Beta-lactam antibiotics: Penicillins

Penicillium chrysogenum + Staph. Aureus

April 15, 2013
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Beta-lactams bind to transpeptidase in place of D-Ala-D-Ala

Transpeptidase (a PBP) normally binds to the D-Ala-D-Ala at the end of peptidoglycan precursors to crosslink the peptidoglycan. Beta-lactams such as penicillin mimic D-Ala-D-Ala, occupying the PBP active site and inhibiting crosslinking of peptidoglycan peptide bridges. Cell wall is weakened and this allows autolytic enzymes that degrade the peptidoglycan network to dominate, leading to lysis of the cells. Bactericidal.
Beta-lactams bind to transpeptidase in place of D-Ala-D-Ala

transpeptidase active site is occupied by beta-lactam and cannot bind D-Ala-D-Ala, thus crosslinking does not take place

bactericidal, provided bacteria is susceptible
Beta-lactam antibiotics

**Amoxicillin** *(penicillin)*

![Amoxicillin structure](image)

**Cephalexin** *(cephalosporin)*

![Cephalexin structure](image)

**Imipenem** *(carbapenem)*

![Imipenem structure](image)

**Aztreonam** *(monobactam)*

![Aztreonam structure](image)
Beta-lactam antibiotics

- **Penicillins**
  - Potent, safe, but alone generally narrow spectrum, and susceptible to beta-lactamases, allergy

- **Cephalosporins**
  - Less susceptible to penicillinases, less allergenic, broader spectrum in later “generations” (increased Gram- coverage)

- **Carbapenem**
  - IV, broad spectrum, resistant to most beta-lactamases; drugs of last resort

- **Monobactam**
  - IV, Gram- coverage (poor for Gram+), resistant to many beta-lactams
Penicillins G and V

- Isolated directly from penicillium molds; fermentation produces large yields
- Fairly narrow spectrum of coverage
- Highly susceptible to beta-lactamase and penicillinase (e.g. *S. aureus*) activity

**Penicillin G**  
(benzylpenicillin)

**Penicillin V**  
*Penicillin VK, potassium salt*  
(phenoxymethylpenicillin)
Semi-synthetic penicillins

- Cleave off natural sidechains with amidase to yield the 6-aminopenicillanic acid (6-APA) core, which can be synthetically substituted with other sidechains.
- Alter the spectrum, stability, bioavailability, resistance to penicillinases.
- Examples: amino-penicillins (amox, amp), nafcillin, dicloxacillin, piperacillin.
Penicillins general features

๏ Still widely prescribed and effective for bacteria that are not resistant

๏ Amoxicillin still one of the most widely used antimicrobials (#5 drug overall)

๏ Very potent, bactericidal, if pathogen is sensitive, MIC~0.02µg/ml

๏ Especially for semi-synthetic derivatives in combination w/ beta-lactamase inhibitors, can get broad spectrum coverage

๏ Generally very safe (good selective toxicity); targets the cell wall, which is unique to bacteria

๏ Penicillin allergies:

๏ One of the highest reported causes of a drug allergy, however actual incidence is believe to be somewhat lower than the 10% reported
Penicillin allergy

- 10% of population claims to be allergic to penicillins
  - Testing reveals that 1-3% in fact have IgE-mediated responses to the drugs

- Older penicillin preparations <1970 included more contaminants that may have contributed frequency of allergic reaction. Newer preparations are more pure.

- Infections themselves, including some viral infections (EBV, HIV, coxsackie, HBV, etc), can have associated rashes.

- Thus, it may not necessarily be the case that every patient who presents with a rash close to penicillin administration necessarily has a penicillin allergy
  - If a patient presents a rash, they are generally considered allergic
  - Fortunately, skin tests are now commercially available again
Penicillin allergy

- Most small, simple drugs such as penicillins do not themselves directly cause allergic reactions; instead they first form covalent adducts (hapten + carrier complexes) with host proteins.

