**Antibiotics II.**

**β-Lactam antibiotics**
Penicillins, cephalosporins, carbapenems, monobactams

- Fleming, 1928 – discovery of the antibiotic action;
- Florey, Chain, Abraham et al. 1938-1944
- Use of natural, biosynthetic penicillins – 1940’s
- 6-Amino-penicillanic acid, semisynthetic penicillins – 1959 –

Wilson & Gisvold p.301

**β-Lactam antibiotics nomenclature**

Instead of systematic names of the bicyclic ring systems, trivial names are often used.

penam = 4-thia-1-azabicyclo[3.2.0]heptan-7-one

**Penicillins**

Three characteristic fundamental structural requirements:
- Highly strained β-lactam ring → nonplanar, reactive -CONH- bond;
- Substituted 6-β-amino side chain;
- Free 3-carboxyl group

**Natural penicillins**

- Penicillium chrysogenum and P. notatum produce penicillins F, G, V, X etc. differing in their acyl side-chain.
Mode of action of β-lactam antibiotics

- Natural penicillins:
  - Precursor acids → directed biosynthesis.

Chemical properties

- Acid-base properties

Chemical reactions

- Reactivity of the β-lactam ring → chemical or enzymatical opening → loss of bioactivity
- Oxidation of the sulfur atoms, esterification of the C-3 carboxyl group results in the loss of activity.

Penicillin G

- Benzylpenicillin (Penicillin G) potassium
  - Benzylpenicillin natricum (Eu. Ph. 5)
  - Penicillin G Sodium
- Benzylpenicillin (Penicillin G) procaine
  - Benzylpenicillin procainum (Eu. Ph. 5)
  - Promptil® suspension for injection
- Benzylpenicillin (Penicillin G) benzathine
  - Benzylpenicillin benzathinum (Eu. Ph. 5)
  - Retarpen® suspension for injection
**Penicillin G**

- The oldest penicillin, still used today.
- Problems:
  - Sensitive to gastric acids (oral administration);
  - Large number of already resistant bacteria;
  - Rapid elimination.
- Use of sparingly soluble salts for depot i.m. injections → slow release.
- Uses: sensitive staphylococcal & streptococcal pharyngitis, meningitis; gonorrhea, syphilis; prophylaxis of bacterial endocarditis.
- All penicillins: allergy!

**Penicillin V**

- More resistant to inactivation by gastric acids
- Uses: similar to penicillin G

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**D-Penicillamine**

- Strong chelating agent, forms soluble complexes with many metal ions → promotes the excretion of metals from the body by the kidney.
- Uses:
  - Treatment of poisoning by heavy metal ions (lead, mercury or arsenic);
  - To remove excess copper in the patients with Wilson’s disease;
  - Effective in many patients with severe rheumatoid arthritis;
  - Preventing stone formation in the urinary tracts in cystinuria.

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**Natural penicillins**

- The drug content is expressed in international units (IU) in the case of old penicillins. ← microbiological assay of impure substances.
  - 1 mg of penicillin G potassium: 1598 IU
  - 1 mg of penicillin G sodium: 1670 IU
  - 1 mg of penicillin G procaine: 1011 IU

International units were and still are used in the case of hormones, antibiotics obtained from natural sources.

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**Semisynthetic penicillins**

- Drawbacks of natural or biosynthetic penicillins:
  - narrow antibacterial spectrum
  - β-lactamase (penicillinase) sensitivity
  - not stable under acidic conditions
- 1957 - 1959 - Isolation of 6-aminopenicillanic acid (6-APA)
Conversion to 6-APA

Chemical conversion to 6-APA

Syntheses of semisynthetic penicillins

Any mild chemical method can be used to acylate the 6-amino group

1) Acid chloride or Schotten-Bauman method

Semisynthetic penicillins

Of the tens of thousands of penicillins synthesized to date only a few are used nowadays.

Classification:

- Narrow or broad spectrum
- β-Lactamase resistant or not
- Acid resistant or not
- Orally or parenterally active

Semisynthetic penicillins

Rough classification

- Gram +, β-lactamase sensitive: penicillin G and V (natural)
- Gram + mainly, β-lactamase resistant: methicillin, oxacillin, cloxacillin, dicloxacillin
- Gram + and Gram -, β-lactamase sensitive: ampicillin, amoxicillin and alike
- Gram + and Gram -, Pseudomonas etc. (extended spectrum), β-lactamase sensitive: carbenicillin, piperacillin, etc.
Semisynthetic penicillins

<table>
<thead>
<tr>
<th>Name</th>
<th>Side chain (R)</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin sodium</td>
<td></td>
<td>Parenteral, oral and β-lactamase sensitive. Used to treat Gram + infections. Resistant Staph. aureus, (MRSA) are spreading.</td>
</tr>
<tr>
<td>Oxaclillin sodium</td>
<td></td>
<td>Oral, acid and β-lactamase sensitive. Used to treat Gram + infections. Resistant Staph. aureus resistant to penicillin. G.</td>
</tr>
<tr>
<td>Ampicillin trihydrate</td>
<td></td>
<td>Parenteral and oral, β-lactamase sensitive. Broad spectrum antibiotic used for urinary tract, G. or respiratory infections (Stapyloalla, gonococci etc.). Allergy.</td>
</tr>
<tr>
<td>Oxacillin trihydrate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ureidopenicillins have extended Gram-negative antibacterial spectra incl. Pseudomonas sp. (azlocillin, mezlocillin, piperacillin)

Comparison of ampicillin and amoxicillin

Active esters of penicillins

- Esters cleavable under physiological conditions by unspecific esterases are 'active esters' - prodrug concept.
- More complete gastrointestinal absorption.
- Active esters strongly bind to plasma → slow release of the active free acid → prolonged action.

