Chapter 19 - Antibacterial Agents

Eukaryote
- Membrane-enclosed nucleus
- Nucleolus
- Mitochondrion
- Ribosomes

Prokaryote
- Nucleoid
- Capsule (some prokaryotes)
- Flagellum
- Cell Wall (in some eukaryotes)
- Cell Membrane

Chemical agents:
- Sulphonamides
- Rifamycins
- Quinolones
- Aminoacridines
- Penicillins
- Cephalosporins
- Cycloserine
- Polymyxins
- Chloramphenicol
- Streptomycin
- Tetracyclines

Structure:
- Nuclear material
- DNA/RNA
- Cytoplasm
- Outer membrane (Gram-negative bacteria only)
- Cell wall
- Periplasmic space
- Ribosomes
- Plasma membrane
- Enzymes
Various classes of antibiotics (there are others)
A prokaryote is a single-celled organism that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle. Prokaryotes can be divided into two domains, Archaea and Bacteria. Species with nuclei and organelles are placed in the domain Eukaryota. Archaea and bacteria are generally similar in size and shape, although a few archaea have very strange shapes. Despite visual similarity to bacteria, archaea possess genes and several metabolic pathways that are more closely related to those of eukaryotes, notably the enzymes involved in transcription and translation. There is no connection between the shape of a bacterium and its color in the gram staining. Prokaryotic cells have various shapes; basic shapes of bacteria are:

The DNA of most bacteria is contained in a single circular molecule, called the bacterial chromosome, which sits in the cytoplasm of the bacterial cell. In addition to the chromosome, bacteria often contain plasmids – small circular DNA molecules. They can pick up new plasmids from other bacterial cells or they can also lose them. Every plasmid has its own ‘origin of replication’ and can copy themselves independently of the bacterial chromosome, so there can be many copies of a plasmid within one bacterial cell.

3 common medicinal targets – they also fight one another using chemical warfare.
1. Bacteria
2. Fungi
3. Viruses
Bacteria are prokaryotic microorganisms, typically a few micrometres in length. Bacteria are among the first life forms to appear on Earth, and inhabit soil, water, acidic hot springs, radioactive waste, and the deep portions of Earth's crust. Bacteria also live in symbiotic and parasitic relationships with plants and animals. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a millilitre of fresh water. There are approximately $5 \times 10^{30}$ bacteria on Earth, forming a biomass which exceeds that of all plants and animals. Bacteria are extremely adaptable to conditions, and survive wherever they are. Most bacteria have not been characterized, and only about half of the phyla of bacteria have species that can be grown in the laboratory. There are approximately ten times as many bacterial cells in the human flora as there are human cells in the body. Several species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, anthrax, leprosy and bubonic plague. The most common fatal bacterial diseases are respiratory infections, with tuberculosis alone killing about 2 million people per year.

Fungi include unicellular microorganisms such as yeasts and molds, as well as multicellular fungi that produce familiar fruiting forms known as mushrooms. Fungi are separate from the other eukaryotic life kingdoms of plants and animals. Fungi perform an essential role in the decomposition of organic matter and have fundamental roles in nutrient cycling and exchange in the environment. Since the 1940s, fungi have been used for the production of antibiotics. Many species also produce bioactive compounds such as alkaloids and polyketides, that are toxic to animals including humans.

Viruses are small infectious agents that replicate only inside the living cells of other organisms. Viruses can infect all types of life forms, including bacteria and archaea. Viruses are found in almost every ecosystem on Earth and are the most abundant type of biological entity. Virions, consist of two or three parts: (i) the genetic material made from either DNA or RNA, long molecules that carry genetic information; (ii) a protein coat, called the capsid, which surrounds and protects the genetic material; and in some cases (iii) an envelope of lipids that surrounds the protein coat when they are outside a cell. Viruses in animals can be spread by blood-sucking insects, by coughing and sneezing, by the faecal–oral route, entering the body in food or water, through sexual contact and by exposure to infected blood. In animals, viruses can provoke an immune response that can eliminate the infecting virus. Vaccines, can also stimulate an immune response. However, some viruses including those that cause AIDS and viral hepatitis evade these immune responses and result in chronic infections. Antibiotics have no effect on viruses, but several antiviral drugs have been developed.
INTRODUCTION TO PENICILLINS (known for ~80 years)

