CHAPTER 19
ANTIBIOTICS AND
ANTIMICROBIAL DRUGS

WHY IS THIS IMPORTANT?

- Antibiotics have drastically reduced the number of deaths due to infection
- They have changed the face of health care

OVERVIEW

Antibiotics and Antimicrobial Drugs

SOURCE OF ANTIBIOTICS
ANTIBiotic SPectRA
ANTIBiotic TARGETS
ANTIMICROBIAL DRUGS
DRUGS FOR PARASITIC INFECTIONS
SOURCE OF ANTIBIOTICS

- The discovery of the first antibiotic was an accident
- Alexander Fleming accidentally contaminated a plate with a fungus
- He observed a clearly defined region of no bacterial growth where the fungi had contaminated the plate
- The area around the fungus was eventually referred to as a zone of inhibition

SOURCE OF ANTIBIOTICS

- It is estimated that over 80 million prescriptions are written in America each year
- 12,500 tons of antibiotics are produced annually
- From 1900 to 1980, mortality from infectious diseases dropped from 797 per 100,000 persons to 36 per 100,000 persons

HISTORICAL PERSPECTIVES
HISTORICAL PERSPECTIVES

- Few major discoveries of natural antibiotic substances have occurred for several years
  - Efforts have now shifted to modifying existing antibiotics
  - Searching in new places for potential antibiotics has also gained in prominence

ANTIBIOTICS ARE PART OF BACTERIAL SELF PROTECTION

- Many antibiotics are produced by microorganisms as part of their survival mechanism
  - They keep other organisms away
  - They protect the supply of nutrients and oxygen

- Microorganisms that produce these substances have molecular mechanisms to control production and prevent self-destruction
- Naturally produced antibiotics are products of secondary metabolic pathways
  - These pathways are not turned on all the time
  - Continuous production could adversely affect the organism
ANTIBIOTICS ARE PART OF BACTERIAL SELF PROTECTION

- Organisms protect themselves in several ways:
  - Some bacteria restrict antibiotic production to the stationary phase
  - Others keep the intracellular concentrations at low levels
    - They regulate rates of production and export

- Antibiotic molecules are exported in an inactive form
  - They become activated by extracellular enzymes
- Some microorganisms modify their own cell walls to ensure their safety

ANTIBIOTIC SPECTRA

- The first molecules that inhibited bacterial growth were natural products
- Over time, these natural molecules have been modified
- Several types of semi-synthetic antibiotics have been derived from these molecules
**ANTIBIOTIC SPECTRA**

- Antibiotics are classified as either broad-spectrum or narrow-spectrum
- The original natural molecules used by humans as antibiotics have a very narrow spectrum
- Natural molecules can be chemically modified making it possible to broaden their spectrum

**ANTIBIOTIC STRUCTURE**

- The structure of penicillin is useful as a template for the development of an entire group of antibiotics (more than 50 so far)
- In its native form, penicillin is composed of a core four-sided ring structure
  - The beta ($\beta$)-lactam ring
**ANTIBIOTIC STRUCTURE**

- All forms of penicillin contain this ring
- Derivatives contain additional specific structures:
  - Side chains attached to the ring
  - Chemically changing the side chain can change:
    - Antimicrobial activity
    - Resistance to stomach acid
    - Overall half-life in body

**ANTIBIOTIC STRUCTURE**

- There are semi-synthetic forms of penicillin
  - They are created through modifications that can be made in a laboratory
- Chemists can create and modify side chains
  - This produces new forms of penicillin
ANTIBIOTIC STRUCTURE

- Natural penicillin has a very narrow spectrum
  - Chemically modifying penicillin broadens the spectrum
- Semi-synthetic penicillins can be further modified to increase the efficiency of inhibiting bacterial growth
  - Ampicillin can be modified to mezlocillin or azlocillin

- The same kind of manipulation can be seen with cephalosporin family
  - Modification of the natural molecule has resulted in several generations of semi-synthetics
- Modifications result from changing the side chains leaving core intact
- Modifications also change reactivity patterns and spectrum