- These complexes present antigens that are recognized by IgE antibodies, leading to immune responses.

- The beta-lactam ring is unstable. It opens up when bound to the PBP active site. Normally this isomer covalently acylates an active site residue to irreversibly shut down the PBP activity.
But an opened ring may also form an adduct with other proteins.

“**Major determinant**” of penicillin allergy involves conjugates forming with lysine side chains of host proteins. Penicilloyl lysyl antigen. IgE mediated response to major antigen.

“**Minor determinants**” include adducts formed with cysteine and carboxyl residues. IgE mediated response directed against “minor determinants”

**Major vs minor** only refer to the amounts of material observed, not to the immunological response.
Penicillin allergy: major determinant

- Small fraction of penicillin is isomerized to penicillenic acid, which reacts with the terminal amino group on lysine side chains, yielding the penicilloyl lysyl major determinant.

- 90% of modified proteins are of this variety.

- Response occurs within 72h.

- Urticaria (hives), rash, pruritus (itch).

- If a patient presents a rash, they are considered allergic.

Penicilloyl determinant (major)

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Penicillin allergy: minor determinant

“Minor determinants”: Result from reaction of other breakdown products with cysteine residues on proteins to form penicillenyl and penicillamine conjugates

These determinants are associated with serious reactions such as anaphylaxis, shock

- Usually appear within 1h of administration; immediate hypersensitivity (IgE)
- Anaphylaxis in 1:5,000-10,000 treatments (0.01-0.02%)
- ~0.0015% fatal outcome
Penicillin allergy: testing

- What is available?
  - PrePen (AllerQuest); back on the market as of May, 2010
    - Skin test (puncture or intradermal) containing the **benzylpenicilloyl major determinant** on a polylsine carrier; those who show a positive test (wheal and flare on skin) to PrePen have IgE against the major determinant and are classified as penicillin allergic
  - PrePen **does not include the minor determinant**. Fresh, diluted PenG may be added to for the minor determinant.

![Chemical structure](image)

- A patient may only exhibit an IgE-mediated response to the minor determinant
- Ideally a penicillin allergy test would include both major and minor determinants
Penicillin allergy

- For patients who have a positive skin test (PrePen+PenG), 40-70% may exhibit an anaphylactic reaction if penicillin were to be administered.

- For patients who have a negative skin test, 1-3% may exhibit a reaction (usually mild) when penicillin is administered.

- If minor determinants are not included (i.e. PenG), 3-10% of allergic patients may be missed by Pre-Pen.

**Desensitization.** If penicillin or beta-lactam is absolutely needed for patient with penicillin allergy, desensitization protocols are available (e.g. oral desensitization with PenV). Must be carefully monitored.

- Examples where this might be indicated:
  - *Streptococcus pyogenes* is still sensitive to penicillins, and they are potent.
  - Syphilis (caused by the spirochete *Treponema pallidum*) in pregnant women.

Penicillin allergy cross-reactivity

Cross-reactivity with other beta-lactams:

- If a patient has had a serious reaction (e.g. anaphylaxis), do not give other beta-lactams, except aztreonam (monobactam), which has no cross-reactivity

- Allergy to penicillins does not necessarily correlate with allergy to cephalosporins, but 10% does occur; if a patient has a rash w/ penicillin, may switch to a cephalosporin

- If a patient has shown an allergy to a cephalosporin, they are presumed to be allergic to penicillins as well

Oral administration gives fewer reactions than IV or IM, probably due to more gradual exposure of immune system to the drug

In general, the semi-synthetic penicillins show fewer allergic reactions

Ampicillin also has a separate rash associated with it; affects 9.5% of patients. Generally more allergenic than PenG or V
Comparative features of penicillins

- Stability of penicillins in stomach acid variable
  - PenG is destroyed by acid, PenV more stable
  - Newer derivatives such as amoxicillin have much better stability and bioavailability