Active esters of penicillins

Synthesis of piperacillin

Janssen & Russel, 1966
**Active esters of penicillins**

- **Penicillin G acetoxyethyl ester**
- **Ampicillin 1-(propionyloxy)ethyl ester**

**Bacampicillin vs. other penicillins**

- **Bacampicillin hydrochloride (Eu. Ph. S)**
- **Penicillin G tablet**

**ß-Lactamase enzymes**

- Intrinsic resistance, induced resistance.
- Classes of ß-lactamases: serine-dependent enzyme classes (classes A, C, and D) and one metal (Zn) dependent (class B).
- Difference between Gram + and Gram - bacteria

- Wilson & Gisvold p. 314

**ß-Lactamase enzymes**

- Old strategy: development of new semisynthetic derivatives which are active against resistant strains because
  - they are resistant toward ß-lactamase (methicillin, 6α-methoxy derivatives);
  - they inhibit ß-lactamase (oxacillin)
- New strategy: development of ß-lactamase inhibitors.

**The final steps of the synthesis of the cell wall:**
**ß-Lactamase inhibitors**

- Clavulanic acid, 1976, *Str. clavuligerus* (Merck)
- Weak antibacterial activity, but strong inhibitor of ß-lactamases
- Synthetic inhibitors: penicillin-sulfone derivatives.

**Combination of amoxycillin and clavulanic acid**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Amoxycillin + clavul. acid (µg/ml)</th>
<th>Clavul. acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacteroides fragilis</em> (28)</td>
<td>0</td>
<td>0.48  0.14  13.1</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (100)</td>
<td>&gt;5000</td>
<td>94.6 13.2  24.8</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em> (15)</td>
<td>150</td>
<td>0.72  0.44  36.8</td>
</tr>
<tr>
<td><em>Klebsiella aerogenes</em> (45)</td>
<td>315</td>
<td>1.75  0.89  33.2</td>
</tr>
<tr>
<td><em>Klebsiella aerogenes</em> (32)</td>
<td>&gt;9000</td>
<td>126   20   33.8</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em> (6)</td>
<td>&gt;40</td>
<td>0.18  -    5.6</td>
</tr>
<tr>
<td><em>Proteus</em> strains (23)</td>
<td>433</td>
<td>11.6  4.2  62.9</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (35)</td>
<td>106</td>
<td>0.72  0.17  17.1</td>
</tr>
</tbody>
</table>

- **ß-Lactam antibiotics + ß-lactamase enzyme inhibitor combinations**
  - Amoxycillin + clavulanic acid (Augmentin, Amil)
  - Ampicillin + sulbactam = sulbactam (Unasyn)

- **Inhibition of ß-lactamase by clavulanic acid**
  - Suicide inhibitor: covalently and irreversibly bonds to the enzyme at two sites → the enzyme cannot regenerate.

- **Natural cephalosporins**
  - Discovery, detection of antibiotic action of *Cephalosporium acremonium* – G. Brotzu, Italy, Sardinia, 1945;
  - First semisynthetic cephalosporin (cefalotin): 1964, Eli Lilly
Natural cephalosporin C

- Cephalosporin C
- Desacetyl cephalosporin C
- Desacetoxy cephalosporin C

- Cephalosporins are more stable than the penicillins, still sensitive to stronger bases or acids.
- α-Amino-adipyl side chain cannot be influenced during the fermentation.

7-Amino-cephalosporanic acid

- The 7-side chain cannot be removed enzymatically.
- The yield of fermentation is much lower than that of the penicillins.
- Chemical conversion of penicillins into cephalosporins.

7-Amino-3-deacetoxy-cephalosporanic acid

- The syn (Z) configuration of the oxim moiety is important (bioactivities of the Z: E isomers ~ 10:1).

Semisynthetic cephalosporins

- Modification at two sites to influence the antibacterial spectrum, the absorption, bioavailability, pharmacodynamics and metabolism.
- Roughly speaking:
  - The 7-NH substituent has more influence on the antibacterial spectra.
  - The 3-substituent has more influence on pharmacokinetics.

7ß-Acylamido side chain

- 3 or 4 generation cephalosporins: methoxyimino-(2-aminothiazolyl) acetyl side chain

7ß-Acylamido side chain

- 2-aminothiazol
- Methoxyimino
- Side chain of cefotaxim
- Side chain of nocardicin A
Generations of cephalosporins

- **First generation**: quickly metabolized (3'-OAc derivs.), sensitive to β-lactamases. Sometimes used against sensitive strains.
- **Second generation**: Extended antibacterial spectrum (E. coli, Proteus, Klebsiella, Haemophilus). 7-Aminobenzyl derivatives are quite sensitive to β-lactamases, newer are more resistant (cefamandol, cefuroxime).
- **Third generation**: Methoxyimino-aminothiazolyl 7-side chain. Good lactamase stability, very good Gram-negative activity, somewhat weaker Gram-positiv activity.
- **Fourth generation**: N⁺-(ammonium) type 3'-substituent. Very broad spectrum and good β-lactamase stability.