ANTIBIOTIC TARGETS

- A fundamental criterion of antibiotics for medical use is selective toxicity
  - The antibiotic should be destructive to the disease-causing organism but have no effect on the human host
- The first antibiotic discovered was most selectively toxic
ANTIBIOTIC TARGETS

- Many chemicals are useful in restricting bacterial growth
  - They are also inherently toxic
  - They cannot be used therapeutically
- Many antibiotic molecules are toxic if administered at high concentrations

ANTIBIOTIC TARGETS

- Antibiotic targets can be subdivided into five major groups:
  - The bacterial cell wall
  - The bacterial plasma membrane
  - Synthesis of bacterial proteins
  - Bacterial nucleic acids
  - Bacterial metabolism
**BACTERIAL CELL WALL**

- It is the most appealing target for antibiotics
  - Found in bacteria but not humans
  - Meets the criterion of selective toxicity
- It is found in both Gram-positive and Gram-negative bacteria

**BACTERIAL CELL WALL**

- The cell wall is built by many enzymatic reactions
  - These enzymes can be used as targets of antibiotic molecules
- The cell wall is made up of the peptidoglycan molecules NAG and NAM
  - They are cross-linked through activity of transglycosylase and transpeptidase enzymes
  - Many antibiotics inhibit the activity of these two enzymes
    - Results in improper cell wall cross-linking
    - Organism is not able to withstand environmental pressures

**BACTERIAL CELL WALL: Penicillins**

- Penicillin-binding proteins (PBPs) are involved in the construction of the cell wall
- β-lactam ring of penicillin binds to these proteins
- New cell wall continuously built during active growth
  - Penicillin prevents the formation of an intact cell wall
  - Penicillin is most effective during this phase
BACTERIAL CELL WALL: Penicillins

- Gram-negative bacteria have markedly less peptidoglycan
  - They are normally less sensitive to penicillin
- Reactivity of penicillin against Gram-negative bacteria has been enhanced by synthetically modifying the core structure

BACTERIAL CELL WALL: Cephalosporins

- Cephalosporins have similar activity to penicillins
  - They prevent the construction of a stable cell wall
- Cephalosporins have a much greater effect on Gram-negative bacteria than penicillins
  - They are naturally broader spectrum antibiotics
  - They are not susceptible to the β-lactamase enzymes

BACTERIAL CELL WALL: Cephalosporins

- There are multiple generations of cephalosporins
  - More than 70 versions are in use
  - They are one of the most widely prescribed antibiotics
- Mechanism of action similar to that of penicillin but cephalosporin penetrates through porin channels
**BACTERIAL CELL WALL: Cephalosporins**

- Side chains can be modified to increase the spectrum of reactivity
- Cephalosporins are frequently used both preoperatively and postoperatively
  - Frequent use has increased resistance

**BACTERIAL CELL WALL: Carbapenems**

- Carbapenems contain a β-lactam ring like penicillin
  - They also inhibit the synthesis of bacterial walls
- The β-lactam ring of carbapenems contains a double bond
  - This prevents β-lactamase cleaving the ring
- Carbapenems have a very broad spectrum of antibacterial activity
- Two are approved for clinical use in humans
  - Both are useful against *Pseudomonas* species

**BACTERIAL CELL WALL: Monobactams**

- Monobactams have a different ring structure
  - They cannot be recognized by β-lactamase
  - They are effective in overcoming bacterial resistance related to β-lactamase
**BACTERIAL CELL WALL: Glycopeptide Antibiotics**

- Glycopeptide antibiotics are derived from *Streptomyces* organisms
  - Vancomycin and teicoplanin are glycopeptide antibiotics
- Glycopeptide antibiotics have serious side effects
  - Toxicity level reduced in recent years by improving purification
- They inhibit cell wall synthesis by forming a complex with the substrates that make up peptidoglycan
- They cannot penetrate the porins of Gram-negative cells
  - Narrow spectrum antibiotics restricted to Gram-positive bacteria

