Development of Chemotherapy

Chemotherapeutic agents are chemicals used to treat disease:
- Destroy pathogenic microbes or inhibit their growth within host
- Most are antibiotics, i.e., microbial products or their derivatives that kill susceptible microbes or inhibit their growth

Paul Ehrlich (1904)
- Developed concept of selective toxicity
- Identified dyes that effectively treated African sleeping sickness
- With Sahachiro Hato (1910), identified arsenic compounds that effectively treated syphilis

Gerhard Domagk, and Jacques and Therese Trefouel (1935) discovered sulfonamides and sulfa drugs
- First discovered by Ernest Duchesne (1896), but the discovery was lost
- Subsequently, accidentally discovered by Alexander Fleming (1928)
- Observed penicillin activity on contaminated bacterial plate
- Did not think could be developed further
Development of Chemotherapy (cont.)

- Effectiveness demonstrated by Florey, Chain, and Heatley (1939)
- Fleming, Florey, and Chain received Nobel Prize in 1945 for discovery and production of penicillin

Characteristics of Antimicrobials

- Selective toxicity – ability of drug to kill or inhibit pathogen while damaging host as little as possible
- Therapeutic dose – drug level required for clinical treatment
- Toxic dose – drug level at which drug becomes too toxic for patient (i.e., produces side effects)

Characteristics of Antimicrobials (cont.)

- Therapeutic index – ratio of toxic dose to therapeutic dose
- Side effects – undesirable effects of drugs on host cells
- Narrow-spectrum drugs – attack only a few different pathogens
- Broad-spectrum drugs – attack many different pathogens

Streptomycin

- Antibiotic active against tuberculosis
- Discovered by Selman Waksman (1944)
- By 1953 chloramphenicol, terramycin, neomycin, and tetracycline isolated

Biological Sciences

Dr. Cooper
Characteristics of Antimicrobials (cont.)

- Effect of an agent may vary with concentration, microbe, host
- Effectiveness expressed in two ways
  - Minimal inhibitory concentration (MIC) - lowest concentration of drug that inhibits growth of pathogen
  - Minimal lethal concentration (MLC) - lowest concentration of drug that kills pathogen

Effectiveness expressed in two ways:

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Table 9.1: Familiarize, don’t memorize

Four Common Types of Inhibitors
- Cell Wall Synthesis
- Protein Synthesis
- Nucleic Acid Synthesis
- Metabolism

Antimicrobial Activity

- Determining the level of antimicrobial activity can be performed in a number of ways to help estimate the proper therapeutic dose and effectiveness
- Dilution susceptibility tests for MIC
- Disk diffusion tests – Kirby Bauer
- The E-test MIC and diffusion

Antimicrobial Activity (cont.)

- Dilution susceptibility tests
  - Involves inoculating media containing different concentrations of drug
  - Broth or agar with lowest concentration showing no growth is MIC
  - If broth used, tubes showing no growth can be subcultured into drug-free medium
  - Broth from which microbe can’t be recovered is MLC
Antimicrobial Activity (cont.)

- Disk Diffusion tests
  - Disks impregnated with specific drugs are placed on agar plates inoculated with test microbe
  - Drug diffuses from disk into agar, establishing concentration gradient
  - Observe clear zones (no growth) around disks

Kirby-Bauer method
- Standardized method for disk diffusion test
- Sensitivity/resistance determined using tables relating zone diameter with microbial resistance
- Table values plotted, used to determine if effective concentration of drug in body can be reached

Etest
- Similar to disk diffusion method, but uses strip rather than disk
- E-test strips contain a gradient of an antibiotic
- Intersection of elliptical zone of inhibition with strip indicates MIC

Antibacterial Drugs
- Inhibitors of cell wall synthesis
  - Penicillins
    - Most are 6-aminopenicillanic acid derivatives and differ in side chain attached to amino group
    - Most crucial feature - β-lactam ring
    - Essential for bioactivity
    - Many penicillin resistant organisms produce β-lactamase (penicillinase) which hydrolyzes a bond in this ring
Antibacterial Drugs (cont.)

- Mode of action
  - Blocks the enzyme that catalyzes transpeptidation (formation of cross-links in peptidoglycan)
  - Prevents the synthesis of complete cell walls leading to lysis of cell
  - Acts only on growing bacteria that are synthesizing new peptidoglycan

Other actions of penicillins

- Bind to periplasmic proteins (penicillin-binding proteins, PBPs)
- May activate bacterial autolysins and murein hydrolases
- Stimulate bacterial holins to form holes or lesions in the plasma membrane

Antibacterial Drugs (cont.)

- Naturally occurring penicillins (penicillin V and G) are narrow spectrum
- Semisynthetic penicillins have a broader spectrum than naturally occurring ones
- Resistance to penicillins, including the semisynthetic analogs, continues to be a problem
- ~1–5% of adults in U.S. are allergic to penicillin (can lead to a violent allergic response and death)

Cephalosporins

- Structurally and functionally similar to penicillins
- Broad-spectrum antibiotics that can be used by most patients that are allergic to penicillin
- Four categories based on their spectrum of activity

Vancomycin

- Glycopeptide antibiotics that inhibit cell wall synthesis by binding to area of peptide bridge formation in peptidoglycan
- Important for treatment of antibiotic-resistant staphylococcal and enterococcal infections
- Previously considered “drug of last resort” so rise in resistance to vancomycin is of great concern
- Teicoplanin – similar to vancomycin, but with fewer side effects

Protein synthesis inhibitors

- Many bind specifically to the bacterial ribosome
- Others inhibit a step in protein synthesis
  - Aminoacyl-tRNA binding
  - Peptide bond formation
  - mRNA reading
  - Translocation
Antibacterial Drugs (cont.)