- Antibacterial agents which inhibit bacterial cell wall synthesis
- Discovered by Fleming from a fungal colony (1928)
- Shown to be non toxic and antibacterial
- Isolated and purified by Florey and Chain (1938)
- First successful clinical trial (1941)
- Produced by large scale fermentation (1944)
- Structure established by X-ray crystallography (1945)
- Full synthesis developed by Sheehan (1957)
- Isolation of 6-APA by Beechams (1958-60) - development of semi-synthetic penicillins
- Discovery of clavulanic acid and β-lactamase inhibitors
STRUCTURE

R = \[
\begin{array}{c}
\text{CH}_2 \\
\text{OCH}_2
\end{array}
\]

Benzyl penicillin (Pen G)

R = \[
\begin{array}{c}
\text{CH}_2 \\
\text{OCH}_2
\end{array}
\]

Phenoxyacetetyl penicillin (Pen V)

6-Aminopenicillanic acid (6-APA)

\[
\begin{array}{c}
\text{N} \\
\text{Me} \text{Me} \\
\text{CO}_2 \text{H}
\end{array}
\]

Thiazolidine ring

\[
\begin{array}{c}
\beta\text{-Lactam ring}
\end{array}
\]

Side chain varies depending on carboxylic acid present in fermentation medium

Present in corn steep liquor

Penicillin G

Penicillin V

(first orally active penicillin)
Biosynthesis

cysteine

valine

6-APA

(+)-6-aminopenicillanic acid

penicillins

Shape of penicillins

Folded 'envelope' shape
Properties of Penicillin G

• Active vs. Gram positive bacilli and some Gram negative cocci
• Non toxic
• Limited range of activity
• Not orally active - must be injected
• Sensitive to β-lactamases
  (enzymes which hydrolyse the β-lactam ring)
• Some patients are allergic
• Inactive vs. Staphylococci

Drug Development

Aims
• To increase chemical stability for oral administration
• To increase resistance to β-lactamases
• To increase the range of activity
SAR = structure activity relationships

Conclusions

• Amide and carboxylic acid are involved in binding
• Carboxylic acid binds as the carboxylate ion
• Mechanism of action involves the β-lactam ring
• Activity related to β-lactam ring strain (subject to stability factors)
• Bicyclic system increases β-lactam ring strain
• Not much variation in structure is possible
• Variations are limited to the side chain (R)
Mechanism of action

- Penicillins inhibit a bacterial enzyme called the transpeptidase enzyme which is involved in the synthesis of the bacterial cell wall
- The β-lactam ring is involved in the mechanism of inhibition
- Penicillin becomes covalently linked to the enzyme’s active site by means of an ester link to a serine residue
- Penicillin is not split in two and acts as a steric shield to prevent access of substrate or water to the active site
- Results in irreversible inhibition

- Covalent bond formed to transpeptidase enzyme
- Irreversible inhibition
Mechanism of action - bacterial cell wall synthesis

Bond formation inhibited by penicillin
Mechanism of action - bacterial cell wall synthesis

D-Alanine

TRANSPEPTIDASE

PENICILLIN

Cross linking
Mechanism of action - bacterial cell wall synthesis

- Penicillin inhibits final crosslinking stage of cell wall synthesis
- It reacts with the transpeptidase enzyme to form an ester linkage with a serine residue
- The ring-opened penicillin acts as a steric shield
- Neither substrate nor water is capable of reaching the ester link
- Results in irreversible inhibition
- Inhibition of transpeptidase leads to a weakened cell wall
- Cells swell due to water entering the cell, then burst (lysis)
- Penicillin thought to mimic D-Ala-D-Ala

Normal Mechanism
Mechanism of inhibition - bacterial cell wall synthesis

Alternative theory - Pencillin mimics D-Ala-D-Ala.

