Antibacterial Agents
Pharmacology

PHPP 516 (IT-II)
Spring 2011
JACOBS

CLASS DATES:
• March 7, 1:00-2:50 PM
• March 8, 1:00-2:50 PM
• March 10, 4:00-4:50 PM
• March 11, 1:00-1:50 PM
Learning objectives

1. Define the concepts of selective toxicity and therapeutic index as applied in antibacterial therapy and provide examples.

2. Explain the difference between bacteriostatic and bactericidal agents.

3. Explain the difference between a minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC) and how these are determined experimentally.

4. Explain the main pharmacokinetic determinants of a clinical response to antibacterial agents, and provide examples.

5. Describe the concept of antibacterial spectrum, and provide examples of broad- and narrow- spectrum agents.
Learning objectives

6. Differentiate between the terms resistant, tolerant, and susceptible as they pertain to pathogenic bacteria and drug therapy.

7. Explain the concept of fractional inhibitory concentration (FIC) and know the significance of an FIC plot for antibacterial agents that are synergistic, additive, or antagonistic.

8. Describe the enzymes and building blocks involved in the biosynthesis of cell walls for both gram (+) and gram (-) bacteria.

9. Recall whether a cell wall biosynthesis inhibitor is a transglycosylase, transpeptidase, or pyrophosphatase inhibitor, and describe their mechanisms of action.
Learning objectives

10. Compare the oral bioavailabilities, metabolism, half-lives, drug interactions, adverse effects, and drug interactions of the cell wall biosynthesis inhibitors.

11. Explain why imipenem is administered with cilastatin, and other carbapenems can be administered alone.

12. Recall the spectrum of activities of cell wall biosynthesis inhibitors.

13. Describe the steps in bacterial protein synthesis and the cellular components involved.

14. Recall whether a specific protein synthesis inhibitor acts on the 30S or 50S ribosomal subunit.

15. Compare the oral bioavailabilities, metabolism, half-lives, drug interactions, adverse effects, and drug interactions of protein synthesis inhibitors.
Learning objectives

16. Recall the spectrum of activities of protein synthesis inhibitors.

17. Recall UNIQUE adverse effects of antibiotics (ALL lectures) and which drugs cause them (such as: bone and teeth deposition; vestibular problems; nephrotoxicity; ototoxicity; cholestatic jaundice; respiratory failure in myasthenia gravis; hepatotoxicity; blood dyscrasias; gray baby syndrome; and achilles tendon rupture).

18. Describe the steps and enzymes involved in bacterial DNA replication.

19. Explain the antibacterial mechanism of action of the fluoroquinolones.

20. Recall the spectrum of activities of the fluoroquinolones.
Learning objectives

21. Compare the oral bioavailabilities, metabolism, half-lives, drug interactions, adverse effects, and drug interactions of the fluoroquinolones.

22. Explain the antibacterial mechanism of action of folate antagonists.

23. Explain the rationale for combining sulfamethoxazole and trimethoprim in antibacterial therapy.

24. Recall the pharmacokinetics, adverse effects, and drug interactions of sulfamethoxazole and trimethoprim.


26. Describe the mechanism of action of gramicidin and daptomycin.
Learning objectives

27. Recall how some antibiotics produce toxic metabolites, including: nitroimidazoles (metronidazole and tinidazole); nitrofurans (nitrofurantoin); and methenamine. Explain their clinical uses and potential toxicities.
Antibacterial PC Topics:

- **Overview of Antibacterial Pharmacology**
- **Drugs by Drug Targets:**
  - Cell Wall Biosynthesis
  - Protein Biosynthesis
  - DNA Synthesis
  - Folate Metabolism
  - Novel Antibiotic Mechanisms
History of Antibacterials

Paul Ehrlich – Theory of the “Magic Bullet” (1910)

Agents that selectively target pathogens could be used to treat infections with high efficacy and minimal detriment to the patient.

Paul Ehrlich and Hata Sahachiro

- Set the framework for modern drug discovery
- Began work with a toxic lead compound, arsanilic acid (atoxyl)
- Made chemical modifications to optimize selectivity
- Tested hundreds of compounds against Treponema pallidum (syphilis)
- Compound “606”, called salvarsan (arsphenamine) worked well – first modern chemotherapeutic (but it did have serious side effects!)
History of Antibacterials

John Scott Burdon-Sanderson (1870)
Mold-infected culture medium does not grow bacteria

Joseph Lister (1871)
Mold-infected urine samples do not grow bacteria

William Roberts (1874)
Bacteria do not grow in the presence of *Penicillium glaucum*

Louis Pasteur (1877)
Anthrax cultures do not grow in the presence of *Penicillium notatum*

Ernest Duchesne (1897)
*Penicillium glaucum* used to cure typhus (in infected guinea pigs)
History of Antibacterials

**Alexander Fleming (1928)**

Absence of *Staphylococcus aureus* growth around mold colonies, coined the term “penicillin” for active component

“I certainly didn't plan to revolutionize all medicine by discovering the world's first antibiotic, or bacteria killer, but I guess that was exactly what I did”.

**Howard Florey  Ernst Chain  Norman Heatley (1939)**

Discovered the chemical structure of penicillin, and how to extract it from mold cultures and use it therapeutically

**Nobel Prize, 1945 (Flemming, Florey and Chain)**
History of Antibacterials

Penicillin – early days

Penicillin was expensive, rare
Often recycled from urine
Purified and re-used

- Penicillin has a short half-life (30 min)
- Rapid urinary excretion by organic anion transporter (OAT)
- Probenecid (anti-gout medication) was used to inhibit the renal OAT, increasing penicillin half-life
History of Antibacterials

Gerhard Domagk (1935)

Developed the first commercially available antibiotic at Bayer laboratories in Germany, sulfanilamide (Prontosil). Identified from screening compound libraries (of dyes). No activity unless converted to active drug in vivo.

Nobel Prize, 1939

Prontosil used widely in both Allied and Axis field kits in WWII

But penicillin only available to Allied Forces
History of Antibacterials

Selman Waksman (1948)

Isolated soil bacteria (actinomycetes) and tested them for the ability to inhibit bacterial growth. He and graduate student, Albert Schatz identified and patented streptomycin (at Rutgers). First clinically effective antibiotic against tuberculosis.

Nobel Prize, 1952

Albert Schatz later sued Waksman and Rutgers for lack of proper credit and was awarded an undisclosed sum.
Basic Concepts in Antibacterial Therapy

**Selective Toxicity**

Agent demonstrates activity against the pathogen, but not the host (magic bullet)

**Therapeutic Index (Window)**

Defined as the toxic dose 50% divided by the effective dose 50%

\[ TI = \frac{TD_{50}}{ED_{50}} \]

Drugs with high (wide) TI are relatively safe
Drugs with low (narrow) TI typically need monitoring

e.g. **Penicillin G** – wide therapeutic index
**Vancomycin** – narrow therapeutic index (nephro-, ototoxicity)
Basic Concepts in Antibacterial Therapy

Bacterial Growth Curves

- **Lag phase**: Initial phase where bacteria adapt to the new environment.
- **Exponential growth phase**: Phase where bacteria multiply rapidly with rich nutrients.
- **Stationary phase**: Phase where growth rate equals death rate, and biomass remains constant.
- **Death phase**: Phase where nutrients and waste are depleted, leading to cell death.

**Log Growth**: Growth rate measured on a log scale, showing different phases of bacterial growth over time.
Basic Concepts in Antibacterial Therapy

Drug Tolerance vs. Drug Resistance

Comparison of different bacterial strains with one antibiotic

- **Resistant** (or no drug added)
- **Tolerant**
- **Susceptible**

Growth (O.D.) vs. Time

antibiotic \((X)\) added
Basic Concepts in Antibacterial Therapy

**Bacteriostatic vs. Bactericidal**

Comparison of different antibiotics in a given strain

![Graph showing the difference between bacteriostatic and bactericidal effects](chart.png)

**Bacteriostatic**: defined by inhibiting the growth of the pathogen.
- Measure of bacteriostatic activity:
  - Minimum Inhibitory Concentration (MIC)

**Bactericidal**: defined by killing the pathogen.
- Measure of bactericidal activity:
  - Minimum Bactericidal Concentration (MBC)
Basic Concepts in Antibacterial Therapy

**Bacteriostatic vs. Bactericidal**

Tube dilution method

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<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Liquid Medium + Ab</th>
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<tr>
<td>128</td>
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<tr>
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MIC

Shaking Incubator “shaker”
Basic Concepts in Antibacterial Therapy

**Bacteriostatic vs. Bactericidal**

**Tube dilution method**

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<th>µg/ml antibiotic</th>
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MIC (will not grow)

MBC (99.9% dead)

Solid Medium (agar) - Ab
Basic Concepts in Antibacterial Therapy

PK Determinants of Clinical Response

**Type I:** PEAK/MIC
- Goal: Maximize drug concentrations
- Examples: Aminoglycosides

**Type II:** TIME > MIC
- Goal: Maximize duration of drug exposure
- Examples: Penicillins, Macrolides, Linezolid

**Type III:** AUC/MIC
- Goal: Maximize amount of drug (AUC)
- Examples: Fluoroquinolones, Tetracyclines, Vancomycin
Basic Concepts in Antibacterial Therapy

Antibacterial Spectrum

**Antibacterial Spectrum** = Range of antibiotic activity

- **Broad-spectrum drug**: active against a wide variety of gram(+) and gram(-) bacteria
- **Narrow-spectrum drug**: only active against a limited range of bacteria.

Methods of Antibiotic Use

**Prophylaxis**: To prevent infections in susceptible patients

**Empiric**: To treat an infection, organism not identified

**Definitive**: Knowledge of organism and drug sensitivity guide use

*This is when knowing the antibiotic spectrum is most useful*
Basic Concepts in Antibacterial Therapy

Susceptibility

Epsilometer test
(Etest)

Vancomycin

MIC
Basic Concepts in Antibacterial Therapy

Susceptibility

Kirby-Bauer Disk Diffusion Method

A – G = Different Antibiotics, standard concentrations

• Diameter correlates with sensitivity
• Antibiotics have different diameters for “zone of inhibition” (in mm) that define ‘susceptible’: sensitive; intermediate; resistant

Etest Method

Different Antibiotics, range of concentrations
Basic Concepts in Antibacterial Therapy

**Susceptibility**

- Serum concentration vs. concentration at site of infection are not always the same
- **Route of administration** can determine local concentrations
- **Local factors** at site of infection that can inhibit efficacy:
  - ✓ low tissue perfusion (drug access)
  - ✓ low pH (drug stability)
  - ✓ high protein concentration (adsorb drug)
  - ✓ low oxygen tension (anaerobic conditions) – dormancy
If you combine antibiotics, it's important to know how they will interact in fighting infections!