**BACTERIAL CELL WALL: Peptide Antibiotics Effective Against Mycobacteria**

- These antibiotics are used against bacteria with modified cell walls
- *Mycobacterium* species (cause TB and leprosy) are a good example
  - Their cell walls are modified by incorporation of mycolic acids
- Isoniazid very effective against these organisms
  - Inhibits the synthesis of mycolic acid

**BACTERIAL CELL WALL: Peptide Antibiotics Effective Against Mycobacteria**

- Ethambutol is given in concert with isoniazid
  - Inhibit the incorporation of mycolic acid into cell wall
- The treatment of choice for tuberculosis is a combination of isoniazid, ethambutol, and rifampin
  - Combinations of drugs lower the potential for development of resistance
BACTERIAL CELL WALL: Polypeptide Antibiotics

- Bacitracin and polymixin B
- Used topically for superficial infections by Gram-positive organisms
  - Staphylococcus and Streptococcus
- They inhibit binding between NAG and NAM
  - Prevents formation of linear strands of peptidoglycan

BACTERIAL PLASMA MEMBRANE

- The plasma membrane is involved with important physiological functions
  - It is a prime target for antibiotics
  - Any disruption of the membrane destroys the bacteria
- Unfortunately the structure of the bacterial plasma membrane is similar to the eukaryotic plasma membrane
  - This does not allow for selective toxicity

SYNTHESIS OF BACTERIAL PROTEINS

- Disruption in the production of protein is devastating to a bacterial cell
- Ribosomes of prokaryotes are not the same as those in the cytoplasm of eukaryotes
  - This allows for selective toxicity
SYNTHESIS OF BACTERIAL PROTEINS

- Eukaryotic cells have mitochondria that contain ribosomes
  - These are the same as the ribosomes in prokaryotic cells
  - There is antibiotic interference in eukaryotic cell function if antibiotics given in excessive amounts

SYNTHESIS OF BACTERIAL PROTEINS

- Antibiotics act at different sites on bacterial ribosomes
  - Streptomycin targets the 30S subunit
  - Chloramphenicol targets the 50S subunit
  - Some antibiotics interfere in peptide elongation
  - Some antibiotics interfere with decoding the message

SYNTHESIS OF BACTERIAL PROTEINS: Macrolides

- Macrolides block elongation of peptide chains in the 50S subunit
  - They include erythromycin, azithromycin, and clarithromycin
  - Narrow spectrum and used only for Gram-positive infections
SYNTHESIS OF BACTERIAL PROTEINS: Tetracyclines

- Tetracyclines have been used since the late 1940s
  - They are bacteriostatic
  - They block the arrival of tRNA at the A site
  - They have been in use for so long many bacteria are resistant
    - Use has steadily declined

SYNTHESIS OF BACTERIAL PROTEINS: Aminoglycosides

- Aminoglycosides have been in use since the 1940s
  - They target the 16S RNA portion of the 30S ribosomal subunit
- Gentamycin and tobramycin are potent against Gram-negative organisms
  - Not very effective against Gram-positive bacteria
  - Used in combination with β-lactam antibiotics
  - Produce significant renal toxicity and ototoxicity

BACTERIAL NUCLEIC ACIDS

- DNA and RNA are universal components
  - Their structure in bacteria is no different from their structure in humans
  - This does not allow for selective toxicity
- Two families of synthetic compounds can target bacterial nucleic acids
  - Quinolones and rifamycins
**BACTERIAL NUCLEIC ACIDS: Quinolones**

- Quinolones target bacterial topoisomerases
  - Bacterial topoisomerases are different to those in eukaryotic cells
  - They are an excellent target
  - Block the movement of the replication fork
  - Used in the treatment of:
    - Urinary tract infections
    - Osteomyelitis
    - Community-acquired pneumonia and gastroenteritis
    - Anthrax