Mechanism inhibited by penicillin

Penicillin can be seen to mimic acyl-D-Ala-D-Ala

d-carbohydrates  L-amino acids

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Gram positive and Gram negative Cell Walls

- Penicillins have to cross peptidoglycan layers in order to reach their target enzyme
- Peptidoglycan layers are porous and are not a barrier
- The peptidoglycan layers of Gram positive bacteria are thicker than Gram negative cell walls, but the former are more susceptible to penicillins
Gram negative bacteria cell walls are thinner, but have extra layers

- Thin peptidoglycan layer
- Hydrophobic outer membrane - acts as a barrier to penicillins
- Gram negative more resistant to penicillins
Gram Positive

Plasma Membrane
Periplasmic space
Peptidoglycan

stain purple

Gram Negative

Plasma Membrane
Periplasmic space
Peptidoglycan
Outer membrane (lipopolysaccharide and protein)

stain pink

Gram Positive Cocci
Gram Negative Bacilli

Color and shape of the cells help classify which type of bacteria are present. Used with permission of AAFP-PT.
Resistance to Penicillins

Factors
• Gram negative bacteria have a lipopolysaccharide outer membrane preventing access to the periplasmic space
  Penicillins can only cross via porins in the outer membrane
  Porins allow small hydrophilic molecules such as zwitterions to cross
• High levels of transpeptidase enzyme may be present
• The transpeptidase enzyme may have a low affinity for penicillins (e.g. PBP 2a for *S. aureus*)
• Presence and concentration of β-lactamases in the periplasmic space
• Efflux mechanisms pumping penicillins out of the periplasmic space
• Transfer of β-lactamases between strains
• Mutations
Penicillin Analogues - Preparation

1) By fermentation
   • Vary the carboxylic acid in the fermentation medium
   • Limited to unbranched acids at the $\alpha$-position i.e. $\text{RCH}_2\text{CO}_2\text{H}$
   • Tedious and slow

2) By total synthesis
   • Only 1% overall yield
   • Impractical

3) By semi-synthetic procedures
   • Use a naturally occurring structure as the starting material for analogue synthesis

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Penicillin Analogues - Preparation

**Problem** - How does one hydrolyse the side chain by chemical means in presence of a labile β-lactam ring?

**Answer** - Activate the side chain first to make it more reactive

**Note** - Reaction with PCl$_5$ requires the involvement of a lone pair of electrons from nitrogen. Not possible for the β-lactam nitrogen.
Penicillin G (R = benzyl), Problem – It’s sensitive to stomach acids

Reasons for sensitivity

1) Ring strain

Relieves ring strain
Problem - It is sensitive to β-lactamases - enzymes which hydrolyse the β-lactam ring

Reasons for sensitivity

2) Reactive β-lactam carbonyl group
Does not behave like a tertiary amide (3° amides = 60/40 resonance split)

Tertiary amide

β-Lactam

• Interaction (resonance) of nitrogen’s lone pair with the carbonyl group is not possible
• Results in a reactive carbonyl group

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Problem - It has a limited range of activity

Reasons for sensitivity

3) Acyl side chain
Neighbouring group participation in the hydrolysis mechanism (self reaction)
Problems - Acid Sensitivity

Conclusions
• The β-lactam ring is essential for activity and must be retained
• Cannot tackle factors 1 and 2 (ring strain, reactive β-lactam)
• Can only tackle factor 3

Strategy
Vary the acyl side group (R) to make it electron-withdrawing to decrease the nucleophilicity of the carbonyl oxygen

![Chemical structure diagram showing electron-withdrawing group (E.W.G.) and decreased nucleophilicity](image)
Examples

Penicillin V
(orally active)

- Better acid stability and orally active
- But sensitive to β-lactamases
- Slightly less active than penicillin G
- Allergy problems with some patients

X = NH₂, Cl, PhOCONH, heterocycles. CO₂H

Very successful semi-synthetic penicillins, e.g. ampicillin, oxacillin
Problem - Sensitivity to β-Lactamases

β-Lactamases

• Enzymes that inactivate penicillins by opening β-lactam rings
• Allow bacteria to be resistant to penicillin
• Transferable between bacterial strains (i.e. bacteria can acquire resistance)
• Important w.r.t. *Staphylococcus aureus* infections in hospitals
• 80% *Staph.* infections in hospitals were resistant to penicillin and other antibacterial agents by 1960
• Mechanism of action for lactamases is identical to the mechanism of inhibition for the target enzyme
• But product is removed (hydrolyzed) efficiently from the lactamase active site
Problem - Sensitivity to β-Lactamases

Strategy

• Use of steric shields
• Block access of penicillin to the active site of the enzyme by introducing bulky groups to the side chain
• Size of shield is crucial to inhibit reaction of penicillins with β-lactamases, but not with the target transpeptidase enzyme (lucky us)
Problem - Sensitivity to β-Lactamases