**FIC** = Fractional Inhibitory Concentration
i.e. SUM of drug conc. A + drug conc B
(ratio of MIC A + ratio of MIC B)

**Synergistic example:**
- **Vancomycin**
  (cell wall biosynthesis inhibition)
- **Kanamycin**
  (protein biosynthesis inhibition)

**FIC < 1 BECAUSE OF COMPLIMENTARY MECHANISMS OF ACTION**
Pathogenic Bacteria
Common Tests for Identification

**Oxidase Test** (Kovaks Oxidase Test)

- **Indophenol oxidase**
  - Colorless reagent
  - Dark purple

**Lactose Fermentation Test**

- **MacConkey agar**
  - Neutral red dye
  - lac+ acidic (Red/Pink)
  - lac- basic (Yellow)

**Catalase Test**

- **catalase**
  - $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$
Pathogenic Bacteria
Common Tests for Identification

**Coagulase Test** (*Staphylococcus*)

- **coagulase**
- *(+) prothrombin*
- **fibrinogen** → **fibrin**

**Hemolysis Test**

- Sheep blood agar
  - **α-hemolysis** – *incomplete*
  - **β-hemolysis** – *complete*
  - **γ-hemolysis** – *absent*
Pathogenic Bacteria

GRAM (+) Pathogens (BACILLI)

Bacilli

Obligate Anaerobes
Clostridium
Propionibacterium
Actinomyces

Aerobic
Bacillus
Corynebacterium
Listeria
Lactobacillus
Gardnerella

Cocci

Next Slide
Pathogenic Bacteria

GRAM (+) Pathogens (COCCI)

- **Bacilli**
  - Previous Slide

- **Cocci**

  - **Obligate Anaerobes**
    - Peptococcus
    - Peptostreptococcus

  - **Aerobic**

    - **Catalase (+)**
      - Coagulase (+)
        - *Staphylococcus aureus*
      - Coagulase (-)
        - *Staphylococcus epidermidis*
        - *Staphylococcus saprophyticus*
      - α-hemolysis
        - *Streptococcus pneumoniae*
        - *Streptococcus pyogenes*
    - β-hemolysis
      - *Streptococcus agalactiae*
    - No hemolysis (γ)
      - *Enterococcus faecium*
      - *Enterococcus faecalis*
Pathogenic Bacteria

**GRAM (−) Pathogens**

- **Bacilli**
  - Obligate Anaerobes
    - Bacteroides
    - Fusobacterium
  - Aerobic
    - Oxidase (−)
      - Lac (−)
        - Salmonella
        - Shigella
        - Yersenia
      - Lac (+/−)
        - Serratia
        - Citrobacter
      - Lac (+)
        - Escherichia
        - Enterobacter
c
- **Cocci**
  - Neisseria
  - Moraxella
- **Spirochetes**
  - Borrelia
  - Leptospira
  - Treponema

**Enterobacteriaceae**

- Oxidase (+)
  - Aeromonas
  - Pseudomonas
  - Vibrio
  - Haemophilus
Pathogenic Bacteria

ATYPICAL Pathogens

Facultative or obligate intracellular bacteria

GRAM (+)

*Bacillus anthracis* (Anthrax)
*Propionibacterium acnes* (Acne)

GRAM (-)

*Yersinia pestis* (Bubonic plague)
*Bordetella pertussis* (Whooping cough)
*Treponema pallidum* (Syphilis)

*Chlamydia tachomatis* (Chlamydia)
*Francisella tularensis* (Tuleremia)
*Legionella pneumophila*
*Mycoplasma, Ureaplasma*

*Ehrlichia* (Erlichiosis, Rocky mountain unspotted fever)
*Rickettsia rickettsii*
Pathogenic Bacteria

ATYPICAL Pathogens

IN THE NEWS

Feb 2011:
About 200 partygoers become ill

Legionella anyone?
Antibacterial PC Topics:

• Overview of Antibacterial Pharmacology
• **Drugs by Drug Targets:**
  • Cell Wall Biosynthesis
  • Protein Biosynthesis
  • DNA Synthesis
  • Folate Metabolism
  • Novel Antibiotic Mechanisms
Cell Wall Biosynthesis

Building Blocks

N-Acetylglucosamine (GlcNAc)

Symbols:

Muramyl pentapeptide

DAP = diaminopimelic acid
Cell Wall Biosynthesis

Enzymes:
1 = MraY translocase, 2 = MurG translocase
3 = Transglycosylase
4 = Pyrophosphatase
Cell Wall Biosynthesis

Lysine – NH$_2$ attack

OR

DAP – NH$_2$ attack

STEP

5

Enzyme:

5. Transpeptidase
Cell Wall Biosynthesis

**Gram (+)**

**Lipoteichoic acid (LTA)**

Peptidoglycan layer "murein"

C.M.
Cell Wall Biosynthesis

Gram (-)

Lipopolysaccharide (LPS)

Porin

Lipoproteins

Periplasmic space

C.M.

Peptidoglycan layer “murein”

Structure of Lipopolysaccharide

Lipid A

O-antigen repeat 40 units

Core polysaccharide

Disaccharide diphosphate

Fatty acids

Gram (‐)
Cell Wall Biosynthesis

Classes of Cell Wall Biosynthesis Inhibitors

• Glycopeptides
• β-Lactams
  ▸ Penicillins
  ▸ Cephalosporins
  ▸ Carbapenems
• Monobactams
• Bacitracin

Transglycosylase Inhibitors

Transpeptidase Inhibitors

Pyrophosphatase Inhibitor

All are Mainly Bactericidal
Cell Wall Biosynthesis
Classes of Cell Wall Biosynthesis Inhibitors

• Glycopeptides
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Cell Wall Biosynthesis

Glycopeptide Antibiotics

Both vancomycin and the lipid glycopeptides (telavancin) bind to terminal alanine residues on bacterial peptidoglycan.

Vancomycin (Vancocin®) – FDA 1964

**MECHANISM:**
Inhibits various steps in cell wall biosynthesis, including transpeptidase and transglycosylase ($IC_{50} = 380$ nM)*

*PNAS (2003) 100(10): 5658-5663*
**MECHANISM:**
Believed to be *mainly* by transglycosylase inhibition*

*Lipoglycopeptide antibiotics*

**Telavancin** (Vibativ™) – FDA 2009

In Development:
- Ramoplanin, Oritavancin
- Dalbavancin (Zevin®)
- Teicoplanin (Targocid®)

*PNAS (2003) 100(10): 5658-5663*
Cell Wall Biosynthesis

CELL WALL BIOSYNTHESIS INHIBITORS: GLYCOPEPTIDES

Vancomycin (Vancocin®) – 1958

ANTIBACTERIAL SPECTRUM:

• HIGH ACTIVITY: broad-spectrum against gram (+)
  ‣ Gram (+) cocci:
    Staphylococci; Streptococci; Enterococci
    MRSA (good); MSSA (moderate)
  ‣ Gram (+) bacilli:
    Clostridium difficile
    (Pseudomembranous colitis) – ORAL use

• POOR Activity Against:
  ‣ Gram (-) bacteria
    But CAN be combined to enhance the activity of aminoglycosides or β-lactams
Cell Wall Biosynthesis

CELL WALL BIOSYNTHESIS INHIBITORS: GLYCOPEPTIDES

Vancomycin

PHARMACOKINETICS:

• Administration: ORAL, INJ (IV)
• Oral bioavailability: VERY POOR
• CNS Penetration:
  • Normal meninges: NIL
  • Inflamed meninges: 20-30%
• Half-life: 4-6 hr (normal renal function)
• Half-life: 200-250 hr (end-stage RENAL disease)
• Excretion:
  • Oral: 99% FECES, unchanged (NO metabolism)
  • IV: 80-90% URINE, unchanged (NO metabolism)
Cell Wall Biosynthesis

CELL WALL BIOSYNTHESIS INHIBITORS: GLYCOPEPTIDES

Vancomycin

ADVERSE EFFECTS:

• Histamine release with fast infusion rate
  • Red Man syndrome (flushing, hypotension) – alleviated with antihistamines, not an allergic reaction
• Superinfection by *C. difficile* (CDAD)
• Ototoxicity (hearing loss – extremely rare)
• Nephrotoxicity at high doses. Rare, however, avoid simultaneous use of nephrotoxic drugs (e.g. avoid gallium nitrate)

RESISTANCE:

• Vancomycin-resistant enterococci (VRE) is a problem, particularly *E. faecium* – conservative use is recommended
Cell Wall Biosynthesis

**TRANSGLYCOSYLASE INHIBITORS: LIPOGLYCOPEPTIDES**

**Telavancin** (Vibativ™) – 2009

**ANTIBACTERIAL SPECTRUM:**
- Similar spectrum to vancomycin
- Approved for treatment of complicated skin and skin structure infections (**cSSSI**) – not approved for *C. dif*

**ADVERSE EFFECTS:**
- **Teratogenic** (Black Box Warning – not for pregnant women!)
- QTc prolongation
- Nephrotoxicity
- Superinfection (CDAD)
Cell Wall Biosynthesis
Classes of Cell Wall Biosynthesis Inhibitors

• Glycopeptides
• β-Lactams
  ▸ Penicillins
  ▸ Cephalosporins
  ▸ Carbapenems
• Monobactams
• Bacitracin
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS

**MECHANISM:**
Inhibition of transpeptidase step of cell wall biosynthesis

- **β-Lactams**
  - Penicillins
  - Cephalosporins
  - Carbapenems
  - Monobactams
  - Aztreonam

Transpeptidase step
Cell Wall Biosynthesis

**TRANSPEPTIDASE INHIBITORS**

\[ \text{TP} \xrightarrow{\text{Ser-OH}} \]

\[ \text{TP} \xrightarrow{\text{NH}_2} + \]

\[ \text{Isopeptide bond} \]

\[ + \text{TP} \]
Cell Wall Biosynthesis

**TRANSPEPTIDASE INHIBITORS**

Why are TP-inhibitors bactericidal when used at high-enough concentrations?