**BACTERIAL NUCLEIC ACIDS: Rifamycins**

- Rifamycins bind to RNA polymerase and prevent it from functioning
  - Binding occurs away from the active site
  - Blocking RNA polymerase means no protein synthesis
    - This is lethal
  - Rifampin is the only rifamycin in use
    - It is used only in combination therapy
    - Resistance develops rapidly if it is used alone

**BACTERIAL METABOLISM**

- Two targets for inhibiting bacterial growth are:
  - Production of nucleic acid precursors
  - Metabolic pathways that occur at the plasma membrane
- Several pathways exclusive to bacteria:
  - Interruption selectively inhibits bacterial growth
  - This allows for selective toxicity
A good example is the metabolism of folic acid. One of the intermediates in the folic acid pathway is para-aminobenzoic acid (PABA). Normal enzyme action incorporates PABA into the pathway. Sulfur drugs competitively inhibit this activity:
- The enzyme is fooled into incorporating the sulfur molecule
- Incorporation of sulfur stops the pathway
- This is a lethal event

Action against the folic acid pathway has selective toxicity:
- Bacteria synthesize folic acid
- Humans obtain folic acid through diet
**BACTERIAL METABOLISM:**

**Folic acid**
- Sulfur drugs have been in use longer than any other antibacterial agent
- Sulfamethoxazole is usually used in combination with trimethoprim to treat urinary tract infections
  - Both of these drugs block a step in folic acid metabolism
  - These drugs have been used for a long time
    - Bacterial resistance continues to increase
    - Their effectiveness continues to decrease

**ANTIVIRAL DRUGS**
- Viruses pose a different set of problems for antibiotic therapy
  - They are obligate intracellular parasites
  - Drugs that can eliminate the virus are dangerous to non-infected cells
  - This makes selective toxicity difficult
- Many viruses difficult to grow
  - It is difficult to test potential antiviral drugs

**ANTIVIRAL DRUGS**
- The lack of rapid tests means it is difficult to differentiate between various viral infections
- Successful antiviral drugs must eliminate all virions
  - The escape of even one virion could restart the infectious cycle
ANTIVIRAL DRUGS

- The first antibiotic to be used against viruses was the sulfa drug derivative thiosemicarbazone.
- Molecular techniques are now used to develop antiviral drugs.

NUCLEOSIDE ANALOGS: Acyclovir

- Acyclovir is a nucleoside analog of guanine produced as a prodrug.
  - It is activated by enzymes once in the patient’s body.
  - These enzymes are found only in infected cells.
  - Acyclovir has selective toxicity.
- It works by blockade and termination of viral DNA replication.
- New variations (Valtrex® and Famvir®) are very effective and widely used.

NUCLEOSIDE ANALOGS: Acyclovir

- Acyclovir is a specific and nontoxic drug.
- It is highly effective against both genital and oral herpes simplex infections.
  - Also been used with some success in treatment of varicella-zoster (chickenpox and shingles).
- It can be taken intravenously or orally or used topically.
NUCLEOSIDE ANALOGS: AZT

- Azidothymidine is a nucleoside analog
- It is used in the treatment of HIV
- It inhibits reverse transcriptase

NUCLEOTIDE ANALOGS

- Similar to nucleoside analogs
- Do not require initial activation step
- Cidofovir is effective against a number of viruses, including cytomegalovirus, papillomavirus, herpesvirus, and poxvirus

NON-NUCLEOSIDE INHIBITORS

- Allosteric inhibitors of DNA polymerase
- Foscarnet is effective against herpesvirus and HIV
INHIBITING VIRAL ASSEMBLY AND RELEASE:
Protease Inhibitors

- Inhibit viral protease and interfere with assembly of virions
- Saquinavir and ritonavir, used for HIV

INHIBITING VIRAL ASSEMBLY AND RELEASE:
Neuraminidase Inhibitors

- Target the neuraminidase proteins of influenza
- Prevent budding of virions from host cell
- Examples are zanamivir and oseltamivir