Examples - Methicillin (Beechams - 1960)

*ortho* groups important

- Methoxy groups block access to β-lactamases but not to transpeptidases
- Binds less readily to transpeptidases compared to penicillin G
- Lower activity compared to Pen G against Pen G sensitive bacteria
- Poor activity vs. some *streptococci*
- Inactive vs. Gram negative bacteria
- Poorer range of activity
- Active against some penicillin G resistant strains (e.g. *Staphylococcus*)
- Acid sensitive since there is no electron-withdrawing group
- Orally inactive and must be injected
Problem - Sensitivity to β-Lactamases

Examples

\[
\begin{align*}
\text{Oxacillin} & \quad R = R' = H \\
\text{Cloxacillin} & \quad R = \text{Cl}, \quad R' = H \\
\text{Flucloxacillin} & \quad R = \text{Cl}, \quad R' = \text{F}
\end{align*}
\]

- Orally active and acid resistant
- Resistant to β-lactamases
- Active vs. \textit{Staphylococcus aureus}
- Less active than other penicillins
- Inactive vs. Gram negative bacteria
- Nature of R & R’ influences absorption and plasma protein binding
- Cloxacillin better absorbed than oxacillin
- Flucloxacillin less bound to plasma protein, leading to higher levels of free drug
Problem - Range of Activity

Factors
1) Cell wall may have a coat preventing access to the cell
2) Excess transpeptidase enzyme may be present
3) Resistant transpeptidase enzyme (modified structure)
4) Presence of β-lactamases
5) Transfer of β-lactamases between strains
6) Efflux mechanisms

Strategy
• The number of factors involved make a single strategy impossible
• Use of trial and error to vary R groups on the side chain
• Successful in producing broad spectrum antibiotics
• Results demonstrate general rules for broad spectrum activity.
Problem - Range of Activity

Results of varying R in Pen G

1) Hydrophobic side chains result in high activity vs. Gram positive bacteria and poor activity vs. Gram negative bacteria
2) Increasing hydrophobicity has little effect on Gram positive activity but lowers Gram negative activity
3) Increasing hydrophilic character has little effect on Gram positive activity but increases Gram negative activity
4) Hydrophilic groups at the α-position (e.g. NH₂, OH, CO₂H) increase activity vs Gram negative bacteria

Can we tell which enzyme is more polar?
Problem - Range of Activity

Examples of Broad Spectrum Penicillins

**Class 1 - NH₂ at the α-position**

Ampicillin and amoxicillin (Beechams, 1964)

![Ampicillin (Penbritin) and Amoxicillin (Amoxil) structures]

**Ampicillin (Penbritin)**
2nd most used penicillin

**Properties**
- Active vs Gram positive bacteria and Gram negative bacteria which do not produce β-lactamases
- Acid resistant and orally active
- Non toxic
- Sensitive to β-lactamases
- Increased polarity due to extra amino group
- Poor absorption through the gut wall
- Disruption of gut flora leading to diarrhoea
- Inactive vs. *Pseudomonas aeruginosa*
Problem - Range of Activity

Prodrugs of Ampicillin (Leo Pharmaceuticals - 1969)

Properties
- Increased cell membrane permeability
- Polar carboxylic acid group is masked by the ester
- Ester is metabolised in the body by esterases to give the free drug
Problem - Range of Activity

Mechanism of prodrug activation (pivampicillin)

- Extended ester is less shielded by the penicillin nucleus
- Hydrolysed product is chemically unstable and degrades
- Methyl ester of ampicillin is not hydrolysed in the body
- Bulky penicillin nucleus acts as a steric shield for methyl ester

Formaldehyde (toxic molecule, is this a problem?)
Problem - Range of Activity
Examples of broad spectrum penicillins

Class 2 - CO$_2$H at the $\alpha$-position (carboxypenicillins)

Examples

- Carbecillin = prodrug for carbenicillin (also makes phenol?)
- Active over a wider range of Gram negative bacteria than ampicillin
- Active vs. *Pseudomonas aeruginosa*
- Resistant to most $\beta$-lactamases
- Less active vs Gram positive bacteria (note the hydrophilic group)
- Acid sensitive and must be injected
- Stereochemistry at the $\alpha$-position is important
- CO$_2$H at the $\alpha$-position is ionised at blood pH ($\text{RCO}_2^-$)
Problem - Range of Activity
Examples of broad spectrum penicillins
Class 2 - CO₂H at the α-position (carboxypenicillins)