Answer: Because even in non-growing (but metabolically active cells) cell wall remodeling still goes on. This remodeling is performed by a class of enzymes called murein hydrolases (aka autolysins). The cell walls are broken-down but not rebuilt, leading to cell lysis (cell death).
Penicillin Binding Proteins (PBPs)

*Transpeptidase* (TP) is a PBP, but it is NOT the only protein that becomes covalently attached to penicillins.
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS

Other PBPs (cell-wall remodeling enzymes)

- **D-alanine carboxypeptidase**
  - Not available for other X-links
  - Ala

- **Peptidoglycan endopeptidase**
  - Contributions to penicillin’s efficacy is unclear
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

GENERAL PROPERTIES

• DIFFER IN:
  ✓ Antibacterial Spectrum
  ✓ Stability to stomach pH
  ✓ Susceptibility to Enzymatic degradation

• EFFECTIVE AGAINST:
  ✓ Replicating OR metabolically active bacteria
    (must be dividing OR actively remodeling their cell walls)
  ✓ Must synthesize a peptidoglycan cell wall
    Inactive against organisms with non-peptidoglycan cell walls (mycobacteria, mycoplasma, fungi)
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

GENERAL PROPERTIES

• SAFETY: Safe, wide therapeutic index

• COMMON ADVERSE EFFECTS
  ▸ Hypersensitivity (allergy)
    • Urticaria (hives)
    • Angioedema (swelling of face, lips, tongue)
  ▸ Diarrhea
    • Killing of normal intestinal microflora, OR
    • *C. difficile* overgrowth (diarrhea-inducing toxins)
  ▸ Superinfection (proliferation of resistant bacteria or fungi)
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

GENERAL PROPERTIES

• ANTIBACTERIAL SPECTRUM:
  • Gram (+) have cell wall that is easily permeated
    • Against enterococci: ONLY Bacteriostatic, unless also combined with an aminoglycoside
  • Gram (-) have outer membrane. Access to PG layer gained through porins – POLAR penicillins have LESS permeability

• DRUG RESISTANCE: (acquired by plasmid transfer)
  1. Drug hydrolysis by $\beta$-lactamase (penicillinase) activity
  2. Decreased permeability to drug
     (altered porins in gram (-) bacteria)
  3. Altered transpeptidases
Cell Wall Biosynthesis

TRANSPERTIDASE INHIBITORS: PENICILLINS

GENERAL PROPERTIES

• PK DISTRIBUTION:
  • WIDE, except:
    • Do NOT enter BONE well – requires prolonged treatment to treat infections in bone
    • Do NOT normally enter CNS, but access is improved if meninges is inflamed

• COMMON DRUG INTERACTIONS
  • Tetracyclines – bacteriostatic: since penicillins are MOST effective against dividing bacteria, co-administration of tetracyclines would reduce their efficacy (What does this imply for the FIC?)
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

Penicillins

1. Natural Penicillins
2. Antistaphylococcal Penicillins
3. Aminopenicillins
4. Extended Spectrum Penicillins (Antipseudomonal Penicillins)
5. β-Lactamase Inhibitors
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

1. Natural Penicillins

   **Penicillin G** (Benzylpenicillin)
   **Penicillin V** (Phenoxyethylpenicillin)
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: PENICILLINS

1. Natural Penicillins

ANTIBACTERIAL SPECTRUM:

• Bactericidal against gram (+) and some gram (-):
  • HIGH ACTIVITY:
    ▶ Gram (+) cocci: most Streptococci
    ▶ Gram (+) bacilli: *Listeria monocytogenes*
    ▶ Gram (-) cocci: *Neisseria meningitidis* (meningitis)
    ▶ Gram (-) spirochetes: *Treponema pallidum* (syphilis)
  • NO ACTIVITY:
    ▶ most Staphylococci
    ▶ *Enterobacteriaceae*
    ▶ *Pseudomonas*
    ▶ *Bacteroides fragilis*
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

1. Natural Penicillins

PHARMACOKINETICS:

• Administration:
  • **G**: (acid unstable)
    • **Aqueous** (IV)
    • **Procaine** penicillin G: (IM), slow absorption
      1-4 hr peak (duration ~ 1 day)
    • **Benzathine** penicillin G: (IM), very slow absorption
      12-24 hr peak (duration ~ 1-4 weeks)
  • **V**: oral (acid stable) – systemic levels much lower
    • Low levels mean less effect against gram (-)
    • Oral bioavailability: 60%
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

1. Natural Penicillins

PHARMACOKINETICS:

• Half-lives:
  • G (aq.), V: SHORT, 30 min

• Protein binding, MODERATE:
  • G: 60%
  • V: 80%

• Metabolism:
  • G: 30% hepatic (hydrolysis of β-lactam)
  • V: 55% hepatic (hydrolysis of β-lactam)

• Excretion: Mostly in urine (unchanged drug and metabolites)
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: PENICILLINS

1. Natural Penicillins

RESISTANCE:
- Staphylococci developed rapid resistance to penicillin
- Resistance, both gram (+) and gram (-): \( \beta \)-Lactamase
- Gram (-) \( \beta \)-Lactamase found in periplasmic space
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

2. Antistaphylococccyl Penicillins

- Dicloxacillin
- Nafcillin
- Oxacillin

• *Not available in US: Floxacillin*
• *No longer available: Methicillin, Cloxacillin*
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

2. Antistaphylococccyl Penicillins

ANTIBACTERIAL SPECTRUM:

• Same spectrum as Penicillin G, but less potent
  • But ALSO active against:
    ‣ MSSA (Resistant to hydrolysis by β-Lactamase)

• NO ACTIVITY:
  ‣ MRSA
  ‣ Enterococci
  ‣ Anaerobic bacteria
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: PENICILLINS

2. Antistaphylococccyl Penicillins

PHARMACOKINETICS:
• Administration:
  • Dicloxacillin: Oral
  • Nafcillin: Injection
  • Oxacillin: Oral, Injection
• Oral Bioavailability
  • Dicloxacillin: 60-80%
  • Nafcillin: LOW, 10-20%
  • Oxacillin: 70-90%
• Protein binding: HIGH, 90-98%
• Half-lives: SHORT, 30-60 min
• Metabolism: Urine and feces
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: PENICILLINS

2. Antistaphylococcal Penicillins

DRUG INTERACTIONS:
• All: Tetracyclines
• Nafcillin: Strong inducer of CYP3A4. Can increase the metabolism of CYP3A4 substrates:
  • Oral contraceptives
  • Calcium channel blockers
  • Chemotherapeutics
  • Opiates
  • etc...
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: PENICILLINS

3. Aminopenicillins

   Amoxicillin
   Ampicillin
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: PENICILLINS

3. Aminopenicillins

ANTIBACTERIAL SPECTRUM:

- **HIGH ACTIVITY:**
  - Gram (+) cocci: most *Streptococci, Enterococci*

- **MODERATE ACTIVITY:**
  - Gram (-) bacilli:
    - *Enterobacteriaceae*
    - *Haemophilus*
    - *Salmonella*

- **NO ACTIVITY:**
  - most *Staphylococci*
  - Anaerobic bacteria
## Cell Wall Biosynthesis

**TRANSPEPTIDASE INHIBITORS: PENICILLINS**

### 3. Aminopenicillins

<table>
<thead>
<tr>
<th>PHARMACOKINETICS</th>
<th><strong>Amoxicillin</strong> vs. <strong>Ampicillin</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADMINISTRATION:</strong></td>
<td>ORAL</td>
</tr>
<tr>
<td><strong>ORAL BIOAVAILABILITY:</strong></td>
<td>95%</td>
</tr>
<tr>
<td><strong>PROTEIN BINDING:</strong></td>
<td>20%</td>
</tr>
<tr>
<td><strong>HALF-LIVES:</strong></td>
<td>1-2 hr</td>
</tr>
<tr>
<td><strong>% METABOLISM:</strong></td>
<td>30%</td>
</tr>
<tr>
<td><strong>EXCRETION:</strong></td>
<td>Urine</td>
</tr>
</tbody>
</table>
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

4. Extended Spectrum Penicillins (Antipseudomonal)

Piperacillin
Carbenicillin
Ticarcillin
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: PENICILLINS

4. Extended Spectrum Penicillins (Antipseudomonal)

ANTIBACTERIAL SPECTRUM:

• HIGH ACTIVITY:
  ▪ Gram (-) bacilli: *Pseudomonas aeruginosa*
  ▪ Gram (+) cocci: most Streptococci, Enterococci

• MODERATE ACTIVITY:
  ▪ Gram (-) bacilli: Enterobacteriaceae, *Haemophilus*

• NO ACTIVITY:
  ▪ Gram (+): most Staphylococci
  ▪ Anaerobic bacteria
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

4. Extended Spectrum Penicillins (Antipseudomonal)

PHARMACOKINETICS:

• ADMINISTRATION:
  • Piperacillin: Injection
  • Carbenicillin: Oral
  • Ticarcillin: Injection

• ORAL BIOAVAILABILITY
  • Piperacillin: NOT ABSORBED
  • Carbenicillin: 40%
  • Ticarcillin: NOT ABSORBED

• PROTEIN BINDING: MODERATE, 20-45%

• HALF-LIVES: SHORT, around 60 min

• METABOLISM: Mostly in URINE
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: PENICILLINS

4. Extended Spectrum Penicillins (Antipseudomonal)

ADVERSE EFFECTS:
Platelet dysfunction
(bind to ADP receptors on platelets and inhibit clotting)

Extended Spectrum Penicillins

Thrombin  →  Morpohology changes
Thromboxane  →  Surface adhesion

ADP → inhibitory  →  Ca²⁺

Platelet

Fibrinogen cross-linking
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: PENICILLINS

5. ß-Lactamase Inhibitors

Clavulanic Acid
Sulbactam
Tazobactam
Cell Wall Biosynthesis

TRANSPERPTIDASE INHIBITORS: PENICILLINS

5. $\beta$-Lactamase Inhibitors

MAJOR $\beta$-Lactamase producing pathogens:
- Staphylococci
- Gram (-) bacilli
- Anaerobes

Penicillin (ACTIVE drug) $\beta$-Lactamase $\rightarrow$ INACTIVE metabolite

$\beta$-Lactamase Inhibitor

- SIMILAR structure to penicillins
- But react IRREVERSIBLY with $\beta$-Lactamase (mechanism based inhibition)
Cell Wall Biosynthesis

TRANSPERPTIDASE INHIBITORS: PENICILLINS

5. β-Lactamase Inhibitors

**COMBINED FORMS:**

- Amoxicillin / Clavulanic Acid (Augmentin®) - ORAL
- Ampicillin / Sulbactam (Unasyn®) - INJ
- Ticarcillin / Clavulanic Acid (Timentin®) - INJ
- Piperocillin / Tazobactam (Zosyn®) - INJ
Cell Wall Biosynthesis

Classes of Cell Wall Biosynthesis Inhibitors

• Glycopeptides
• β-Lactams
  ▸ Penicillins
  ▸ Cephalosporins
  ▸ Carbapenems
• Monobactams
• Bacitracin
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

GENERAL PROPERTIES

• First isolated from fungus *Cephalosporium acremonium* (1948)
• Effective against a β-lactamase producing *Salmonella typhi*
• Inhibit bacterial TRANSPEPTIDASES (like penicillins)
• More RESISTANT to β-Lactamase than penicillins
• β-Lactamases that hydrolyze penicillins, but NOT cephalosporins are called *penicillinases*
• ONLY about 5-10% cross-hypersensitivity with penicillins
• NOT effective against Enterococci or *Listeria*
• Adverse effects are similar to penicillins, UNLESS NOTED
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