<table>
<thead>
<tr>
<th>drug</th>
<th>oral absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>PenG</td>
<td>15-30%</td>
</tr>
<tr>
<td>PenV</td>
<td>60%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>30-50%</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>70-80%</td>
</tr>
</tbody>
</table>

- Susceptibility Gram+ penicillinases (narrow spectrum beta-lactamase) significant for PenG, PenV, amoxicillin, ampicillin
  - Dicloxacillin considered a “penicillinase resistant” penicillin

- Highly susceptibility to Gram- beta-lactamases; combine with beta-lactamase inhibitors to get coverage of Gram- bugs
General features of penicillins

- Not significantly metabolized. Significant amounts are excreted in unmodified, active form in urine. PenG ~80% excreted within 3-4h.
- Can be effective therapeutic for urinary tract infections (UTI)
- Frequent dosing required
- Co-administered with probenicid to inhibit renal excretion. Increases plasma concentrations of penicillins and cephalosporins ~2x.
- Low penetration to cerebrospinal fluid unless inflammation is present
## Properties of Penicillins

- **R-group side chain affects spectrum, activity, beta-lactamase susceptibility, immunogenicity**

<table>
<thead>
<tr>
<th>type of penicillin</th>
<th>R group structure</th>
<th>oral absorption</th>
<th>route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. &quot;Natural:&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin G</td>
<td><img src="image1" alt="Structure" /></td>
<td>poor</td>
<td>IV, IM</td>
</tr>
<tr>
<td>Penicillin V</td>
<td><img src="image2" alt="Structure" /></td>
<td>60-70%</td>
<td>PO</td>
</tr>
<tr>
<td>2. Amino</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td><img src="image3" alt="Structure" /></td>
<td>30-50%</td>
<td>PO, IV, IM</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td><img src="image4" alt="Structure" /></td>
<td>70-90%</td>
<td>PO</td>
</tr>
</tbody>
</table>

- **Amino penicillins, more polar, better Gram- porin penetration**
Penicillinase-resistant penicillins: “anti-\textit{Staph}” (MSSA) penicillins

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</thead>
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<tr>
<td>3. Penicillase resistant</td>
<td><img src="Image" alt="Structure" /></td>
<td>poor</td>
<td>IV, IM</td>
</tr>
<tr>
<td>Nafcillin</td>
<td><img src="Image" alt="Structure" /></td>
<td>poor</td>
<td>IV, IM</td>
</tr>
<tr>
<td>Oxacillin</td>
<td><img src="Image" alt="Structure" /></td>
<td>poor</td>
<td>IV, IM</td>
</tr>
<tr>
<td>Dicloxacillin</td>
<td><img src="Image" alt="Structure" /></td>
<td>good</td>
<td>PO</td>
</tr>
</tbody>
</table>

- Bulky side chains reduce binding of resistant penicillins to the penicillinase
- Methicillin was of this class, but no longer in use
# Extended spectrum penicillins: “anti-
\textit{Pseudomonas}” penicillins

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<th>R group structure</th>
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<th>route of administration</th>
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<tbody>
<tr>
<td>4. Extended spectrum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbenicillin (indanyl)</td>
<td><img src="image" alt="Carbenicillin" /></td>
<td>OK</td>
<td>PO</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td><img src="image" alt="Ticarcillin" /></td>
<td>poor</td>
<td>IV, IM</td>
</tr>
<tr>
<td>Piperacillin</td>
<td><img src="image" alt="Piperacillin" /></td>
<td>poor</td>
<td>IV, IM</td>
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- Charged, polar R-group improved permeability through Gm-porins
- Improved binding to PBP3 in \textit{Pseudomonas}
- Used synergistically with aminoglycosides

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\item Improved binding to PBP3 in \textit{Pseudomonas}
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\end{itemize}
## Antimicrobial spectrum of penicillins