Examples

- Administered by injection
- Identical antibacterial spectrum to carbenicillin (thiophene vs. Phenyl)
- Smaller doses required compared to carbenicillin
- More effective against *P. aeruginosa*
- Fewer side effects
- Can be administered with clavulanic acid (inhibits β-lactamase)
Problem - Range of Activity

Examples of broad spectrum penicillins

Class 3 - Urea group at the $\alpha$-position (ureidopenicillins)

Examples

Azlocillin
Mezlocillin
Piperacillin

• Administered by injection
• Generally more active than carboxypenicillins vs. streptococci and *Haemophilus* species
• Generally have similar activity vs Gram negative aerobic rods
• Generally more active vs other Gram negative bacteria
• Azlocillin is effective vs *Pseudomonas aeruginosa*
• Piperacillin can be administered alongside tazobactam
Introduction - Cephalosporins

- Antibacterial agents which inhibit bacterial cell wall synthesis
- Discovered from a fungal colony in Sardinian sewer water (1948)
- Cephalosporin C identified in 1961 (X-ray crystallography)

Structure of Cephalosporin C

7-Aminoadipic side chain, (backwards attachment)

β-Lactam ring

Dihydrothiazine ring

7-Aminocephalosporinic acid (7-ACA)
Properties of Cephalosporin C

Disadvantages
• Polar due to the side chain - difficult to isolate and purify
• Low potency - limited to the treatment of urinary tract infections where it is concentrated in the urine
• Not absorbed orally

Advantages
• Non toxic
• Lower risk of allergic reactions compared to penicillins
• More stable to acid conditions
• More stable to β-lactamases
• Ratio of activity vs Gram negative and Gram positive bacteria is better

Conclusion
• Useful as a lead compound
Biosynthesis of Cephalosporins – similar to penicillins

SAR of Cephalosporins

• Similar to penicillins
• The β-lactam ring is crucial to the mechanism
• The carboxylic acid at position 4 is important to binding
• The bicyclic system is important in increasing ring strain
• Stereochemistry is important
• The acetoxy substituent is important to the mechanism

Possible modifications

• 7-Acylamino side chain
• 3-Acetoxyethylene side chain
• Substitution at C-7
Mechanism of Action

Note
The acetoxy group acts as a good leaving group and aids the mechanism
Variation of the 7-Acylamino Side Chain

- Not possible to generate analogues by fermentation
- Not possible to generate analogues by a full synthesis
- Restricted to semi-synthetic procedure

7-ACA

- 7-ACA not available by fermentation
- 7-ACA not available by enzymatic hydrolysis of cephalosporin C
- 7-ACA can be generated by a chemical hydrolysis
Variation of the 7-Acylamino Side Chain

Generation of 7-ACA

- Need to hydrolyse a relatively unreactive secondary amide in the presence of a labile β-lactam ring

![Chemical structures and reaction pathways](image)

- Range of cephalosporins becomes possible
First Generation Cephalosporins

Cephalothin

• More active than penicillin G vs. some Gram negative bacteria
• Less likely to cause allergic reactions
• Useful vs. penicillinase producing strains of *S. aureus*
• Not active vs. *Pseudomonas aeruginosa*
• Poorly absorbed from GIT
• Administered by injection
• Metabolised to give a free 3-hydroxymethylene group (deacetylation)
• Metabolite is less active

Cephalothin - drug metabolism

Strategy
Replace the acetoxy group with a metabolically stable leaving group

• Less active
• OH is a poorer leaving group
First-generation Cephalosporins

**Cephaloridine**

- The pyridine ring is stable to metabolism
- The pyridine ring is a good leaving group (neutralisation of charge)
- Cephaloridine exists as a zwitterion and is soluble in water
- Poorly absorbed through the gut wall
- Administered by injection

**Cefalexin**

- The 3-methyl group is not a leaving group
- Methyl group is bad for activity but aids oral absorption
- Can be administered orally
- A hydrophilic amino group at the $\alpha$-carbon of the side chain helps to compensate for the loss of activity due to the methyl group
First Generation Cephalosporins