Cephalosporins

1. First Generation
2. Second Generation
3. Third Generation
4. Fourth Generation
# Cell Wall Biosynthesis

**TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS**

## GENERATIONS: DIFFERENT SPECTRA, DIFFERENT USES

<table>
<thead>
<tr>
<th>Gen</th>
<th>Common Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>Surgical prophylaxis</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>URI, Pneumonia (Community-acquired), Gonorrhea, Surgical Prophylaxis</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>Pneumonia (Nosocomial), Pylonephritis, Meningitis, cSSSI</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>Pneumonia (Nosocomial), Meningitis (post-surgical), Febrile neutropenia</td>
</tr>
</tbody>
</table>
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

Cephalosporins

1. First Generation
   - Cefazolin (Ancef®)
   - Cephalexin (Keflex®)
   - Cefadroxil (Duricef®)
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

- First Generation

ANTIBACTERIAL SPECTRUM:

- **HIGH ACTIVITY:**
  - Gram (+) cocci: MSSA, most Streptococci
  - Gram (-) bacilli: PEcK
    - *Proteus mirabilis*
    - *E. coli*
    - *Klebsiella Pneumoniae*

- **NO ACTIVITY:**
  - MRSA
  - *Pseudomonas*
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

Cephalosporins

2. Second Generation

- **Cefuroxime** (Ceftin®, Zinacef®)
- **Cefprozil** (Cefzil®)
- **Cefaclor** (Ceclor®)
- **Loracarbef** (Lorabid®)
- **Cefotetan** (Cefotan®)
- **Cefoxitin** (Mefoxin®)
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

- Second Generation

ANTIBACTERIAL SPECTRUM:

- **HIGH ACTIVITY:**
  - Gram (-) bacilli: HENPEcK
    - *Haemophilus influenzae*
    - *Enterobacter aerogenes*
    - *Neisseria*
    - *Proteus mirabilis*
    - *E. coli*
    - *Klebsiella Pneumoniae*

- **MODERATE ACTIVITY:**
  - Streptococci

- **NO ACTIVITY:**
  - MRSA
  - *Pseudomonas*

*Cefoxitin* is *technically* a cephapalcymcin and is also effective against *B. fragilis* (a Gram (-) anaerobe)
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

Cephalosporins

3. Third Generation

- **Cefdinir** (Omnicef®)
- **Cefditoren** (Spectracef®)
- **Cefixime** (Suprax®)
- **Cefotaxime** (Claforan®)
- **Cefpodoxime** (Vantin®)
- **Ceftibuten** (Cedax®)
- **Ceftriaxone** (Rochephin®)
- **Ceftizoxime** (Cefizox®)
- **Ceftazidime* (Fortaz®, Tazicef®)
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

- Third Generation

ANTIBACTERIAL SPECTRUM:

- **HIGH ACTIVITY:**
  - Gram (-) bacilli: HENPEcKS
    - *Haemophilus influenzae*
    - *Enterobacter aerogenes*
    - *Neisseria*
    - *Proteus mirabilis*
    - *E. coli*
    - *Klebsiella pneumoniae*
    - *Serratia marcescens*

- **MODERATE ACTIVITY:**
  - Streptococci

- **NO ACTIVITY:**
  - MRSA
  - *Pseudomonas*
  - ESBL-producing Gram (-) bacilli

*Ceftazidime* is ALSO effective against *Pseudomonas aeruginosa*
Cell Wall Biosynthesis

TRANSEPTIDASE INHIBITORS: CEPHALOSPORINS

Cephalosporins

4. Fourth Generation
   • Cefepime (Maxipime®)
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

- Fourth Generation

ANTIBACTERIAL SPECTRUM:
  - **HIGH ACTIVITY:**
    - Gram (-) bacilli:
      - Enterobacteriaceae
      - *Pseudomonas aeruginosa*
      - HACEK:
        - *Haemophilus*
        - *Actinobacillus*
        - *Cardiobacterium*
        - *Eikenella*
        - *Kingella*

- **NO ACTIVITY:**
  - MRSA
  - *B. fragilis*
**Cell Wall Biosynthesis**

**TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS**

**PHARMACOKINETICS**

**Orally Available Cephalosporins**

<table>
<thead>
<tr>
<th>Gen</th>
<th>Agent (Bioavailability)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>Cefadroxil (90%); Cephalexin (75%)</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>Cefprozil (95%); Cefaclor (95%); Cefuroxime (<strong>40-50%</strong>); Loracarbef (90%)</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>Cefdinir (<strong>15-20%</strong>); Cefditoren (15%); Cefixime (<strong>40-50%</strong>); Cefpodoxime (50%); Ceftibuten (80%);</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*If not listed, agent is injected*
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

PHARMACOKINETICS

• DISTRIBUTION: Some penetrate CNS enough to be effective
  3rd (ceftriaxone, cefotaxime) and 4th (cefepime)

• METABOLISM: MINIMAL hepatic metabolism, for most agents
  most of the dose is excreted unchanged

• ELIMINATION: Primarily RENAL*
  (adjust dose for renal insufficiency)

*except ceftriaxone (50% renal; 50% biliary)
  no dose adjustment unless BOTH hepatic
  and renal failure
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

PHARMACOKINETICS

• HALF-LIVES
  MOST cephalosporins: 1-2 hr
  Cefotetan: 3-5 hr
  Ceficixime: 2-4 hr
  *Ceftriaxone: 5-9 hr

*Long half-life means can be given ONCE daily
  (except for CNS infections, where
  higher drug concentrations are needed)
Cell Wall Biosynthesis
TRANSPEPTIDASE INHIBITORS: CEPHALOSPORINS

ADVERSE EFFECTS

- Hypersensitivity – Although cross-hypersensitivitiy with penicillin is LOW (5-10%), cephalosporins are contraindicated if there is a history of anaphylaxis
- Diarrhea, nausea
- Renal toxicity (RARE)
- Disulfiram-like effect: cefotetan
- Hyperprothrombinemia (hypercoagulability)
- Thrombocytopenia, Platelet dysfunction (hypocoagulability)
Cell Wall Biosynthesis

Classes of Cell Wall Biosynthesis Inhibitors

• Glycopeptides
• β-Lactams
  ▸ Penicillins
  ▸ Cephalosporins
  ▸ Carbapenems
• Monobactams
• Bacitracin
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: CARBEPENEMS

Imipenem and Cilastatin (Primaxin®)
Doripenem (Doribax™)
Meropenem (Merrem®)
Ertapenem (Invanz®)

GENERAL PROPERTIES:

• VERY BROAD spectrum (less for ertapenem)
• \(\beta\)-Lactam containing structures, similar to penicillins, but LACKING ring sulfur
• Synthetic derivatives of thienamycin (a natural product from *Streptomyces*)
• Inhibit transpeptidases (like other \(\beta\)-Lactams)
• Highly resistant to most \(\beta\)-Lactamases (SERINE-TYPE)
• Sensitive to hydrolysis by METAL-TYPE \(\beta\)-Lactamases
• HIGH potential for penicillin cross-hypersensitivity
Cell Wall Biosynthesis

**TRANSPEPTIDASE INHIBITORS: CARBEPENEMS**

**ANTIBACTERIAL SPECTRUM:**

- **HIGH ACTIVITY:**
  - Gram (+) cocci: MSSA, most Streptococci
  - Enterococci (NOT ertapenem)
  - *Pseudomonas* (NOT ertapenem)
  - Gram (-): ESBL-producing Enterobacteriaceae
    - (ESBL = extended spectrum β-Lactamase)*
  - Anaerobic bacteria

- **NO ACTIVITY:**
  - MRSA
  - Penicillin-resistant Streptococci

*ESBL* can cleave third-generation cephalosporins, so these drugs can be useful in cephalosporin-resistant strains
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: CARBEPENEMS

PHARMACOKINETICS

• ADMINISTRATION: INJ (IV, IM)
• METABOLISM:

Imipenem and Doripenem:
RENAL metabolism by *Dehydropeptidase I* (*DHP-1*)

![Diagram]

- **Imipenem** → **DHP-1** → NEPHROTOXIC metabolite
- **Cilastatin**
- **Doripenem** → **DHP-1** → NON-TOXIC metabolite
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: CARBEPENEMS

PHARMACOKINETICS

• EXCRETION: Urine, 70-80% UNCHANGED drug (some adjustments with renal impairment)
• HALF-LIFE: 1 hr (except ertapenem, 4 hr)
• PROTEIN BINDING:
  
<table>
<thead>
<tr>
<th>Drug</th>
<th>Protein Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>20%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>2%</td>
</tr>
<tr>
<td>Doripenem</td>
<td>8%</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>95%</td>
</tr>
</tbody>
</table>

  HIGH BINDING gives it a longer half-life

• DISTRIBUTION:
  
  Meropenem has EXCELLENT CNS penetration
  Useful for meningitis (while other carbapenems are NOT)
Cell Wall Biosynthesis

Classes of Cell Wall Biosynthesis Inhibitors

• Glycopeptides
• β-Lactams
  ▸ Penicillins
  ▸ Cephalosporins
  ▸ Carbepenems
• Monobactams
• Bacitracin
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: MONOBACTAMS

Aztreonam (Azactam®, Cayston®)

GENERAL PROPERTIES:

• Synthetic derivative of a natural product from a Gram (-) bacterium, *Chromobacterium violaceum*
• Contains ONLY a β-Lactam ring (i.e. monobactam)
• Useful mainly for Gram (-) infections
• Alternative in patients with known penicillin allergies
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: MONOBACTAMS

ANTIBACTERIAL SPECTRUM:

• HIGH ACTIVITY:
  ▸ Gram (-) bacilli
    • Pseudomonas aeruginosa

• NO ACTIVITY:
  ▸ Gram (+) bacteria
  ▸ Anaerobic bacteria
Cell Wall Biosynthesis

TRANSPEPTIDASE INHIBITORS: MONOBACTAMS

PHARMACOKINETICS

• ADMINISTRATION: INJ (IV, IM); INHALATION (nebulizer)
• EXCRETION: RENAL, 60-70% UNCHANGED drug in urine
  Injected doses adjusted with renal impairment
• HALF-LIFE: 2-3 hr
• PROTEIN BINDING: 50%
• DISTRIBUTION:
  WIDE into most tissues
  Normal CNS penetration LOW (1%),
  but inflamed meninges MODERATE (10-40%)

ADVERSE EFFECTS

Similar to β-lactams, except:
  • Lower incidence of cross-hypersensitivity
  • Inhalation causes high incidence of cough
Cell Wall Biosynthesis
Classes of Cell Wall Biosynthesis Inhibitors

• Glycopeptides
• β-Lactams
  ▸ Penicillins
  ▸ Cephalosporins
  ▸ Carbepenems
• Monobactams
• Bacitracin
Cell Wall Biosynthesis

PYROPHOSPHATASE INHIBITORS: BACITRACIN

Bacitracin

PPase

C55 C55 C55
Cell Wall Biosynthesis
PYROPHOSPHATASE INHIBITORS: BACITRACIN

ANTIBACTERIAL SPECTRUM:
• HIGH ACTIVITY:
  ▶ Gram (+) bacteria
    • *C. diff* – pseudomembranous colitis (ORAL)
    • *Staphylococci*
      ▸ Infant pneumonia (IM)
      ▸ Skin, eye infections (TOPICAL)
• NO ACTIVITY:
  ▶ most Gram (-) bacteria
Cell Wall Biosynthesis

PYROPHOSPHATASE INHIBITORS: BACITRACIN

ADVERSE EFFECTS:

• Typical use: TOPICAL (skin, eye)
  • Can cause contact dermatitis
  • Very Rare: anaphylaxis
• IM use for *Staph* pneumonia:
  • Renal failure: *glomerular and tubular necrosis*

TOXIC!