<table>
<thead>
<tr>
<th>penicillin type</th>
<th>Staph penicillinase resistance?</th>
<th>Gram- beta-lactamase resistance?</th>
<th>useful coverage</th>
<th>route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Penicillin G</strong></td>
<td>no</td>
<td>no</td>
<td>Gram+; <em>S. pyogenes</em>, some <em>neisseria</em> (Gm-)</td>
<td>IV</td>
</tr>
<tr>
<td><strong>Penicillin V</strong></td>
<td>no</td>
<td>no</td>
<td>Gram+; some <em>neisseria</em></td>
<td>PO</td>
</tr>
<tr>
<td><strong>Ampicillin</strong></td>
<td>no</td>
<td>no</td>
<td>Gram+; some Gram- due to better porin penetration</td>
<td>IV, IM</td>
</tr>
<tr>
<td>(aminopenicillin)</td>
<td></td>
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<td><strong>Amoxicillin</strong></td>
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<td>(aminopenicillin)</td>
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<td></td>
</tr>
<tr>
<td><strong>Nafcillin</strong></td>
<td>yes</td>
<td>no</td>
<td><em>Staph</em> (not MRSA)</td>
<td>IV, IM</td>
</tr>
<tr>
<td>(penicillinase-resistant)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oxacillin</strong></td>
<td>yes</td>
<td>no</td>
<td><em>Staph</em> (not MRSA)</td>
<td>IV, IM</td>
</tr>
<tr>
<td>(penicillinase-resistant)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dicloxacillin</strong></td>
<td>yes</td>
<td>no</td>
<td><em>Staph</em> (not MRSA)</td>
<td>PO</td>
</tr>
<tr>
<td>(penicillinase-resistant)</td>
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</tr>
</thead>
</table>
| **Piperacillin** (extended spectrum) | no                              | bit better than ticarcillin; usually combined w/ a lactamase inhibitor e.g. tazobactam | Gram+ anaerobes
*Pseudomonas*
Enterobacteriaceae
{Enterobacter, E.Coli, Klebsiella, Serratia, Salmonella, Shigella, Providencia, Citrobacter, Proteus} | IV, IM                  |
| **Ticarcillin** (extended spectrum) | no                              | no; usually combined w/ a lactamase inhibitor, e.g. clavulanic acid | similar to pip.                                                               | IV, IM                  |
Beta-lactamase inhibitors

- Used to overcome beta-lactamase-related resistance to beta-lactams; **not** effective against all beta-lactamases, however
- Bind to beta-lactamase active site and irreversibly inactivate them by forming a covalent linkage to the active site serine
- Minimal antibiotic activity by themselves
- Combination therapy with beta-lactam antibiotics
  - Example: Augmentin (amoxicillin + clavulanic acid)

**clavulanic acid**

**sulbactam**

**tazobactam**
## Penicillins combined with beta-lactamase inhibitors

<table>
<thead>
<tr>
<th>penicillin type</th>
<th>Staph beta-lactamase (penicillinase) resistance?</th>
<th>Gram- beta-lactamase resistance?</th>
<th>useful coverage</th>
<th>route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin + clavulanate (Augmentin ®)</td>
<td>yes</td>
<td>yes</td>
<td>broad</td>
<td>PO</td>
</tr>
<tr>
<td>Ampicillin + sulbactam (Unisyn ®)</td>
<td>yes</td>
<td>yes</td>
<td>broad</td>
<td>IM, IV</td>
</tr>
<tr>
<td>Ticarcillin + clavulanate (Timentin ®)</td>
<td>yes</td>
<td>yes</td>
<td>broad</td>
<td>IV, IM</td>
</tr>
<tr>
<td>Piperacillin + tazobactam (Zosyn ®)</td>
<td>yes</td>
<td>yes</td>
<td>broad</td>
<td>IV, IM</td>
</tr>
</tbody>
</table>
Penicillins combined with beta-lactamase inhibitors

- Generally would like to use as narrow spectrum of antibiotic as possible so as to reduce impact on non-pathogenic flora and so as to maintain balance, prevent overgrowth (e.g. *C. difficile, Candida* yeast)

- But often we do not know the bacterial target, or may have a super-infection involving multiple species