INHIBITING VIRAL UNCOATING

- Binds a viral protein and inhibits uncoating
- Amantadine was used against influenza
- Influenza strains are now resistant to amantadine
INHIBITING VIRAL ENTRY

- An exciting area of research and development
- Focused on small molecules that inhibit one of the proteins the virus uses to fuse with plasma membranes

DRACO

- Double-stranded RNA Activated Caspase Oligomerizer
- Potentially effective against many infectious viruses
- Causes apoptosis in infected cells, but leaves uninfected cells unharmed

ANTIFUNGAL DRUGS: Polyenes

- Polyenes are produced by the soil bacterium *Streptomyces*
- They interact with sterols and increase the permeability of the plasma membrane
- They must be used with caution because of side effects
  - Amphotericin B has high renal toxicity
ANTIFUNGAL DRUGS:
Azoles

- Azoles (imidazole and triazole) inhibit the production of ergosterol
- Clotrimazole and miconazole are derivatives of imidazole
  - Sold without a prescription
  - Routinely used topically against athlete’s foot and vaginal yeast infection

ANTIFUNGAL DRUGS:
Azoles

- Ketoconazole is a broad spectrum derivative used for systemic fungal infections
  - Can be taken orally
  - Less toxic than amphotericin B
- Fluconazole and itraconazole are the least toxic azoles
  - Widely used for systemic fungal infections

ANTIFUNGAL DRUGS:
Other Antifungals

- Echinocandins inhibit glucan synthesis
  - Narrow spectrum and very specific
- Flucytosine interferes with DNA and RNA synthesis
  - It is taken up preferentially by fungi
  - Has a high level of toxicity in kidney and bone marrow
DRUGS FOR PARASITIC INFECTIONS

- The development of drugs for parasitic infections has lagged behind, because:
  - Parasitic infections do not occur often in developed nations
  - There was no money to be made

ANTI-PROTOZOAN DRUGS: Antimalarials

- Quinine has been used as a treatment for malaria since the 1600s
- It has been chemically modified into several synthetic forms
  - Chloroquine and mefloquine have been widely used

ANTI-PROTOZOAN DRUGS: Antimalarials

- Another quinine derivative:
  - Diiodohydroxyquin
    - Used for treatment of intestinal amebic disease
    - Found to be toxic to the optic nerve
ANTI-PROTOZOAN DRUGS:
Antimalarials

- *Plasmodium* develops resistance to many antimalarial drugs
- Artemisinin, primaquine, and quinacrine are more recently developed antimalarials

ANTI-PROTOZOAN DRUGS:
Folate Agonists

- Disrupt folic acid metabolism and block nucleic acid synthesis and repair
- Pyrimethamine and proquanil are antimalarial and used to treat toxoplasmosis

ANTI-PROTOZOAN DRUGS:
Drugs for Anaerobic Protozoa

- Metronidazole is one of the most widely used anti/protozoan drugs
  - Sold under the name Flagyl®
- It is the drug of choice for:
  - Vaginitis resulting from *Trichomonas vaginalis*
  - Giardiasis
  - Amebic dysentery
  - *Clostridium difficile*
ANTI-PROTOZOAN DRUGS:
Drugs for Anaerobic Protozoa

- Metronidazole and iodoquinol used to treat amebiasis caused by *Entamoeba histolytica*

ANTI-PROTOZOAN DRUGS:
Drugs for *Trypanosoma* and *Leishmania*

- Eflornithine has greatly reduced infection by *Trypanosoma brucei* (cause of African sleeping sickness)
- Melarsoprol is used to treat African sleeping sickness and Chagas’ disease
- Sodium stibogluconate is used to treat leishmaniasis

ANTHELMINTHICS

- Anti-helminthic drugs have also been largely ignored until recently
  - Affected populations were not found in developed countries
  - The popularity of sushi has led to an increase in tapeworm infestations
  - Increased world travel has also increased helminth infections
ANTHELMINTHICS: Nematodes

- Ivermectin is used for a wide variety of internal nematode infections
- Piperazine and tetrahydropyrimidine are also used for nematode infections