†
Antibacterial PC Topics:

• **Overview of Antibacterial Pharmacology**

• **Drugs by Drug Targets:**
  • Cell Wall Biosynthesis
  • **Protein Biosynthesis**
  • DNA Synthesis
  • Folate Metabolism
  • Novel Antibiotic Mechanisms
Bacterial Protein Synthesis

**REVIEW**

**Bacterial Genes**

- DNA (chromosomal or plasmid)
  - Transcription factors, RNA polymerase

- mRNA
  - Ribosomes, tRNA

- Proteins

**Examples:**

- **Borrelia:** 1 chromosome (1Mb) + several plasmids (5Kb – 200Kb)
- **E. coli:** 1 chromosome (4.6Mb)
- **Pseudomonas aeruginosa:** 1 chromosome (6.3 Mb)
- **Vibrio cholerae:** 2 chromosomes (2.9Mb + 1.1Mb)
Bacterial Protein Synthesis

REVIEW

Steps of mRNA translation (protein synthesis)

1. **INITIATION** – ribosome + mRNA assembly
2. **ELONGATION** – peptide chain synthesis
3. **TERMINATION** – ribosome + mRNA disassembly
4. **RIBOSOME RECYCLING**

Bacterial Ribosome

- 16S rRNA
- 5S rRNA
- 23S rRNA
- 30S subunit
- 50S subunit
- 70S “Polysome”
Bacterial Protein Synthesis

**INITIATION**

**Small subunit (30S)**

Initiation factors (IF1-IF3)

**Large subunit (50S)**

- **Acceptor (A) site**
- **Peptide (P) site**
- **tRNA**
- **AUG = initiator codon**
- **fMet = first amino acid**

**direction of mRNA movement**

**direction of codon reading**
1. "activated" tRNA binds A-site

2. "peptidyl transfer"

3. mRNA movement

3. "naked" tRNA diaplace
Bacterial Protein Synthesis
Classes of Protein Synthesis Inhibitors

Small subunit (30S) inhibitors
- Tetracyclines
- Glycylcyclines
- Aminocyclitols
- Aminoglycosides → Bactericidal

Large subunit (50S) inhibitors
- Macrolides
- Ketolides
- Lincosamides
- Oxazolidinones
- Chloramphenicol → Bactericidal
Bacterial Protein Synthesis

Classes of Protein Synthesis Inhibitors

Small subunit (30S) inhibitors
  • Tetracyclines
  • Glycylcyclines
  • Aminocyclitols
  • Aminoglycosides

Large subunit (50S) inhibitors
  • Macrolides
  • Ketolides
  • Lincosamides
  • Oxazolidinones
  • Chloramphenicol
Bacterial Protein Synthesis

**TETRACYCLINES**

- **Tetracycline** (Sumycin®, etc.)
- **Doxycycline** (Vibramycin®, Vibra-Tabs®, etc.)
- **Minocycline** (Solodyne®, etc.)
- **Demeclocycline** (Declomycin®)

**GENERAL PROPERTIES:**

- Natural products (tetracycline) or derivatives (others) from POLYKETIDES derived from *Streptomyces*
- BROAD-spectrum antibiotics (but resistance has increased dramatically)
- Bind REVERSIBLY to the 16S rRNA on the 30S ribosomal subunit
- Block access of activated tRNAs (inhibits protein synthesis)
Bacterial Protein Synthesis
TETRACYCLINES

ANTIBACTERIAL SPECTRUM:

• **BROAD** spectrum against Gram (+) and Gram (-)

• Effective against many “atypical” pathogens
  
  • *Bacillus anthracis* (Anthrax)
  • *Yersinia pestis* (Bubonic plague)
  • *Francisella tularensis* (Tularemia)
  • *Bordetella pertussis* (Whooping cough)
  • *Treponema pallidum* (Syphilis)
  • *Chlamydia tachomatis* (Chlamydia)
  • *Propionibacterium acnes* (Acne)
  • *Mycoplasma, Ureaplasma*
  • *Ehrlichia* (Erlichiosis, Rocky mountain unspotted fever)
  • *Rickettsia rickettsii* (Rocky mountain spotted fever)
  
   Bioterrorism
  
   STDs
Bacterial Protein Synthesis
TETRACYCLINES

PHARMACOKINETICS:

• ADMINISTRATION:
  Tetracycline (ORAL) – 80% absorbed
  Doxycycline (ORAL, IV) – 80-95% absorbed
  Minocycline (ORAL, IV) – 100% absorbed
  Demeclocycline (ORAL) – 65% absorbed

• ABSORPTION:
  • Chelate multivalent cations (Ca$^{2+}$, Mg$^{2+}$, Al$^{3+}$, Fe$^{3+}$)
  • Chelates are NOT ABSORBED in GI tract
  • Absorption inhibited by:
    • Milk, cheese, antacids, high iron diet (or supplements)
Bacterial Protein Synthesis

TETRACYCLINES

PHARMACOKINETICS:
• DISTRIBUTION:
  • WIDE distribution
  • Bind to calcium in teeth and bones
  • Minocycline (most lipophilic tetracycline) has GOOD access to CNS (best tetracycline for meningitis)

• METABOLISM:
  • Only minocycline undergoes significant hepatic metabolism (about 70%) – several metabolites
  Other tetracyclines excreted mostly unchanged

• ELIMINATION (HALF-LIVES range from around 8-20 hr):
  • Tetracycline: mainly in URINE (beware w/renal insuff.)
  • Minocycline: metabolized, products in feces
  • Others eliminated in BOTH urine and feces
Bacterial Protein Synthesis

TETRACYCLINES

ADVERSE EFFECTS

GI upset, diarrhea
- Worsened by consumption of dairy

Deposition in bone and teeth.
- Avoid use in pregnancy and in children
- DISCOLORATION and hypoplasia of teeth, stunted growth

Photosensitivity (esp. doxycycline)
- Easy sunburn (avoid intense sunlight)

Vestibular problems (esp. minocycline)
- Accumulates in the endolymph of the ear
- Causes vertigo, nausea, and vomiting

Superinfection (due to broad-spectrum activity)
- May occur with Candida (vaginal) or Staph, C. Diff. (intestinal)
Bacterial Protein Synthesis
Classes of Protein Synthesis Inhibitors

Small subunit (30S) inhibitors
• Tetracyclines
• Glycylcyclines
• Aminocyclitols
• Aminoglycosides

Large subunit (50S) inhibitors
• Macrolides
• Ketolides
• Lincosamides
• Oxazolidinones
• Chloramphenicol
Bacterial Protein Synthesis

GLYCYLICYCLINES

Tigecycline (Tygacil®)

GENERAL PROPERTIES:

• Only a single FDA approved drug in this class (2005)
• Similar in structure to minocycline
• Like tetracyclines, binds 16S rRNA (on 30S subunit)
• Blocks protein synthesis
Bacterial Protein Synthesis

GLYCYLICYCLINES

ANTIBACTERIAL SPECTRUM:

• BROAD spectrum against Gram (+) and Gram (-)
  • **MRSA**
  • **VRE**

• Alternative therapy for many “atypicals” (similar to tetracyclines)
Bacterial Protein Synthesis
GLYCICYCLINES

PHARMACOKINETICS:
• ADMINISTRATION: IV
• ORAL BIOAVAILABILITY: POOR
• METABOLISM: 40% hepatic (glucuronidation)
• HALF-LIFE: 24-48 hr
• ELIMINATION: 60% fecal (unchanged)
  40% urine (as glucuronide conjugate)
Bacterial Protein Synthesis

Classes of Protein Synthesis Inhibitors

Small subunit (30S) inhibitors
- Tetracyclines
- Glycylcyclines
- **Aminocyclitols**
- Aminoglycosides

Large subunit (50S) inhibitors
- Macrolides
- Ketolides
- Lincosamides
- Oxazolidinones
- Chloramphenicol
Bacterial Protein Synthesis

AMINOCYCLITOLS

Spectinomycin (Trobicin®)

GENERAL PROPERTIES:
• Only a single FDA approved drug in this class (1971)
• Often grouped with aminoglycosides, but different chemically
• Like tetracyclines, binds 16S rRNA (on 30S subunit)
• Blocks protein synthesis
• No longer widely used

ANTIBACTERIAL SPECTRUM:
• Gram (-) cocci
  • *Neisseria gonorrhoeae*
    (mainly to treat gonorrhea in penicillin-sensitive patients)
    Given by IM injection (gluteal)
Bacterial Protein Synthesis

Classes of Protein Synthesis Inhibitors

Small subunit (30S) inhibitors
- Tetracyclines
- Glycylcyclines
- Aminocyclitols
- Aminoglycosides

Large subunit (50S) inhibitors
- Macrolides
- Ketolides
- Lincosamides
- Oxazolidinones
- Chloramphenicol
Bacterial Protein Synthesis

**AMINOGLYCOSIDES**

*from Streptomyces (-MYCIN)*

- Streptomycin
- Kanamycin (Kantrex®)
- Tobramycin (TOBI®)
- Neomycin
- Paromomycin (Humatin®)
- Amikacin (Amikin®) – derived from Kanamycin

*from Micromonospora (-MICIN)*

- Gentamicin
Bacterial Protein Synthesis
AMINOGLYCOSIDES

GENERAL PROPERTIES:
• Natural products or semi-synthetic derivatives
• **NARROW** therapeutic index
• RESERVED for Serious infections
• **DUAL MECHANISM:**
  • Bind TIGHTLY to the **30S ribosomal subunit**
    a. Blocks polysome formation;
    b. Inhibits movement of mRNA
  • ALSO INHIBIT bacterial **electron transport chain**
    (only useful in aerobic bacteria)
• **BACTERICIDAL**
• Synergize well with β-lactams to kill resistant bacteria
• High **POSITIVE** charge (amino groups)
Bacterial Protein Synthesis

AMINOGLYCOSIDES

ANTIBACTERIAL SPECTRUM:

• HIGH ACTIVITY:
  ▸ Gram (-) bacilli
    ▶ *Pseudomonas aeruginosa*
    ▶ Enterobacteriaceae (*E. Coli*, *Klebsiella*, etc.)