Synthesis of cephalosporins with a 3-methyl substituent
First-generation Cephalosporins

Summary

• Generally lower activity than comparable penicillins
• Better range of activity than comparable penicillins
• Best activity is against Gram positive cocci
• Useful against some Gram negative infections
• Useful against *S. aureus* and streptococcal infections when penicillins have to be avoided
• Poorly absorbed across the gut wall (except for 3-methyl substituted cephalosporins)
• Most are administered by injection
• Resistance has appeared amongst Gram negative bacteria (presence of more effective β-lactamases)
Second-generation Cephalosporins

Cephemycins

- Isolated from a culture of *Streptomyces clavuligerus*
- First β-lactam to be isolated from a bacterial source
- Contains a 7-methoxy group
- Modifications carried out on the 7-acylamino side chain

Cephamycin C

Cefoxitin

- Broader spectrum of activity than most first-generation cephalosporins
- Greater resistance to β-lactamase enzymes
- The 7-methoxy group may act as a steric shield
- The urethane group is stable to metabolism
Second-generation Cephalosporins

Oximinocephalosporins

- Much greater stability against some β-lactamases
- Resistant to esterases due to the urethane group
- Wide spectrum of activity
- Useful against organisms that have gained resistance to penicillin
- Not active against *Pseudomonas aeruginosa*
- Used clinically against respiratory infections
Third-generation Cephalosporins
Oximinocephalosporins

- Aminothiazole ring enhances penetration of cephalosporins across the outer membrane of Gram negative bacteria
- May also increase affinity for the transpeptidase enzyme
- Good activity against Gram negative bacteria
- Variable activity against Gram positive cocci
- Variable activity vs. *Pseudomonas aeruginosa*
- Lack activity vs MRSA
- Generally reserved for troublesome infections
Third-generation Cephalosporins

Oximinocephalosporins

Ceftazidime

- Injectable cephalosporin
- Excellent activity vs. *P. aeruginosa* and other Gram negative bacteria
- Can cross the blood brain barrier
- Used to treat meningitis
Fourth-generation Cephalosporins

Oximinocephalosporins

- Zwitterionic compounds
- Enhanced ability to cross the outer membrane of Gram negative bacteria
- Good affinity for the transpeptidase enzyme
- Low affinity for some β-lactamases
- Active vs. Gram positive cocci and a broad array of Gram negative bacteria
- Active vs. Pseudomonas aeruginosa
Thienamycin

Acylamino side chain absent
Opposite stereochemistry to penicillins

Plays a role in β-lactamase resistance

Carbon

Double bond leading to high ring strain and an increase in β-lactam ring reactivity

Carbapenam nucleus

Merck 1976

- Isolated from *Streptomyces cattleya*
- Potent and wide range of activity vs Gram positive and Gram negative bacteria
- Active vs. *Pseudomonas aeruginosa*
- Low toxicity
- High resistance to β-lactamases
- Poor stability in solution (ten times less stable than Pen G)
Thienamycin analogues used in the clinic

**Imipenem**

mipenem is an intravenous β-lactam antibiotic discovered in 1980. It was the first member of the carbapenem class of antibiotics. Carbapenems are highly resistant to the β-lactamase enzymes and thus plays a key role in the treatment of infections not readily treated with other antibiotics. It was discovered via a lengthy trial-and-error search for a more stable version of the natural product thienamycin, which is unstable in aqueous solution.

**Meropenem**

Meropenem is an ultra-broad-spectrum injectable antibiotic used to treat a wide variety of infections. It belongs to the subgroup of carbapenem, similar to imipenem and ertapenem. Meropenem gained US FDA approval in July 1996. It penetrates well into many tissues and body fluids, including cerebrospinal fluid, bile, heart valve, lung, and peritoneal fluid.