- Aminoglycosides
  - Large group, all with a cyclohexane ring, amino sugars
  - Bind to 30S ribosomal subunit.
    - Interferes with protein synthesis by directly inhibiting the process
    - Causes misreading of the messenger RNA to produce abnormal proteins, which lead to hydroxyl radical formation
  - Bactericidal effect

- Tetracyclines
  - All have a four-ring structure to which a variety of side chains are attached
  - Are broad spectrum, bacteriostatic
  - Combine with 30S ribosomal subunit to inhibit the binding of aminoacyl-tRNA molecules to the A site of the ribosome

- Macrolides
  - Contain 12- to 22-carbon lactone rings linked to one or more sugars
  - Erythromycin
    - Broad spectrum, usually bacteriostatic
    - Inhibits peptidyl chain elongation
  - Chloramphenicol
    - Now is chemically synthesized
    - Binds to 23s rRNA on 50S ribosomal subunit and inhibits peptidyl transferase reaction
    - Toxic with numerous side effects so only used in life-threatening situations

- Metabolic antagonists
  - Act as antimetabolites that antagonize or block functioning of metabolic pathways by competitively inhibition of key enzymes
  - Are structural analogs, i.e., molecules that are structurally similar to, and compete with, naturally occurring metabolic intermediates
Antibacterial Drugs (cont.)

- Sulfonamides (sulfa drugs)
  - An analog of paminobenzoic acid (PABA)
  - PABA used for the synthesis of folic acid and is made by many pathogens
  - Selectively toxic due to competitive inhibition of folic acid synthesis enzymes.

Trimethoprim
- Synthetic antibiotic that also interferes with folic acid production
- Broad spectrum
- Can be combined with sulfa drugs to increase efficacy of treatment; combination blocks two steps in folic acid pathway
- Has a variety of side effects including abdominal pain and photosensitivity reactions

Quinolones
- Broad-spectrum, synthetic drugs containing the 4-quinolone ring
- Nalidixic acid first synthesized quinolone (1962)
- Act by inhibiting bacterial DNA-gyrase and topoisomerase II
- Broad spectrum, bactericidal, wide range of infections

Antifungal Drugs

- Fewer effective agents because of similarity of eukaryotic fungal cells and human cells
- Many are toxic when applied systemically
- Fungal infections are typically divided into different groups which are generally treated different from one another
- Fungal infections: superficial, subcutaneous, and systemic

- Treatment of superficial infections
  - Imidazole drugs – disrupt cell membrane permeability and inhibits sterol synthesis
  - Nystatin – polypeptide that damages the cell membrane and inhibits sterol synthesis
  - Griseofulvin – disrupts mitotic spindle

Elizabeth Hazen and Rachael Brown: Co-discoverers of Nystatin (1950)

http://www.wadsworth.org/images/BrownHazen03.jpg
Antifungal Drugs (cont.)
- Treatment of subcutaneous and systemic infections
  - Amphotericin – polyene drug that damages the cell membrane and inhibits sterol synthesis
  - 5-flucytosine – disrupts RNA function
  - Azoles – inhibits cell membrane synthesis
  - Atovaquone – ubiquinone analog that suppresses electronic transport during respiration

Antiviral Drugs
- Drug development has been slow because it is difficult to specifically target viral replication
- Drugs currently used inhibit virus-specific enzymes and life cycle processes
- Amantidine
  - Used to prevent influenza infections
  - Blocks penetration and uncoating of influenza virus

Antiviral Drugs (cont.)
- Adenine arabinoside (vidarabine) - inhibits herpes virus enzymes involved in DNA and RNA synthesis and function
- Tamiflu
  - Anti-influenza agent that is a neuraminidase inhibitor
  - Not a cure for influenza, but has been shown to shorten course of illness

Antiviral Drugs (cont.)
- Acyclovir - inhibits herpesvirus DNA polymerase
- Valacyclovir - prodrug form of acyclovir
- Ganciclovir - anti-herpesvirus drug
- Foscarnet - inhibits herpes virus DNA polymerase

Antiprotozoal Drugs
- The mechanism of drug action for many antiprotozoal drugs is not known
- Some antibiotics that inhibit bacterial protein synthesis are used against protozoa
- Examples of available drugs
  - Chloroquine and mefloquine – malaria
  - Metronidazole – Entamoeba infections
  - Atovaquone – Toxoplasma gondii
Drug Resistance

- An increasing problem
- Once resistance originates in a population, it can be transmitted to other bacteria
- A particular type of resistance mechanism is not confirmed to a single class of drugs
- Microbes in abscesses or biofilms may be growing slowly and not be susceptible
- Resistance mutants arise spontaneously and are then selected

Drug Resistance (cont.)

- Mechanisms of drug resistance
  - Prevent entrance of drug
  - Drug efflux (pump drug out of cell)
  - Inactivation of drug by chemical modification of drug by pathogen
  - Modification of target enzyme or organelle
  - Use of alternative pathways or increased production of target metabolite

Drug Resistance (cont.)

- Origin and transmission of drug resistance
  - Immunity (resistance) genes exist in nature to protect antibiotic producing microbes from their own antibiotics
  - Horizontal gene transfer transferred immunity genes from antibiotic producers to non-producing microbes

Drug Resistance (cont.)

- Resistance genes can be found on:
  - Bacterial chromosomes - resistance from (rare) spontaneous mutations (usually result in a change in the drug target)
  - Plasmids - can be transferred to other cells by conjugation, transduction, and transformation
  - Transposons - can move rapidly between plasmids and through a bacterial population