• NO ACTIVITY:
  ▸ Anaerobic bacteria
  ▸ most Gram (+)
  ▸ Atypical

Active carrier transport across cytoplasmic membrane

Gram (-)  Gram (+)

Cannot get easily past the thick PG layer
Bacterial Protein Synthesis

AMINOGLYCOSIDES

PHARMACOKINETICS:

• ADMINISTRATION:
  • TOPICAL
    Neomycin
  • ORAL
    Neomycin (to kill bacterial flora prior to surgery)
    Paromomycin (for parasitic infections)
  • INJ
    Streptomycin, Kanamycin, Tobramycin, Amikacin, Gentimycin

• ORAL BIOAVAILABILITY: POOR (good for killing GI organisms)
Bacterial Protein Synthesis
AMINOGLYCOSIDES

PHARMACOKINETICS:

• DISTRIBUTION: POOR (LOW $V_d$)
  Low tissue accumulation
  (POOR access to CNS, Lungs)
• METABOLISM: NO hepatic metabolism (already water sol. !)
• HALF-LIFE: 2-4 hr (accumulate with renal failure)
• ELIMINATION: URINE (most unchanged, beware in renal insufficiency)
Bacterial Protein Synthesis

AMINOGLYCOSIDES

ADVERSE EFFECTS:

1. **Nephrotoxicity** (typically reversible, dose-related)
   
   - Aminoglycosides filtered through glomerulus
   - Bind to phosphotidylinositol (PI) in filtrate
   - Reabsorbed along with PI in proximal tubules by endocytosis – accumulates in these cells, causing tubular damage (mitochondrial inhibition)
   - Also inhibits tubular prostaglandin biosynthesis
   - **Deficiency in** $\text{PGE}_2$, $\text{PGI}_2$ **levels causes unopposed angiotensin II – vasoconstriction**
   - Vasoconstriction causes **decreased renal filtration**
Bacterial Protein Synthesis
AMINOGLYCOSIDES

ADVERSE EFFECTS:

2. Ototoxicity
   - Aminoglycosides accumulate in endolymph of ear
   - Causes cochlear (auditory) toxicity and vestibular (balance) toxicity
   - Combination with loop diuretics (furosemide) is not advised due to increased risk of ototoxicity

3. Neuromuscular blockade (RARE)
   *But can occur in AT-RISK patients:*
   - With myesthenia gravis
   - If also given NMJ blockers
Bacterial Protein Synthesis
Classes of Protein Synthesis Inhibitors

Small subunit (30S) inhibitors
• Tetracyclines
• Glycylcyclines
• Aminocyclitols
• Aminoglycosides

Large subunit (50S) inhibitors
• Macrolides
• Ketolides
• Lincosamides
• Oxazolidinones
• Chloramphenicol
Bacterial Protein Synthesis

MACROLIDES

Erythromycin
Clarithromycin (Biaxin®)
Azithromycin (Zithromax®)

GENERAL PROPERTIES:
• Erythromycin is a natural product polyketide derived from an actinomycete (Saccharopolyspora erythraea) in 1949
• Others are semisynthetic derivatives of erythromycin
• Inhibit 50S ribosomal subunit (Bind to 23S rRNA) (prevent ribosomal transpeptidase reaction)
• BROAD BUT NOT DEEP spectrum – i.e. effective against large variety of bacteria, but RESISTANCE is common (particularly to erythromycin) – penicillin alternatives
• Commonly used for outpatient respiratory infections
• Also used in combination therapy against *H. pylori*
Bacterial Protein Synthesis
MACROLIDES

ANTIBACTERIAL SPECTRUM:

- **HIGH ACTIVITY:**
  - Some Gram (+) bacteria
    - *Streptococcus* (some resistance)
  - Some Gram (-) bacteria
    - *Haemophilus*
    - *Moraxella*
    - *H. pylori* (azithromycin is active)
  - Atypical pneumonias
    - *Chlamydia*
    - *Legionella*
    - *Mycoplasma*

- **NO ACTIVITY:**
  - Staphylococci, enterococci, anaerobes
Bacterial Protein Synthesis

MACROLIDES

PHARMACOKINETICS:

• ADMINISTRATION (oral bioavailability):
  Erythromycin:
    TOPICAL, OPHTHALMIC, ORAL (30-65%), INJ
  Clarithromycin:
    ORAL (50%)
  Azithromycin:
    OPHTHALMIC, ORAL (40%), INJ

• DISTRIBUTION:
  Wide in most tissues, but POOR access to CNS
  Azithromycin accumulates 10X more in tissues than others
  Macrolides accumulate in macrophages:
  They can “piggy-back” to sites of infection!
Bacterial Protein Synthesis

MACROLIDES

PHARMACOKINETICS

• METABOLISM
  
  Erythromycin and Clarithromycin: **CYP3A4** (major substrates)
  
  Azithromycin (minor metabolism)

• HALF-LIVES
  
  Erythromycin: 1-2 hr
  
  Clarithromycin: 3-7 hr
  
  Azithromycin: **72 hr** (3 days) – tissue accumulation

• EXCRETION (major routes):
  
  Feces: Erythromycin and Azithromycin (unchanged)
  
  Urine: Clarithromycin (dose adjustment in renal failure)
Bacterial Protein Synthesis
MACROLIDES

ADVERSE EFFECTS:
• GI UPSET, DIARRHEA (causes poor compliance)
  • Caused mainly by salt forms of erythromycin
    (estolate, ethylsuccinate and stearate salts)
  • Free base is less offensive
  • Can be used as a prokinetic agents!
• Cholestatic jaundice
  • Hypersensitivity reaction to etolate salt forms
• Ototoxicity
  • HIGH DOSES of erythromycin can cause deafness
• Long QTc
Bacterial Protein Synthesis
MACROLIDES

DRUG INTERACTIONS:

**CYP3A4 INHIBITION: erythromycin, clarithromycin**

Inhibit the metabolism of other CYP3A4 substrates:
Can cause HIGH concentrations of, examples:
- Terfenadine, astemizole (arrhythmias)
- Digitoxin (arrhythmias)
- Ca\(^{2+}\) channel blockers (cardiac depression)
- Statins (rhabdomyalosis)
- Opiates (opiate toxicity)

**QTc-prolonging drugs:**

Combination with known QTc prolonging drugs can cause re-entry arrhythmias
Bacterial Protein Synthesis
Classes of Protein Synthesis Inhibitors

Small subunit (30S) inhibitors
• Tetracyclines
• Glycylcyclines
• Aminocyclitols
• Aminoglycosides

Large subunit (50S) inhibitors
• Macrolides
• **Ketolides**
• Lincosamides
• Oxazolidinones
• Chloramphenicol
Bacterial Protein Synthesis
KETOLIDES

Telithromycin (Ketek®)

GENERAL PROPERTIES:
• Semi-synthetic derivative of erythromycin
• FDA approved in 2004
• Inhibit 50S ribosomal subunit (Interact with 23S rRNA)
  Prevent ribosomal transpeptidase reaction
  Binds stronger to 50S than macrolides

ANTIBACTERIAL SPECTRUM:
• Macrolide spectrum + \textit{S. pneumoniae}
Bacterial Protein Synthesis
KETOLIDES

PHARMACOKINETICS:
• ADMINISTRATION: ORAL (57% bioavailable)
• METABOLISM: Mainly CYP3A4 (also inhibits CYP3A4)
• HALF-LIFE: 10 hr
• ELIMINATION: Urine, as metabolites

ADVERSE EFFECTS:
• GI Upset,
• QTc prolongation
• HEPATOTOXICITY (rare)
• Can exacerbate symptoms of myasthenia gravis
• RESPIRATORY FAILURE in myasthenia gravis patients (BOXED warning!)
Bacterial Protein Synthesis

Classes of Protein Synthesis Inhibitors

Small subunit (30S) inhibitors
- Tetracyclines
- Glycylcyclines
- Aminocyclitols
- Aminoglycosides

Large subunit (50S) inhibitors
- Macrolides
- Ketolides
- Lincosamides
- Oxazolidinones
- Chloramphenicol
Bacterial Protein Synthesis

LINCOSAMIDES

Lincomycin (Lincocin®)
Clindamycin (Cleocin®) – more commonly used

GENERAL PROPERTIES:
- Isolated from Streptomyces sample
- First used in mid-1960’s
- Inhibit 50S ribosomal subunit (Interact with 23S rRNA)
  - Prevent ribosomal transpeptidase reaction
- Active against stationary-phase bacteria
  - Including bacteria that cause necrotizing fasciitis, which express virulence factors (toxins) and resistance genes during stationary phase
Bacterial Protein Synthesis
LINCOSAMIDES

ANTIBACTERIAL SPECTRUM:

- **MOST Active against:**
  - Gram (+) bacteria
    - *EXCEPT: C. dif., Enterococci*
  - Gram (-) anaerobic bacteria
    - *Bacteroides fragilis*
- **NOT Active against:**
  - Gram (-) aerobic bacteria
  - *Clostridium difficile*
  - Enterococci
Bacterial Protein Synthesis

LINCOSAMIDES

PHARMACOKINETICS:

• Administration: Topical, Oral (95-100% bioavailable!) Vaginal, Injection
• Distribution: wide, but NOT to CNS
• Metabolism: Hepatic
• Half-life:
  • Lincomycin: 5 hr
  • Clindamycin: 2-3 hr
• Elimination: Feces (70-80%), Urine

ADVERSE EFFECTS:

• CDAD – because they are wide spectrum, but do NOT inhibit growth of C. dif., can cause C. dif overgrowth and diarrhea (BOXED warning)
Bacterial Protein Synthesis
Classes of Protein Synthesis Inhibitors

Small subunit (30S) inhibitors
  • Tetracyclines
  • Glycylcyclines
  • Aminocyclitols
  • Aminoglycosides

Large subunit (50S) inhibitors
  • Macrolides
  • Ketolides
  • Lincosamides
  • Oxazolidinones
  • Chloramphenicol
Bacterial Protein Synthesis
OXAZOLIDINONES

Linezolid (Zyvox®)

GENERAL PROPERTIES:
• The only totally SYNTHETIC protein synthesis inhibitor
• Approved by FDA in 2000 (first novel class in 3 decades!)
• Inhibit 50S ribosomal subunit
  Studies show it interacts at both the A-site and P-site
• Extremely EXPENSIVE!
• Bacteriostatic in vitro
  But some reports suggest it to be bactericidal in vivo
Bacterial Protein Synthesis
OXAZOLIDINONES

ANTIBACTERIAL SPECTRUM:

• HIGH ACTIVITY:
  - Gram (+) cocci
    - Enterococci (including **VRE**)
    - Staphylococci (including **MRSA**)
    - Streptococci
  - Gram (+) bacilli
    - *Corynebacterium*
    - *Listeria monocytogenes*