**Ertapenem**

Ertapenem is a carbapenem antibiotic. It is a broadly active antibacterial that is used for infections caused by difficult to treat or multidrug-resistant bacteria. Ertapenem differs from other carbapenems in having a somewhat less broad spectrum of activity and in that its serum half-life allows it to be administered once every 24 hours.
Monobactams

Nocardicins (Fujisawa 1975)

- Monocyclic β-lactam ring
- Moderately active \textit{in vitro} vs narrow group of Gram negative bacteria
- Active vs. \textit{Pseudomonas aeruginosa}
- Inactive vs. Gram positive bacteria
- Different spectrum of activity from penicillins
- Thought to operate by a different mechanism from penicillins
- Low toxicity (can include skin rash and occasional abnormal liver functions)

Clinically useful monobactam

- Administered by intravenous injection
- Can be used for patients with allergies to penicillins and cephalosporins
- No activity vs. Gram positive or anaerobic bacteria
- Active vs. Gram negative aerobic bacteria
**β-Lactamase Inhibitors**

**Clavulanic acid (Beechams 1976)**

![Chemical structure of clavulanic acid]

- Isolated from *Streptomyces clavuligerus*
- Weak, unimportant antibacterial activity
- Powerful irreversible inhibitor of β-lactamases - suicide substrate
- Used as a sentry drug for ampicillin
- Augmentin = ampicillin + clavulanic acid
- Allows less ampicillin per dose and an increased activity spectrum
- Timentin = ticarcillin + clavulanic acid
Propose a possible mechanism?
Clavulanic acid - mechanism of action

Irreversibly blocked
β-Lactamase Inhibitors

Penicillanic acid sulfone derivatives

- Suicide substrates for β-lactamase enzymes
- Sulbactam has a broader spectrum of activity vs β-lactamases than clavulanic acid, but is less potent
- Unasyn = ampicillin + sulbactam
- Tazobactam has a broader spectrum of activity vs β-lactamases than clavulanic acid, and has similar potency
- Tazocin or Zosyn = piperacillin + tazobactam
Sulfphonamides – A Lead Compound

Notes
• Prontosil - red dye
• Antibacterial activity in vivo (1935)
• Inactive in vitro
• Metabolised to active sulphonamide
• Acts as a prodrug
• Sulphanilamide - first synthetic antibacterial agent acting on a wide range of infections
Structure-Activity Relationships

- Primary amino group is essential (R¹=H)
- Amide groups (R¹=acyl) are allowed
  - inactive *in vitro*, but active *in vivo*
  - act as prodrugs
- Aromatic ring is essential
- para-Substitution is essential
- Sulphonamide group is essential
- Sulphonamide nitrogen must be primary or secondary
- R² can be varied
Prodrugs of sulfonamides

Notes
- Amide group lowers the polarity of the sulphonamide
- Amide cannot ionise
- Alkyl group increases the hydrophobic character
- Crosses the gut wall more easily
- Metabolised by enzymes (e.g. peptidases) \textit{in vivo}
- Metabolism generates the primary amine
- Primary amine ionises and can form ionic interactions
- Ionised primary amine also acts as a strong HBD
Sulphanilamide analogues

\[ \text{R}^1\text{H}\text{N} - \text{SO}_{\text{NHR}^2} \]

Notes

• \(\text{R}^2\) is variable
• Different aromatic and heteroaromatic rings are allowed
• Affects plasma protein binding
• Determines blood levels and lifetime of the drug
• Affects solubility
• Affects pharmacokinetics rather than pharmacodynamics
Sulphanilamides - applications

Notes
• Antibacterial drugs of choice prior to penicillins (1930s), thousands of variations have been made
• Superseded by penicillins

Current uses
• Treatment of urinary tract infections
• Eye lotions
• Treatment of gut infections
• Treatment of mucous membrane infections
Mechanism of action

para-Aminobenzoic acid

Dihydropteroate synthetase

Reversible inhibition

Sulphonamides

L-Glutamic acid

Dihydrofolate

Dihydrofolate reductase

NADPH

Trimethoprim

Tetrahydrofolate (coenzyme F)
Mechanism of action

Target enzyme
• Dihydropteroate synthetase - bacterial enzyme
• Not present in human cells
• Important in the biosynthesis of the tetrahydrofolate cofactor
• Cofactor is crucial to pyrimidine and DNA biosynthesis
• Crucial to cell growth and division

Sulphonamides
• Competitive enzyme inhibitors
• Bacteriostatic agents
• Not ideal for patients with weakened immune systems
• Mimic the enzyme substrate - para-aminobenzoic acid (PABA)
• Bind to the active site and block access to PABA
• Reversible inhibition
• Resistant strains produce more PABA
Mechanism of action

