridine and trifluridine, both analogs of thymine, are administered in eye drops to treat inflammation of the cornea caused by a herpesvirus. They should not be used internally because they suppress bone marrow.

Vidarabine , an analog of adenine, has been used effectively to treat viral encephalitis, an inflammation of the brain caused by herpesviruses and by cytomegaloviruses. It is not effective against cytomegalovirus infections acquired before birth. Vidarabine is less toxic than either idoxuridine or cytarabine, but it sometimes causes gastrointestinal disturbances.

Ribavirin (Virazole), a synthetic nucleotide analog of guanine, blocks replication of certain viruses. In an aerosol spray, it can combat influenza viruses; in an ointment, it can help to heal herpes lesions. Although it has low toxicity, it can induce birth defects and should not be given to pregnant women. It has been found to be effective against hantaviruses, such as those that caused the deadly outbreak of respiratory disease on the Navajo reservation in the Four Corners region of the American Southwest in 1993. Ribavirin has shown activity against a wide variety of unrelated viruses, raising hopes of finding a broad-spectrum antiviral agent.

Acyclovir (Zovirax), an analog of guanine, is much more rapidly incorporated into virus-infected cells than into normal cells. Thus, it is less toxic than other analogs. It can be applied topically or given orally or intravenously. It is especially effective in reducing pain and promoting healing of primary lesions in a new case of genital herpes. It is given prophylactically to reduce the frequency and severity of recurrent lesions, which appear periodically after a first attack. It does not, however, prevent the establishment of latent viruses in nerve cells. Acyclovir is more effective than vidarabine against herpes encephalitis and neonatal herpes, an infection acquired at birth, but is not effective against other herpesviruses.

Ganciclovir is an analog of guanine similar to acyclovir. The drug is active against several kinds of herpesvirus infections, particularly cytomegalovirus eye infections in patients with AIDS. Zidovudine (AZT) interferes with reverse transcriptase making DNAfrom RNA. It is used in treatingAIDS.

Amantadine

The tricyclic amine amantadine prevents influenza A viruses from penetrating cells. Given orally, it is readily absorbed and can be used from a few days before to a week after exposure to influenza A viruses to reduce the incidence and severity of symptoms. Unfortunately, it causes

insomnia and ataxia (inability to coordinate voluntary movements), especially in elderly patients,who also are often severely affected by influenza infections. Rimantadine, a drug similar to amantadine, may be effective against a wider variety of viruses and may be less toxic as well.

Reference:

Microbiology,7th Edition, Principles and Explorations,
Jacquelyn G. Black, Marymount university, Arlington, Virginia contributor: laura j. black