• NO ACTIVITY:
  - Gram (-) bacteria
  - Anaerobes
Bacterial Protein Synthesis
OXAZOLIDINONES

PHARMACOKINETICS:

• ADMINISTRATION: Oral (95-100% bioavailable!), INJ (IV)
• DISTRIBUTION: Wide, but NOT well to CNS or bone
  ACCUMULATES up to 4X in lower respiratory tract
• METABOLISM: Hepatic (NON-P450 oxidation)
• HALF-LIFE: 4-5 hr
• ELIMINATION: URINE, mainly as METABOLITES
  (no dose adjustment needed for renal insufficiency – WHY?)
Bacterial Protein Synthesis

OXAZOLIDINONES

ADVERSE EFFECTS:
- GI Upset (nausea, diarrhea)
- Lactic acidosis
- Bone marrow suppression (with long-term use)
- Neuropathies (with long-term use)
  - Peripheral
  - Optic (vision loss)

DRUG INTERACTIONS:
- INHIBITS monoamine oxidase (MAO)
  Combination with SSRIs, Triptans and can cause serotonin syndrome
Bacterial Protein Synthesis

Classes of Protein Synthesis Inhibitors

Small subunit (30S) inhibitors
• Tetracyclines
• Glycylcyclines
• Aminocyclitols
• Aminoglycosides

Large subunit (50S) inhibitors
• Macrolides
• Ketolides
• Lincosamides
• Oxazolidinones
• Chloramphenicol
Bacterial Protein Synthesis

CHLORAMPHENICOL

Chloramphenicol (Chloromycetin®)

GENERAL PROPERTIES:

• Discovered in 1940’s in *Streptomyces* sample
• Inhibit 50S ribosomal subunit
  Prevent ribosomal transpeptidase reaction
• Once widely used, but highly toxic
• Bactericidal
  (but usually used *at bacteriostatic concentrations*,
  MIC not MBC to prevent toxicity!)
Bacterial Protein Synthesis

CHLORAMPHENICOL

ANTIBACTERIAL SPECTRUM:

• HIGH ACTIVITY:
  – Bacterial meningitis
    (ALTERNATIVE to 3rd Gen Cephalosporins)
    • Haemophilus influenzae
    • Neisseria meningitidis
    • Streptococcus pneumoniae
  – Anaerobic bacteria
    (Abdominal and pelvic infections)
    • Bacteroides fragilis
  – Some atypicals
    • Chlamydia
    • Mycobacteria
    • Rickettsia rickettsii
Bacterial Protein Synthesis

CHLORAMPHENICOL

PHARMACOKINETICS:

• Administration: IV
  (but does have 80% oral bioavailability)
• Distribution: wide, even to CNS
• Metabolism: **Glucuronidation** (UDP-glucuronyl transferase)
• Half-life: 4 hr
• Elimination: Urine, *as inactive glucuronide metabolite*
  (renal failure does not significantly increase half-life of the active drug)
Bacterial Protein Synthesis

CHLORAMPHENICOL

ADVERSE EFFECTS:

Blood Dyscrasias: Aplastic and Hemolytic anemias
High risk in glucose-6-phosphate dehydrogenase (G6PD) DEFICIENCY: because G6PD protects erythrocytes from oxidative stress

Gray Baby Syndrome
• Neonates, infants do not express much UDP-glucuronyl transferase, so drug ACCUMULATES
• Symptoms:
  ✓ Ash-gray coloration
  ✓ Vomiting
  ✓ Hypotension
  ✓ Cardiovascular collapse
Antibacterial PC Topics:

• Overview of Antibacterial Pharmacology

• **Drugs by Drug Targets:**
  • Cell Wall Biosynthesis
  • Protein Biosynthesis
  • DNA Synthesis
  • Folate Metabolism
  • Novel Antibiotic Mechanisms
Bacterial DNA Synthesis

• Bacteria do not have a distinct cell cycle
• Replication occurs constantly in growing cultures

Critical proteins:
• DNA Helicase
• DNA Gyrase (Topoisomerase II)
• Topoisomerase IV
• DNA Primase
• DNA Polymerase
Bacterial DNA Synthesis

**REVIEW**

**Origin of Replication**

Several “initiator” proteins bind to ORI and melt (separate) DNA strands.

Then DNA helicase binds.

And continues unwinding DNA.

“strand melting”
Bacterial DNA Synthesis REVIEW

Unwinding (Helicase)

DNA Gyrase

• DNA synthesis (unwinding) causes positive supercoiling
• DNA Gyrase introduces negative supercoils
DNA Gyrase
Catalytic cycle

1. G strand
2. T strand

Double strand cut
(Type II Topoisomerase)

2.

161

E. coli
DNA Gyrase
Crystal structures
Bacterial DNA Synthesis

**REVIEW**

- **RNA primer**
- Primase
- DNA Polymerase
- New DNA Strand
Bacterial DNA Synthesis

**REVIEW**

Holliday Junction

\[ \text{Intertwined chromosomes} \]

- Similar to DNA Gyrase, Topo IV is a bacterial Type II Topoisomerase

**Topoisomerase IV**

\[ \text{Intertwined chromosomes} \]
Bacterial DNA Synthesis
Classes of DNA Synthesis Inhibitors

• Fluoroquinolones
  • Norfloxacin (Noroxin®)
  • Ciprofloxacin (Cipro®)
  • Ofloxacin (Floxin®)
  • Levofoxacin (Levaquin®)
  • Moxifloxacin (Avelox®)
  • Gemifloxacin (Factive®)

• Aminocoumarins
  • Novobiocin (Albamycin®) – discontinued
Bacterial DNA Synthesis

**FLUOROQUINOLONES**

- Working on the synthesis of antimalarial drugs (chloroquine) at Sterling Drug (Bayer)
- **Nalidixic acid** was produced as a by-product (ONLY a quinolone – NOT a fluoroquinolone)
- **DID NOT** have strong anti-malarial activity
- **DID** have decent anti-bacterial activity (mainly against gram (-) bacteria)
- Main use: *uncomplicated, gram (-) UTI*
- **QUICKLY APPROVED** by FDA:
  - Discovery – 1962
  - FDA Approval – **1963**!
- Several historical marketed (brand) names:
  - Nevigramon, Neggram, Wintomylon
Bacterial DNA Synthesis

**FLUOROQUINOLONES**

**GENERAL PROPERTIES:**

- **Quinolones** (nalidixic acid, cinoxacin, oxolinic acid) only inhibit **DNA GYRASE**
  - ✓ Only useful for some Gram (-)
- **Fluoroquinolones** inhibit **DNA GYRASE AND TOPO IV**
  - ✓ Useful for both Gram (-) and Gram (+)
- **Nalidixic acid** had high toxicity: nausea, vomiting, abdominal pain, hyperglycemia
- **Fluoroquinolones** are **BROAD SPECTRUM** and **BACTERICIDAL**
- Cross-resistance to other drug classes is **LOW** because mechanism of action is quite unique
Bacterial DNA Synthesis

Fluoroquinolones

Mechanism

Stabilize the cleavage complexes of DNA gyrase AND Topo IV:
- Disrupt DNA synthesis
- Lead to lethal double strand breaks
Bacterial DNA Synthesis

**FLUOROQUINOLONES**

Dates of FDA APPROVALS

- **Norfloxacin** (Noroxin®) – 1986
- **Ciprofloxacin** (Cipro®) – 1987
- **Ofloxacin** (Floxin®) – 1990
- **Levofloxacin** (Levaquin®) – 1996
- **Moxifloxacin** (Avelox®) – 1999
- **Gemifloxacin** (Factive®) – 2003
Bacterial DNA Synthesis

FLUOROQUINOLONES

ANTIBACTERIAL SPECTRUM

Gram (-)
Enterobacteriaceae
Nalidixic Acid
Pseudomonas
Norfloxacin
*S. pneumoniae*
Ciprofloxacin, Ofloxacin
Atypicals
Levofloxacin, Gemifloxacin**
Anaerobes
Moxifloxacin*

* Not active against Pseudomonas or UTIs
* Mainly used for Streptococcus pneumoniae
Bacterial DNA Synthesis
FLUOROQUINOLONES

ANTIBACTERIAL SPECTRUM: Bioterrorism

Biological Warfare Agents:

• *Bacillus anthracis* (Anthrax)
  Widened mediastinum, pleural effusion, rapid severe respiratory distress and failure, shock (not usually hemoptysis).

• *Yersenia pestis* (Bubonic plague)
  Fulminant (quick) pneumonia, hemoptysis, rapid respiratory failure, septicemia and shock.

• *Francisella tularensis* (Tularemia)
  Pneumonitis, ARDS, pleural effusion, hemoptysis, sepsis. Ocular lesions, skin ulcers, oropharyngeal or glandular disease.
Bacterial DNA Synthesis

FLUOROQUINOLONES

PHARMACOKINETICS:

ADMINISTRATION

• Norfloxacin – ORAL
• Ciprofloxacin – ORAL, IV
• Ofloxacin – ORAL, OPTHALMIC, OTIC
• Levofloxacin – ORAL, OPTHALMIC, IV
• Moxifloxacin – ORAL, OPTHALMIC, IV
• Gemifloxacin – ORAL

ORAL BIOAVAILABILITY:

• **GOOD** for all fluoroquinolones (80-100%)
• However: Multivalent **CATIONS** *(milk, antacids, iron, etc.)* significantly **DECREASE** absorption! – should separate consumption of these from drug.
Bacterial DNA Synthesis
FLUOROQUINOLONES

PHARMACOKINETICS:

METABOLISM:
- Some NOT metabolized (e.g. Ofloxacin, Levofloxacin)
- Others are CONJUGATED in liver
  - Glucuronidation (urinary elimination)
  - Sulfation (fecal elimination)
- NONE are major substrates of P450’s

HALF-LIVES:
- Range from 3-8 hr
  (Moxifloxacin considerably longer, 12-16 hr)

EXCRETION:
- MOST are excreted in BOTH Urine and Feces
- Except: Ofloxacin, Levofloxacin (URINE, UNCHANGED)
  (dose adjustments for renal failure)
Bacterial DNA Synthesis

**FLUOROQUINOLONES**

**ADVERSE EFFECTS:**
- GI Upset (nausea, diarrhea)
- Headache
- **Photosensitivity**
- **Achilles tendon** pain, rupture (rare – BOXED warning)
- CNS reactions (dizziness, confusion, vivid dreams)
- Long QTc

**DRUG INTERACTIONS**
1. QTc-prolonging drugs
2. **Ciprofloxacin**: Strong CYP1A2 inhibitor
   - Can increase concentrations of CYP1A2 substrates (caffeine, some antidepressants)
Novobiocin (Albamycin®)

MECHANISM
Inhibits DNA gyrase
• Bind to GyrB subunits
• Inhibit ATPase function
  (necessary for “loading” of the enzyme with DNA strands)