Binding interactions
Mechanism of action

Metabolic differences between bacterial and mammalian cells
Dihydropteroate synthetase is present only in bacterial cells
Transport protein for folic acid is only present in mammalian cells

Sulphonamides - Drug Metabolism

Notes
• Sulphonamides are metabolised by $N$-acetylation
• $N$-Acetylation increases hydrophobic character
• Reduces aqueous solubility
• May lead to toxic side effects
Sulfonamides with reduced toxicity

Notes
• Thiazole ring is replaced with a pyrimidine ring
• Pyrimidine ring is more electron withdrawing
• Sulphonamide NH proton is more acidic and ionisable
• Sulphadiazine and its metabolite are more water soluble
• Reduced toxicity
• Silver sulphadiazine is used topically to prevent infection of burns

\[ \text{Sulphathiazole} \rightarrow \text{Sulphadiazine} \]

\[ \text{pK}_a \text{ 6.48} \]

86% Ionized
Examples of Sulphonamides

Sulphadoxine

• Belongs to a new generation of sulphonamides
• Long lasting antibacterial agent
• Once weekly dosing regime
• Sulphadoxine + pyrimethamine = Fanisdar
• Used for the treatment of malaria
Examples of Sulphonamides

Succinyl sulphathiazole

Notes
• Acts as a prodrug of sulphathiazole
• Ionised in the slightly acidic conditions of the intestine
• Too polar to cross the gut wall
• Concentrated in the gut
• Slowly hydrolysed by enzymes in the gut
• Used versus gut infections
Examples of Sulphonamides

Benzoyl prodrugs

- Too hydrophobic to cross gut wall
- Slowly hydrolysed by enzymes in gut
- Used versus gut infections
Examples of Sulphonamides

- Sulphamethoxazole + trimethoprim = co-trimoxazole
- Agents inhibit different enzymes in the same biosynthetic pathway
- Strategy of sequential blocking
- Allows lower, safer dose levels of each agent

Sulphones

- Thought to inhibit dihydropteroate synthetase
- Used in the treatment of leprosy
First sold in 1957 in West Germany (as Countergan) as a sedative or for anxiety, insomnia, tension. Began use as anti-nausea drug for morning sickness (October, 1957). Approximately 10,000 infants were born in 46 countries with malformation of the limbs (only about half survived). Other problems included deformed eyes, hearts, alimentary and urinary tracts, blindness and deafness. Thalidomide was banned.

However, in 1964 in Isreal, thalidomide was administered to a critically ill patient with leprosy. Afterwards the patient was able to sleep and get out of bed the next day. Thalidomide was revived as a treatment for leprosy. Women or reproductive age are required to use 2 forms of birth control, submit to regular pregnancy tests and undergo a patient education program. In Brazil, from 2005-2010 over 5,000,000 pills were dispensed with 100 reported cases of embreopathy.

In 1997, the desperate family of a critically ill cancer patient with multiple myeloma requested help from a Dr. Folkman. He could not get his preferred drug (TNP-470) and substituted thalidomide with dramatic improvement. Since then thalidomide had been used in combination with dexamethasone. In 2006 the combination was approved by the FDA for treatment of multiple myeloma.

dexamethasone

...has all sorts of uses:

- inflammatory and autoimmune conditions, such as arthritis, before and after dental surgery (wisdom teeth), plantar fasciitis, counter anaphylactic shock, used after eye surgery, as nasal spray, ear drops, etc.

\[2^8 = 256 \text{ possible stereoisomers}\]
Thalidomide is a strong angiogenesis inhibitor in vivo, but has no effect in cell culture. What might this suggest? It was felt that modification of thalidomide and its ability to racemize might make it more configurationally stable. Researchers in this paper wanted a similar electronic group to the imide carbonyl group next to the chiral center that would not allow racimization. They incorporated an oxetane ring at that position and found that it did alter the in vivo stability and metabolism. Explain how oxetane group is electronically similar to a carbonyl group, but does not allow racemization.

Lenalidomide is one of the novel drug agents used to treat multiple myeloma. Multiple myeloma is a cancer of the blood, which inhibits tumor angiogenesis, tumor secreted cytokines and tumor proliferation through the induction of apoptosis. Lenalidomide has significantly improved overall survival in myeloma (which formerly carried a poor prognosis), although toxicity remains an issue for users. It costs $163,381 per year for the average patient.