ANTIBACTERIAL SPECTRUM:
  Bactericidal
  Effective against MRSA, but no longer available
  Available as a veterinary medication
Bacterial DNA Synthesis
AMINOCOUMARINS

ADVERSE EFFECTS (Novobiocin)
• GI upset (nausea, vomiting)
• Fever
• Hepatotoxicity
• Blood dyscrasias

POSSIBLE FUTURE APPLICATIONS
1. Alternative therapy for MRSA
2. Cancer (Hsp90 inhibitor)
Antibacterial PC Topics:
• Overview of Antibacterial Pharmacology
• **Drugs by Drug Targets:**
  • Cell Wall Biosynthesis
  • Protein Biosynthesis
  • DNA Synthesis
  • **Folate Metabolism**
  • Novel Antibiotic Mechanisms
Folate Metabolism
Classes of Antibacterial Folate Antagonists

Dihydropteroate Synthetase Inhibitors
  • **Sulfamethoxazole** (SMZ)
  • **Dapsone**

Dihydrofolate Reductase (DHFR) Inhibitors
  • **Trimethoprim** (TMP)

Sulfamethoxazole + Trimethoprim (**Co-Trimoxazole**)  
(Bactrim™, Septra®, generic)  
**5:1 SMZ:TMP** – theoretically **synergistic, bacteriostatic**  
(target different steps in folate metabolism)
Folate Metabolism
SULFAMETHOXAZOLE/TRIMETHOPRIM

GENERAL PROPERTIES
• Due to increasing resistance, is becoming LESS useful
• Use in limited by ALLERGY to sulfonamides in some patients (i.e. Sulfa Drug allergy)

ANTIBACTERIAL SPECTRUM:
✓ Some pneumonia pathogens (community acquired)
✓ UTIs and prostate pathogens (incl. prophylaxis)
✓ Nocardia sp.
✓ Alternative for:
  *Salmonella typhii* (typhus)
  MRSA
  *Listeria* (meningitis)
Folate Metabolism
SULFAMETHOXAZOLE/TRIMETHOPRIM

PHARMACOKINETICS:

• ADMINISTRATION (SMX-TMP): ORAL, INJ (IV)
• ORAL BIOAVAILABILITY: Very GOOD (90-100%)
• METABOLISM:
  SMX: Primary hepatic: CYP2C9 (oxidation)
    Secondary hepatic: N-acetylation + glucuronidation
  TMP: Hepatic: CYP2C9 (oxidation)
    forms REACTIVE METABOLITES (hepatotoxic?)

• HALF-LIVES:
  SMX: 9 hr
  TMP: 6-17 hr

• ELIMINATION: RENAL (beware in renal failure)
Folate Metabolism
SULFAMETHOXAZOLE/TRIMETHOPRIM

ADVERSE EFFECTS:

Rash (to sulfamethoxazole) – usually NOT severe
   (BUT, Stevens-Johnson syndrome has been documented)
Bone marrow suppression (at high doses – like chemotherapy!)
Crystaluria (renal damage) – caused by precipitation of
   N-acetylated sulfamethoxazole
Hyperkalemia – trimethoprim is similar in structure to
   triamterene (K⁺ sparing duretic)
Folate Metabolism
SULFAMETHOXAZOLE/TRIMETHOPRIM

Drug Interactions:

SMX and TMP are BOTH CYP2C9 Inhibitors
Can increase levels of CYP2C9 Substrates:
   Phenytoin
   Antidiabetic sulfonylureas
   NSAIDs (ibuprofen, diclofenac, celecoxib)
   Fluvastatin (Lescol®)
   Losartan (Cozaar®)
   Warfarin – can cause bleeding

Leucovorin (5-formyl-THF) – used in chemotherapy “rescue” will inhibit the antibacterial effect of SMX-TMP
Folate Metabolism

DAPSONE

GENERAL PROPERTIES:
• Also inhibits *dihydropteroate synthetase* (like sulfamethoxazole)
• Also inhibits *myeloperoxidase (MPO)* (anti-inflammatory)
• Has a **bacteriostatic** activity (like sulfamethoxazole)
• Has LIMITED usefulness against common infections
• Most useful against:
  - Pneumocystic pneumonia (yeast-like)
  - *Mycobacterium leprae* (leprosy)
• **Will be covered in later lectures**
Antibacterial PC Topics:

• Overview of Antibacterial Pharmacology

• **Drugs by Drug Targets:**
  • Cell Wall Biosynthesis
  • Protein Biosynthesis
  • DNA Synthesis
  • Folate Metabolism
  • **Novel Antibiotic Mechanisms**
Other Antibiotic Mechanisms

1. Inhibition of Transcription
2. Disruption of Ion Gradients
3. Production of Toxic Metabolites
Other Antibiotic Mechanisms

1. Inhibition of Transcription

<table>
<thead>
<tr>
<th>RNA Polymerase</th>
<th>RNA Strand</th>
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**Rifampin**

- Discovered in 1950’s, soil microbe, *Amycolatopsis rifamycinica*
- Bactericidal
- Very WIDE SPECTRUM of antibacterial activity, however...
- Mostly reserved for mycobacterial infections: tuberculosis, leprosy
Other Antibiotic Mechanisms

2. Disrupt Ion Gradients

How Bacteria Get Energy
(aerobically)

\[
\text{glucose} \rightarrow \text{H}_2\text{O} \quad \text{O}_2 
\]

proton gradient
Other Antibiotic Mechanisms

2. Disrupt Ion Gradients

**Gramicidin**
(topical use)
- 15 amino acid
- Linear peptide
- “punches holes in bacterial membranes”

**Daptomycin**
(IV use)
- Acts similar to gramicidin
- Useful for cSSSI, and for certain Staph infections (MSSA and MRSA)
Other Antibiotic Mechanisms

3. Production of Toxic Metabolites

A. Nitroimidazoles (antibacterial + antiparasitic)
   - Metronidazole (Flagyl®)
   - Tinidazole (Fasigyn®)

B. Nitrofurans
   - Nitrofurantoin (Furadantin®, Macrobid®, Macrodantin®)

• Taken up mainly by anaerobic bacteria
• Reduced by iron-sulfur proteins (ferridoxins)
• Reduction forms radical intermediates
• These generate superoxide anion (O$_2^-$) – highly reactive
• Cause oxidative damage inside the cell (DNA, proteins, etc.)
• Accumulation of cellular damage = ☠
Other Antibiotic Mechanisms

3. Production of Toxic Metabolites

Metronidazole

ANTIBACTERIAL SPECTRUM:

• MOST Active against:
  ▪ Gram (+) and gram (-) anaerobes
    • Bacteroides fragilis
    • Clostridium
  ▪ H. pylori (anaerobe-like)
  ▪ Parasitic infections

• NOT Active against:
  ▪ Aerobic Gram (+) and gram (-)
Other Antibiotic Mechanisms

3. Production of Toxic Metabolites

Metronidazole

PHARMACOKINETICS:
- ADMINISTRATION: Topical, Vaginal, Oral, Injection
- ORAL BIOAVAILABILITY: EXCELLENT (100%)
- METABOLISM: Minimal
- HALF-LIFE: 6-8 hr
- ELIMINATION: URINE (most unchanged)
  Beware with renal insufficiency

ADVERSE EFFECTS:
- GI Upset (nausea, vomiting)
- Metallic taste
- Disulfiram-like reaction
Other Antibiotic Mechanisms

3. Production of Toxic Metabolites

Nitrofurantoin

GENERAL PROPERTIES:

- UTIs caused by E. coli
- Action is dictated by pharmacokinetics:
  - ADMIN: Oral
  - HALF-LIFE: Only 20-60 min
- (however, rapidly ACCUMULATES IN URINE)
Other Antibiotic Mechanisms

3. Production of Toxic Metabolites

Nitrofurantoin

ADVERSE EFFECTS:

GI distress (drug should be taken with food)

Acute pneumonitis (seen after chronic therapy, may cause interstitial fibrosis!)

CNS effects: headaches, nystagmus, neuropathy with demyelination!

Hemolytic anemia
As usual, occurs most in patients with glucose-6-phosphate dehydrogenase deficiency and in pregnant women)
Other Antibiotic Mechanisms

3. Production of Toxic Metabolites

C. Hexamine

• **Methenamine** (Urex®)

**MECHANISM:**
- DECOMPOSES in *acidic pH* (< 5.5) to form **FORMALDEHYDE**
- **Bactericidal**
- Formulated as a weak acid (usually as mandelic acid) which lowers the urine pH and permits decomposition

**GENERAL PROPERTIES:**
- Suppression of chronic UTIs
- Toxic – **NO RESISTANCE** seen to develop
- However, some bacteria (e.g. *Proteus*) alkalinize the urine and are not killed
Other Antibiotic Mechanisms

3. Production of Toxic Metabolites

C. Hexamine
  • **Methenamine** *(Urex®)*

Pharmacokinetics:

Administration: **Oral** (BUT stomach pH is acidic!)
  10-30% degrades in stomach (unless enteric coating)
  Difficult to control release of formaldehyde
  Systemic toxicity is low (must be below pH 5.5 to be active)

**Ammonium ion** *(NH₄⁺)* is also produced
Converted to urea (nontoxic) by liver.
Contraindication = hepatic dysfunction
  (high NH₄⁺ is toxic, especially to CNS!)
Other Antibiotic Mechanisms

3. Production of Toxic Metabolites
   C. Hexamine
     • Methenamine (Urex®)

Adverse effects:
   1. GI distress (low pH = formaldehyde), major side effect
   2. High doses can cause hematuria (blood in urine), rash

Contraindications:
   1. Renal failure (mandelic acid may ppt. in the kidney)
   2. Hepatic dysfunction (NH₄⁺ accumulates, causes CNS toxicity)
   3. Sulfonamides (sulfonamides react chemically with formaldehyde and inactivate both agents)
Drug Resistance: Beta-Lactamases

**Molecular Classifications (A, B, C, D)**

A,C,D = **Serine-type**

- **Class A** (Includes ESBLs*) – Example genes (TEM-1; SHV-1; CTX-M)
  - Very common in Gram (-), some can also cleave cephalosporins, monobactams (aztreonam)
- **Class C** – Example genes (AmpC; ABA-1) – can also cleave cephalosporins and are *NOT inhibited by clavulanic acid*
- **Class D** – Oxacillinase (OXA-2) – can cleave oxacillin (and other antistaph PCNs)

B = **Metal-type** (zinc) aka Metallo-beta-lactamases (MBLs) – Example genes: (NDM-1)**

- Very broad activity and confer resistance against all beta-lactam antibiotics (except monobactams)

* **Extended Spectrum Beta Lactamases (ESBL)**
  - Cleave (inactivate) Penicillins + 3rd Gen Cephalosporins + Aztreonam
  - *THEY ARE inhibited by clavulanic acid*

** **New-Delhi Metallo Beta-Lactamase** (identified Dec 2009)